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(54) Titre : PROCEDE DE VERIFICATION DE METHODOLOGIES D'ESSAI POUR METHODES ANALYTIQUES
(54) Title: ASSAY VERIFICATION CONTROL FOR ANALYTICAL METHODS

(57) **Abrégé/Abstract:**

A method for verifying that an assay methodology is properly performed that assay reagents employed possess the necessary potency for accurately performing such assay methodology, and whether or not test samples or assay reagents have been tampered with or are adulterated, is described. The method is performed by employing an assay verification sample, comprising a positive analyte component and the test sample under analysis, wherein the assay verification sample is analyzed employing the same assay reagents and essentially the same assay methodology employed to analyze the test sample. The method is particularly useful for performing heterogeneous immunoassays on an automated continuous and random access analytical system.

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(54) Title: ASSAY VERIFICATION CONTROL FOR ANALYTICAL METHODS (57) Abstract A method for verifying that an assay methodology is properly performed that assay reagents employed possess the neces- sary potency for accurately performing such assay methodology, and whether or not test samples or assay reagents have been tampered with or are adulterated, is described. The method is performed by employing an assay verification sample, comprising a positive analyte component and the test sample under analysis, wherein the assay verification sample is analyzed employing the same assay reagents and essentially the same assay methodology employed to analyze the test sample. The method is particularly useful for performing heterogeneous immunoassays on an automated continuous and random access analytical system.		

ASSAY VERIFICATION CONTROL FOR ANALYTICAL METHODS

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Field of the Invention

10 The present invention relates to assay controls for the analysis of a test sample. In particular, the present invention relates to methods of providing assay verification controls in an analytical system for determining the presence or amount of an analyte in a test sample.

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Background of the Invention

 Generally, analysis of a test sample involves the reaction of test samples with one or more reagents with
20 respect to one or more analytes wherein it is frequently desired that the analysis be performed on a selective basis with respect to each test sample. Typically, such analysis involves forming a reaction mixture comprising the test sample and one or more assay reagents, and the reaction
25 mixture is then analyzed by an apparatus for one or more characteristics of the test sample. In order to meet the growing demands of the modern clinical laboratory to provide consistently reliable and accurate results, various devices and automated apparatus for performing such analysis have been
30 provided.

 For example, automated clinical analyzers are presently available for automatically performing such analysis of a test sample. Such analyzers typically include a transport or conveyor system designed to transport containers of sample
35 liquids between various operating stations. For example, a reaction tube or cuvette containing a test sample may pass through a reagent filling station, mixing station, reaction

forming station, detection stations, analysis stations, and the like. In particular, various automated immunoassay analyzers have been provided such as the Abbott IMx® analyzer and the Abbott TDx® analyzer (Abbott Laboratories, Abbott Park, Illinois, USA) which utilize procedures involving a variety of different assay steps which rely on detection and measurement of optical changes in a reaction mixture during the assay process. Random access analyzers have also been described which not only can analyze multiple test samples, but multiple analytes may be analyzed from each test sample. In addition, presently available sequential and random access analyzers include various reagents within the apparatus itself or placed near the apparatus for pipetting purposes. Liquid reagents, in bulk form, are selected for the various types of tests which are to be performed on the test sample, and are stored in or near the apparatus. The reagent delivery units, such as pumps and the like, along with valves, control and pipette mechanisms, are included in these automated analyzers so that different reagents can be mixed according to the type of test to be performed. Recently, automated apparatus and methods have been proposed for performing, selectively on the same sample, various homogeneous and heterogeneous assays concurrently in a random access fashion. Such apparatus and methods provide for the analysis of a plurality of liquid samples wherein each sample is analyzed with respect to at least one analyte utilizing both homogeneous and heterogeneous assay techniques.

When performing various assay methodologies on such automated instruments, various analytes under determination may occur at substantially low frequencies to thereby provide a large number of negative results. In addition, prolonged storage of assay reagents are often required due to the large number of test samples which are analyzed by such automated instruments according to various assay methodologies during the course of a day. However, such prolonged storage of assay reagents could cause loss of assay reagent potency or

capability to provide accurate results due to such prolonged storage.

Although the use of positive control samples have been previously described to demonstrate that assay reagents are properly performing, the use of such positive control samples is time consuming and not cost effective because, in addition to the test samples being analyzed, separate assays are required to be performed employing such positive control samples. Accordingly, there exists a need to provide verification of a negative assay result when performing such assay methodologies to ensure that assay reagents employed therein possess the required potency, and that such negative results are not due to inferior assay reagents or test samples which have been tampered with.

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Summary of the Invention

The present invention provides a method for verifying that an assay methodology is properly performed, and that the assay reagents employed therein possess the necessary potency for accurately performing such assay methodology, by employing a assay verification sample therein. According to the method, such verification is accomplished by forming an assay verification sample, which can be formed in situ, comprising a positive analyte component, the test sample under analysis, and one or more assay reagents, wherein the assay verification sample is analyzed employing the same assay reagents and essentially the same assay methodology employed to analyze the test sample. When performing the method of the present invention, such assay verification sample can be analyzed simultaneously with or subsequent to the analysis of the test sample.

The method according to the present invention verifies that a negative result is due to the absence of analyte rather than (i) assay reagents which lack potency or which are otherwise not capable of performing to provide accurate results, (ii) test samples or assay reagents which have been

tampered with or adulterated, such as the addition of blocking agents or inhibitors to a test sample for the analysis of such test sample for drugs of abuse and other controlled substances, (iii) incorrect processing of a test sample, such as incorrect pipetting steps or addition of reagents, or (iv) the presence of contaminants which may be present in, for example, test tubes, reaction vessels, assay containers, assay devices, and the like, as the result of a manufacturing process. The method of the present invention is particularly useful in an automated instrument which typically does not perform the same pipetting sequence consecutively, such as the automated continuous and random access analytical system described herein.

15 Brief Description of the Drawings

FIGURE 1 is an isometric view of an automated analytical system described herein illustrating the system cabinetry, exposed front end carousel, computer screen and keyboard.

FIGURE 2 is an isometric view of the automated analytical system apparatus frame and cabinet.

FIGURE 3 is a top plan view of the automated analytical system in section with component covers removed to show the automated analytical system apparatus in detail and relative position.

FIGURE 4 is a front elevational view of the automated analytical system in isolation and partial section of elements of the front end carousel.

FIGURES 4A and 4B represent a perspective side elevational view and partial end view of a reagent pack and reagent pack cover means for use with the automated analytical system.

FIGURE 5 is a top view in isolation and partial section of drive and guide elements of the front end carousel of the automated analytical system being removed.

FIGURE 6 is a cross-sectional side view of a process carousel of the automated analytical system in isolation with two reaction vessels in place, one of which is in position for an FPIA read.

5 FIGURE 7 is an isometric view of the probe, probe arm and pipettor of the automated analytical system in isolation.

FIGURE 8 is a schematic side view of the probe arm wiring and sensor means of the automated analytical system.

10 FIGURE 9 is a cross-sectional side elevational view of an automatic bubble flushing syringe apparatus of the automated analytical system.

FIGURE 9A is a sectional side view in isolation of the syringe bore end portion of the automatic bubble flushing syringe with the reciprocating piston near the end of travel
15 toward the bore end portion.

FIGURE 9B is a sectional end view in isolation of the piston and bore of the automatic bubble flushing system syringe taken along line 9B-9D.

20 FIGURES 10 and 10A represent a top plan view of a reaction vessel and a side view of the reaction vessel for use with the automated analytical system, respectively, with reaction vessel compartments labeled where appropriate for FPIA processing.

25 FIGURES 10B and 10C present a top plan view and a side view of the reaction vessel, respectively, labeled and presented for MEIA processing.

30 FIGURE 11 is a sectional side view of the transfer element of the automated analytical system engaging a reaction vessel for transfer from the main carousel into the transfer station.

FIGURE 12 is a perspective side elevational view of the transfer station of the automated analytical system.

35 FIGURE 13 is a top plan view in section illustrating in isolation the controlled environment portion of the automated analytical system.

FIGURE 14 is a top plan view in section of the lower cabinet of FIGURES 1 and 2 illustrating water and/or buffer

supply station as well as liquid and solid waste containers of the automated analytical system.

FIGURE 15 is a schematic view illustrating the system control environment airflow and temperature control of the automated analytical system.

FIGURE 16 is a side elevational view in partial section of a MEIA cartridge for use with the automated analytical system.

FIGURE 17 is a side elevational view in section of a MEIA cartridge feeder of the automated analytical system.

FIGURE 18 is a side sectional view in isolation of the MEIA cartridge feeder-cartridge orientation pin mechanism of the automated analytical system.

FIGURE 19 is a side sectional view in isolation of the MEIA cartridge ejector of the automated analytical system.

FIGURE 20 is a box diagram of the optics signal processor of the automated analytical system.

FIGURE 21 is a schematic of the FPIA optical system of the automated analytical system.

FIGURE 22 is a schematic of the FPIA read[er] sequence of the automated analytical system.

FIGURE 23 is a side sectional view in isolation of a MEIA cartridge carousel of the automated analytical system, MEIA cartridge and MEIA reader.

FIGURE 24 is a schematic of the MEIA system optical assembly of the automated analytical system.

FIGURE 25 is a schematic of the MEIA read sequence of the automated analytical system.

FIGURE 26 is a schematic reaction sequence of a FPIA for T4 performed on the automated analytical system.

FIGURE 27 is a schematic reaction sequence of a one-step sandwich MEIA performed on the automated analytical system.

FIGURE 28 is a schematic reaction sequence of a two-step sandwich MEIA performed on the automated analytical system.

FIGURE 29 illustrates the steps for verifying the results in a MEIA procedure according to one embodiment of the present invention.

FIGURE 30 illustrates the steps for verifying the results in a MEIA procedure according to another embodiment of the present invention.

Description of the Invention

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Definitions

The following definitions are applicable to the present invention:

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The term "spectrophotometric assay", as used herein, refers to the interaction in an assay solution between the analyte to be determined and a reagent system specific for the analyte to produce a detectable change in the transmittive properties of the assay solution. The change in the transmittive properties refers to the amount of light absorbed or scattered by an assay solution within a particular wavelength band when a beam of light of known intensity is passed through the assay solution. The change in the transmittive properties of an assay solution is measured by passing monochromatic light having a known intensity through the assay solution and determining the ratio of the intensity of the transmitted or scattered light to the intensity of the incident light. Nearly all analytes either absorb energy of a specific wavelength or interact in an assay solution with a particular reagent system to produce a detectable change in the transmittive properties of the assay solution, characteristics which have resulted in the development of numerous specific spectrophotometric assays.

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Spectrophotometric assays which rely upon the measurement of the change in the transmittive properties of an assay solution as a measure of an analyte in the assay solution include, for example, assays wherein there is a change in the

color of the assay when there is a change in the turbidity of the assay solution, that is, turbidimetric or nephelometric assays.

5 The term "colorimetric assay", as used herein, refers to the change in the transmittive properties of an assay solution which is generally referred to as the absorbance of the assay solution and is dependent upon the change in the color of the assay solution due to the interaction of the analyte to be determined and reagent system specific for the analyte. The
10 absorbance of the assay solution is related to the concentration of the analyte in the assay solution. A colorimetric assay utilizes a chromogenic reagent system capable of interacting in an assay solution with the particular analyte of interest, to produce a detectable change in the
15 transmittive properties, specifically the color, of the assay solution. Numerous chromogenic reagent systems useful in the determination of specific analytes have been developed and are commercially available.

The term "turbidimetric assay", as used herein, refers to
20 the determination of the amount of light scattered or blocked by particulate matter as light passes through an assay solution. The analyte of interest interacts with a reagent system specific for the analyte to form a suspension of particles in the assay solution. As a beam of light having a known
25 intensity is passed through an assay solution, the suspension of particles formed by the interaction of the analyte reagent system blocks or scatters the incident light, thereby reducing the intensity of the light transmitted through the assay solution. The change of the transmittive properties in a
30 turbidimetric assay refers to the decrease in the intensity of the light transmitted through an assay solution and is related to the amount of incident light that is scattered or blocked by the suspension of particles, and depends upon the number of particles present and the cross-sectional area of such
35 particles.

The term "nephelometric assay", as used herein, is similar to a turbidimetric assay in that the analyte of

interest interacts with a reagent system specific for the ligand to form a suspension of particles in the assay solution. The change in the transmittive properties of the assay solution is also related to the amount of incident light
5 scattered or blocked by the suspension of particles. Unlike a turbidimetric assay wherein the intensity of the light transmitted through the assay solution is measured, the scattered or blocked light is measured at an angle to the light incident to the assay solution. Therefore, in a nephelometric
10 assay the change in the transmittive properties refers to the difference in intensities of light incident to the assay solution and light scattered at an angle to the incident light.

- The term "fluorometric assay", as used herein, refers to the determination of an analyte in an assay solution which is
15 chemically or immunologically transformed into a fluorescent complex or conjugate thereby producing a detectable change in the fluorescent properties of the assay solution. The change in the fluorescent properties of the assay solution is measured by exciting the fluorescent complex or conjugate
20 properties produced with monochromatic light of a wavelength within the excitation wavelength band of the fluorescer, and measuring the intensity of the emitted light at a wavelength within the emission wavelength band of the fluorescer. The fluorescent intensity of the emitted light is related to the
25 concentration of the analyte. However, the intensity of the fluorescence emitted by the assay solution may be inhibited when the analyte to be determined complexes with nonfluorescent interferences such as protein or phosphates present in the sample, or when the sample containing the
30 ligand to be determined has sufficient color so as to act as a filter and thereby reduce the intensity of the emitted fluorescence. It is well recognized that in order to maximize the sensitivity and specificity of a fluorometric assay, these inhibiting factors, if present, must be overcome either by
35 removal of the nonfluorescent interferences or color producing material prior to the analysis, or by compensating for the presence of such factors using an internal standard

added to a second aliquot of sample and carrying out the entire assay procedure using the aliquot containing the internal standard.

The term "homogeneous immunoassays", as used herein, refers to a competitive immunoassay format involving a competition between an analyte from a test sample and a tracer for a limited number of receptor binding sites on an antibody to the analyte. The tracer comprises the analyte or analog thereof labeled with a detectable moiety wherein the concentration of analyte in the test sample determines the amount of the tracer that will specifically bind to the antibody. The amount of the tracer-antibody conjugate produced by such binding may be quantitatively measured and is inversely proportional to the amount of analyte present in the test sample. For example, fluorescent polarization techniques for making such determination, such as in fluorescent polarization immunoassays as described herein, are based on the principle that a fluorescently labeled compound when excited by linearly polarized light will emit fluorescence having a degree of polarization inversely related to its rate of rotation. When a molecule such as a tracer-antibody conjugate having a fluorescent label is excited with a linearly polarized fluorescent molecule it is constrained from rotating between the time light is absorbed and emitted. When a "free" tracer molecule (i.e., unbound to an antibody) is excited by linearly polarized light, its rotation is much faster than the corresponding tracer-antibody conjugate and the molecules are more randomly orientated, therefore, the emitted light is polarized. Accordingly, when plane polarized light is passed through a solution containing the aforementioned reagents, a fluorescent polarization response is detected and correlated to the amount of analyte present in the test sample. Various fluorescent compounds which can be employed for performing fluorescent polarization assays include, but are not intended to be limited to, aminofluoresceins, such as described in U.S. Patent No. 4,510,251 and U.S. Patent No. 4,614,823,

triazinylaminofluoresceins, such as described in U.S. Patent No. 4,420,668 and U.S. Patent No. 4,593,089; carboxyfluoresceins, such as described in U.S. Patent No. 4,668,640 , and the like.

The term "heterogenous immunoassays", as used herein, refers to immunoassay formats involving a labeled reagent or tracer comprising an analyte, an analog of the analyte, or an antibody thereto, labeled with a detectable moiety, to form a free species and a bound species. In order to correlate the amount of tracer in one of such species to the amount of analyte present in the test sample, the free species must first be separated from the bound species, which can be accomplished according to methods known in the art employing solid phase materials for the direct Immobilization of one of the binding participants in the binding reaction, such as the antibody, analyte or analog of the analyte, wherein one of the binding participants is immobilized on a solid phase material, such as a test tube, beads, particles, microparticles or the matrix of a fibrous material, and the like, according to methods known in the art. Heterogenous immunoassays can be performed in a competitive immunoassay format wherein, for example, the antibody can be immobilized to a solid phase material whereby upon separation, the amount of the tracer which is bound to such solid phase material can be detected and correlated to the amount of analyte present in the test sample. Another form of a heterogeneous immunoassay employing a solid phase material is referred to as a sandwich immunoassay, which involves contacting a test sample containing, for example, an antigen with a protein such as an antibody or another substance capable of binding the antigen, and which is Immobilized on a solid phase material. The solid phase material typically is treated with a second antigen or antibody which has been labeled with a detectable moiety. The second antigen or antibody then becomes bound to the corresponding antigen or antibody on the solid phase material and, following one or more washing steps to remove any

unbound material, an indicator material such as a chromogenic substance which reacts with the detectable moiety (e.g., where the detectable moiety is an enzyme, a substrate for such enzyme is added) to produce a color change. The color change is then detected and correlated to the amount of antigen or antibody present in the test sample. For example, the teachings of the present invention can be employed in a heterogeneous immunoassay which can be performed by the automated analytical system described herein, in either a competitive or sandwich immunoassay format, or in a microparticle capture enzyme immunoassay, such as that described in Clinical Chemistry, Volume 34, No. 9, pages 1726-1732 (1988), employing microparticles as the solid phase material.

The term "test sample", as used herein, refers to a material suspected of containing the analyte. The test sample can be used directly as obtained from the source or following a pretreatment to modify the character of the sample. The test sample can be derived from any biological source, such as a physiological fluid, including, blood, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, raucous, synovial fluid, peritoneal fluid, amniotic fluid or the like. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can involve filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for the performance of environmental or food production assays. In addition, a solid material suspected of containing the analyte can be used as the test sample. In some instances it may be beneficial to modify a solid test sample to form a liquid medium or to release the analyte.

The term "analyte" or "analyte of interest", as used herein, refers to the compound or composition to be detected or measured and which has at least one epitope or binding site. The analyte can be any substance for which there exists a

naturally occurring binding member or for which a binding member can be prepared. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), virus particles and metabolites of or antibodies to any of the above substances. In particular, such analytes include, but are not intended to be limited to, ferritin; creatinine kinase MIB (CK-MB); digoxin; phenytoin; phenobarbital; carbamazepine; vancomycin; gentamycin; theophylline; valproic acid; quinidine; leutinizing hormone (LH); follicle stimulating hormone (FSH); estradiol, progesterone; IgE antibodies; vitamin B2 micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella-IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis B e antigen (HBeAg); antibodies to hepatitis B e antigen (Anti-HBe); thyroid stimulating hormone (TSH); thyroxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryonic antigen (CEA); and alpha fetal protein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines such as librium and valium; cannabinoids such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. The term "analyte" also includes any antigenic substances,

haptens, antibodies, macromolecules and combinations thereof.

The term "analyte-analog", as used herein, refers to a substance which cross-reacts with an analyte-specific binding member, although it may do so to a greater or lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog has at least one epitopic site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to an analyte-specific binding member.

The term "binding member", as used herein, refers to a member of a binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to antigen and antibody binding pair members, other binding pairs include, as examples without limitation, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g., ribonuclease, S-peptide and ribonuclease S-protein), and the like. Furthermore, binding pairs can include members that are analogs of the original binding member, for example, an analyte-analog or a binding member made by recombinant techniques or molecular engineering. If the binding member is an immunoreactant it can be, for example, a monoclonal or polyclonal antibody, a recombinant protein or recombinant antibody, a chimeric antibody, a mixture(s) or fragment(s) of the foregoing, as well as a preparation of such antibodies, peptides and nucleotides for which suitability for use as binding members is well known to those skilled in the art.

The term "detectable moiety", as used herein, refers to any compound or conventional detectable chemical group having a detectable physical or chemical property and which can be used to label a binding member to form a conjugate therewith. Such detectable chemical group can be, but is not intended to be limited to, enzymatically active groups such as enzymes, enzyme substrates, prosthetic groups or coenzymes; spin labels; fluorescers and fluorogens; chromophores and chromogens; luminescers such as chemiluminescers and bioluminescers; specifically bindable ligands such as biotin and avidin; electroactive species; radioisotopes; toxins; drugs; haptens; DNA; RNA; polysaccharides; polypeptides; liposomes; colored particles and colored microparticles; and the like.

The term "continuous access", as used herein, refers to the ability to add additional test samples or reagents to the automated analytical system described herein without the interruption of assays which are being performed by the automated analytical system at the time of such addition.

The term "random access", as used herein, refers to the ability of the automated analytical system described herein to simultaneously perform more than one scheduled assay in any order in which such plurality of scheduled assays are presented into the automated analytical system described herein.

The term "simultaneous", as used herein, refers to the ability of the automated analytical system described herein to independently perform two or more scheduled assays at the same time.

The term "kitting", as used herein, refers to the ability of the automated analytical system described herein to create a unit dose disposable by separately transferring test samples and reagents to a reaction vessel described herein without initiation of an assay reaction sequence.

The term "quat" refers to a polycationic material solution for assays.

The term "flexible protocols" refers to the variety of different assay protocols capable of being processed in

accordance with the inventive system. Examples include MEIA formats configured in 1-step and 2-step sandwich and competitive assay formats; order of activity processing, including the ability to initiate sample processing for both
5 MEIA formats and FPIA formats on the front-end carousel prior to transfer onto the process carousel; variable incubation periods; optical read formats and wash sequences. This contrasts to some prior art, known random access systems which force all assay protocols to adhere to a strict
10 "lock-step" format, in which assay configuration (i.e. 1-step versus 2-step formats), activity order, incubation timing, and other similar protocols are fixed by the instrument.

Assay Verification

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The method of the present invention can be employed in the various assay methods described herein, whether such methods are performed manually or with the various assay detection systems and instruments described herein. The
20 method of the present invention employs an assay verification sample comprising a positive analyte component, the test sample under analysis, and one or more assay reagents, wherein the assay verification sample is analyzed employing the same assay reagents and essentially the same assay
25 methodology employed to analyze the test sample. The assay verification sample is prepared by adding the positive analyte component to the test sample under analysis for the separate analysis thereof. Such separate analysis can be performed by either (i) first removing a portion of the test sample for the
30 separate analysis thereof, and then forming the assay verification sample with the remaining portion thereof for the separate analysis thereof, or (ii) the test sample can first be independently analyzed, and then the assay verification sample can be formed with the independently analyzed test
35 sample together with any assay reagents present during such first analysis. The positive analyte component is the analyte under determination, or analog thereof, which is present in the

assay verification sample at a known amount or concentration to provide a detectable signal or response in the particular assay methodology being performed.

5 In particular, when analyzing a test sample employing an assay verification sample according to the present invention, the test sample is analyzed according to a desired assay protocol or methodology employing the necessary assay reagents therefor, and the assay verification sample is independently analyzed according to essentially the same
10 assay protocol or methodology and employing the same assay reagents which were utilized to analyze the test sample. Accordingly, if the test sample provides a negative result, an appropriate signal difference with the assay verification sample verifies that such negative result is due to the
15 absence of analyte. On the other hand, if the assay verification sample does not provide an appropriate detectable signal or response, then the negative result for the test sample could be due to a number of reasons. For example, the assay reagents could lack potency or are otherwise not
20 capable of performing the particular assay to provide accurate results; the test samples or assay reagents could have been tampered with or adulterated, such as by the addition of blocking agents or inhibitors to a test sample for the analysis of such test sample for drugs of abuse and other controlled
25 substances; the test sample could have been incorrectly processed, such as incorrect pipetting steps or incorrect addition of reagents; contaminants or other substances which could interfere with assay performance may be present as the result of, for example, manufacturing, sterilization, washing, and the like processes, of test tubes, reaction vessels, assay
30 containers, assay devices, and the like.

The method of the present invention is particularly useful when analyzing test samples for the presence of analytes which are typically present in low frequencies. Such
35 analytes include, but are not intended to be limited to, hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM

(Anti-HBC), human immune deficiency virus 1 and 2 (HIV 1 and 2), human T-cell leukemia virus 1 and 2 (HTLV), hepatitis B e antigen (HBeAg), antibodies to hepatitis B e antigen (Anti-HBe), and the like analytes. Since such analytes occur in low frequencies, particularly when performing assays therefor on hundreds of test samples during the course of a day, the majority of such assay results may be negative. Accordingly, when performing such analyses employing the assay verification sample according to the present invention, potency of assay reagents employed therein and proper performance of the assay methodologies is verified.

According to one embodiment, the method of the present invention is particularly useful when performing a heterogeneous immunoassay format. According to such assay format, a reaction mixture is formed by contacting the test sample containing the analyte under determination with a labeled reagent or tracer comprising an analyte, an analog of the analyte, or an antibody thereto, labeled with a detectable moiety, to form a free species and a bound species thereof. In order to correlate the amount of tracer in one of such species to the amount of analyte present in the test sample, the free species is separated from the bound species. Such separation is accomplished by contacting the reaction mixture, either simultaneously or sequentially, with a solid phase material for the direct immobilization of one of the binding participants in the binding reaction, such as the antibody, analyte or analog of the analyte, wherein one of the binding participants is immobilized on the solid phase material, such as a test tube, beads, particles, microparticles or the matrix of a fibrous material, and the like, according to methods known in the art. Following, for example, one or more washing steps to remove any unbound material, an indicator material is added to produce a detectable response which can be detected and correlated to the amount of antigen or antibody present in the test sample. Another form of a heterogeneous immunoassay employing a solid phase material is referred to as a sandwich immunoassay, which involves contacting a test

sample containing, for example, an antigen with a protein such as an antibody or another substance capable of binding the antigen, and which is immobilized on a solid phase material. The solid phase material typically is treated with a second
5 antigen or antibody which has been labeled with a detectable moiety. The second antigen or antibody then becomes bound to the corresponding antigen or antibody on the solid phase material.

When performing such heterogeneous immunoassays
10 according to the present invention, the reaction mixture containing the free and bound species is divided into first and second portions, wherein the free and bound species of the first portion are separated as described above and an indicator reagent added thereto to provide a first result. A positive
15 analyte component is added to the second portion to form the assay verification sample according to the present invention, and is similarly analyzed independently to provide a second result. Alternatively, subsequent to obtaining the first result, the second portion containing the positive analyte component
20 can be added to the first portion to provide the second result. In either case, if the first result is negative, an appropriate second result, i.e., an appropriate detectable signal or response, verifies that such negative result of the test sample is due to the absence of analyte in the test sample. On the
25 other hand, if the first result is negative, absence of an appropriate second result indicates that one or more of the events described could have occurred.

Analytical Systems

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According to the present invention, the various known assay techniques and formats described herein, can be performed manually or can be performed employing various analytical apparatus described herein, as well as other
35 analytical apparatus known in the art. It is to be understood that when such assay techniques according to the present invention are performed with an automated analytical system

utilizing one or more automated pipetting steps, the various pipette additions of assay reagents and formation of the assay verification sample as described herein can be performed sequentially or simultaneously. In addition, the method of the present invention is particularly useful in the automated continuous and random access analytical system described below, which typically does not necessarily perform the same pipetting sequence consecutively for the determination of the same analyte in a different test sample, such as with batch analyzers known in the art. Generally, such assay techniques and formats include, but are not intended to be limited to, spectrophotometric absorbance assays such as end-point reaction analysis and rate of reaction analysis, turbidimetric assays, nephelometric assays, radiative energy attenuation assays (such as those described in U.S. Patent No. 4,496,293 and U.S. Patent No. 4,743,561),

ion capture assays, colorimetric assays, fluorometric assays, electrochemical detection systems, potentiometric detection systems, amperometric detection systems, and immunoassays. Immunoassays include, but are not intended to be limited to, heterogeneous immunoassays such as competitive immunoassays, sandwich immunoassays, immunometric immunoassays, and the like, where the amount of a detectable moiety employed therein can be measured and correlated to the amount of analyte present in a test sample.

When performing a heterogeneous immunoassay as described herein, separation of the bound and free species is preferably accomplished by capture of the microparticles on a glass fiber matrix of an MEIA cartridge, a process that relies on the high affinity of glass fibers for the microparticles, wherein the microparticles adhere to the surface of the fibers irreversibly, and nonspecifically bound material can be effectively removed by washing the matrix. The matrix also provides a precisely located mechanical support for the microparticles during the optical quantification phase of the assay protocol as described herein. In particular, microparticle reagent comprising microparticles coated with

antibody to the analyte in the test sample are incubated with the test sample containing the analyte of interest to form a capture complex with the analyte from the test sample. A conjugate comprising antibody to the analyte labeled with a detectable moiety, preferably an enzyme, is then incubated with the capture complex to form the second of a sandwich complex. When performing a competitive immunoassay, microparticles coated with antibody to the analyte in the test sample are incubated with the test sample containing the analyte of interest and a conjugate comprising the analyte or analog thereof labeled with a detectable moiety, preferably an enzyme. Removal of unbound conjugate is accomplished with the glass fiber matrix of an MEIA cartridge and, where the detectable moiety is an enzyme, a substrate for the enzyme capable of providing a detectable signal is added and the signal provided thereby is measured and correlated to the amount of analyte present in the test sample. Preferably, the enzyme-substrate system employed by the competitive and sandwich MEIA formats is alkaline phosphatase and 4-methylumbelliferyl phosphate (MUP), although other enzyme-substrate systems known in the art can be employed as well. As would be understood by one skilled in the art, such assay format can be followed for the determination of an antibody present in a test sample wherein the microparticle reagent comprises microparticles coated with a specific binding partner for the antibody and the conjugate comprises such specific binding partner labeled with a detectable moiety.

The MEIA cartridge comprises a reaction well for retaining and immobilizing microparticle-analyte complexes. The reaction well has an entrance port and means for holding a quantity of sample and assay reaction mixtures positioned over a fibrous matrix which retains and immobilizes microparticle-analyte complexes as described above. The fibrous matrix is composed of fibers having an average spatial separation greater than the average diameter of the microparticles. Preferably, the average fiber spatial separation is greater than 10 microns. The reaction well

further comprises an absorbant material positioned below the fibrous matrix to enhance the flow of sample and assay reaction mixtures through the fibrous matrix. Preferably, the absorbant material is a fibrous material whose fibers

5 predominantly lie in a plane perpendicular to the lower surface of the fibrous matrix. The absorbant material is in fluid communication with the fibrous matrix. Generally, the absorbant material is in physical contact with the lower surface of the fibrous matrix. The interior of the reaction

10 well, therefore, is generally sized or contains positioning means to maintain the fluid communication between the absorbant material and the fibrous matrix. Preferably, a spike located at the bottom of the reaction well can be used to force the absorbant material into contact with the lower surface of

15 the fibrous matrix. Additionally, it is preferable to vent to the atmosphere the gases displaced in the absorbant material by the liquids absorbed therein during the performance of an immunoassay.

When performing a heterogeneous immunoassay

20 employing an MEIA cartridge as described above, a reaction mixture comprising the test sample, the microparticle reagent, and other reagents, if needed, are aliquoted and allowed to incubate. The reaction mixture is then divided into first and second portions. As shown in FIGURE 29, the first

25 portion is deposited onto the matrix of the MEIA cartridge to obtain the test sample result. While the test sample result is being obtained, the assay verification sample is independently formed by adding a positive analyte component to the second portion of the reaction mixture which is also incubated,

30 wherein the necessary assay reagents are pipetted simultaneously or sequentially to the first portion and the second portion to complete the immunoassay in the first and second portions. The second portion is then deposited onto the matrix of the same MEIA cartridge to obtain a second result.

35 Alternatively, the first and second portions are formed as described above prior to obtaining the first and second result

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and, once both have been formed, the first and second results are obtained (FIGURE 30).

The teachings of the present invention are particularly useful when performing the various assay techniques

5 described herein on a continuous and random access analytical system apparatus as described below and as shown in the Figures hereof. Such instrument comprises a front end carousel assembly inclusive of a sample cup carousel, an assay reagent pack carousel and a reaction vessel carousel

10 mounted concentrically and serviced by a transfer pipetting means suitable for kitting and/or mixing reagents with a test sample. The reagent pack carousel includes a plurality of reagent packs containing one or more containers which are capable of holding assay reagents for performing various

15 assays described herein and as known in the art. The kitted and pipetted reaction vessels are transferred through a transfer station which provides means for transferring the kitted and pipetted reaction vessels to a processing work station which includes a controlled environment for

20 maintaining temperature and provides timing for mixing of reagents and incubation. At least two assay procedural apparatus are provided which are scheduled for the various samples and kitted reagents in a unit dose disposable means for analyzing the incubated reaction mixtures. The unit dose

25 disposable reaction vessels are removed from the process carousel by operation of the transfer station, which includes means for removing the disposable reaction vessel from the system. According to such analytical system apparatus, a system scheduler generates and optimizes the workload for

30 the system's mechanical resources from all the tests ordered to run on the system. The main goal of the scheduler is to keep the system's resources from sitting idle while there are tests remaining to be processed by the system. Keeping each of the resources busy also minimizes the time required by the

35 instrument to perform the tests.

A high-level view of the scheduling process can be broken into two steps: (1) proper scheduling of each of the

activities in a test is ensured before the test is kitted, and
(2) an attempt to perform each test activity prior to its
original scheduled execution time, to minimize resource idle
time and increase test throughput in the system. To enable
5 scheduling a test in advance of its performance in the system,
each test's assay protocol contains several timing parameters
used in the scheduling process. Each activity of the test
contains time values which the scheduler uses to determine
which resources the activity requires and the time period that
10 these resources are needed. Also, each activity in the test can
be tied to other activities by incubation periods. These
incubation periods, which are dictated by the chemistry of the
assay, help the scheduler determine the amount of time that
must elapse between the execution of two activities. Each
15 incubation period in the assay protocol provides for the
minimum and maximum time that may elapse between the
execution of each activity. These limits are referred to in the
scheduling process as the incubation window for the
activities.

20 When operating such analytical apparatus system, the
operator chooses the order that tests are prepared to run on
the instrument by selecting the placement of samples on the
instrument. The sample placed closest to the pipette station
is the first sample prepared to run on the instrument. To guard
25 against evaporation, a test will not be prepared until the
scheduler ensures that all resources used by the test's
activities will be available at the required times set forth in
the test's assay protocol. Preparation of a particular test will
be postponed whenever an activity of another test already in
30 the instrument has a resource scheduled at the time it is
needed by an activity on that test. The sample preparation
area of the instrument will remain idle until the test can be
scheduled without conflicting with tests already in the
instrument. When proper scheduling of the test can be
35 achieved, the test will be prepared and transferred into the
process area.

The second step in the scheduling process is to optimize the workload for each system resource to minimize both the resource's idle time and the time required to perform the resource's workload. once tests are transferred into the process area, the scheduler optimizes the existing schedule for each resource. At predetermined intervals, the scheduler examines the next interval of work for each resource. If there is any idle time in this interval, the scheduler attempts to minimize the idle time by rearranging the resource's workload to eliminate idle time, providing the activities remain within their allowed incubation windows. When optimization of this interval is complete, this section of the workload is performed by the resource at the designated times. The scheduler continues to prepare samples as long as there are samples on the instrument that have tests ordered to be run. optimization of the resources' workloads will continue until all tests transferred into the system have finished processing. The analytical apparatus system described herein allows special priority handling of specific samples identified by the user as being stat samples. A stat sample is a sample that must be processed by the instrument in the shortest amount of time possible. Special handling of stat samples occurs both in the front sample entry area and in the processing area of the instrument.

When performing a stat procedure, the operator chooses the order that tests are prepared to run on the instrument by selecting the placement of samples on the instrument. The sample placed closest to the pipette station is the first sample prepared to run on the instrument. This pattern of sample preparation is interrupted whenever the user places a stat test on the instrument. Whenever a stat test is ordered, the system will finish preparing the test on the current sample, and then move directly to the stat sample to prepare all its tests. To guard against evaporation, sample preparation will not begin for a test before proper scheduling of the test's activities in the processing area is ensured. The system scheduling algorithm is also modified for stat processing. The

scheduling algorithm used for normal tests attempts to maximize the number of tests processed in the instrument each hour. This occurs by allowing sufficient time between test activities to enable other tests' activities to be performed in these gaps. The scheduling approach used for stat tests attempts to process this one test in the shortest amount of time possible. Each activity of a stat test is scheduled at the earliest possible time of execution as defined in the test's assay definition. When all activities of a test are guaranteed proper scheduling in the instrument, sample preparation of the test will begin. After all tests on the stat sample are prepared, the system will return to the sample it was working on before it serviced the stat.

Stat tests receive special consideration in the processing area when there is idle time in a resource's workload. At predetermined intervals, the scheduler examines the next interval of work allocated to each resource in the processing area of the system. If there is any idle time during this interval, the scheduler attempts to minimize it by rearranging the resource's workload. Test activities scheduled for this resource that can be performed earlier than they are currently scheduled, as defined by their assay protocols, are moved forward to fill the idle time. Stat test activities are the first candidates to be pulled forward in the workload, thus further decreasing the amount of time needed to process the stat test in the instrument. The system stat test handling algorithms have been shown to allow stat tests to be processed in the minimum amounts of time possible, without having a negative effect on the instrument's overall throughput of tests per hour.

According to the immunoassay methodologies described above, standard solutions of the analyte of known concentrations covering the clinical concentration range are typically prepared and assayed as is the test sample to be assayed. This blank assay provides a series of signal measurements corresponding to the known concentrations from which a standard curve is drawn. The optical signal

corresponding to the unknown sample is correlated in a concentration value through interpretation from the blank or standard curve.

Automated analytical methodology for effecting analysis of a plurality of test samples according to analytical apparatus system described herein is achieved by introducing reagent packs, test sample container and reaction vessels onto concentric carousels of a main carousel. The test sample container can be a test tube, cuvette, vacutainer tube, and the like, for holding a test sample. The reagent packs and test sample containers are identified and aligned respectively with a reaction vessel for transfer and kitting of the reaction vessel by transfer of test sample and specific reagents from the reagent pack for preparation of a predetermined test. The reaction vessel containing the test sample and one or more reagents is transferred to a process carousel wherein controlled environment conditions exist for incubation once the sample has been appropriately mixed with various reagents to form a reaction mixture. When all assay processing steps have been completed, the reaction mixture is identified and transferred to at least, for example, one of a fluorescent polarization immunoassay reader or a microparticle enzyme immunoassay cartridge positioned on a separate cartridge wheel or carousel for further preparation before reading. The processed test samples are read and the readings are calculated with the resulting data being recorded and/or printed.

The methodology of the automated immunoassay analytical system is achieved through the use of a self-contained, fully automated, continuous and random access instrument comprising a main carousel assembly consisting of the reagent pack carousel, a reaction vessel carousel and a test sample container carousel concentrically and independently rotatable. The main carousel assembly is provided with a transfer pipette operated by a boom arm for transferring and kitting test sample and reagents into the reaction vessel automatically following a predetermined test

schedule. The main carousel assembly is provided with bar code readers for reagent packs and test sample containers and has the capability of aligning the reagent pack carousel and test sample container carousel and a reaction vessel for
5 pipette transfer operations. Once the assay to be performed is scheduled, the reaction vessel carousel, the reagent pack carousel and the test sample container carousel are rotated until the reaction vessel, a reagent pack and a test sample
10 container, respectively, are determined to be in the transfer pipette access position. The transfer pipette then transfers the test sample from the test sample container and, depending upon the assay to be performed, the reagents from the reagent pack are transferred to the reaction vessel. The reaction
15 vessel carousel is then rotated to a transfer station position which contacts the reaction vessel with a transfer mechanism and pulls the reaction vessel into the transfer station. The reaction vessel is then loaded onto the process carousel by the transfer mechanism.

When performing a fluorescent polarization
20 immunoassay (FPIA) with the automated analytical system, various pipetting activities are performed by a second transfer pipette apparatus which is in service for the process carousel, and the process carousel is rotated so that the reaction vessel, when properly pipetted with, for example,
25 FPIA reagents, is at the read station of the FPIA processing stations and the FPIA determination on reading, is made on the reaction vessel. The process carousel is then rotated so that the read reaction vessel is at the transfer station. The reaction vessel is again contacted and transferred
30 by the transfer station. The transfer station is rotated and pushes the reaction vessel into a release container opening.

For a microparticle enzyme immunoassay (MEIA) according to the present invention performed with the automated analytical system described herein, after the
35 various pipetting activities, which can be completed at the main carousel assembly, the reaction vessel is transferred to the process carousel as described in the FPIA process.

Pipetting can also be accomplished in the process carousel or jointly between the two carousels. To complete the MEIA, the reaction mixtures, including first and second portions as described herein, are then transferred from the reaction vessel to a matrix of an MEIA cartridge as described above on a cartridge carousel with the second transfer pipette. The matrix is washed with a buffer and a substrate, such as MUP (defined earlier), or other suitable substrate known in the art. The cartridge carousel is then rotated so that the MEIA cartridge is positioned at an MEIA processing assembly and the MEIA determination is made. The MEIA reaction vessel is ejected into the waste container as described for the FPIA reaction vessel. The MEIA cartridge is independently ejected from the cartridge wheel by an ejector at an appropriate ejector station into a waste container.

Preferably, two distinct analytical technologies as described above, FPIA and MEIA, are incorporated into the automated analytical system described herein and with which the method of the present invention can be employed. However, more than two distinct analytical technologies can be incorporated into the analytical system. These methods are complimentary and share a commonality of apparatus and procedural steps, with the FPIA generally being the method of choice for analytes of low molecular weight and MEIA for molecules such as protein hormones, antibodies or analytes of low molecular weight requiring higher sensitivity. The two technologies share system components including the operator control panel, pipetting boom assemblies, fluidic systems, air and liquid reagent heaters, printers, bar code reader and stepper motors. Such commonality of use of system components allows for a compact instrument despite the dual FPIA and MEIA capability.

The FPIA optic systems (such as described in U.S. Patent No. 4,269,511) can utilize a polarizing filter which is an electrically switched liquid crystal, maintaining a compact size and avoiding complex and potentially unreliable moving parts. When

performing FPIA assays utilizing the automated analytical system described herein, the FPIA reagent packs will typically include a tracer comprising the analyte or analog thereof, coupled to a detectable moiety, an antibody specific to that analyte, and a specimen pretreatment reagent. In a preferred FPIA format, the analyte being determined competes with the tracer for a limited number of binding sites on the antibodies specific to the portion or portions of the analyte and tracer. The detectable moiety component of the tracer is preferably a fluorescent moiety selected from the group consisting of fluoresceins, aminofluoresceins, carboxyfluoresceins, fluoresceinamines, and the like, more preferably carboxymethyl-aminomethyl-fluorescein, carboxyethylaminomethyl-carboxyfluorescein, 6-carboxyfluorescein, 5-carboxyfluorescein, succinylanimomethyl-fluorescein, thiourea-aminofluorescein, methoxytrianolylaminofluorescein, aminofluorescein, and the like.

MEIA results can be determined by quantifying the rate of fluorescence developed when fluorogenic substrate is converted by the action of an enzyme labeled conjugate. For example, when performing either a competitive MEIA or sandwich MEIA, the specifically bound alkaline phosphatase on the microparticles is detected by addition of the fluorogenic substrate MUP to the matrix. The alkaline phosphatase catalyzes hydrolysis of the MUP to inorganic phosphate and fluorescent 4-methylumbelliferone (4-MU). The liberated 4-mu is detected by the MEIA optics assembly front surface fluorometer which is designed to detect fluorescence of low concentrations of 4-MU without interference by fluorescence of 4-MUP at a wavelength of 367 nm. A system of lenses and optical filters focus filtered light (wavelength = 365 nm) from a mercury arc lamp on to the surface of the matrix and focus emitted fluorescence from 4-MU (wavelength = 448 nm) on to a photo multiplier tube. Like the FPIA optics assembly, the MEIA optics system is compact and has no moving parts. About five percent of the excitation light is detected by a

photodiode, allowing normalization of the fluorescence data and generation of a control signal used by the lamp power supply to maintain the intensity of the excitation light within five percent over the useful life of the lamp. The MEIA post-processor uses linear regression analysis to convert the data from multiple successive determinations of 4-MU fluorescence to a rate which is proportional to the concentration of alkaline phosphatase conjugate specifically bound to the microparticles.

MEIA formats can be run with a multi-position MEIA auxiliary carousel and process carousel as well as a MEIA reagent pack containing an alkaline phosphatase conjugate and, in some cases, a dilute buffer specific for the assay being performed. The effective surface area of polystyrene latex microparticles is several fold greater than that of a large diameter polystyrene bead (e.g., one quarter inch beads) commonly used in commercial immunoassays. Because of this large surface area and the very small diffusion distance between analyte and the capture molecules on the surface of the microparticles, the capture phase employed in many of the MEIA methods being performed reaches equilibrium within several minutes, allowing for a full carousel of test samples to be completed in a very short time frame.

Unlike an FPIA, the heterogeneous immunoassays, such as a MEIA, require a separation step as described above. In particular, after incubation of the microparticles with a test sample, the microparticles are separated from the reaction mixture by transfer to the matrix contained in the MEIA cartridge as described above. The matrix provides a precisely located mechanical support for the microparticles during the subsequent optical read phase-of the assay. This precisely located mechanical support, i.e. the cartridge, is fit into the auxiliary carousel at a predetermined spacing from the reader apparatus by camming means.

Referring to the drawings, FIGURES 1 and 2 present isometric views of an automatic immunoassay analytical system apparatus with which the teachings of the present

invention are particularly useful. It is to be understood that the automated immunoassay analytical system described herein is presented only with those components of primary interest with respect to the assay cuvette of the present invention. The drawings do not illustrate all of the mechanical and electrical elements for driving and controlling the various components of the system, wherein an of such omitted elements may have various known forms which can be readily realized by one of ordinary skill in the art having knowledge of the information provided herein with regard to the mode of operation of the system and the various components and related processes utilized for treating samples and determining analytical results.

The system apparatus as it appears in FIGURE 1 presents the system apparatus as used by the technician, with FIGURE 2 illustrating an isometric view of the frame and cabinetry with component parts removed. The system apparatus described herein is identified generally by the reference numeral 2 in FIGURE 1. The system apparatus 2 has an exposed front end carousel 4 which is serviced by a first transfer pipette mechanism 6 for kitting scheduled tests along with samples into a reaction vessel. The system provides a computer screen 8 and computer keyboard 10 along with access panels 12 for accessing storage and waste compartments. The system apparatus 2 is provided with rollers 14 for movement of the system apparatus within a laboratory complex as required. The freedom of movement of the system apparatus through rollers 14 is allowed since the system is fully self-contained but for power requirements.

In FIGURE 2, the system apparatus 2 cabinet frame 16 is illustrated with substantially all functioning components of the system apparatus removed. A controlled environment zone 18 is a closed unit during operation with light shielding and rigid control of airflow as well as temperature as opposed to the open front end carousel 4. The front end carousel 4 communicates with the controlled environment zone 18 through a transfer port 20. The front end carousel 4 is

mounted to an aluminum base plate which rests on a support platform 22 and the first transfer pipette mechanism is mounted on means 24.

The top plan view in section of FIGURE 3 presents the functioning component system apparatus in some detail with relative positioning of the system apparatus to further illustrate the process flow of the system apparatus. For example, sample cups 26 are mounted on a sample cup carousel 28 which is concentrically fitted within the front end carousel 4 along with reagent pack carousel 32 and reaction vessel carousel 36. The reagent pack carousel 32 is concentrically fitted between the sample cup carousel 28 and the reaction vessel carousel 36. The reagent pack carousel carries reagent packs 30 and the reaction vessel carousel 36 carries reaction vessels 34. The front end carousel 4 has an operable bar code reader 38 for automatically identifying reagent pack carousel 32 and sample carousel 28. A wash cup 40 is provided for the first transfer pipette mechanism 6 for washing as required between transfer of various sample and reagents. The first transfer pipette mechanism 6 is utilized in kitting the various reagent pack liquid materials and sample into a reaction vessel 34. The reagents and the sample are properly kitted through means of the first transfer pipette mechanism 6 inclusive of pump means. The various carousels are rotated and aligned for kitting at the pipetting station. The kitted reaction vessel 34 is positioned by reaction vessel carousel 36 into the proper position for transfer to the transfer station 42. The reaction vessel 34 is transferred to the transfer station 42 through transfer means wherein the transfer station 42 is then rotated to move the reaction vessel onto process carousel 46. As shown, the process carousel is driven by a stepper motor 48 and is serviced by a second transfer pipette mechanism 50. Both the FPIA and MEIA procedures utilize the system apparatus commonly up through and including the process carousel 46. The process carousel 46 includes FPIA processing 52 and FPIA processing lamp 54 for direct reading of FPIA analysis of kitted, pipetted and

properly reacted reagents sample from the reaction vessel 34. The controlled environmental zone 18, which includes the transfer station 42 and process carousel 46, provides FPIA processing with air circulation under temperature control by cabinet air circulation fan 56. A wash cup 58 for the second transfer pipette mechanism 50 is provided. The second transfer pipette 50 is utilized for adding reagents (pipetting) under conditions of incubation and timing to the sample in the FPIA test schedule reaction vessel 34 for FPIA processing.

10 MEIA processing can also utilize the second transfer pipette 50 for adding reagents to the sample before the reaction mix is added to MEIA cartridges 68 which are mounted on the cartridge wheel carousel 64. The transfer of the MEIA reagent mixed sample to the MEIA cartridge 68 is by the function of

15 the second transfer pipette 50. A motor 60 drives the cartridge wheel 64. The cartridge wheel 64 is provided with MEIA cartridges 68 through the operation of a cartridge hopper 66 which automatically feeds and positions the MEIA cartridges 68 onto the cartridge wheel 64. The process area

20 includes the second transfer pipette mechanism 50 and heater/pump 44. The cartridge wheel carousel 64 is further serviced by a MEIA buffer heater and dispenser 70, MUP heater and dispenser probe 72, and MEIA reader 74. The MEIA cartridges 68 are removed from the cartridge wheel 64 by a

25 cartridge ejector 62 after the MEIA read has been completed.

It is to be understood that the utilization of the first transfer pipette mechanism 6 and the second transfer pipette mechanism 50 as described herein provide a safety mechanism to ensure that test samples and reagents are pipetted to

30 thereby prevent false negative results in the event there are incorrect amounts of the respective sample and reagents for a particular assay.

Approaching the operable elements of the system apparatus in greater detail, FIGURE 4 provides a front

35 elevational view in isolation and partial section of elements of the front end carousel 4. FIGURES 4A and 4B illustrate a reagent pack with a cover means 31 which is opened and

closed pivoting along axis 37. A return notched drive arm 35 is utilized to open and close the cover means 31 by contact with the cover contact surface 33.

FIGURE 5 provides a top view in isolation and partial
5 section of elements of the drive and guide systems of the main carousel 4 with the various carousels removed. In FIGURE 5 a sample cup carousel stepper motor 76 is shown mounted with mounting spring 78. The reagent pack carousel motor 80 is also shown with a mounting spring 82. The reaction vessel
10 carousel motor 84 and mounting spring 86 are positioned to the exterior of the two inner carousels, i.e. the sample cups carousel 28 and the reagent pack carousel 32. Roller guides 88 are provided for the sample cup carousel 28 and a tensioning spring 90. The reagent pack carousel is provided with roller
15 guides 92 and tensioning means 94. The reaction vessel roller guides 96 are also provided with spring elements 98, the purposes of the guide and these various spring elements being to maintain very finite tracking of the concentric carousels when motivated by the individual stepper motors.

20 The front end carousel 4 inclusive of the three front end carousels, the sample cup carousel 28, reagent pack carousel 32 and reaction vessel carousel 36 can by example contain the following capacities. The sample cup carousel 28 can hold 60 blood collection tubes, such as Vacutainer® blood collection
25 tubes, or 90 sample cups which are injection molded as one piece and can be provided with standalone base mounts. Standalone base mounts are suitable for technician handling and pipetting of samples into the sample cups. The reagent pack carousel 32 provides for 20 different reagent packs 30.
30 The reaction vessel carousel 36 provides 90 reaction vessels 34.

The process carousel 46 as shown in FIGURE 6 is an isolational cross-sectional side view. One reaction vessel 34 is at rest or nonoperative position and a second reaction
35 vessel 34 is in position for FPIA read. The process carousel 46 is capable of bidirectional motion for timely movement of the various reaction vessels 34 to pipettor action, read, or

transfer to and from the carousel. Up to about 36 or more reaction vessels 34 can be processed at one time on the process carousel 46 depending on diameter and sizing of the reaction vessels 34.

- 5 The first transfer pipette mechanism 6 of FIGURE 7 includes a transfer pipette Z axis motor 102 which moves the probe arm 104, probe 106 and probe tip 108 in a vertical direction while transfer pipette R axis motor 100 drives the probe arm 104, probe adjustment means 106 and probe tip 108
- 10 in a horizontal motion. The first transfer pipette mechanism 6, sometimes labeled "Sample Probe Arm Mechanism", moves the probe between the sample cup 26, the reagent pack 30, the reaction vessel 34 and the wash cup 40. The wash cup 40 is used to wash the interior and exterior surfaces of the first
- 15 transfer pipettor mechanism 6 probe. The drive of the first transfer pipette mechanism is a rack-and-pinion drive means along the Z and R axis by two- stepper motor drivers. A brake is provided to hold the Z axis position when power is lost, thus avoiding damage to the system apparatus. For example,
- 20 the first transfer pipette mechanism can be designed to have a Z axis travel of about 3 inches and an R axis travel of about 11-1/2 inches.

- The first transfer pipette mechanism 6 and the second transfer pipette mechanism 50 are closely related in general
- 25 system apparatus function and design, with variation on travel and size being the only substantial differences. Both units have a probe arm circuit 110 as illustrated by the schematic side view of FIGURE 8. The schematic illustrates the R axis motor 100 and the Z axis motor 102 in relationship to an upper
- 30 PCB 112 and a R axis home sensor 114. A lower PCB 116 is illustrated in relationship to the Z axis home sensor 118 with a coil cable 120 connecting the various elements.

- Various elements of syringe 122 which provides automatic bubble flushing and fluids to the various pipetting
- 35 mechanisms is provided in various views in FIGURES 9, 9A and 9B. The ability of diagnostic instrumentation to accurately perform an assay is critically dependent on the precision and

accuracy with which syringes, i.e. pipetting, can aspirate and dispense reagents and samples. The precision and accuracy of a syringe is severely degraded by the presence of small air bubbles inside a syringe. Bubbles, unfortunately, are all too common and are difficult to remove or avoid. Syringe 122 avoids these problems by automatically flushing bubbles completely out of the fluidics system. The syringe 122 is configured such that a piston 124 reciprocates through a seal 126 and into a close-fitting bore 128. The end of the bore 130 is closed. The piston 124 has a piston end 132 which approximates the geometry of the closed bore end 130. Two ports to the bore are 180° apart and are located near the seal and are comprised of a fluid entry port 134 and a fluid exit port 136. An annulus 138 exists between the piston 124 and bore 128. Pressurized line diluent is introduced to the fluid entry port 134. The fluid flows out into the annulus 138 around both sides of the piston 124 and then into the fluid exit port 136. This crossflow flushes bubbles from the area near the seal. While the crossflow is occurring, the piston 124 is reciprocated inside the bore 128. This reciprocation causes high fluid flow velocities in the annulus 138 between the piston 124 and the bore 128. The high flow velocity dislodges any bubbles that may be adhering to the piston 124 or bore wall. The inward stroke of the piston 124 pushes these dislodged bubbles across the crossflow area where they are swept out of the syringe. The piston end 132 and the bore end 130 have similar spherical shapes. When the piston 124 strokes to its full inward extension, it comes very close to the bore end 130. Any bubble that may be stuck on the bore end 130 is disrupted and dislodged. Likewise, when the piston strokes to its full outward extension, its end is flush with the seal 126. The sequence of reciprocating the piston while crossflowing can be automatically executed any time by the system apparatus.

Once the fluid leaves the fluid exit port 136 of the syringe 122, it must travel through a tube fitting, through a length of tubing, through another tube fitting, into a probe 106

and out the probe tip 108. It is at the probe tip 108 that the aspirating and dispensing of reagents actually occurs. Any bubbles trapped between the syringe and the probe tip will also degrade performance, so there must be no place for the bubbles flushed out of the syringe to lodge. It is therefore necessary to use zero dead volume tubing fittings on the tubing between the syringe and the probe.

The reaction vessel 34 is discussed in detail relative to either the MEIA scheduling or the FPIA scheduling in FIGURES 10, 10A, 10B and 10C. FIGURES 10 and 10A present the FPIA kitting utilization. The reaction vessel is illustrated in both the top plan view (FIGURE 10) and the side view (FIGURE 10A). S reagent antiserum is deposited in well 142 while T reagent tracer is deposited in well 144 with P reagent popper being deposited in well 146. Wells 150 and 152 can serve for providing a variety of reagents, buffers and/or dilution liquids to the apparatus. The sample is deposited in well 148 and predilution liquid in well 154. The utilization of the transfer pipettor in depositing the required reagents into a reaction vessel along with the sample is called kitting. The depositing of the various required reagents and the like into a single reaction vessel along with a sample to be analyzed is called pipetting.

The MEIA reaction vessel as shown in top and side views of FIGURES 10B and 10C, respectively, contains prediluent in well 156; microparticle materials being deposited in well 158; conjugate directly in the reaction well 166; assay diluent in well 162; and the sample in well 164. The buffer well is 168 and predilution well is 170. Once kitting is complete, many of the subsequent FPIA and MEIA pipetting steps can be performed either in the main carousel or in the process carousel utilizing the pipetting mechanisms of both carousels. This is possible because the kitted reaction vessel, once kitted, is transferred immediately into the transfer station and thus into the process carousel which exists in a controlled temperature environment.

The transfer station 42 plays a key role in apparatus and process function. In FIGURE 11, a sectional side view of the transfer element of the transfer station 42 is shown engaging reaction vessel 34 by means of a reaction vessel transfer projection 172. The transfer arm 173 is projected out between reaction vessel elements of the reaction vessel carousel 36 and, by rotation of the transfer station 42, engages the reaction vessel transfer projection 172. By means of a transfer arm drive gear 174, the transfer arm 173 rack gear 176 moves the transfer arm 173 out and in relationship to the transfer station 42. The transfer station 42 has a rotation axis 178. In FIGURE 11A, a reaction vessel is shown in phantom as would be mounted on the front end carousel 4, reaction vessel carousel 36 engaged by the transfer arm 173 by means of reaction vessel transfer projection 172. The reaction vessel 34 in FIGURE 11 is illustrated onboard the transfer station by reaction transfer station 42 moves the reaction vessel 34 between the front end carousel 4 and the process carousel 46. The transfer station 42 moves the discarded reaction vessel 34 from the process carousel 46 to the waste ejection station (not shown). The transfer station 42 is driven by a stepper motor drive and is supported by precision linear ball bearings and axis of rotation ball bearings.

The process carousel 46 holds, for example, 36 reaction vessels 34 and has a carousel diameter of about 12.5 inches. The process carousel 46 moves the reaction vessel 34 between the transfer station 42, the second transfer pipettor mechanism 50, the point of pipetting, and the FPIA reader processing 52. The process carousel 46 is driven by a stepper motor and supported by three wheels for height control and control of any radial movement caused by irregularly shaped carousel elements.

The second transfer pipette mechanism 50 moves the pipette probe between the wells in the reaction vessel 34 on the process carousel 46 to the MEIA cartridge 68 on the auxiliary carousel 64 and to the wash cup 58. A rack-and-

pinion drive through two axis stepper motor drives achieves precision drive on both the R and Z axis. Travel, for example, on the Z axis can be about 3 inches and on the R axis about 4.5 to 5.0 inches.

5 The auxiliary carousel 64 holds, for example, 32 MEIA cartridges 68 and has a diameter of about 9.5 inches. The auxiliary carouser 64 moves the MEIA cartridges 68 between various stations including the second transfer pipettor mechanism pipette point, the MUP dispense station 72, the
10 MEIA washstation 70 and the MEIA reader 74 and the MEIA cartridge ejection point 62. The auxiliary carousel 64 is stepper motor driven and is carried by three wheels with one wheel located at the Z axis height control at the cartridge insertion point, the second wheel at the pipette point, and the
15 third wheel at the MEIA reader in order to maintain the auxiliary carousel 64 within desired geometric relationships to these various functions.

MEIA cartridges 68 are loaded into a cartridge hopper 66 which feeds the MEIA cartridges 68 into the auxiliary carousel
20 64. The automatic feeding of the MEIA cartridges 68 is provided with a proper height adjustment of the cartridge 68 into the auxiliary carousel 64 as required by MEIA reading. The cartridge hopper 66 feeds individual cartridges 68 to the auxiliary carousel 64 and changes the axis of orientation of
25 the cartridge 68 from horizontal to vertical by automatic means. Removal of the MEIA cartridges 68 is achieved through the use of an ejector 62 which operates through an ejection rod and forces the MEIA cartridge 68 from the auxiliary carousel 64 which is dropped into a solid waste container.

30 Buffer supply stations are presented in FIGURE 14 which is a top plan view in section of the apparatus showing the cabinet frame 16, front end carousel 4 in partial phantom and a power supply element 192 along with diluent system or buffer pressurization means 194. A supply bottle 196 is also
35 mounted in the lower cabinet of frame 16 as well as solid waste 198 and liquid waste 200 containers for receiving processed liquids and solid waste.

A schematic view illustrating the environmental airflow and temperature control system is shown in FIGURE 15 wherein make up air 204 enters and hot air exits at exhaust 206. Airflow 202 is indicated by arrows and the controlled environmental airflow schematic 214 is provided with at least one heater element 208 and fan element 210. At least one temperature sensor 212 is provided for control of the air temperature and can be correlated with the airflow 202 control.

10 The MEIA cartridge 68 is shown in a side elevational view in FIGURE 16. The MEIA cartridge 68 has a funnel throat 216 and a cartridge opening 218. The MEIA cartridge 68 contains support matrix material 222.

15 A MEIA cartridge 68 and cartridge hopper 66 are shown in a side elevational view in FIGURE 17. The MEIA cartridges are positioned horizontally in the cartridge hopper 66 and are manipulated from the bottom of the V-shaped cartridge hopper 66 one-by-one through a cartridge shuttle 222. The cartridge feeder has a cartridge cam block 224 and a cartridge orientation shoot 226 which functions through cartridge orientation pin 228 and cartridge orientation pin 230 for providing the MEIA cartridge 68 in vertical alignment= for insertion into the auxiliary carousel 64. The orientation pins 228 and 230 are illustrated in FIGURE 18 which is a side sectional view in isolation of the MEIA cartridge feeder cartridge orientation mechanism. The MEIA cartridge 68 is shown in an enlarged view in FIGURE 18 as being engaged and disengaged by cartridge orientation pin 228 and cartridge orientation pin 230. The cartridge orientation pin 230 is shown in engagement position at position 232 against the base 236 of the MEIA cartridge 68 while cartridge orientation pin 228 is shown in engagement position 234 of the cartridge funnel throat portion 216. Upon withdrawal of these pins from the engaging positions, the MEIA cartridge 68 is released from the bottom portion first, i.e. the withdrawal of cartridge orientation pin 230, thus allowing the bottom of a cartridge 68 to drop by gravity before the top of the cartridge is

released which is engaged by cartridge orientation pin 228 in the cartridge funnel throat 216. The rounded or semicircular holding surfaces of the orientation pin allow the release of the bottom of the MEIA cartridge and the rolloff of the funnel throat portion 216 from the cartridge orientation pin 228. The vertically aligned MEIA cartridge 68 is then inserted into the auxiliary carousel 64 to a controlled height by the action of an insertion cam means 227 as shown in FIGURE 17.

A side view of a MEIA cartridge ejector 62 is illustrated in FIGURE 19. The cartridge ejector 62 functions through an ejector rod 240 and can be driven by manual or automatic drive means 242. The ejected MEIA cartridge is ejected through an ejection passage to the solid waste 198 container.

A box diagram of the optics signal processor of the apparatus is provided in FIGURE 20 wherein the signal from the FPIA optics 248 is fed to a DSP A/D 250 which also sends serial bus signal 252 from an optic signal processor 8-bit microcontroller 254. The controller 254 is connected to computer elements through 256. Signal from the MEIA optics 258 are fed into a DSP A/D element 260 which also sends serial bus 262 from the controller 254. Signal is fed to the FPIA optics through 264 from high voltage power supply 266 and serial bus 268 which is in communication between the microcontroller 254 and the optics power supply board 270A. The FPIA tungsten lamp power supply FPIA 270 is in electronic communication with the FPIA optics 272. Signal is sent to the MEIA optics through 274 from high voltage power supply 276 which is in communication through serial bus 268 to the microcontroller 254 and mercury lamp power supply MEIA 280. The MEIA mercury lamp power supply 280 is also in electronic communication with MEIA optics through 282.

A schematic view of the FPIA optical system 284 is shown in FIGURE 21. The FPIA optical system 284 has a tungsten halogen source lamp 286 which focuses light through a heat reflector 288, an aperture 290 and heat absorber 292 to a lens 293 for introduction into an excitation filter 294. The light energy is then contacted with a beam splitter 296 which

presents part of the beam to a polarizer 298 and liquid crystal 300. The light continues into another lens 301 before being focused on the cuvette 140 containing the FPIA reaction mixture. Light is emitted from the cuvette through lens means 5 303 before entering an emission filter 302. The reflected light from the emission filter 302 passes through a polarizer 304 before going to a focusing lens 306 and being focused for feed into photo multiplier tube 308. The beam splitter 296 splits out part of the light from the original source through 10 lens 310 into a reference detector 312 which, in turn, controls the tungsten halogen source lamp.

A schematic view of the FPIA read sequence 314 is presented in FIGURE 22. The FPIA read sequence 314 has a preread time 316 divided into carousel move time 318 and 15 carousel settle time 320. Sub-read interval 340 is divided into a horizontal sub-read 342, A/D converter settle time 344, and a liquid crystal activation time 346. A vertical sub-read interval is identified by 348 which is inclusive of A/D converter settle time 350. Liquid crystal relaxation time is 20 indicated by 352. The liquid crystal relaxation time 352 is illustrated in a preread time sequence. High voltage settle time 324 is further illustrated by lamp settle time 326 that shows the lamps in a sinner 328 and full burn 330 activation. Activities of the FPIA read sequence 314 provide for 25 activities where scheduling windows 332 as exemplified by read prep 334, read parameter 336 during which the lamps are at full burn, and collection results 338 during the lamp settlement time and liquid crystal relaxation time 352.

FIGURE 24 is a schematic view of the MEIA system 30 optical assembly 364. An MEIA light source is provided by mercury source lamp 364 which passes light through an excitation filter 362 to a filter reflector 360 before being fed through lens 358 into MEIA cartridge 68. Reflected fluorescent light is fed back through the filter 360 to a photomultiplier 35 tube 374 after passing through a wide band-pass emission filter 370 and narrow band-pass emission filter 372. Part of the light energy from the mercury source lamp 364 passes

directly through filter 360 to a bandpass filter 368 before influencing the photo diode 366.

An MEIA read sequence schematic is presented in FIGURE 25 wherein the MEIA read sequence 376 has a preread time 378 inclusive of carousel move time 380 and carousel settle time 382. High voltage settle time is indicated by graph 384 which is coincident with the lamp settlement time 386 showing lamp simmer 388 and lamp full burn 390. MEIA read sequence 376 has activities with scheduling windows 392 inclusive of read prep 394, read parameter 396 and collection results 398. The actual MEIA read sequence 376 is inclusive of sub-read interval 400 having a sub-read 402 and a dwell time 404. Another segment of the MEIA read sequence 376 is indicated by sub-read interval 406 inclusive of sub-read number to 408 and dwell time 410 with additional sub-reads 412 as indicated by number 3 through (N-1) and partial sub-read interval 414 inclusive of sub-read number N-416. The next possible preread time is indicated by 418.

In addition to the continuous and random access analytical system apparatus described herein, immunoassay formats described above can be performed on the Abbott IMx® analyzer and the Abbott TDx® analyzer (Abbott Laboratories, Abbott Park, Illinois, USA) according to the teachings of the present invention. The Abbott IMx® analyzer utilizes MEIA technology for high and low molecular weight analytes requiring greater sensitivity, and FPIA technology, such as that used on the Abbott TDx® analyzer, is used primarily for lower molecular weight analytes. A front surface fluorometer is used to quantify a fluorescent product generated in the MEIA assays, while a fluorescence polarization optical system is used to quantify the degree of tracer binding to antibody in the FPIA assays. The test samples are automatically processed by a robotic arm with a pipetting probe and a rotating carousel which positions the samples for processing and permit the analysis of multiple samples and provides for access to the test samples for the formation of subsequent reaction mixtures. In particular, test samples are

automatically processed with a robotic arm comprising a pipetting probe and a rotating carousel which positions the samples for processing. The assay reagents for performing MEIA and FPIA procedures are stored in a stationary reagent
5 pack from which the pipetting probe removes the appropriate assay reagents for performance of a particular assay. The reagent pack typically includes a plurality of containers which separately contain various assay reagents such as, for example, an antibody reagent, a labeled reagent, buffer,
10 diluent, and the like, for performing MEIA and FPIA methodologies.

Other assay formats, such as homogeneous assays, the detection of precipitate formed by reaction between antigens and antibodies in a test sample-cell to form light scattering
15 centers, and methods and apparatus for detecting immunological agglutination reactions known in the art can also be performed according to the teachings of the present invention. Such apparatus and methods include, for example, the steps of measuring light absorption of the liquid medium
20 with antibody before and after the antigen-antibody reaction by using light which is absorbable by the antibody, and calculating the difference of the absorptions. In this way, the presence or absence of agglutination can be detected based on the fact that the agglutination reaction reduces the
25 concentration of antibody, which affects the light absorption of the liquid medium. As is typical of methods and apparatus for performing homogeneous assays, these procedures do not require separation of a solid phase from the reaction mixture for further analysis. Spectrophotometric assays can also be
30 performed according to the teachings of the present invention on the Abbott Spectrum clinical analyzer and the Abbott Spectrum Series II clinical analyzer (Abbott Laboratories, Abbott Park, IL, USA). In addition, turbidimetric and nephelometric assays according to the teachings of the
35 present invention can be utilized in the analysis of blood, urine, spinal fluid, and the like, for the determination of analytes such as proteins wherein there is no comparable

colorimetric assay due to the lack of an effective chromogenic reagent system. Yoe and Klimman, Photoelectric Chemical Analysis, Vol. II: Nephelometry, Wiley & Sons, Inc., New York, 1929, describe various nephelometric assays. various reagents and reagent systems which can be employed for performing spectrophotometric assays on the automated analytical systems described herein include, but are not intended to be limited to, those for the simultaneous determination of glucose and urea, such as described in U.S. Patent No. 5,037,738. The simultaneous determination of calcium and phosphorous; the simultaneous determination of cholesterol and triglycerides; determining isoenzymes; determining blood ammonia levels, and the like, can be also be performed according to the teachings of the present invention.

It is to be understood that although automated instruments for performing various assay formats and techniques have been described herein, such assay techniques and formats or portions thereof can be performed manually in accordance with the teachings of the present invention. In addition, the various assay formats and methodologies described herein can be performed according to the present invention utilizing various analytical elements or test devices, such as test strips, known in the art wherein the advantages of the present invention can be readily appreciated by those skilled in the art. For example, analytical elements and test devices have been described for performing various immunoassay formats, such as those described herein, which, for example, inherently perform the required separation step in a heterogeneous immunoassay. In general, such devices include zones, typically in the form of, for example, one or more pads which are assembled either as layers or in as an elongate strip wherein such pads are assembled with the edges thereof in fluid communication with each other. The zones of such devices include the necessary reagents for carrying out a particular immunoassay as described herein

upon the application of a test sample and assay verification sample thereto.

The present invention will now be illustrated, but is not intended to be limited by, the following examples.

5

Example 1

Description Of Kitting And Process Area Activities For
Performing An FPIA FPIA On The Continuous And Random
Access Analytical System

10

SYSTEM DESCRIPTION OF KITTING AREA FOR PHENOBARBITAL
ASSAY

15 A. ASSUMPTIONS

1. Analyzer is in Standby/Ready mode when sample is loaded. System has been previously initialized (All motors are homed, syringe and pumps are purged, all electronics and sensors are checked.)

20

2. Waste has been emptied, Diluent, MEIA buffer, MUP, and Quat bulk liquid consumables have been checked for sufficient volume.

3. All Consumable inventory files have been updated.

25

B. PREPARATION STEPS

1. User loads empty Reaction Vessel (RV) into RV carousel.

30

2. To load a reagent pack(s), the user must first pause the front end carousels. The system will complete kitting of the current test and transfer the test to the process area.

3. User opens the reagent carousel cover, loads reagent pack(s) into reagent carousel, closes the reagent carousel cover, then resumes the front-end.

35

4. Instrument automatically scans all reagent packs onboard to verify reagent status.

- 5 (a) Each reagent pack is positioned in front of the reagent pack barcode reader by rotation of the reagent carousel.
- (b) Reagent pack barcode reader reads barcode to identify assay type and carousel location.
- (c) If the barcode is unreadable, the system will request a barcode override.
- 10 (d) If the barcode is good or override complete, the system will check the system inventory. The user will be notified if the pack is found to be empty, invalid or outdated. Once the reagent pack is found to be good, it is ready
- 15 to use.

C. REQUESTING A TEST

1. User has two options for requesting a test or group
20 of tests on one or more patient samples.

- (a) User may download the test request loadlist from a host computer to create an order list.
- (b) User enters test request or creates an order list on the System directly.

25 2. If sample cups (no barcode) are used, the following scenario occurs:

- (a) User refers to order list for segment ID and position number to place sample.
- (b) User loads a sample cup into referenced
30 position in segment.
- (c) User transfers patient sample from blood collection tube into sample cup.
- (d) Segment is placed into sample carousel.
- (e) Indication is made to instrument that
35 samples have been loaded.
- (f) Instrument checks consumable inventories, waste status, cal status, etc.

(g) Sample carousel rotates segment to segment identification reader.

(h) Instrument reads segment identification.

5 3. If primary tubes (with barcode) are used, the following scenario occurs (two types of carriers are used for primary tubes: one for tubes with heights of 75 mm and a second for tubes with heights of 100 mm.):

(a) User loads primary tube into next available segment location on sample carousel.

10 (b) Indication is made to instrument that samples are available to be run.

(c) Instrument checks consumable inventories, waste status, cal status, etc.

15 D. SCHEDULING A TEST

1. When the sample is presented to the pipettor, the System attempts to schedule the tests ordered on that sample for processing. Each test ordered for the sample will be
20 scheduled separately.

(b) The System checks for adequate inventory (reagent packs, cartridges, buffer, MUP), system resources, sample time to complete the test.

25 (c) The System checks for valid calibration or orders for them on the order list.

(d) If all test requirements are met, the test is scheduled for processing.

30 (e) If all test requirements are not met, the test request is moved to the exception list. Once the test requirements have been met, the test request is moved back to the order list by the user.

2. When a test has been scheduled, the System moves
35 it to the processing list and attempts to schedule other tests ordered for that sample.

3. When all tests for the current sample have been kitted, the System advances to the next sample on the sample carousel.

5 E KITTING A TEST

1. Once a test is scheduled, it is immediately kitted. (No tests are kitted until the scheduler ensures that the test can be transferred onto the process carousel immediately and processed within the timing requirements of the assay.)

2. RV carousel is rotated clockwise until an RV is detected in pipette axis position.

3. Reagent pack carousel is rotated until reagent pack for test ordered is at the actuator position. The actuator opens the reagent cartridge caps and the reagent pack carousel is then rotated until a reagent pack for test ordered is in the pipette axis position. After all pipetting steps have been completed, the reagent pack carousel is rotated back to the actuator position where the reagent cartridge caps are closed.

4. Sample carousel is rotated until sample cup (or primary tube) is in pipette axis position.

5. Pipette is always at "HOME" position (Pipette R-axis is parked over wash station and Pipette Z-axis is at the Z-clear position) when not in use.

6. Sample kitting.

(a) Sample aspirate.

(i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.

(ii) Pipette R-axis is moved over sample cup.

(iii) Pipette Z-axis is moved down to the Z-above position.

(iv) LLS is enabled to ensure that no liquid is currently detected.

(v) Pipette Z-axis is moved down at constant speed until fluid is detected

or until Z-Asp limit has been reached
(It will be assumed that fluid is
detected)

- 5 (vi) Based on the Z-height position at which
fluid is detected and the Z-
height/volume table, the System
calculates the volume of fluid in the
well and compares it to the volume
10 specified in the pipetting description.
If sufficient volume is present in the
well, the aspiration sequence is
initiated (If insufficient volume is
present, the test is aborted and the
test request moved to the exception
15 list. The exception list provides
notice to an operator of tests which
cannot be completed).
- 20 (vii) The following occur simultaneously
until the total volume of sample
required is aspirated:
- (1) Pipette Z-axis motor is moved
down at a rate of "X" steps/sec.
 - (2) Syringe motor aspirates "X" uL at
a rate of "X" ul/sec.
 - 25 (3) LLS is checked to ensure probe
still in liquid Liquid Level Sense
(LLS) is disabled. Pipette Z-axis
is moved up to Z-clear position.
 - (4) Pipette R-axis is moved over the
RV sample well.
 - (5) Pipette Z-axis is moved down to
the dispense position within the
RV sample well.
 - (6) Syringe dispenses "X" uL of
sample at a rate of "X" ul/sec.
 - 30 (7) Pipette Z-axis is moved up to Z-
clear position.
- 35

(b) Probe Post-Wash

5 The probe is washed to ensure that it is free from contamination. It is to be understood that all pipette activities (in both kitting and process areas) are followed with a probe post-wash to minimize carryover from one fluid aspirate to another. In some cases, pipette activities may be preceded with a probe prewash if necessary to guarantee the validity of the next fluid aspirate. For this assay description, it will be assumed that only a post-wash is used.

10 (i) The inside of the probe is cleaned first.

15 (1) Pipette R-axis is moved over waste area.

(2) Pipette Z-axis is moved down to appropriate position within the waste area.

20 (3) The wash valve is opened for the amount of time specified in the assay protocol.

(4) Wash valve is closed.

(5) Pipette Z-axis is moved up to the Z-clear position.

25 (ii) The outside of the probe is cleaned next.

(1) Pipette R-axis is moved over wash cup.

30 (2) Pipette Z-axis is moved down to wash position within the wash cup.

(3) The wash valve is opened for the amount of time specified in the assay protocol.

35 (4) Wash valve is closed.

(iii) Pipette is returned to "HOME" position.

7. Popper kitting ("Popper" is defined as a substance which eliminates in general interfering substances in assays such as, for example, those discussed and claimed in U.S. Patent 4,492,762 issued January 8, 1985).

5

(a) Popper aspirate.

10

(i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.

(ii) Pipette R-Axis is moved over the popper reagent bottle in the Reagent Pack.

(iii) Pipette Z-axis is moved down to the Z-above position.

15

(iv) LLS is enabled to ensure no liquid currently detected.

20

(v) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-aspiration-lower (Z-Asp) limit is reached (it will be assumed that fluid is detected).

25

(vi) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present in the well, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).

30

(vii) The following occur simultaneously until the total volume of popper required is aspirated:

35

(1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.

- 5 (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (3) LLS is checked to ensure probe still in liquid.
- (4) LLS is disabled.
- (5) Pipette Z-axis is moved up to Z-clear position.
- (6) Pipette R-axis is moved over the RV reagent 1 well.
- 10 (7) Pipette Z-axis is moved down to the dispense position within the RV reagent 1 well.
- (8) Syringe dispenses "X" uL of popper at a rate of "X" ul/sec.
- 15 (9) Pipette Z-axis is moved up to Z-clear position.

(b) Probe post-wash.

The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample Kitting).

20

8. Antiserum kitting

(a) Antiserum aspirate

- (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- 25 (ii) Pipette R-Axis is moved over the antiserum reagent bottle in the Reagent Pack.
- (iii) Pipette Z-axis is moved down to the Z-above position.
- 30 (iv) LLS is enabled to ensure no liquid currently detected.
- (v) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 35 (vi) Based on the Z-height position at which fluid is detected and the Z-

5

height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present in the well, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).

10

(vii) The following occur simultaneously until the total volume of antiserum required is aspirated:

15

- (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" micro liter (uL) at a rate of "X" ul/sec. LLS is checked to ensure probe still in liquid.

20

- (3) LLS is disabled.
- (4) Pipette Z-axis is moved up to Z-clear position.
- (5) Pipette R-axis is moved over the RV reagent 2 well.

25

- (6) Pipette Z-axis is moved down to the dispense position within the RV reagent 2 well.
- (7) Syringe dispenses "X" uL of antiserum at a rate of "X" ul/sec.

30

- (8) Pipette Z-axis is moved up to Z-clear position.

(b) Probe post-wash.

The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample Kitting).

35

9. Tracer kitting.

(a) Tracer aspirate.

- 5
- (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- (ii) Pipette R-Axis is moved over the tracer reagent bottle in the Reagent Pack.
- (iii) Pipette Z-axis is moved down to the Z-above position.
- (iv) LLS is enabled to ensure no liquid currently detected.
- 10
- (v) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 15
- (vi) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present in the well, the aspiration sequence is initiated. (if sufficient volume not is present, the test is aborted and the test request moved to the exception list).
- 20
- 25
- (vii) The following occur simultaneously until the total volume of tracer required is aspirated:
- 30
- (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (3) LLS is checked to ensure probe still in liquid.
- 35
- (4) LLS is disabled.
- (5) Pipette Z-axis is moved up to Z-clear position.

- 5 (6) Pipette R-axis is moved over the RV reagent 3 well.
- (7) Pipette Z-axis is moved down to the dispense position within the RV reagent 2 well.
- (8) Syringe dispenses "X" uL of tracer at a rate of "X" ul/sec.
- (9) Pipette Z-axis is moved up to Z-clear position.
- 10 (b) Probe post-wash.
- The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample Kitting).
- 15 F. TRANSFER OF REACTION VESSEL (RV) INTO PROCESS AREA
1. RV carousel is rotated to transfer station.
2. Process carousel is rotated so that the empty position is aligned with the transfer station.
- 20 3. Transfer mechanism O-axis is rotated to sample entry area.
4. Transfer mechanism R-axis grabs the RV and pulls it into the transfer mechanism.
5. Transfer mechanism O-axis is rotated so that RV
- 25 is aligned with the empty position on the process carousel.
6. RV is loaded onto process carousel.

SYSTEM DESCRIPTION OF FPIA PROCESS AREA FOR
PHENOBARBITAL

30

- A. Wait for temperature equilibration time and evaporation window to expire.
- B. FIRST PIPETTE ACTIVITY (preparation of sample blank comprising diluted sample and popper).
- 35 1. Incubation timer is set according to assay file specifications.

2. Precision diluent aspirate. The following activities are performed simultaneously:

- (a) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (b) Wash valve is opened.
- (c) Wait "n" seconds.
- (d) Wash valve is closed.

3. Sample aspirate.

- (a) Pipette R-axis is moved over the RV sample well.
- (b) LLS is enabled to ensure no liquid currently detected.
- (c) Pipette Z-axis is moved down at constant speed until fluid is detected OR until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- (d) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- (e) The following occur simultaneously until the total volume of sample required is aspirated:
 - (i) Pipettor Z-axis motor is moved down at a rate of "X" steps/sec.
 - (ii) Syringe aspirates "x" uL of sample at a rate of "X" ul/sec.
 - (iii) LLS is checked to ensure probe still in liquid.
 - (iv) LLS is disabled.
 - (v) Pipette Z-axis is moved up to Z-above position.

4. Diluent/sample dispensed to the RV predilute well.
 - (a) Pipette R-axis is moved over the RV predilute well.
 - (b) Pipette Z-axis is moved down to the dispense position within the RV predilute well.
 - (c) Syringe dispenses "X" uL of diluent/sample at a rate of "X" ul/sec.
 - (d) Pipette Z-axis is moved up to Z-clear position.
5. Probe post-wash.

The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting).
6. Precision diluent aspirate. The following activities are performed simultaneously:
 - (a) Syringe aspirates "X" uL at a rate of "X" ul/sec.
 - (b) Wash valve is opened.
 - (c) Wait "n" seconds.
 - (d) Wash valve is closed.
7. Popper aspirate.
 - (a) Pipette R-axis is moved over the RV Reagent (popper) well.
 - (b) LLS is enabled to ensure no liquid currently detected.
 - (c) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
 - (d) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test

request moved to the exception list).

(e) The following occur simultaneously until the total volume of popper required is aspirated:

5

(i) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.

(ii) Syringe aspirates "X" uL at a rate of "x" ul/sec.

(iii) LLS is checked to ensure probe still in liquid.

10

(iv) LLS is disabled.

(v) Pipette Z-axis is moved up to the Z-above position.

8. Diluted sample aspirate.

15

(a) Pipette R-axis is moved over the RV predilute well.

(b) LLS is enabled to ensure no liquid currently detected.

20

(c) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).

25

(d) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).

30

(e) The following occur simultaneously until the total volume of diluted sample required is aspirated:

35

(i) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.

(ii) Syringe aspirates "X" uL at a rate of "x" ul/sec.

- (iii) LLS is checked to ensure probe still in liquid.
- (iv) LLS is disabled.
- (v) Pipette Z-axis is moved up to the Z-above position.

5

11. Diluted sample/popper diluent dispensed to RV cuvette.

- (a) Pipette R-axis is moved over to the RV cuvette position.
- (b) Pipette Z-axis is moved down to the dispense position in the RV cuvette.
- (c) Syringe dispenses "X" uL of diluted sample/popper/diluent at a rate of "X" uL/sec.
- (d) Pipette Z-axis is moved up to the Z-above position.

10

15

12. Probe post-wash.

The probe is again washed to ensure that it is free from contamination as described in section 6 (sample kitting) to complete first pipette activity

20

C. BLANK READ PREPARATION

When incubation timer expired, the following activities are started:

25

1. The FPIA reader is prepared to take a read; lamp intensity is brought from simmer state to burn state.
2. Photomultiplier tube (PMT) gain is set.

30 D. BLANK READ (BACKGROUND)

1. Incubation timer is set according to assay file specifications.
2. Process carousel is rotated so that the RV is at the read station.
3. Horizontal intensity is read for "X.XX" seconds.
4. The crystal is flipped for the vertical read.

35

5. Wait "n" seconds until the crystal settles.
6. Vertical intensity is read for "X.XX" seconds.
7. The raw reads are converted to normalized reads (light intensity hitting detector/lamp intensity) by the optics
5 microprocessor.
8. Background reads are stored.
9. System calculates BLANK I to complete blank read.
10. Next activity started when incubation timer expires.

10

E SECOND PIPETTE ACTIVITY (for reaction between diluted sample, popper, tracer and antiserum).

1. Incubation timer is set according to assay file
15 specifications.
2. Precision diluent aspirate.
 - (a) The following activities are performed simultaneously:
 - (i) Syringe aspirates "X" uL at a rate of "X"
20 ul/sec.
 - (ii) Wash valve is opened.
 - (iii) Wait "n" seconds.
 - (iv) Wash valve is closed.
3. Antiserum aspirate.
 - (i) Pipette R-axis is moved over the RV Reagent
25 2 (antiserum) well.
 - (ii) LS is enabled to ensure no liquid currently detected.
 - (iii) Pipette Z-axis is moved down at constant
30 speed until fluid is detected OR until the Z-Asp limit is reached (it will be assumed that fluid is detected).
 - (iv) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If
35

sufficient volume is present, the aspiration sequence is initiated. (If sufficient volume is not present, the test is aborted and the test request moved to the exception list.)

5 (v) The following occur simultaneously until the total volume of antiserum required is aspirated:

- 10 (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (3) LLS is checked to ensure probe still in liquid.
- (4) LLS is disabled.
- 15 (5) Pipette Z-axis is moved up to the Z-above position.

4. Tracer aspirate.

- (a) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- 20 (b) Pipette R-axis is moved over the RV Reagent 3 (tracer) well.
- (c) LLS is enabled to ensure no liquid currently detected.
- (d) Pipette Z-axis is moved down at constant speed until fluid is detected OR until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 25 (e) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- 30
- 35

- (f) The following occur simultaneously until the total volume of tracer required is aspirated:
- (i) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
 - (ii) Syringe aspirates "X" uL at a rate of "X" ul/sec.
 - (iii) LLS is checked to ensure probe still in liquid.
 - (v) LLS is disabled.
 - (vi) Pipette Z-axis is moved up to the Z-above position.
- 5.
- Diluted sample aspirate.
- (a) Pipette R-axis is moved over the RV predilute well.
 - (b) LLS is enabled to ensure no liquid currently detected.
 - (c) Pipette Z-axis is moved down at constant speed until fluid is detected OR until the Z-Asp limit is reached (it will be assumed that fluid is detected).
 - (d) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list.)
 - (e) The following occur simultaneously until the total volume of diluted sample required is aspirated:
 - (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
 - (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.

65

- 5 (3) LLS is checked to ensure probe still in liquid.
- (4) LLS is disabled.
- (5) Pipette Z-axis is moved up to the Z-above position.
6. Diluted sample/tracer/aspirate/antiserum/diluent dispensed to RV cuvette.
- (a) Pipette R-axis is moved over to the RV cuvette position.
- 10 (b) Pipette Z-axis is moved down to the dispense position in the RV cuvette.
- (c) Syringe dispenses "X" uL of diluted sample/tracer/air/antiserum/diluent at a rate of "X" ul/sec.
- 15 (d) Pipette Z-axis is moved up to the Z-above position.
7. Probe post-wash.
- The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting)
- 20 to complete the second pipette activity.
8. Next activity started when incubation timer expires.

E FINAL READ PREPARATION

25

1. The FPIA reader is prepared to take a read; lamp intensity is brought from simmer state to burn state.
2. PMT gain is set.

30 F. FINAL READ

1. Process carousel is rotated so that the RV is at the read station.
2. Horizontal intensity is read for "X.XX" seconds.
- 35 3. The crystal is flipped for the vertical read.
4. The System delays "n" seconds until the crystal settles.

5. Vertical intensity is read for "X.XX" seconds.
 6. The raw reads are converted to normalized reads (light intensity hitting detector/lamp intensity) by the optics microprocessor.
 - 5 7. Reads are stored.
 8. System calculates NET intensity (I) and milipolarization (mP).
 9. mP value is fitted to calibration curve to yield a concentration result.
- 10
- G RV UNLOAD (this activity occurs when resources are not in use. The following are performed simultaneously:
1. Process carousel is rotated so that the empty position is at the transfer station. Transfer mechanism 0-axis
 - 15 is moved to process carousel.
 2. RV is grabbed with the transfer mechanism R-axis and pulled into the transfer mechanism.
 3. Transfer mechanism 0-axis is rotated so that RV is aligned with the waste container.
 - 20 4. RV is pushed into the waste container.

Example 2

25 Description Of Kitting And Process Area Activities For Performing An MEIA On The Continuous And Random Access Analytical System

SYSTEM DESCRIPTION OF KITTING AREA FOR CEA ASSAY

30 A. ASSUMPTIONS

1. Analyzer is in Standby/Ready mode when sample is loaded. System has been previously initialized (All motors are homed, syringe and pumps are purged, all electronics and sensors are checked).
- 35 2. Waste has been emptied, dilution, MEIA buffer, MUP, and Quat bulk liquid consumables have been checked for sufficient volume.

3. Cartridges have been placed into hopper and are available for loading onto auxiliary carousel when needed (for MEIA assays only).

4. All Consumable inventory files have been updated.

5

B. PREPARATION STEPS

1. User loads empty RVs into RV carousel.

10 2. To load a reagent pack(s), the user must first pause the front end carousels. The system will complete kitting of the current test and transfer the test to the process area.

15 3. User opens the reagent carousel, loads reagent pack(s) into reagent carousel, closes the reagent carousel cover, then resumes the front-end.

4. Instrument automatically scans all reagent packs onboard to verify reagent status.

20 5. Each reagent pack is positioned in front of the reagent pack barcode reader by rotation of the reagent carousel.

6. Reagent pack barcode reader reads barcode to identify assay type and carousel location. If the barcode is unreadable, the system will request a barcode override.

25 7. If the barcode is good or override complete, the system will check the system inventory. The user will be notified if the pack is found to be empty, invalid or outdated. Once the reagent pack is found to be good, it is ready to use.

C. REQUESTING A TEST

30

1. User has two options for requesting a test or group of tests on one or more patient samples.

(a) User may download the test request loadlist from a host computer to create an order list.

35 (b) User enters test request or creates an order list on the System directly.

2. If sample cups (no barcode) are used, the

following scenario occurs:

- (a) User refers to order list for segment ID and position number to place sample.
- 5 (b) User loads a sample cup into referenced position in segment.
- (c) User transfers patient sample from blood collection tube into sample cup.
- (d) Segment is placed into sample carousel.
- 10 (e) Indication is made to instrument that samples have been loaded.
- (f) Instrument checks consumable inventories, waste status, assay calibration, etc.
- (g) Sample carousel rotates segment to segment identification reader.
- 15 (h) Instrument reads segment identification.

3. If primary tubes (with barcode) are used, the following scenario occurs:

- (a) User loads primary tube into next available segment location on sample carousel (two types of carriers are used for primary tubes: one for tubes with heights of 75 mm and a second for tubes with heights of 100 mm.).
- 20 (b) Indication is made to instrument that samples are available to be run.
- 25 (c) Sample carousel rotates segment to segment identification reader.

D. SCHEDULING A TEST

- 30 1. When the sample is presented to the pipettor, the System attempts to schedule the tests ordered on that sample for processing. Each test ordered for the sample will be scheduled separately.

- 35 (a) The System checks for adequate inventory (reagent packs, cartridges, buffer, MUP), system resources, sample time to complete the test.

- (b) The System checks for valid calibration or orders for them on the order list.
- (c) If all test requirements are met, the test is scheduled for processing.
- 5 (d) If all test requirements are not met, the test request is moved to the exception list. Once the test requirements have been met, the test request is moved back to the order list by the user.
- 10 2. When a test has been scheduled, the system moves it to the processing list and attempts to schedule other tests ordered for that sample.
- 15 3. When all tests for the current sample have been kitted, the System advances to the next sample on the sample carousel.

E KITTING A TEST

1. Once a test is scheduled, it is immediately kitted.
- 20 (no tests are kitted until the scheduler ensures that the test can be transferred onto the process carousel immediately and processed within the timing requirements of the assay).
2. RV carousel is rotated clockwise until an RV is detected in pipette axis position.
- 25 3. Reagent pack carousel is rotated until reagent pack for test ordered is at the actuator position. The actuator opens the reagent cartridge caps and the reagent pack carousel is then rotated until reagent pack for test ordered is in the pipette axis position. After all pipetting steps have been
- 30 completed, the reagent pack carousel is rotated back to the actuator position where the reagent cartridge caps are closed.
4. Sample carousel is rotated until sample cup (or primary tube) is in pipette axis position.
5. Pipette is always at HOME position (Pipette R-axis
- 35 is parked over wash station and Pipette Z-axis is at the Z-clear position) when not in use.
6. Sample kitting.

- 5
- (a) Sample aspirate.
- (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- (ii) Pipette R-axis is moved over sample cup.
- (iii) Pipette Z-axis is moved down to the Z-above position.
- (iv) Pipette Z-axis is moved down to the Z-LLS position.
- 10 (v) LLS is enabled to ensure that no liquid is currently detected.
- (vi) Pipette Z-axis is moved down at constant speed until fluid is detected or until Z-Asp limit has been reached (it will be assumed that fluid is detected).
- 15
- (vii) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present in the well, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- 20
- (viii) The following occur simultaneously until the total volume of sample required is aspirated:
- 25 (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- 30 (3) LLS is checked to ensure probe still in liquid.
- 35

- 5
- (4) LLS is disabled.
- (5) Pipette Z-axis is moved up to Z-clear position.
- (6) Pipette R-axis is moved over the RV sample well.
- (7) Pipette Z-axis is moved down to the dispense position within the RV sample well.
- 10
- (8) Syringe dispenses "X" uL of sample at a rate of "X" uL/sec.
- (9) Pipette Z-axis is moved up to Z-clear position.
- 15
- (b) Probe post-wash.
- The probe is washed to ensure that it is free from contamination. It is to be understood that pipette activities in both kitting and process areas are generally followed with a probe post-wash to minimize carryover from one fluid aspirate to another. In some cases, pipette activities may be preceded with a probe prewash if necessary to guarantee the validity of the next fluid aspirate. For this assay description, it will be assumed that only a post-wash is used .
- 20
- 25
- (i) The inside of the probe is cleaned first.
- (1) Pipette R-axis is moved over waste area.
- 30
- (2) Pipette Z-axis is moved down to appropriate position within the waste area.
- (3) The wash valve is opened for the amount of time specified in the assay protocol.
- 35
- (4) Wash valve is closed.

- (ii) Pipette Z-axis is moved up to the Z-clear position.
 - (iii) The outside of the probe is cleaned next.
 - (1) Pipette R-axis is moved over wash cup.
 - (2) Pipette Z-axis is moved down to wash position within the wash cup.
 - (3) The wash valve is opened for the amount of time specified in the assay protocol.
 - (4) Wash valve is closed.
 - (5) Pipette is returned to "HOME" position.
- 5
- 10
- 15
7. Microparticle kitting.
- (a) Microparticle aspirate (microparticles are pipetted directly into the RV incubation well to save on volume, as this is the most costly MEIA reagent).
- 20
- (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
 - (ii) Pipette R-Axis is moved over the microparticle reagent bottle in the Reagent Pack.
 - (iii) Pipette Z-axis is moved down to the Z-above position.
 - (iv) Pipette Z-axis is moved down to the Z-LLS position.
 - (v) LLS is enabled to ensure no-liquid currently detected.
 - (vi) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected)
 - (vii) Based on the Z-height position at which
- 25
- 30
- 35

5 fluid is detected and the Z-
height/volume table, the System
calculates the volume of fluid in the
well and compares it to the volume
specified in the pipetting description.
If sufficient volume is present in the
well, the aspiration sequence is
initiated (if sufficient volume is not
present, the test is aborted and the
test request moved to the exception
list).

10 (viii) The following occur simultaneously
until the total volume of
microparticles required is aspirated:

15 (1) Pipette Z-axis motor is moved
down at a rate of "X" steps/sec.
(2) Syringe aspirates "X" uL at a rate
of "X" ul/sec.
(3) LLS is checked to ensure probe
still in liquid.

20 (ix) LLS is disabled.
(x) Pipette Z-axis is moved up to Z-clear
position.
(xi) Pipette R-axis is moved over the RV
incubation well.

25 (xii) Pipette Z-axis is moved down to the
dispense position within the RV
incubation well.
(xiii) Syringe dispenses "X" uL of
microparticles at a rate of "X" ul/sec.
Pipette Z-axis is moved up to Z-clear
position.

30 (b) Probe post-wash.
The probe is again washed to ensure that it
is free from contamination as described in
section 6 (Sample kitting).

35

8. Conjugate kitting.

- 5 (a) Conjugate aspirate (conjugate, special wash fluid, and/or specimen diluent are pipetted into either RV reagent wells or RV predilution well, depending on volume requirements).
- 10 (i) Syringe aspirates "X": uL of air at a rate of "X" ul/sec.
- (ii) Pipette R-Axis is moved over the conjugate reagent bottle in the Reagent Pack.
- (iii) Pipette Z-axis is moved down to the Z-above position.
- (iv) Pipette Z-axis is moved down to the Z-LLS position.
- 15 (v) LLS is enabled to ensure no liquid currently detected.
- (vi) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 20 (vii) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present in the well, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- 25 (viii) The following occur simultaneously until the total volume of conjugate required is aspirated:
- 30 (1) Pipette Z-axis motor is moved down at a rate of "x" steps/sec.
- 35

75

- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (3) LLS is checked to ensure probe still in liquid.
- 5 (ix) LLS is disabled.
- (x) Pipette Z-axis is moved up to Z-clear position.
- (xi) Pipette R-axis is moved over the RV reagent well.
- 10 (xii) Pipette Z-axis is moved down to the dispense position within the RV r reagent well.
- (xiii) Syringe dispenses "X" uL of conjugate at a rate of "X" ul/sec.
- 15 (xiv) Pipette Z-axis is moved up to Z-clear position.
- (b) Probe post-wash.
- The probe is again washed to ensure that it is free from contamination as described in
- 20 section 6 (Sample kitting).
9. MEIA Buffer Kitting.
- (a) RV Carousel is rotated until RV buffer well is under the MEIA buffer dispenser at buffer kitting station.
- 25 (b) "X" uL of MEIA buffer is dispensed into the buffer well at a rate of "X" ul/sec

F. TRANSFERRING RV INTO PROCESS AREA

- 30 1. RV carousel is rotated to transfer station.
2. Process carousel is rotated so that the empty position is aligned with the transfer station.
3. Transfer mechanism O-axis is rotated to sample entry area.
- 35 4. Transfer mechanism R-axis grabs the RV and pulls it into the transfer mechanism.

5. Transfer mechanism 0-axis is rotated so that RV is aligned with the empty position on the process carousel.

6. RV is loaded onto process carousel.

5 SYSTEM DESCRIPTION OF MEIA PROCESS AREA FOR CEA

A. System waits for temperature equilibration time and evaporation window to expire.

10 B. FIRST PIPETTE ACTIVITY (microparticle/sample reaction)

- 1. Incubation timer is set according to assay file specifications.

15 2. MEIA buffer aspirate.

(a) The process carousel is moved so that the RV is at the pipetting station.

(b) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.

20 (c) Pipette R-axis is moved over the RV buffer well.

(d) Pipette Z-axis is moved down to the Z-above position over the RV buffer well.

25 (e) Pipette Z-axis is moved down to the Z-LLS position.

(f) LLS is enabled to ensure no liquid currently detected.

30 (g) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).

35 (h) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration

sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).

5

- (i) The following occur simultaneously until the total volume of MEIA buffer required is aspirated:

10

(1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.

(2) Syringe aspirates "X" uL at a rate of "X" ul/sec.

(j) LLS is checked to ensure probe still in liquid.

(k) LLS is disabled.

(l) Pipette Z-axis is moved up to Z-above position.

15

3. Sample aspirate

(a) Pipette R-axis is moved over the RV sample well.

(b) Pipette Z-axis is moved down to the Z-LLS position.

20

(c) LLS is enabled to ensure no liquid currently detected.

(d) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).

25

(e) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the system calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).

30

35

(f) The following occur simultaneously until the total volume of sample required is aspirated:

- (1) Pipettor Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- 5 (g) LLS is checked to ensure probe still in liquid.
- (h) LLS is disabled.
- (i) Pipette Z-axis is moved up to Z-above position.
4. MEIA buffer and sample are added to
- 10 microparticles in incubation well.
- (a) Pipette Z-axis is moved down to the dispense position within the RV incubation well.
- (b) Syringe dispenses "X" uL of MEIA buffer and sample at a rate of "X" ul/sec.
- 15 (c) Pipette Z-axis is moved up to Z-clear position.
5. Probe post-wash.
- The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting).
- 20
- C. CARTRIDGE LOAD (This activity occurs when resources are not in use)
1. Move the auxiliary carousel so that reserved
- 25 position is under feeder.
2. Cycle trap-door mechanism to load flashlight into carousel.
3. Cycle shuttle mechanism to place another MEIA cartridge on trap door (for next tab load).
- 30 4. Check incubation timer. When expires start next pipetting.
- D. SECOND PIPETTE ACTIVITY (transfer of reaction mixture
- 35 to matrix)

1. Incubation timer is set according to assay file specifications.
2. Buffer aspirate.
 - (a) The process carousel is moved so that the RV is at the pipetting station.
 - (b) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
 - (c) Pipette R-axis is moved over the RV buffer well.
 - (d) Pipette Z-axis is moved down to the Z-above position.
 - (e) Pipette Z-axis is moved down to the Z-LLS position.
 - (f) LLS is enabled to ensure no liquid currently detected.
 - (g) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
 - (h) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the system calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
 - (i) The following occur simultaneously until the total volume of buffer required is aspirated:
 - (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
 - (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
 - (j) LLS is checked to ensure probe still in liquid.
 - (k) LLS is disabled.

- (l) Pipette Z-axis is moved up to the Z-above position.
3. Reaction mixture aspirate.
- 5 (a) Pipette R-axis is moved over the RV incubation well.
- (b) Pipette Z-axis is moved down to the Z-LLS position.
- (c) LLS is enabled to ensure no liquid currently detected.
- 10 (d) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 15 (e) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the system calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- 20 (f) The following occur simultaneously until the total volume of reaction mixture required is aspirated:
- 25 (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (2) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- 30 (g) LLS is checked to ensure probe still in liquid.
- (h) LLS is disabled.
- (i) Pipette Z-axis is moved up to the Z-clear position.
4. Reaction mixture dispense onto matrix.
- 35 (a) The following are performed simultaneously and concurrently with the reaction mixture aspirate (above):

- 5 (i) The auxiliary carousel is moved so that the cartridge is at the pipetting station.
- (ii) Pipette R-axis is moved over the MEIA cartridge (matrix) surface.
- (iii) Pipette Z-axis is moved down to the matrix dispense position.
- 10 (iv) Syringe dispenses "X" uL of reaction mixture at a rate of "X" ul/sec.
- (v) System delays "X" seconds until reaction mixture has been absorbed by matrix.
5. Buffer wash of matrix.
- 15 (a) Syringe dispenses "X" uL of buffer at a rate of "X" ul/sec.
- (b) Pipette Z-axis is moved up to the Z-clear position.
6. Probe post-wash.
- 20 The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting).
7. When incubation timer expires, next pipette activity begins.

25 E THIRD PIPETTE ACTIVITY (conjugate addition)

1. Incubation timer is set according to assay file specifications.
2. Conjugate aspirate.
- 30 (a) The process carousel is moved so that the RV is at the pipetting station.
- (b) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- (c) Pipette R-axis is moved over the RV reagent 1 (conjugate) well.
- 35 (d) Pipette Z-axis is moved down to the Z-above position.

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- 5 (e) LLS is enabled to ensure no liquid currently detected.
- (f) Pipette Z-axis is moved down at constant speed until fluid is detected or until the Z-Asp limit is reached (it will be assumed that fluid is detected).
- 10 (g) Based on the Z-height position at which fluid is detected and the Z-height/volume table, the System calculates the volume of fluid in the well and compares it to the volume specified in the pipetting description. If sufficient volume is present, the aspiration sequence is initiated (if sufficient volume is not present, the test is aborted and the test request moved to the exception list).
- 15 (h) The following occur simultaneously until the total volume of conjugate required is aspirated:
- 20 (i) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- (ii) Syringe aspirates "X" uL at a rate of "X" ul/sec.
- (i) LLS is checked to ensure probe still in liquid.
- (j) LLS is disabled.
- 25 (k) Pipette Z-axis is moved up to the Z-clear position.
3. Conjugate dispense (performed simultaneously).
- (a) The auxiliary carousel is moved so that the cartridge is at the pipetting station.
- 30 (b) Pipette R-axis is moved over the cartridge (matrix) surface.
- (c) Pipette Z-axis is moved down to the matrix dispense position.
- (d) Syringe dispenses "X" uL of conjugate at a rate of "X" ul/sec.
- 35 (e) Pipette Z-axis is moved up to the Z-clear position.

(f) Wait "X" seconds until reaction mixture has been absorbed by matrix.

4. Probe post-wash.

5 The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting).

F. RV UNLOAD (This activity occurs when resources are not in use)

- 10 1. The following are performed simultaneously:
- (a) Process carousel is rotated so that the empty position is at the transfer station.
 - (b) Transfer mechanism 0-axis is moved to process carousel.
- 15 2. RV is grabbed with the transfer mechanism R-axis and pulled into the transfer mechanism.
3. Transfer mechanism 0-axis is rotated so that RV is aligned with the waste container.
4. RV is pushed into the waste container.
- 20 5. Check incubation timer. When expires start next activity.

G MEIA READ PREPARATION

- 25 1. Lamp intensity is brought from simmer state to burn state.
2. PMT gain is set.

30 H. MATRIX WASH

1. Auxiliary carousel is rotated so that the cartridge is at the matrix wash station.
2. The following steps are repeated until all the
- 35 buffer specified in the assay file for cartridge wash has been dispensed.

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- (a) "X" uL of heated MEIA buffer are dispensed in 50uL cycles at a rate of "X" uL/sec onto the matrix.
- (b) Wait "n" seconds.

5

I. MUP DISPENSE

- 1. Auxiliary carousel is rotated so that the cartridge is at the MUP station.
- 10 2. 50uL of heated MUP are dispensed at a rate of "X" uL/sec onto the matrix.
- 3. Wait "n" seconds.

J. MEIA READ

15

- 1. Auxiliary carousel is rotated so that the cartridge is at the read station.
- 2. The following steps are repeated until the number of micro-reads specified in the assay file have been taken
20 (usually 8)
 - (a) Read for "X.XX" seconds.
 - (b) Wait "X.XX" seconds.
- 3. The reader is returned to its idle state.
 - (a) Lamp intensity is turned to simmer state.
 - 25 (b) PMT gain is set.
- 4. The raw reads are converted to normalized reads (light intensity hitting detector/lamp intensity) by the optics microprocessor.
- 5. A rate is calculated by the System from the
30 normalized reads vs time.
- 6. For quantitative assays, the rate is fitted to a calibration curve to yield a concentration result.
- 7. For qualitative assays, the sample rate is compared to an index or cutoff rate to determine if the sample
35 is positive or negative (or reactive or nonreactive).

K. CARTRIDGE UNLOAD (This activity occurs when resources are not in use)

1. Auxiliary carousel is rotated so that cartridge is
5 at the ejector station.
2. Ejector is cycled to place cartridge into waste container.

Schematic reaction sequences are presented in FIGURES-
10 26, 27 and 28 which are typical of assays that can be handled by the automated immunoassay analytical system of the invention. In FIGURE 26, a T4 assay, FPIA sequence 420, is presented wherein Step 1, T4 bound by thyroxine binding protein (TBP) 424, is reacted with T4 displacing agent 426 to
15 yield TBP 428 plus unbound T4 (430). In step 2, the T4 (430) is added to T4 antibody 432 which yields a reaction product 434 (T4 antibody-T4 complex). In Step 3, the T4 antibody-T4 complex 434 is treated with T4 tracer (fluorescent) 436 which yields a fluorescent polarization measurable reaction
20 product 438.

In FIGURE 27, a schematic reaction sequence 440 for a 1-step sandwich MEIA determination (ferritin) is presented. In Steps 1 and 2 an anti-ferritin alkaline phosphatase conjugate is mixed with ferritin sample 444 and anti-ferritin
25 microparticles 446 to yield a ferritin antibody-antigen-antibody complex 448. In step 3, the antibody-antigen-antibody complex 448 is reacted with 4-methylumbelliferyl phosphate (MUP) 450 which yields methylumbelliferone (MU) which is fluorescent. The rate of MU production is measured.

30 In FIGURE 28, the schematic reaction sequence 456 for a 2-step sandwich MEIA is provided for HTSH assay. Anti-hTSH specific microparticles 458 are added to the HTSH sample 460 which provides a reaction product HTSH antibody-antigen complex 462. In Steps 2 through 4, the complex 462 is
35 combined with an anti-hTSH alkaline phosphatase 464 yielding hTSH antibody-antigen-antibody complex 466. In step 5, the complex 466 is reacted with MUP 450 to yield MU which is

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fluorescent. The rate of MU production is measured. In accordance with the embodiments, the automated immunoassay analytical system provides apparatus, software, hardware and process technology for performing a multitude of assays continuously and with random access being available to the operator. The utilization of carousel pipettor technology for kitting and pipetting operations at either the main carousel or the process carousel, depending on the scheduled test, provides scheduling flexibilities heretofore unachievable. The inventive system allows for a commonality of kitting and pipetting for either immuno precipitation or competitive immunoassay technologies utilizing a common main carousel, transfer station, first kitting and pipetting probe and process carousel as well as a second pipetting probe before separating into respective apparatus and process requirements. Also shared is the commonality of cabinetry disposal and supply materials as well as a common computer network for scheduling, testing, kitting and pipetting.

It will be seen that multiple assays can be performed with a minimum of operator input or handling on the system and the system can be utilized for other processes and assays which have not been directly discussed but will be readily apparent to one practiced in the art in view of the above invention disclosure and the claims.

25

Example 3

MEIA For Hepatitis B Surface Antigen (HBsAg) Employing Assay Verification Sample

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An MEIA for HBsAg employing a HBsAg assay verification sample according to the present invention on the continuous and random access analytical system apparatus described herein as follows:

35

Reagents And Materials

- 5 (1) Microparticles coated with monoclonal anti-HBs(IgM) antibodies at a final concentration of 200 ug/ml antibody, stored in storage buffer comprising (1 L): 7.87 g Tris, 13.32 g Tris-HCl, 5.82 g NaCl, 136 g Sucrose, 1 ml Tween-20*, 2 g azide.
- 10 (2) Biotinylated goat anti-HBs probe at a working concentration of 1.25 ug/ml (diluent (1 L) = 0.535 g Tris, 0.897 g Tris-HCl, 1.76 g NaCl, 5 g Sodium Citrate, 5 g Galactose, 1 g Saponin, 5 ml Triton* X-100, 1 g Azide, 5 mg A56620, 1 g Sodium Alkyl Paraben, 400 ml Calf Serum, 50 ml Goat Serum, 12.5 ml Mouse Serum, 10 ml Rabbit Serum).
- 15 (3) Rabbit anti-biotin periodate-calf intestinal alkaline phosphatase conjugate at a working concentration of 1.25 ug/ml (diluent = 12.1 g Tris, 29.22 g NaCl, 0.203 g MgCl₂, 0.0138 ZnCl₂, 29 ml Brij 35, 1 g Azide, 24.3 ml Fish Skin Gelatin, 5 g Carnation* Nonfat Dry Milk, 30 mg Rabbit IgG).
- 20 (4) Recombinant Hepatitis B Surface antigen at 2 ug/mL in recalcified HBsAg plasma was employed as the positive analyte component in the assay verification sample.

Method

25

A heterogeneous immunoassay for HBsAG was performed (i) on a test sample alone and (ii) a test sample and an assay verification sample of the present invention as follows:

- 30 (1) Kitting Of Reagents And Test Sample

(a) Microparticles (22 uL) were deposited into the incubation vial of a reaction vessel as described herein.

- 35 (b) Probe (73 uL + dead volume) was deposited into reagent 1 incubation reaction vessel.

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(c) Conjugate (100ul + dead volume) was deposited into reagent location of reaction vessel.

5 (d) Test sample (73ul + dead volume) was deposited into a sample cup as described herein.

(e) Positive analyte component (50ul + dead volume) was deposited into predilute location.

10 (2) Pipetting Steps

(a) Probe (73ul) and test sample (73ul) are pipetted into incubation well [P1].

15 (b) Reaction mixture (110ul) from step 2(a) is transferred from incubation well [P2]. Conjugate for test sample reaction and assay verification sample reaction is aspirated substantially simultaneously (75uL). Conjugate (50ul) is added to matrix of MEIA cartridge. Positive analyte component (50uL) is aspirated and both
20 excess conjugate and positive analyte component (75uL) are added to the remaining reaction mixture (dead volume) in the incubation well.

(3) The MEIA cartridge matrix is washed with buffer (6X50ul) and MUP (50ul) is added, followed by Read 1 [R1].

25 (4) The positive analyte component and excess reaction mixture are transferred from incubation to the MEIA cartridge [P3].

(5) The MEIA cartridge matrix is washed with buffer (4X50ul) and MUP (50ul) is added, followed by Read 2 [R2].

30

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Table 1
INCUBATION DESCRIPTION

	INCUBATION			WINDOW	
				+	-
5	P1	P2	1100	0	0
	P2	R1	400	0	0
	R1	P3	20	0	0
	P3	R2	2	0	0
10					

Table 2
READ DESCRIPTIONS

15	TYPE	GAIN	I.TIME	D.TIME	#READS	T.TIME
	MEIA	15	0.5	0.5	8	4.9
	MEIA	15	0.5	0.5	3	1.8
20	[equilibration time = 30 seconds]					
	[evaporation time = 180 seconds]					

Table 3
IMMUNOASSAY DATA (C/SS)

	(i) ASSAY WITHOUT ASSAY VERIFICATION SAMPLE	(ii) ASSAY WITH ASSAY VERIFICATION SAMPLE	
		<u>TEST SAMPLE</u>	<u>ASSAY VERIFICATION SAMPLE</u>
30	10.4	10.6	237
	25.5	26.7	268
	21.2	21.8	273
	28.8	32.4	276
35			

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As shown by Tables 1-3 above, results obtained using this procedure demonstrated that the negative results were verified in all cases. In particular, the first read result [R1] is the test sample result, and the second read result [R2] is the positive analyte component result of the assay verification sample which verifies that the test employed potent assay reagents which were capable of providing accurate results, that the reaction vessel, sample cup and MEIA cartridge were free from contaminants or interfering substances, and that the pipet sequences and MEIA cartridge properly performed to provide a positive result where the samples contained HBsAg.

It will also be appreciated that although particular embodiments of the present invention have been disclosed, various changes and adaptations to the apparatus and methods can be made without departing from the teachings of the specification and scope of the invention as set out in the following claims.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for verifying a result in a heterogeneous immunoassay for determining the presence or amount of an analyte present in a test sample, comprising the steps of:

- (a) forming a reaction mixture comprising said test sample and a labeled reagent comprising a substance that specifically binds to said analyte labeled with a detectable moiety to form a bound species of said labeled reagent and a free species of said labeled reagent, wherein said bound species of said labeled reagent comprises said analyte and said labeled reagent and said free species of said labeled reagent does not contain said analyte;
- (b) dividing said reaction mixture containing said bound species and free species into a first portion and a second portion;
- (c) forming an assay verification sample comprising said second portion of said reaction mixture and a positive analyte component, in a known amount, capable of providing a detectable response as an indication of the presence or amount of said positive analyte component present in said assay verification sample;
- (d) independently analyzing said first portion of said reaction mixture to provide a first result indicative of the presence or amount of said analyte present in said test sample, said step of analyzing said first portion comprises the steps of (i) separating said free species from said bound species by means of a solid phase material, said solid phase material having a substance that specifically binds to said bound species but not capable of binding said free species, brought into contact with said reaction mixture, and (ii) correlating the amount of labeled reagent in said free species or said bound species to the amount or presence of said analyte in said test sample;
- (e) independently analyzing said assay verification sample to provide a second result indicative of the presence or amount of said positive analyte component in said assay verification sample, said step of analyzing said assay verification sample comprising the steps of (i) separating said free species from said bound species by means of a solid phase material, said solid phase material having a substance that specifically binds to said bound species but not capable of

binding said free species, brought into contact with said assay verification sample, and (ii) correlating the amount of labeled reagent in said free species or said bound species to the amount or presence of said analyte present in said assay verification sample; and

- (f) determining if said second result is as expected, given knowledge that said assay verification sample contains said positive analyte component or said positive analyte component in said known amount, to verify said first result.

2. The method of claim 1, wherein the steps of forming said reaction mixture and said assay verification sample are performed sequentially.

3. The method of claim 1, wherein said positive analyte component is said analyte or an analog of said analyte.

4. The method of claim 1, wherein said solid phase material is selected from the group consisting of beads, particles, and microparticles.

5. A method for verifying a result in a heterogeneous immunoassay for determining the presence or amount of an analyte present in a test sample, wherein said heterogeneous assay is a competitive immunoassay, comprising the steps of:

- (a) forming a mixture comprising said test sample and a labeled reagent comprising said analyte or an analog of said analyte labeled with a detectable moiety;
- (b) dividing said mixture into a first portion and a second portion;
- (c) forming an assay verification sample comprising said second portion of said reaction mixture and a positive analyte component, in a known amount, capable of providing a detectable response as an indication of the presence or amount of said positive analyte component present in said assay verification sample;
- (d) independently analyzing said first portion of said reaction mixture to provide a first result indicative of the presence or amount of said analyte present in said test sample, said step of analyzing said first portion comprising the steps of (i) contacting said mixture with a solid phase material, said solid phase material having a substance that specifically binds to said analyte or said labeled reagent to form

a bound species of said labeled reagent and a free species of said labeled reagent, and (ii) correlating the amount of said bound species of said labeled reagent or said free species of said labeled reagent to the amount or presence of said analyte present in said test sample;

- (e) independently analyzing said assay verification sample to provide a second result indicative of the presence or amount of said positive analyte component in said assay verification sample, said step of analyzing said assay verification sample comprising the steps of (i) contacting said mixture with a solid phase material, said solid phase material having a substance that specifically binds to said analyte or said labeled reagent to form a bound species of said labeled reagent and a free species of said labeled reagent, and (ii) correlating the amount of said bound species of said labeled reagent or said free species of said labeled reagent to the amount or presence of said analyte present in said assay verification sample; and
- (f) determining if said second result is as expected, given knowledge that said assay verification sample contains said positive analyte component or said positive analyte component in said known amount, to verify said first result.

6. A method for verifying a result obtained in an analytical procedure for determining the presence or amount of an analyte in a test sample, comprising the steps of:

- (a) forming a reaction mixture comprising said test sample and at least one appropriate assay reagent suitable for analysis of said test sample for said analyte;
- (b) independently analyzing said reaction mixture to provide a first result indicative of the presence or amount of said analyte in said test sample;
- (c) forming an assay verification sample comprising said independently analyzed reaction mixture of step (b) and a positive analyte component capable of providing a detectable response as an indication of the presence or amount of said positive analyte component present in said assay verification sample;

- (d) independently analyzing said assay verification sample to provide a second result indicative of the presence or amount of said positive analyte component in said assay verification sample; and
- (e) determining if said second result is as expected, given knowledge that said assay verification sample contains said positive analyte component or said positive analyte component in said known amount, to verify said first result.

7. The method of claim 6, wherein said second result indicates said first result could be inaccurate if said second result is not as expected given knowledge that said assay verification sample contains said positive analyte component.

8. The method of claim 6, wherein said second result indicates said first result is accurate if said second result is as expected given knowledge that said assay verification sample contains said positive analyte component.

9. The method of claim 6, wherein the steps of forming said reaction mixture and said assay verification sample are performed simultaneously.

10. The method of claim 6, wherein the steps of forming said reaction mixture and said assay verification sample are performed sequentially.

11. The method of claim 6, wherein said positive analyte component is selected from the group consisting of said analyte and analogs of said analyte.

12. The method of claim 6, wherein said analytical procedure is an immunoassay.

13. The method of claim 12, wherein said immunoassay is a homogeneous immunoassay.

14. The method of claim 12, wherein said immunoassay is a heterogeneous immunoassay.

15. A method for verifying a result in a heterogeneous immunoassay for determining the presence or amount of an analyte present in a test sample, comprising the steps of:

- (a) forming a reaction mixture comprising said test sample and a labeled reagent comprising a substance that specifically binds to said analyte labeled with a detectable moiety to form a bound species of said labeled reagent and a free species of said labeled reagent, wherein said bound species of said labeled reagent comprises said analyte and said labeled reagent and said free species of said labeled reagent does not contain said analyte;
- (b) dividing said reaction mixture containing said bound species and said free species into a first portion and a second portion;
- (c) forming an assay verification sample comprising said second portion of said reaction mixture and a positive analyte, in a known amount, capable of providing a detectable response as an indication of the presence or amount of said positive analyte component present in said assay verification sample;
- (d) independently analyzing said first portion of said reaction mixture to provide a first result indicative of the presence or amount of said analyte present in said test sample, said step of analyzing said first portion comprising the steps of (i) separating said free species from said bound species by means of a solid phase material, said solid phase material having a substance that specifically binds to said bound species but not capable of binding said free species, brought into contact with said reaction mixture, and (ii) correlating the amount of labeled reagent in said free species or said bound species to the amount or presence of said analyte present in said test sample;
- (e) adding assay verification sample to said independently analyzed first portion of said reaction mixture to provide a second result indicative of the presence or amount of said positive analyte component in said assay verification sample, said step of analyzing said assay verification sample comprising the steps of (i) separating said free species from said bound species by means of a solid phase material, said solid phase material having a substance that specifically binds to said bound species but not capable of binding said free species, brought into contact with said assay verification sample, and (ii) correlating the amount of labeled reagent in said

free species or said bound species to the amount or presence of said analyte present in said assay verification sample; and

- (f) determining if said second result is as expected, given knowledge that said assay verification sample contains said positive analyte component or said positive analyte component in said known amount, to verify said first result.

16. The method of claim 15, wherein the steps of forming said reaction mixture and said assay verification sample are performed sequentially.

17. The method of claim 15, wherein said positive analyte component is said analyte or an analog of said analyte.

18. The method of claim 15, wherein said solid phase material is selected from the group consisting of beads, particles, and microparticles.

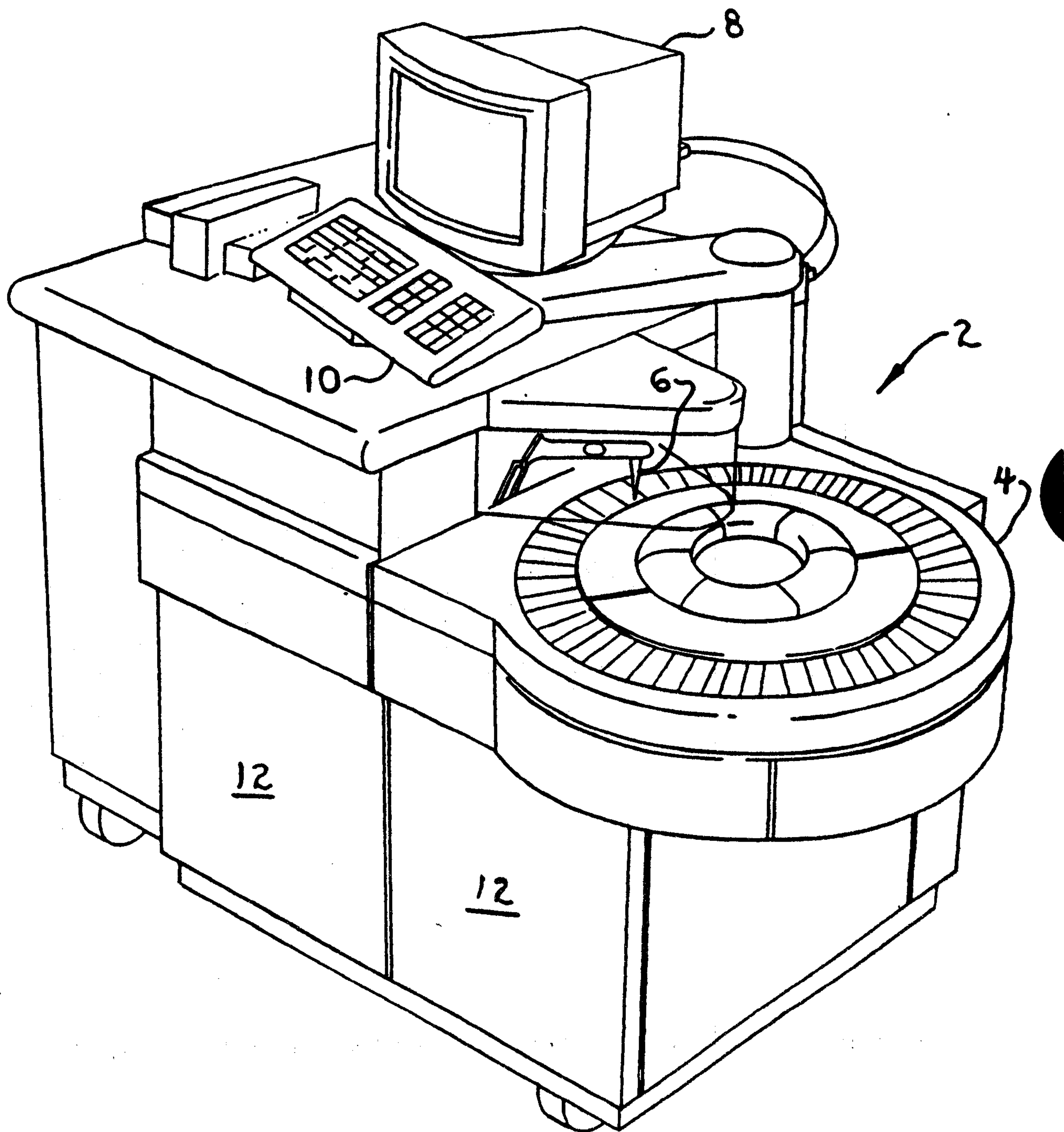
19. A method for verifying a result in a heterogeneous immunoassay for determining the presence or amount of an analyte present in a sample, wherein said heterogeneous immunoassay is a competitive heterogeneous immunoassay, comprising the steps of:

- (a) forming a mixture comprising said test sample and a labeled reagent comprising said analyte or an analog of said analyte labeled with a detectable moiety;
- (b) dividing said mixture into a first portion and a second portion;
- (c) forming an assay verification sample comprising said second portion of said reaction mixture and a positive analyte component, in a known amount, capable of providing a detectable response as an indication of the presence or amount of said positive analyte component present in said assay verification sample;
- (d) independently analyzing said first portion of said reaction mixture to provide a first result indicative of the presence or amount of said analyte present in said test sample, said step of analyzing said first portion comprising the steps of (i) contacting said mixture with a solid phase material, said solid phase material having a substance that specifically binds to said analyte or said labeled reagent to form a bound species of said labeled reagent and a free species of said labeled reagent, and (ii) correlating the amount of said bound species of said labeled reagent or said free species of said labeled

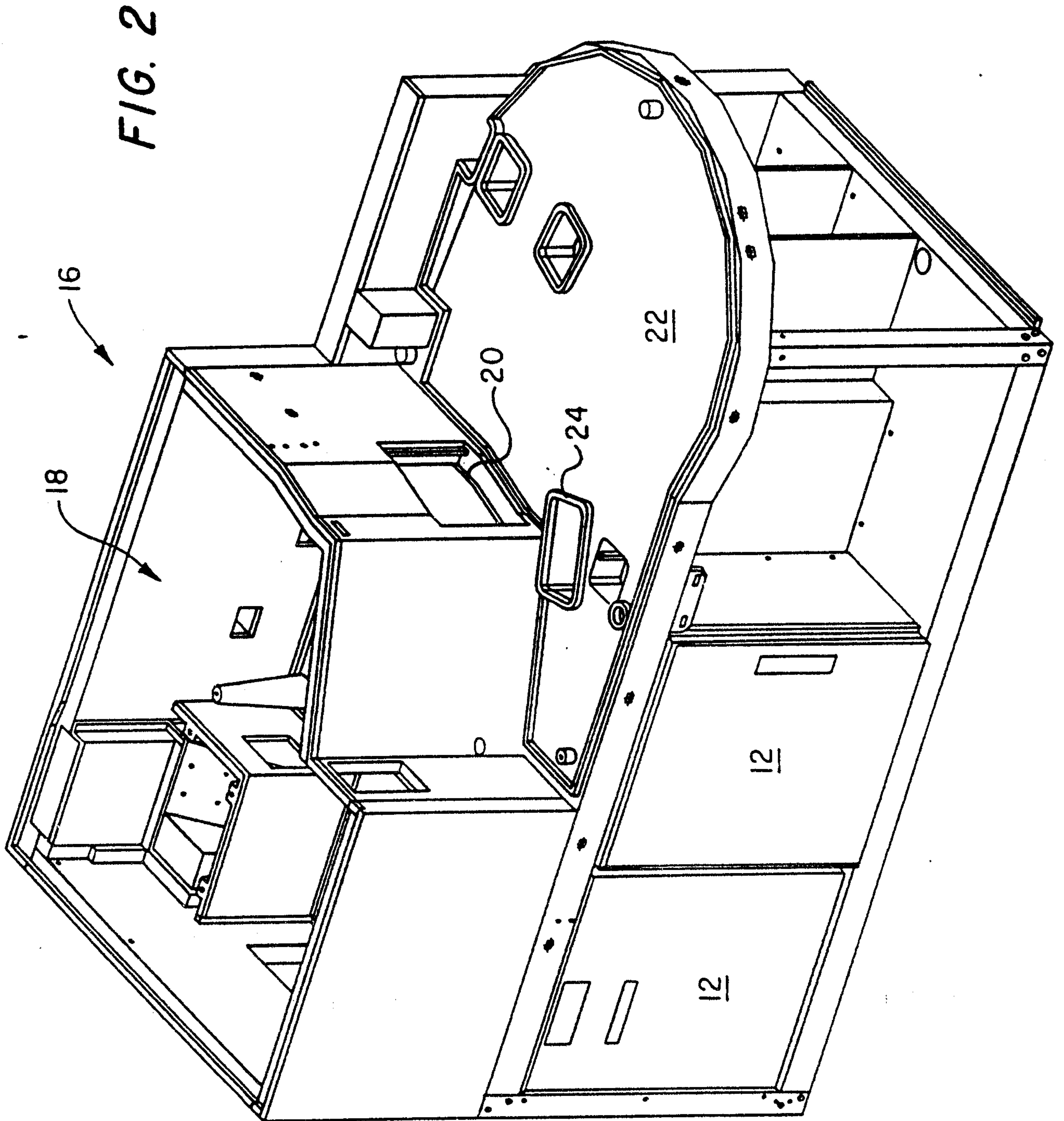
reagent to the amount or presence of said analyte present in said test sample;

- (e) independently analyzing said assay verification sample to provide a second result indicative of the presence or amount of said positive analyte component in said assay verification sample, said step of analyzing said assay verification sample comprising the steps of (i) contacting said mixture with a solid phase material, said solid phase material having a substance that specifically binds to said analyte or said labeled reagent to form a bound species of said labeled reagent and a free species of said labeled reagent, and (ii) correlating the amount of said bound species of said labeled reagent or said free species of said labeled reagent to the amount or presence of said analyte present in said assay verification sample; and
- (f) determining if said second result is as expected, given knowledge that said assay verification sample contains said positive analyte component or said positive analyte component in said known amount, to verify said first result.

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SUBSTITUTE SHEET

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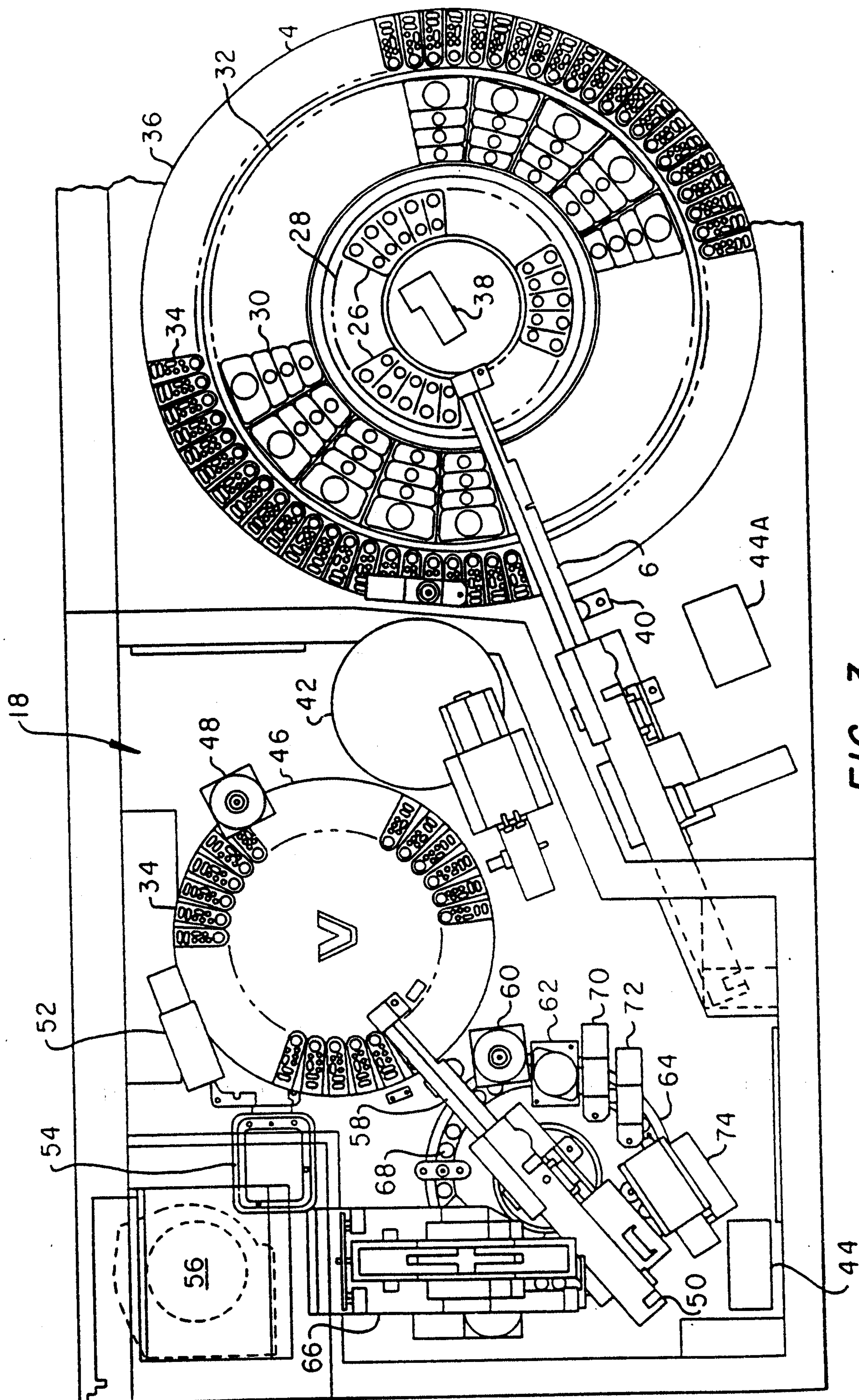


FIG. 3

SUBSTITUTE SHEET

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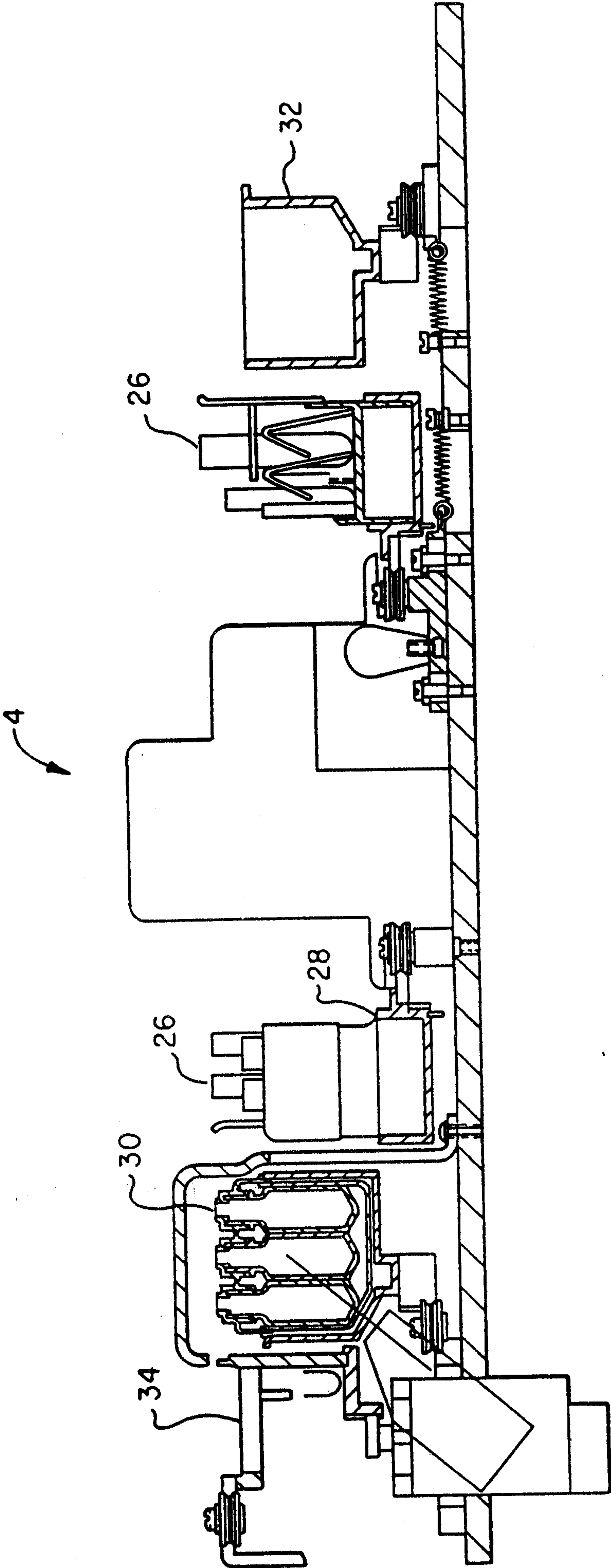
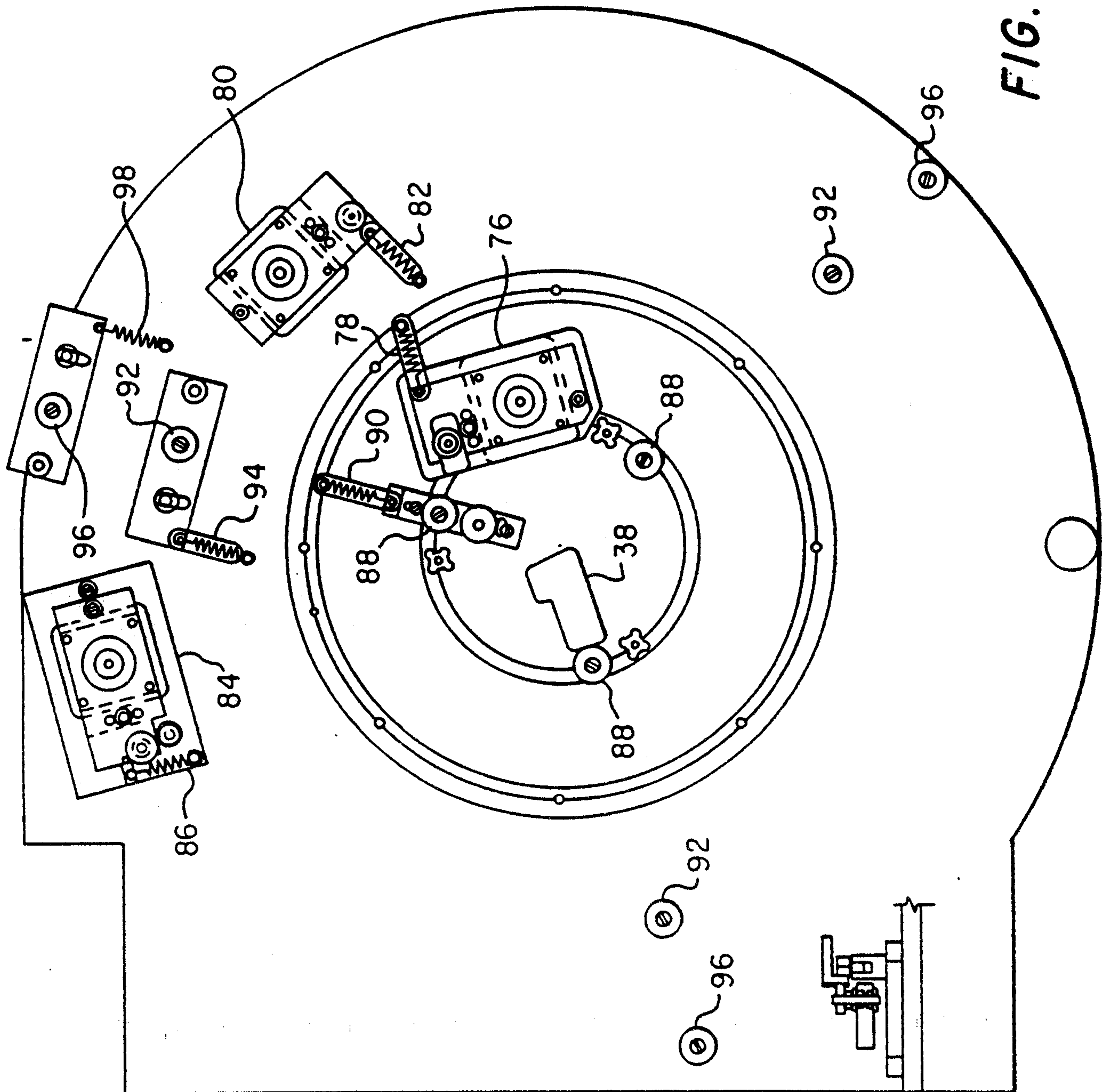


FIG. 4

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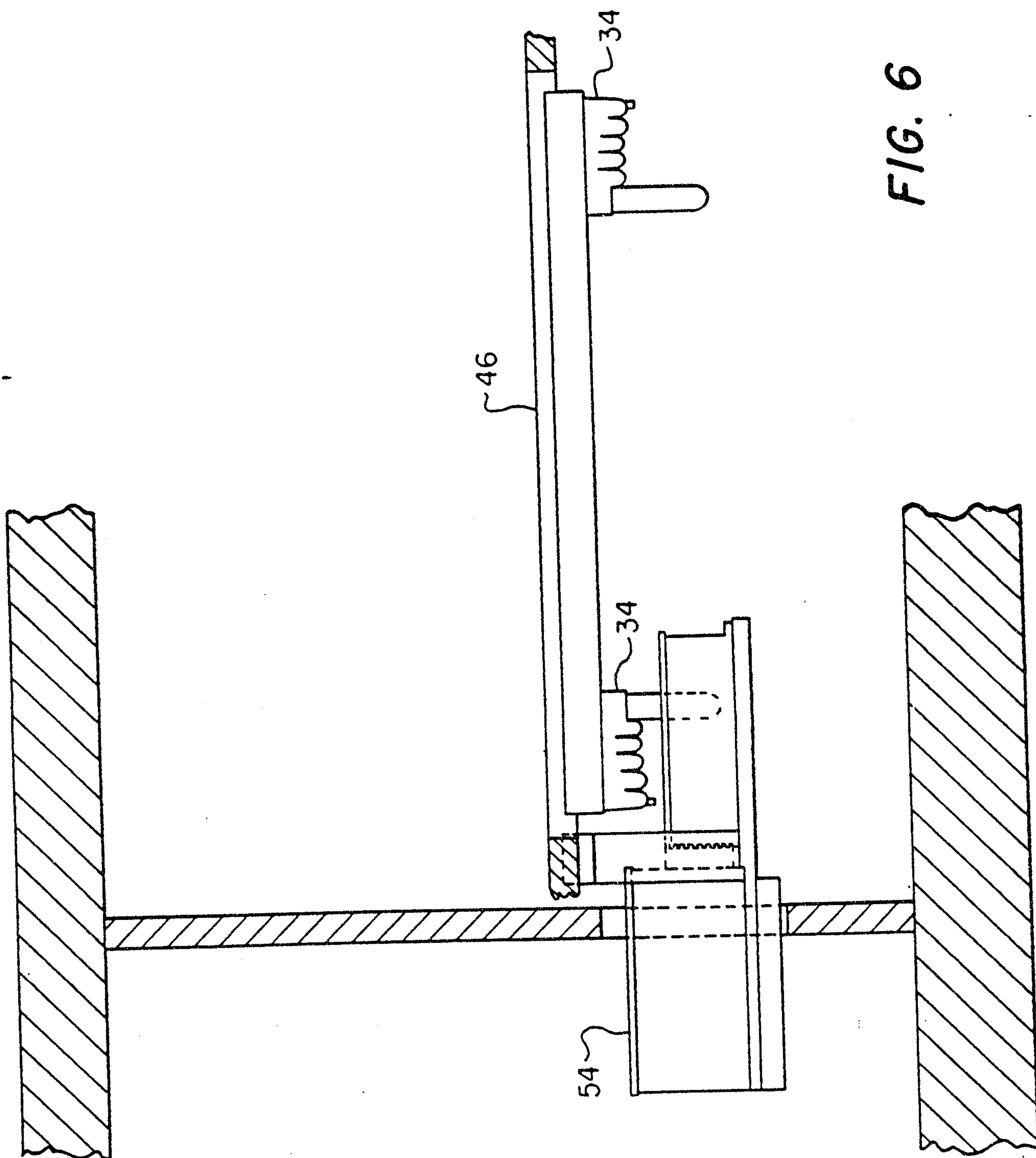
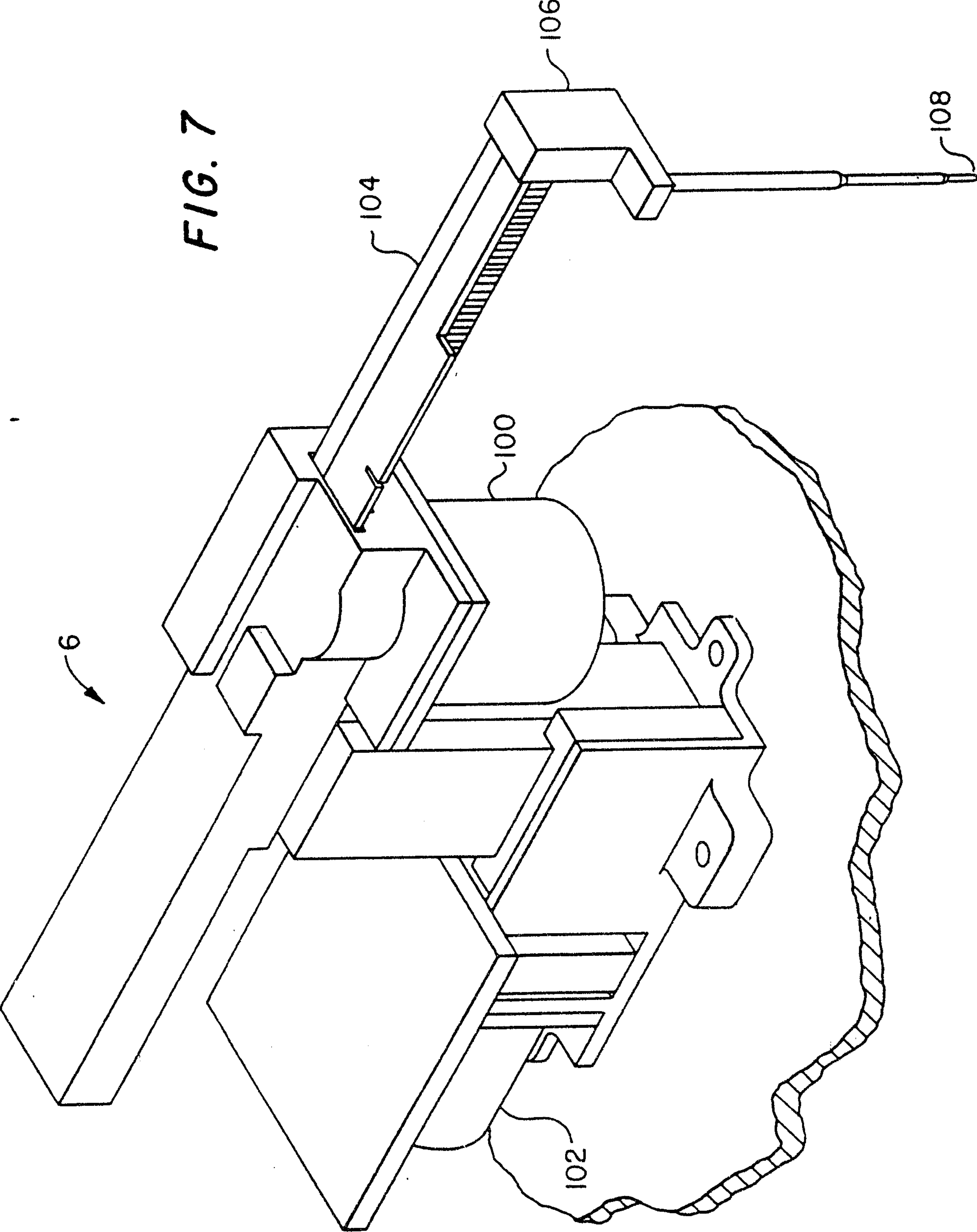
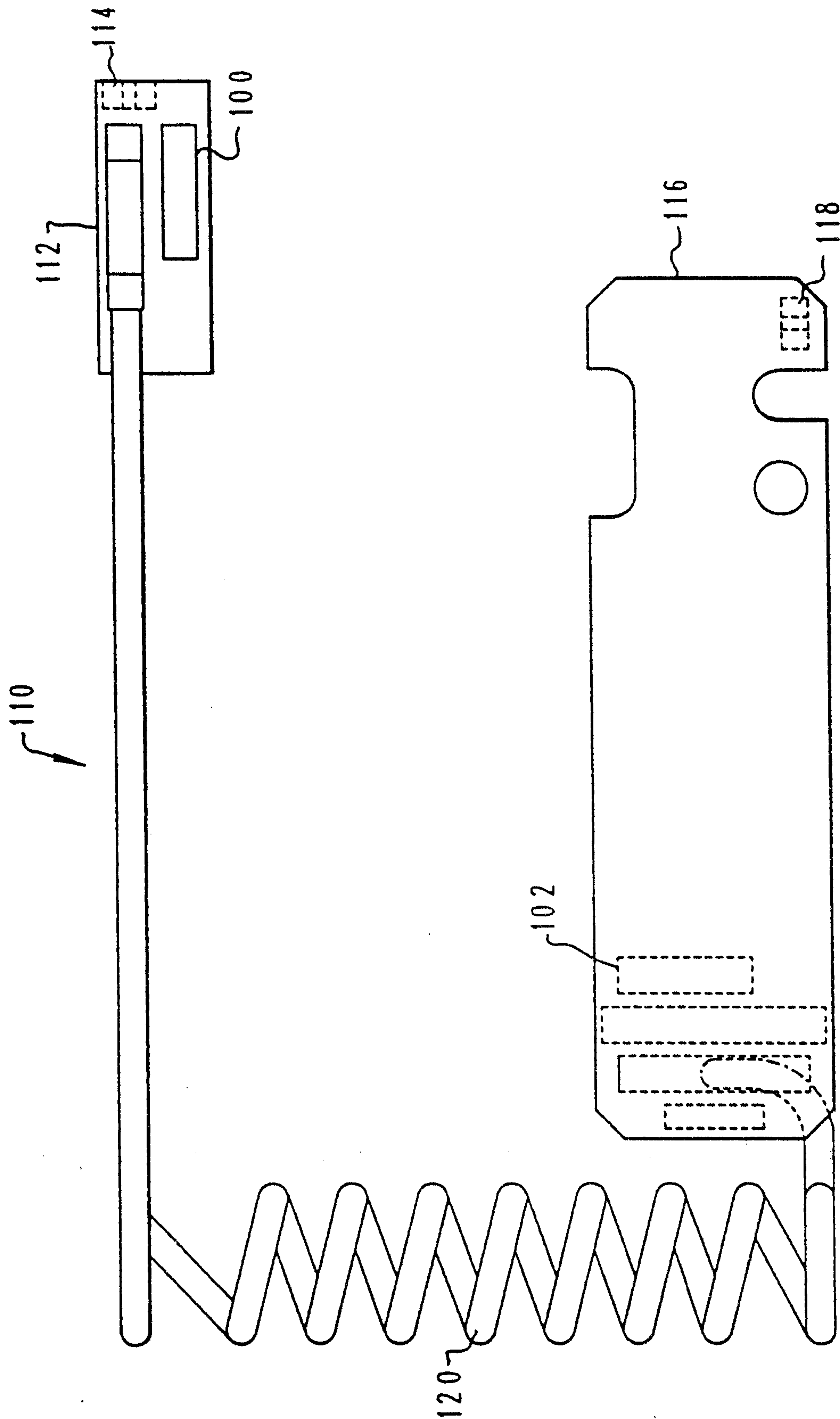


FIG. 6



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*Fig. 8*

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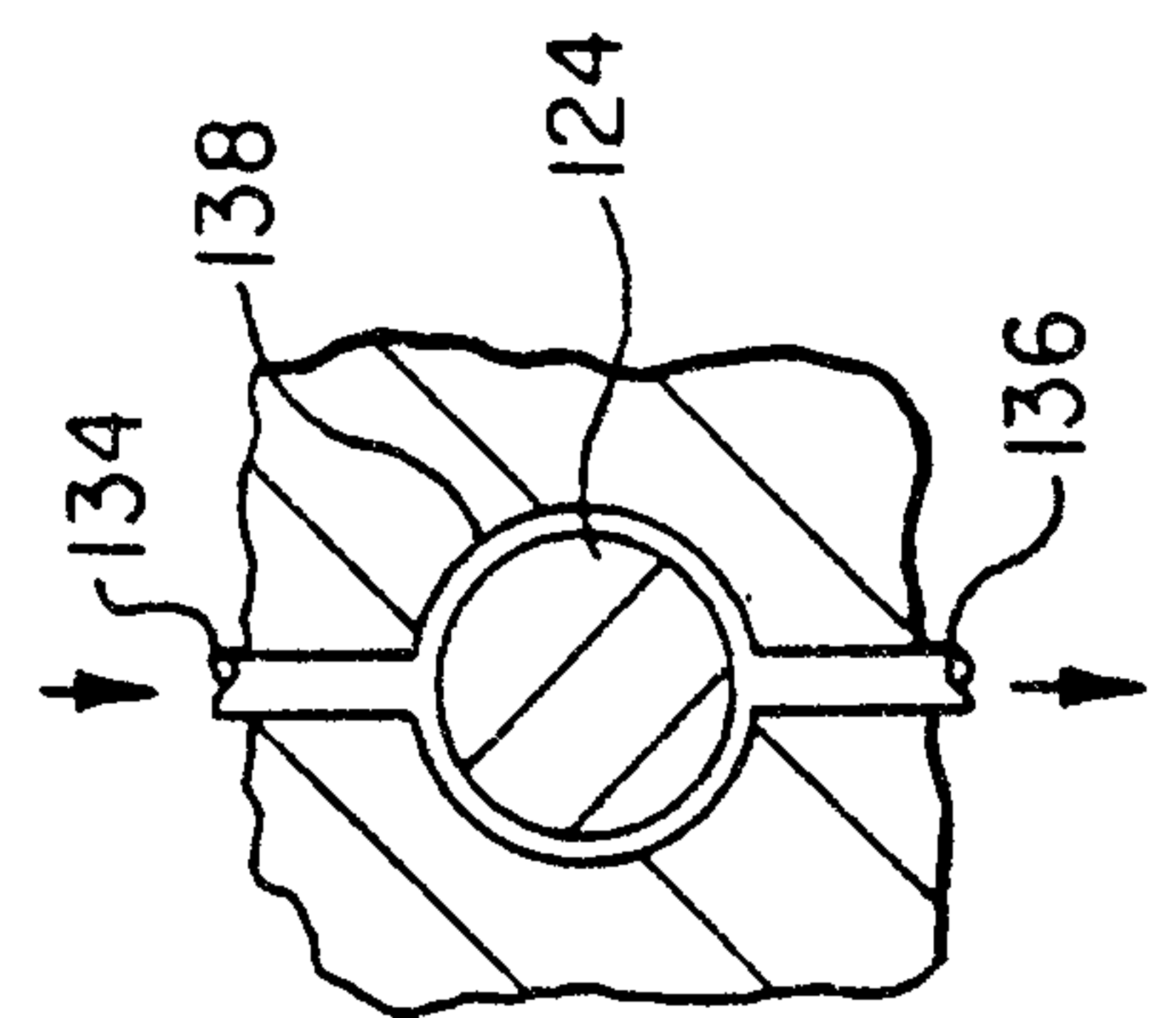
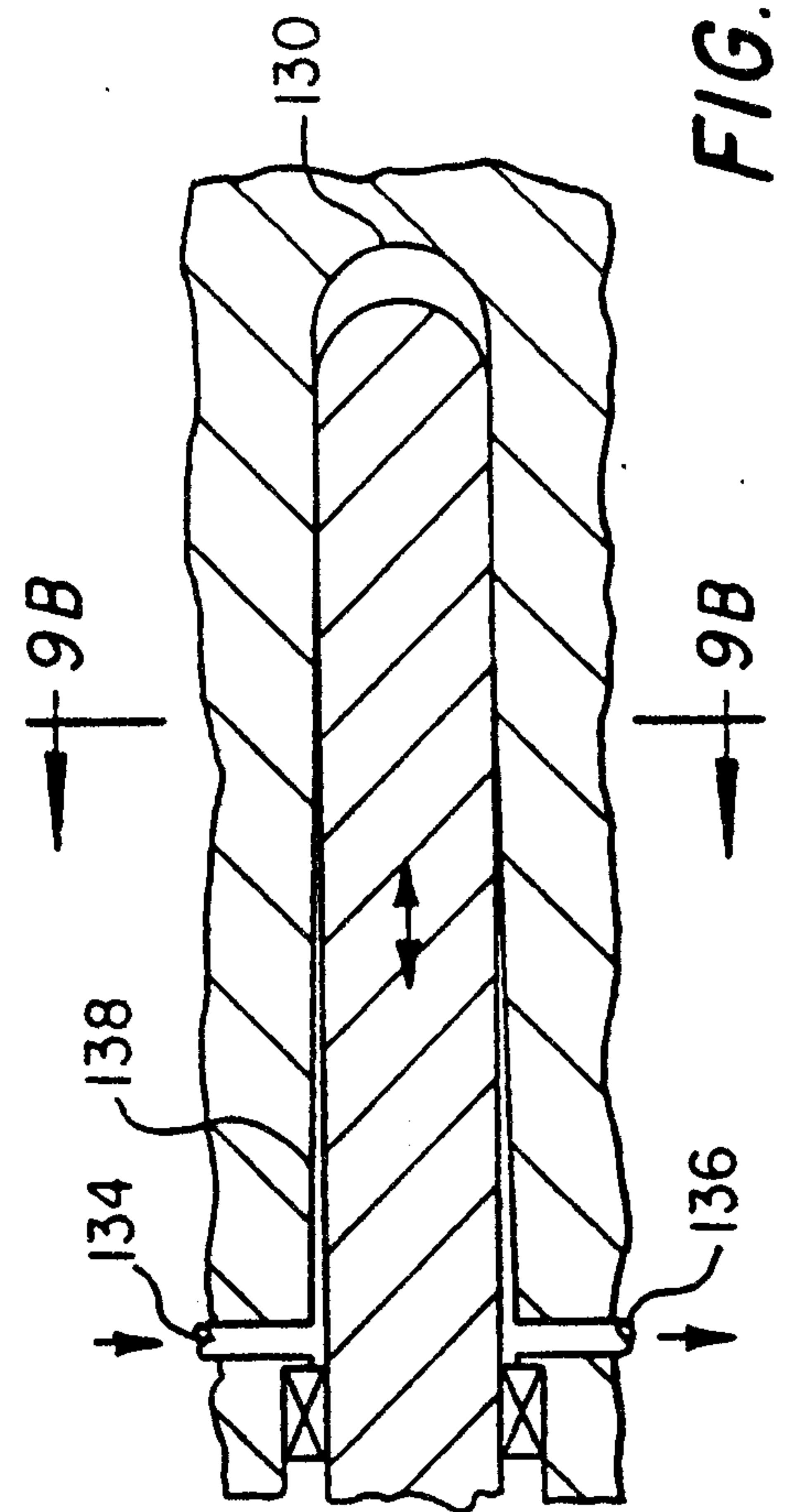
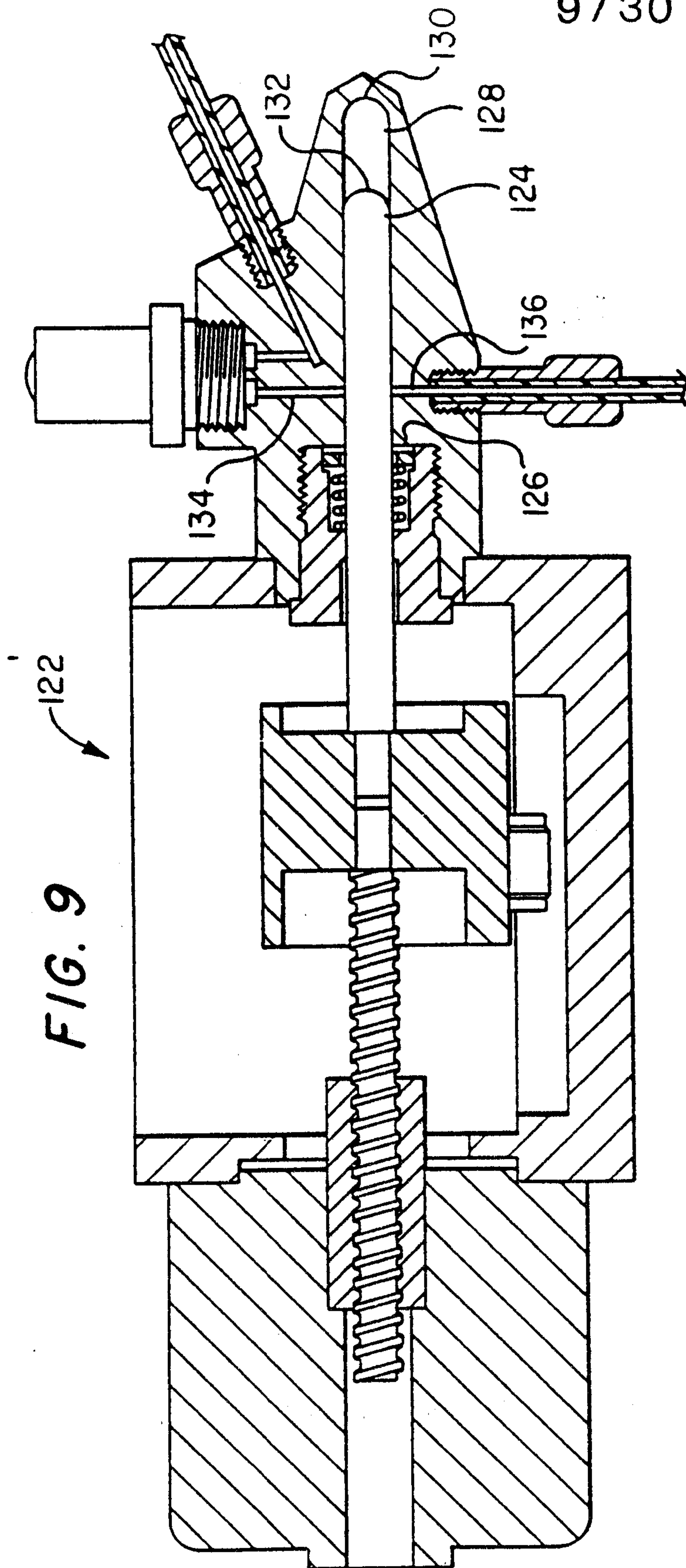


FIG. 9B

FIG. 9A

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FIG. 10

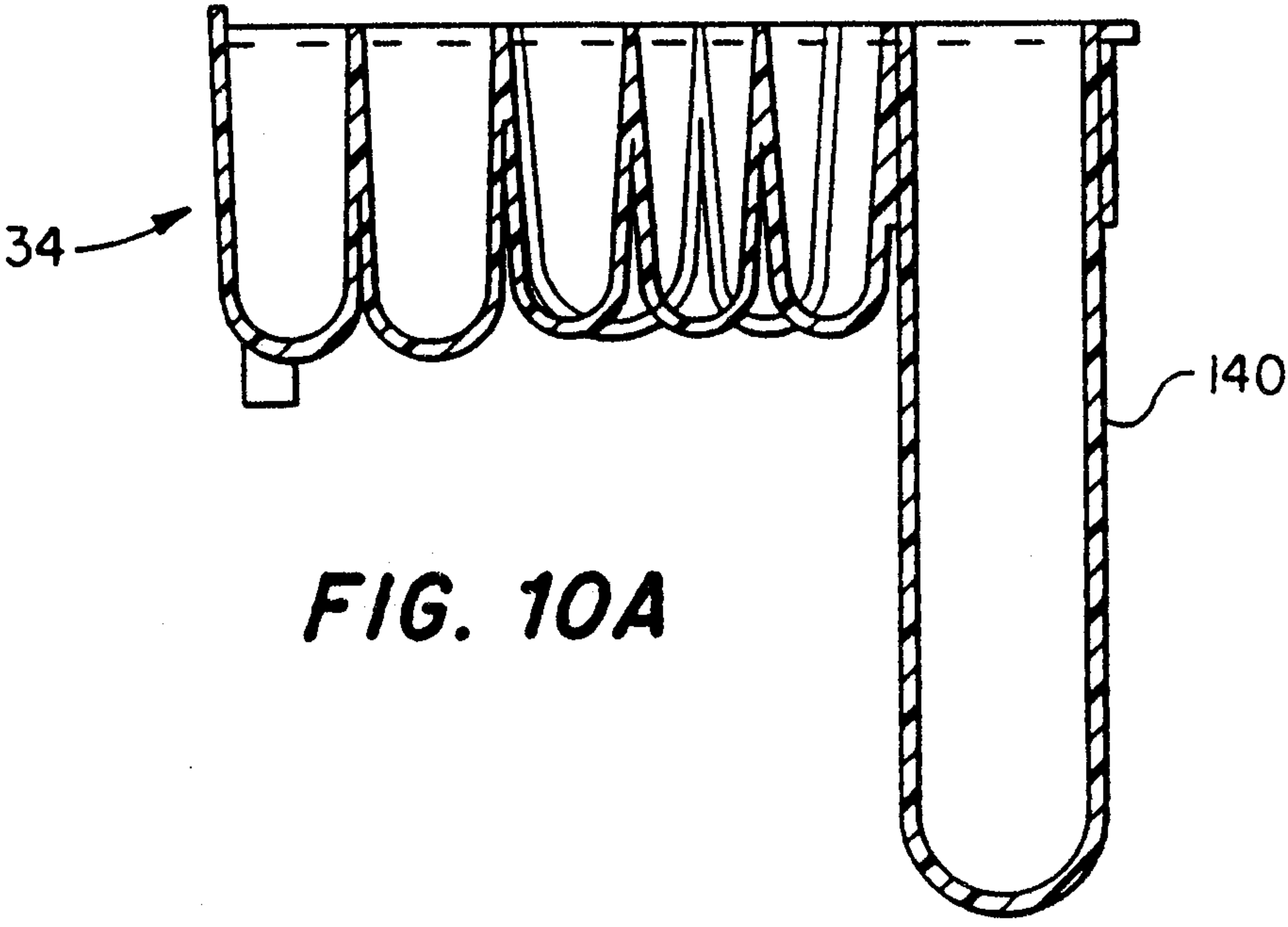
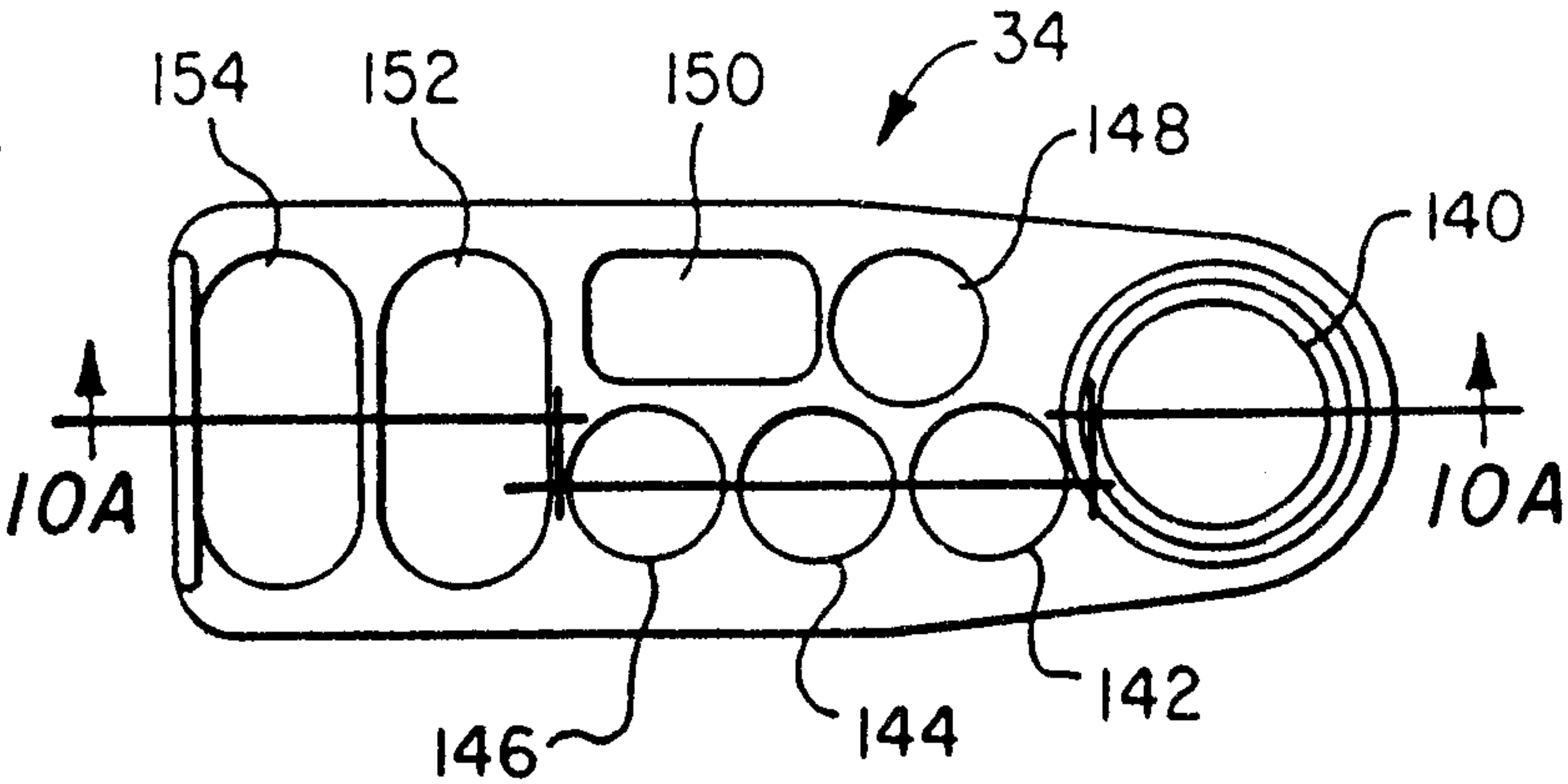


FIG. 10A

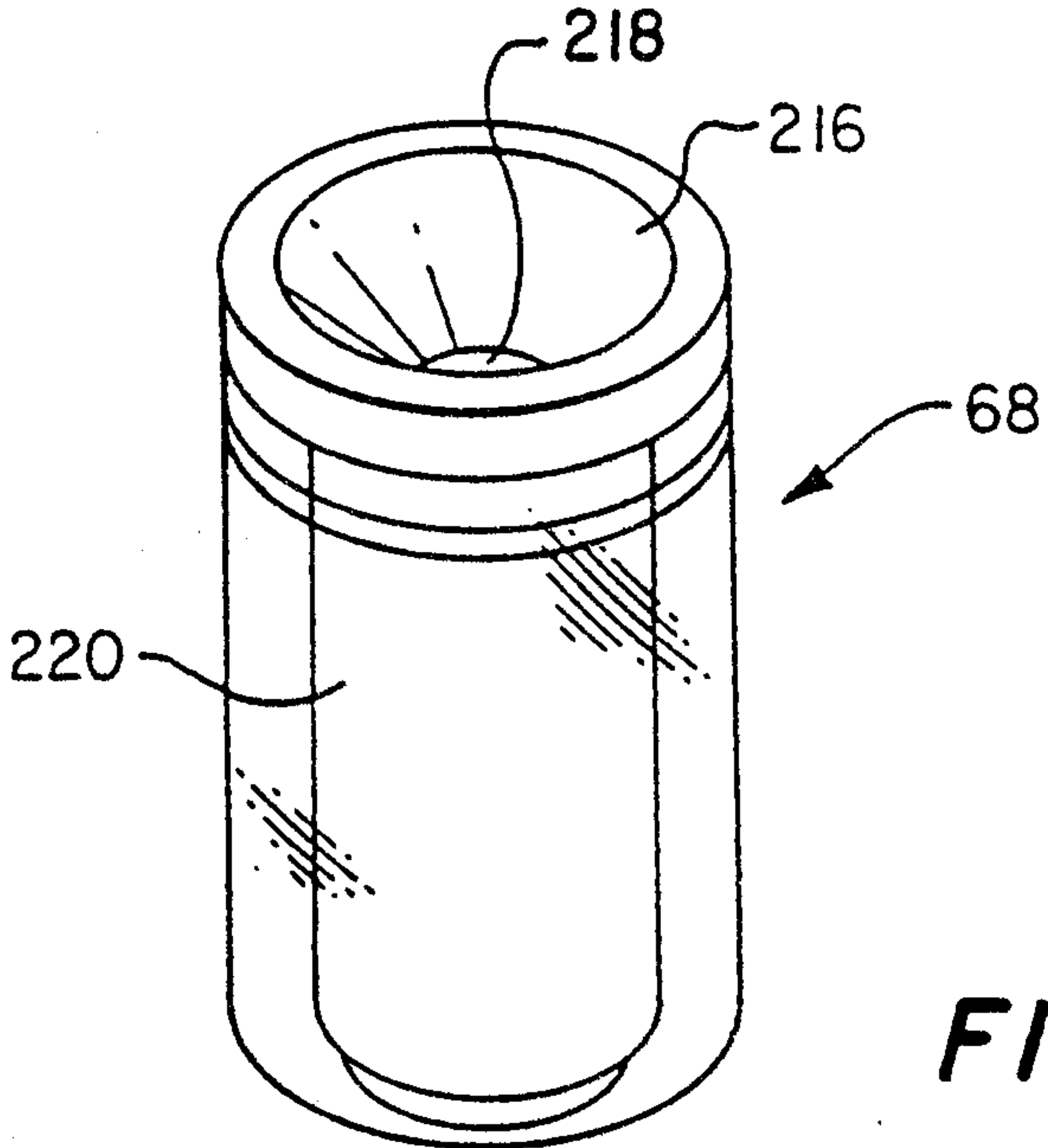
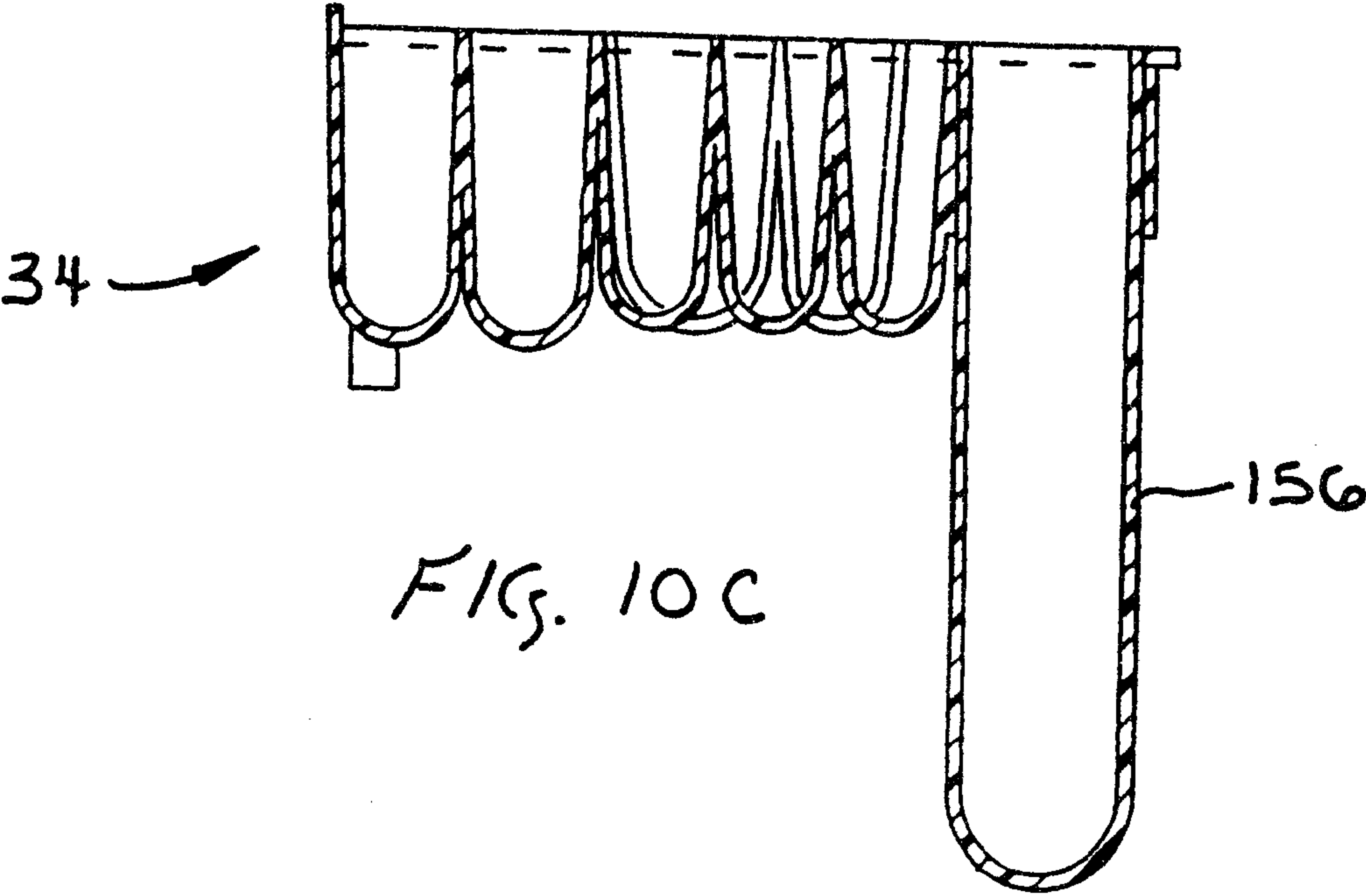
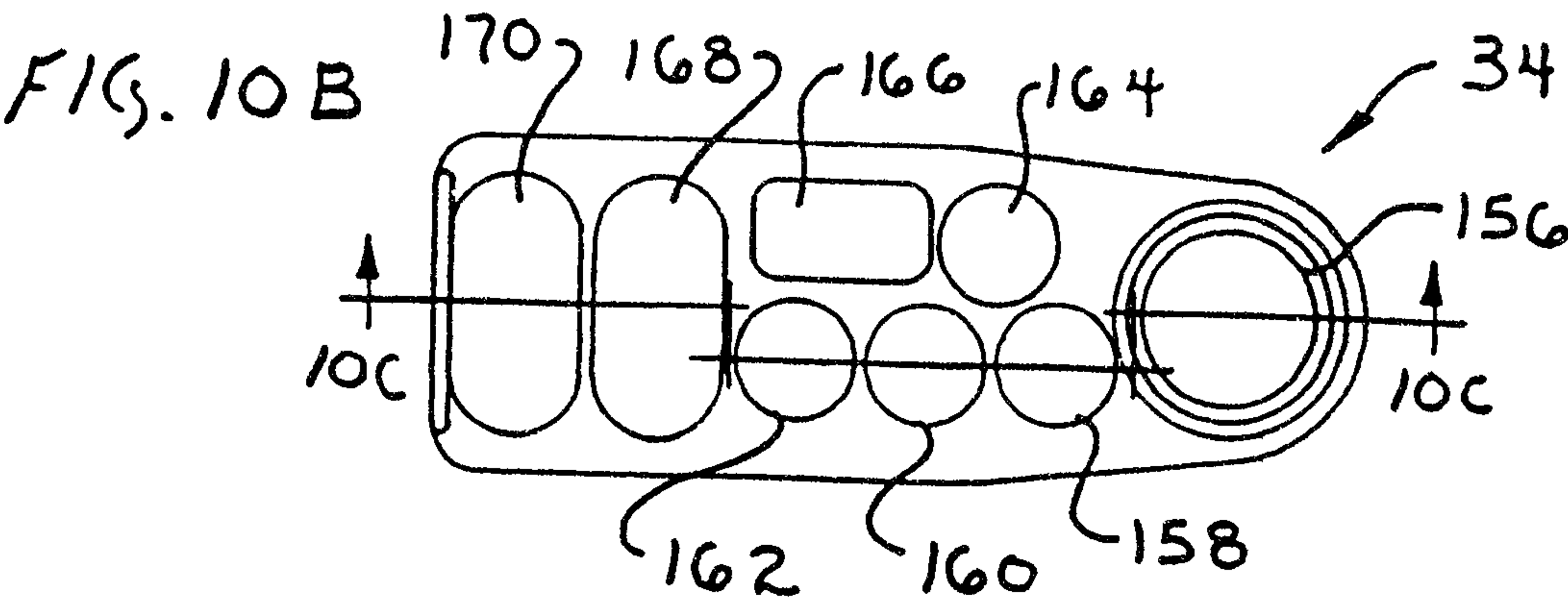


FIG. 16



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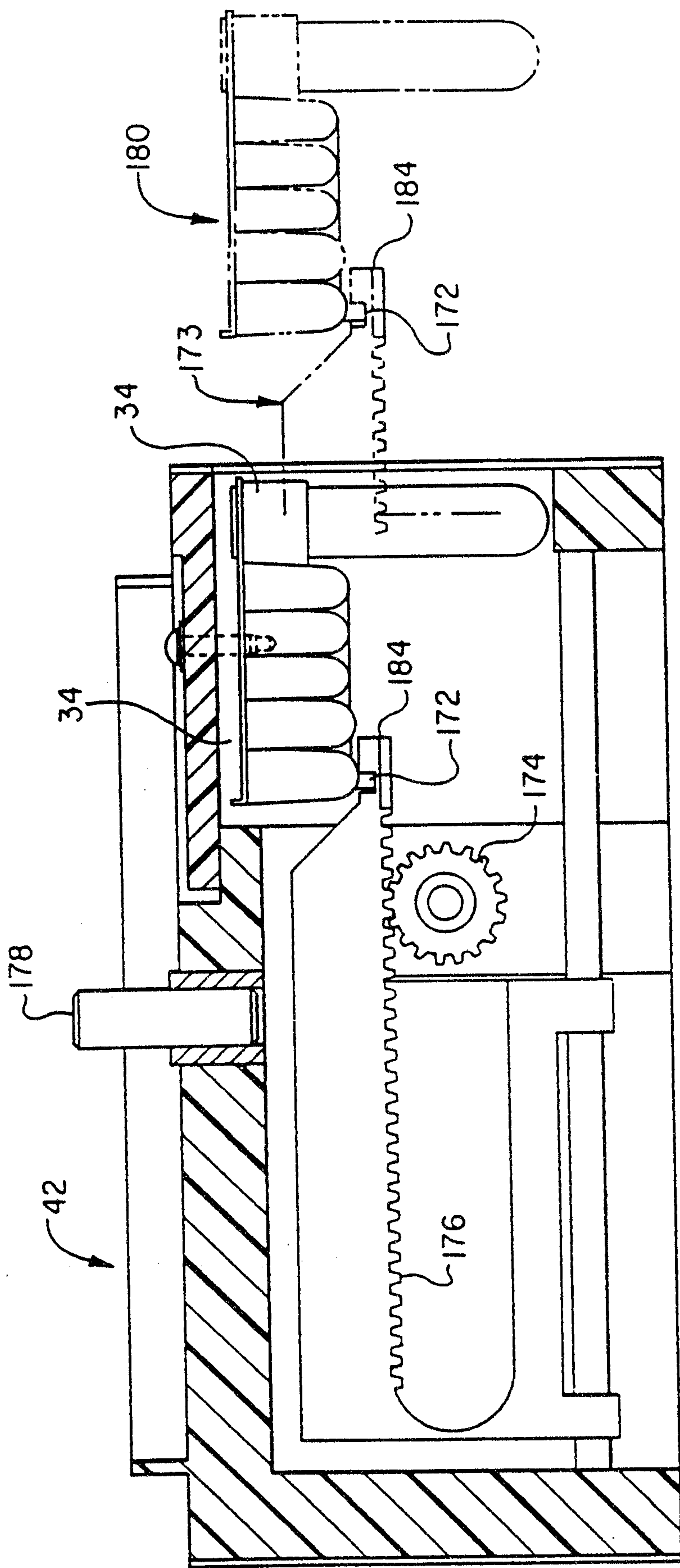


FIG. 11

FIG. 4A

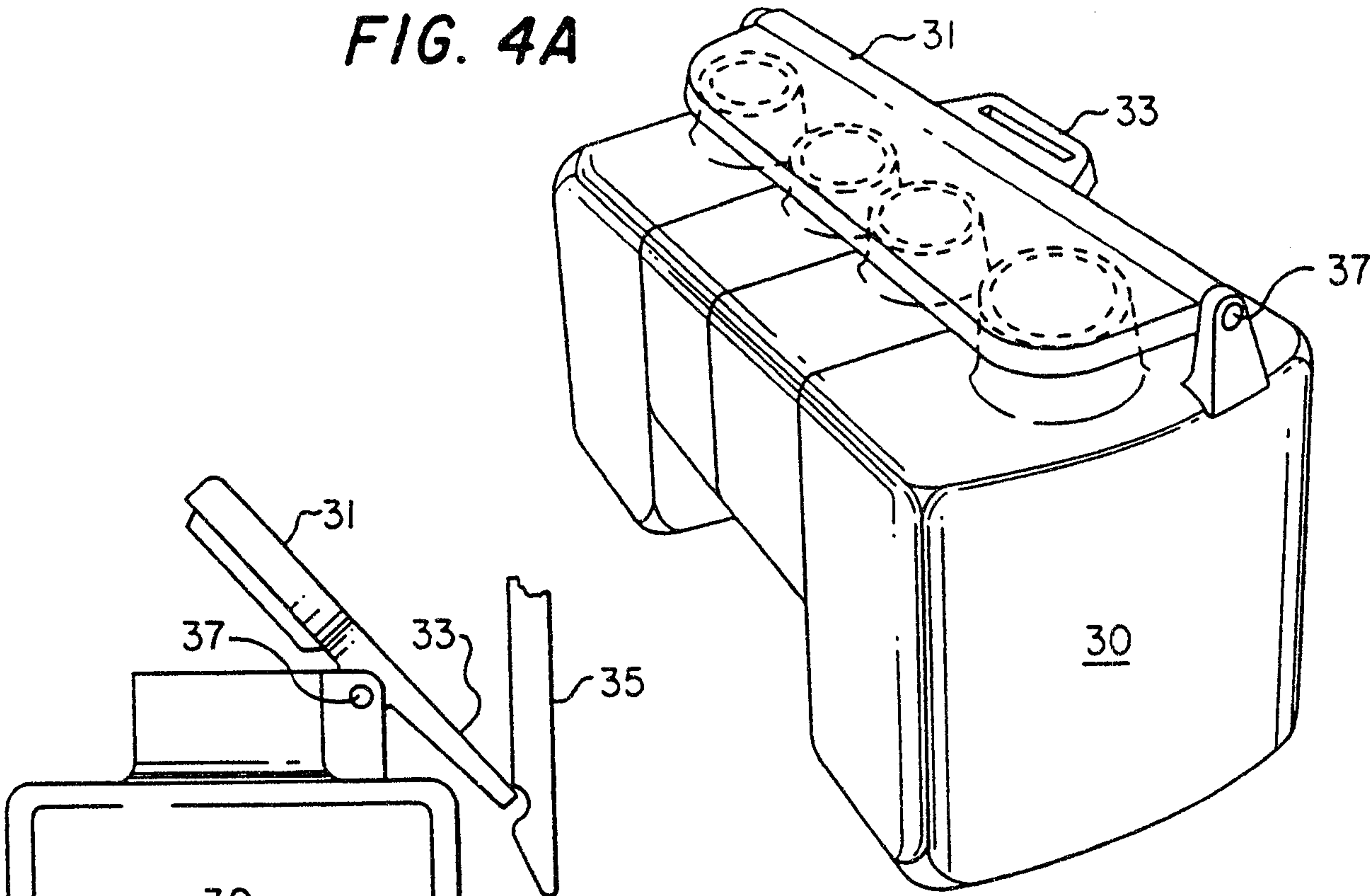


FIG. 4B

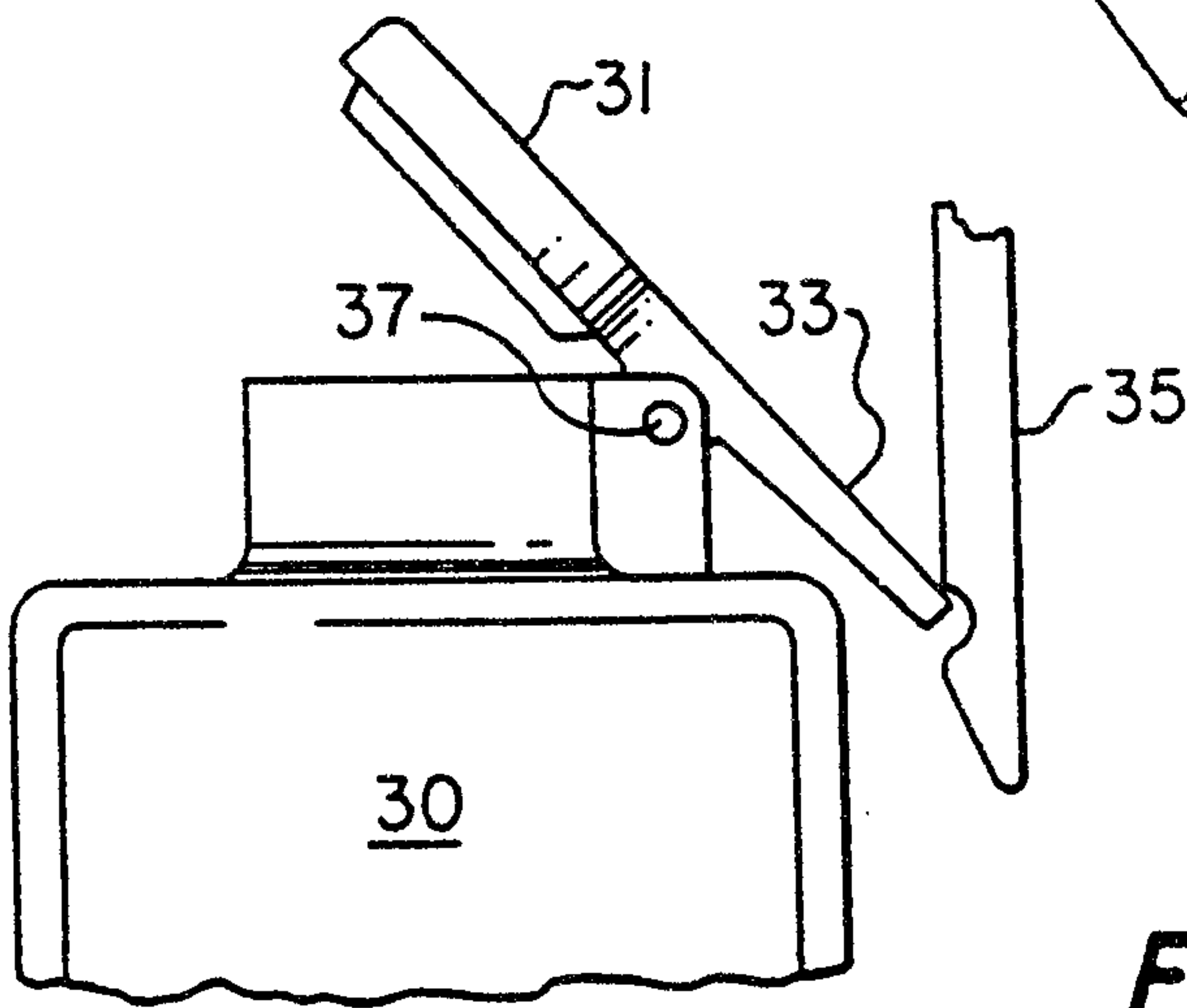


FIG. 12

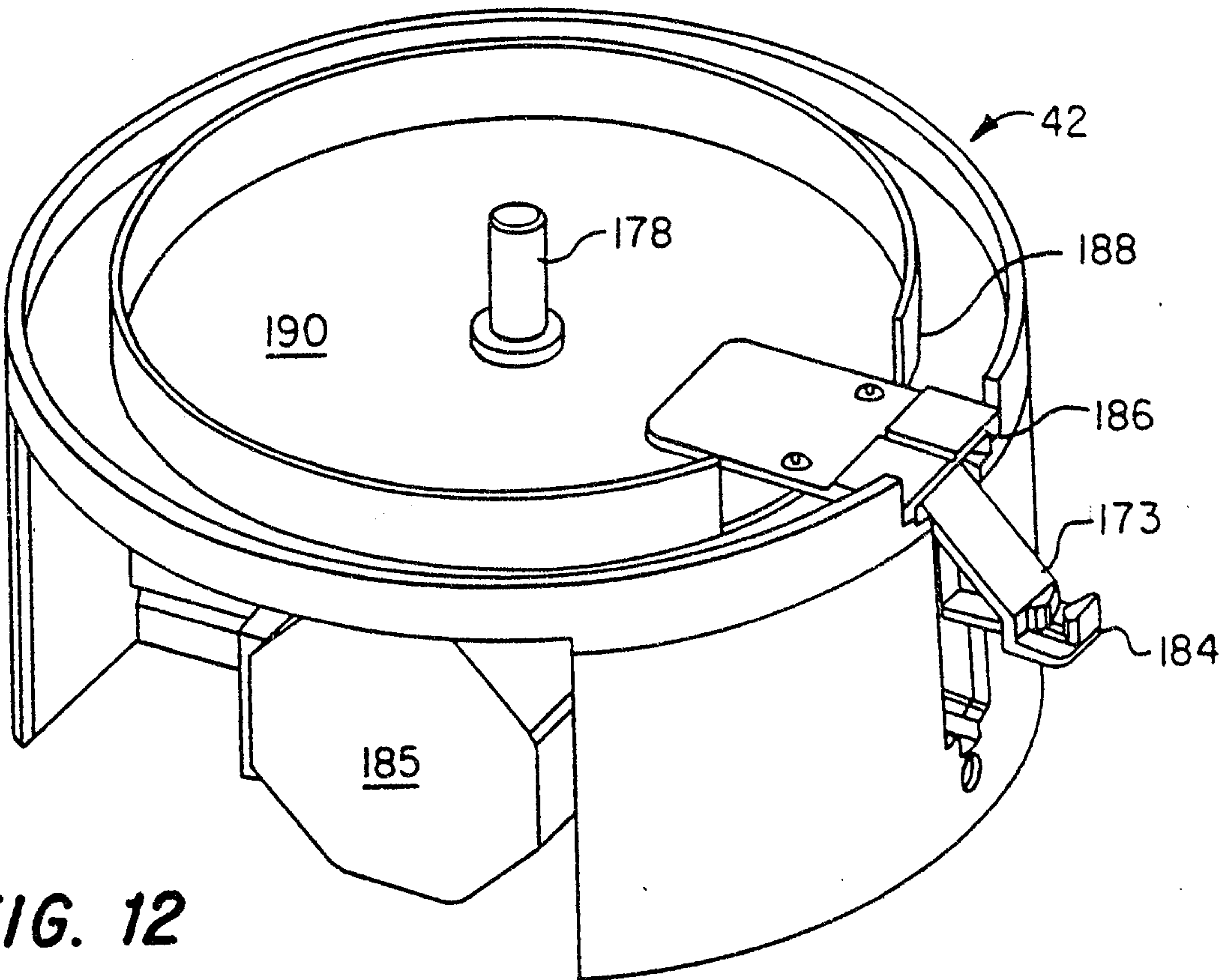
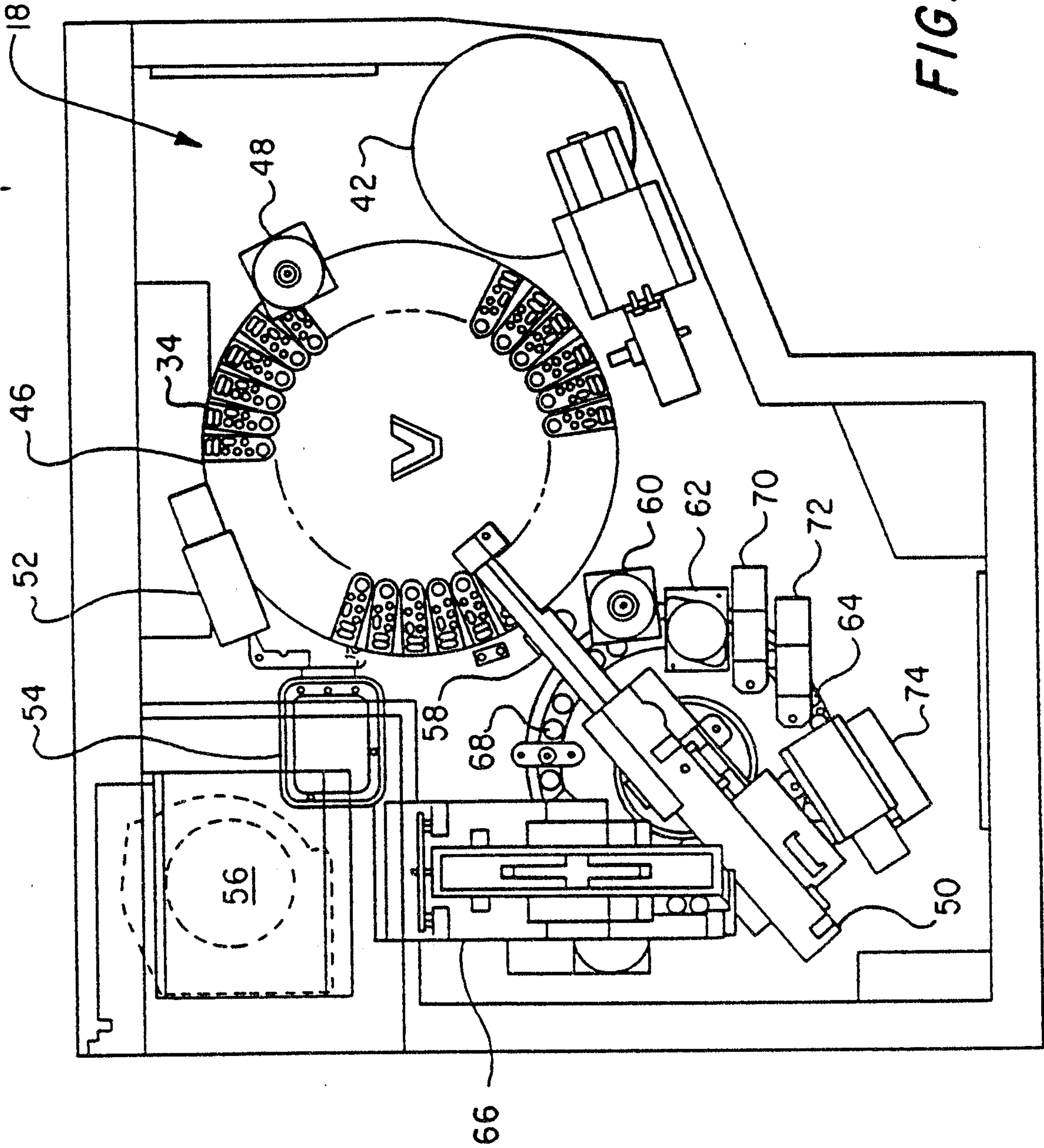


FIG. 13



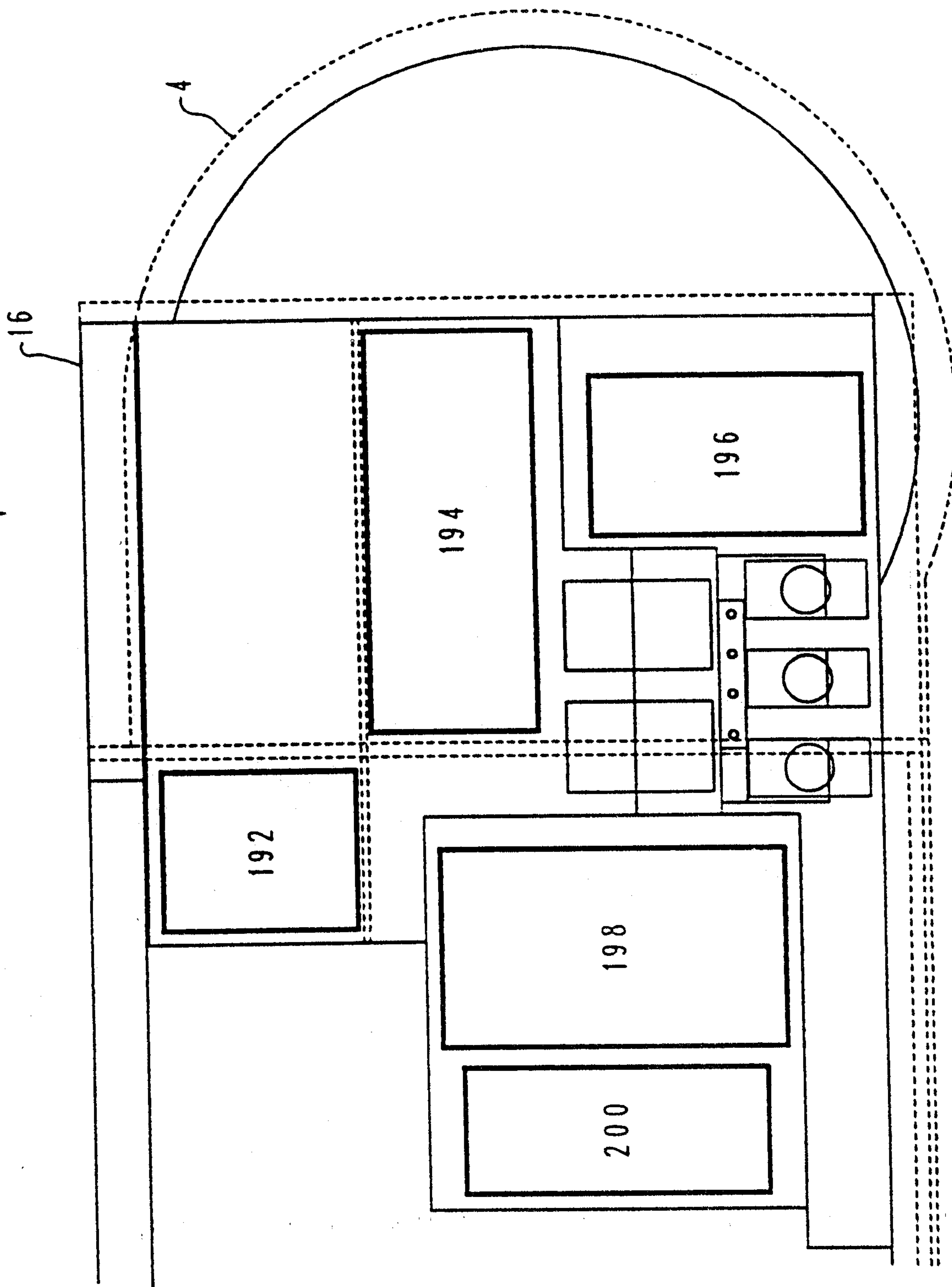


Fig. 14

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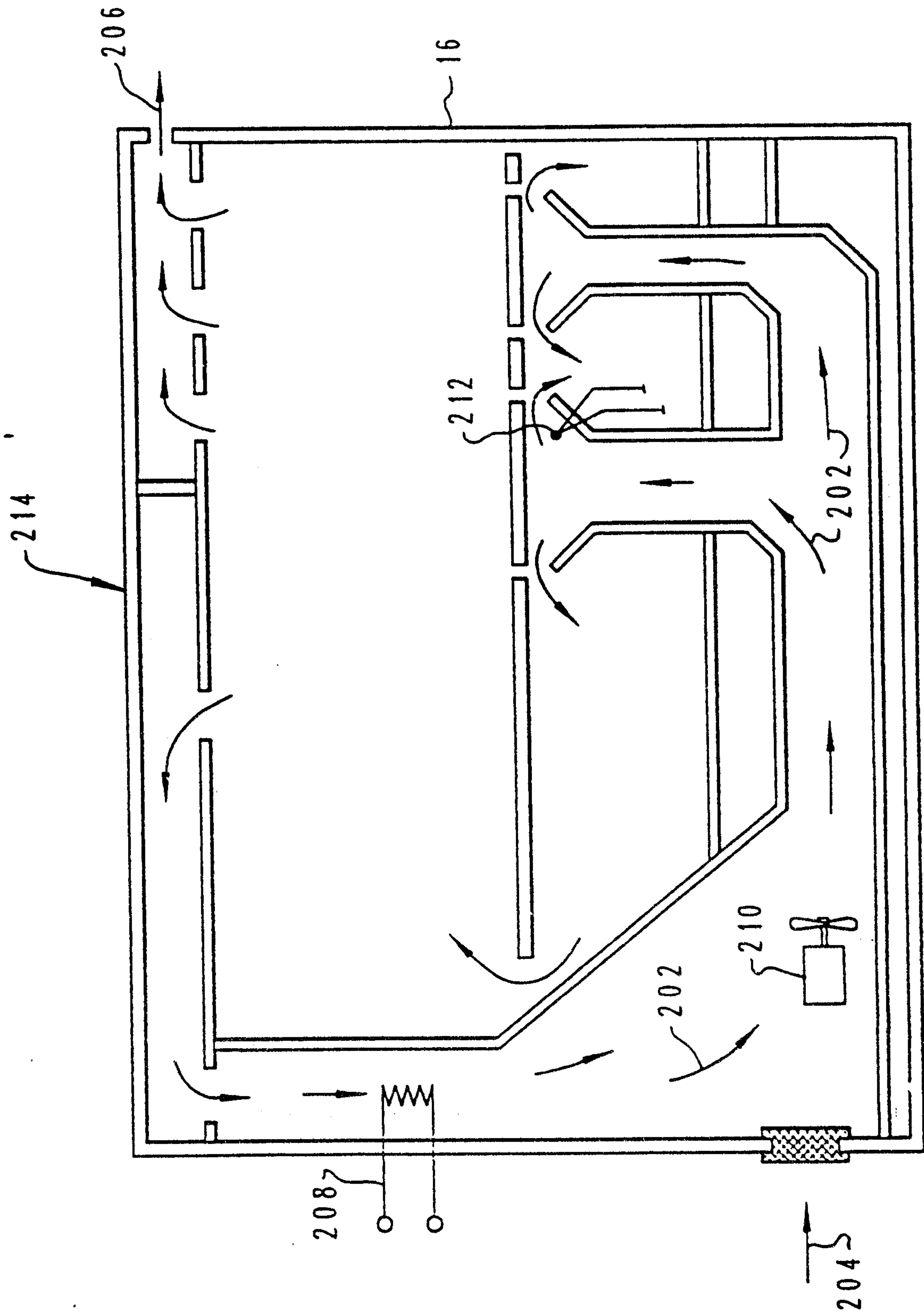
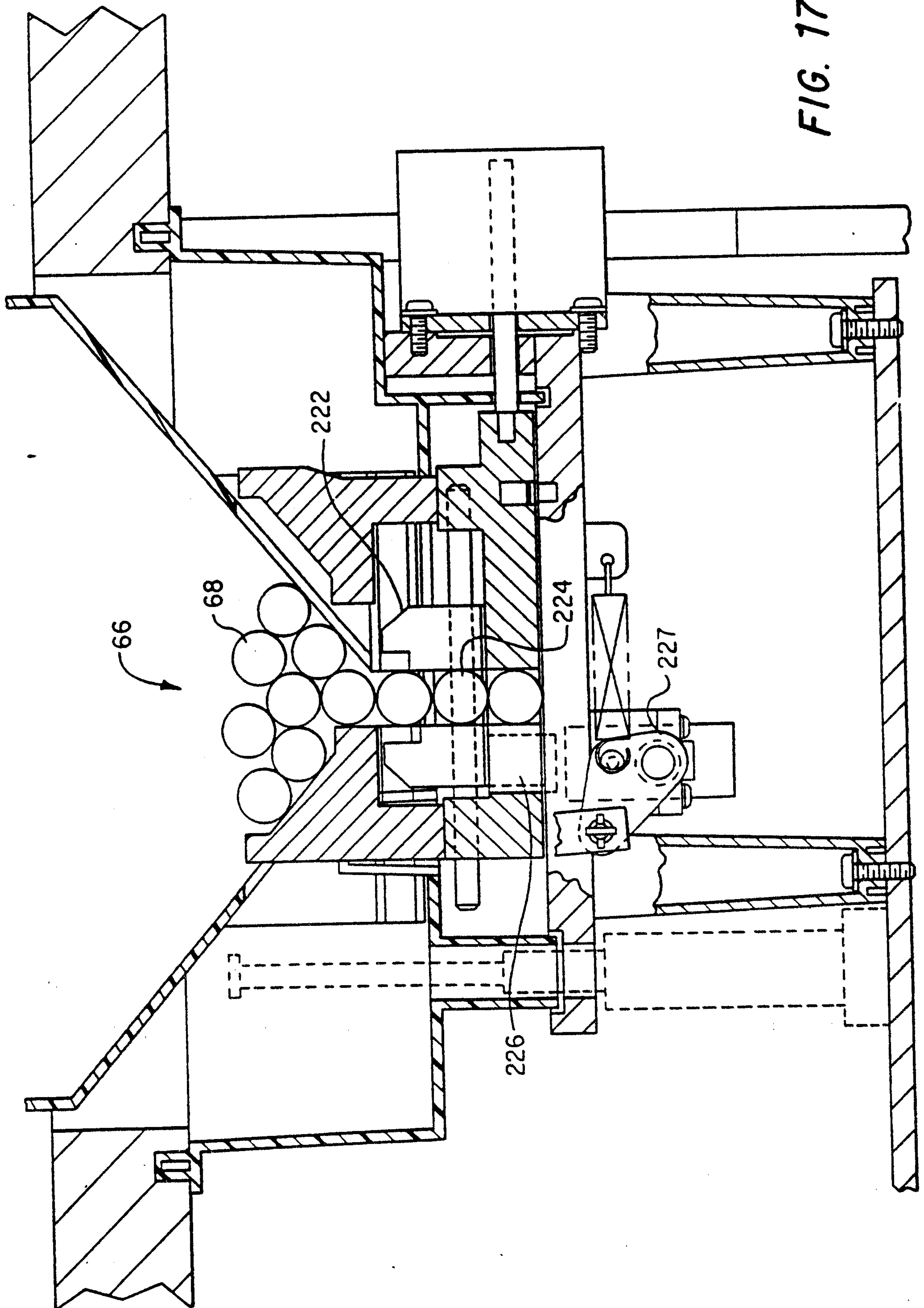


Fig. 15

FIG. 17



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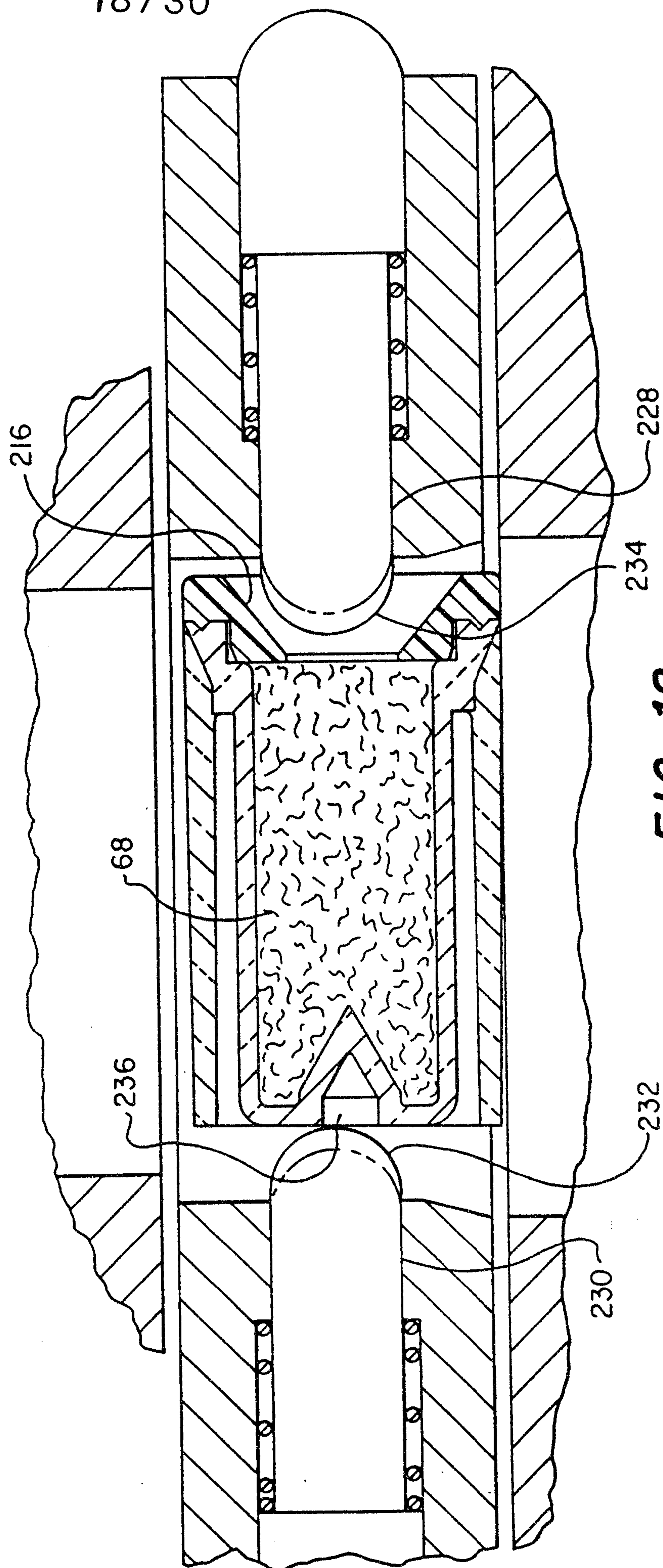


FIG. 18

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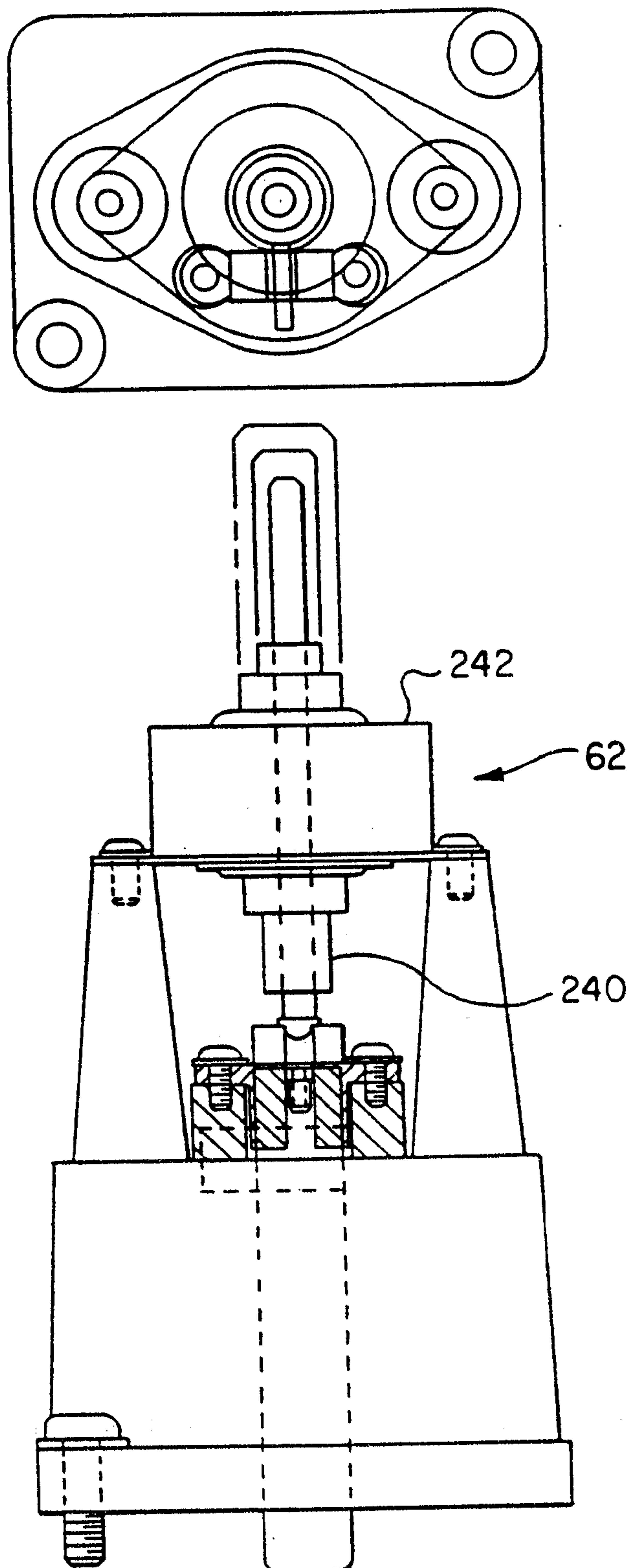


FIG. 19

SUBSTITUTE SHEET

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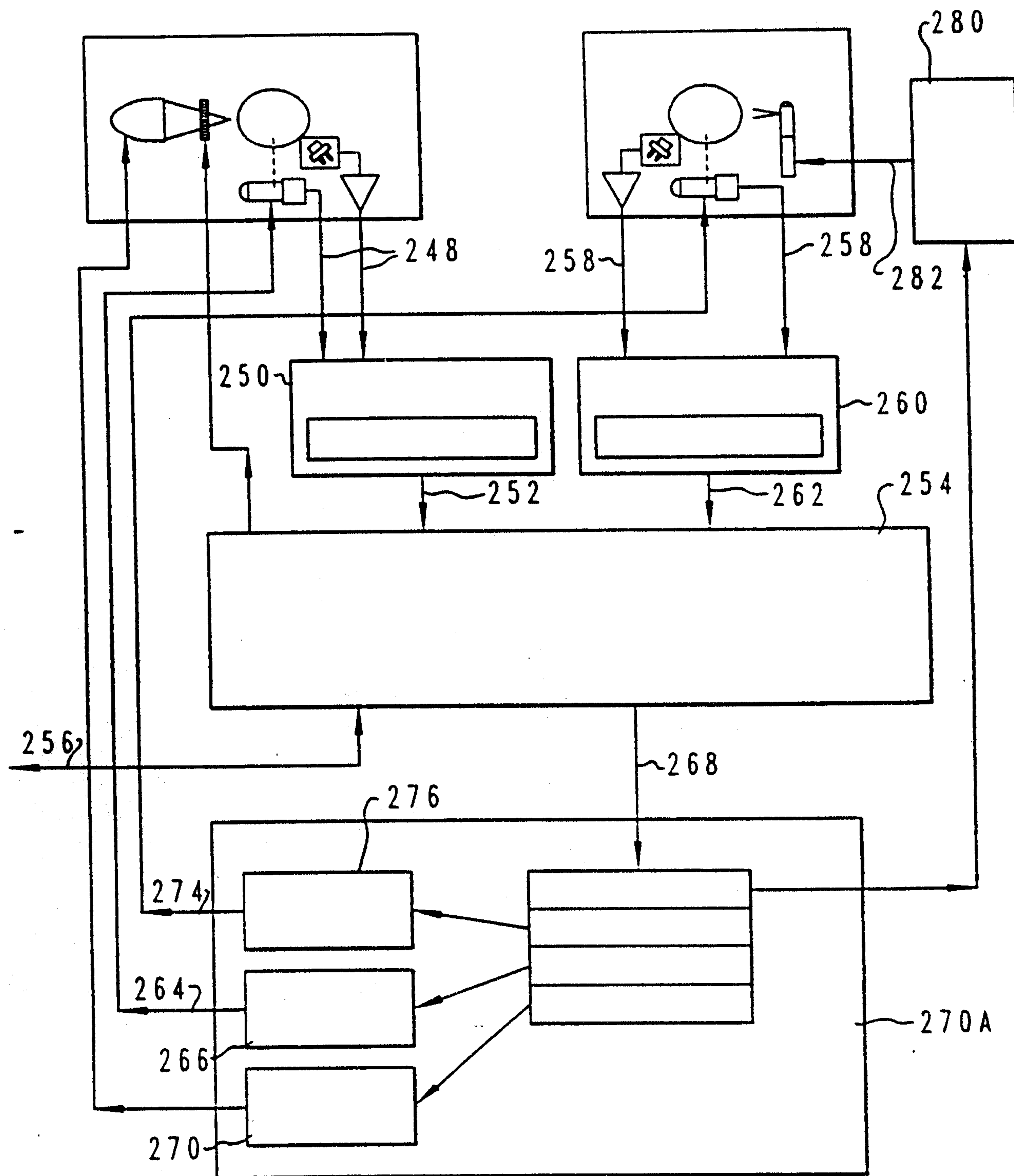


Fig. 20

SUBSTITUTE SHEET

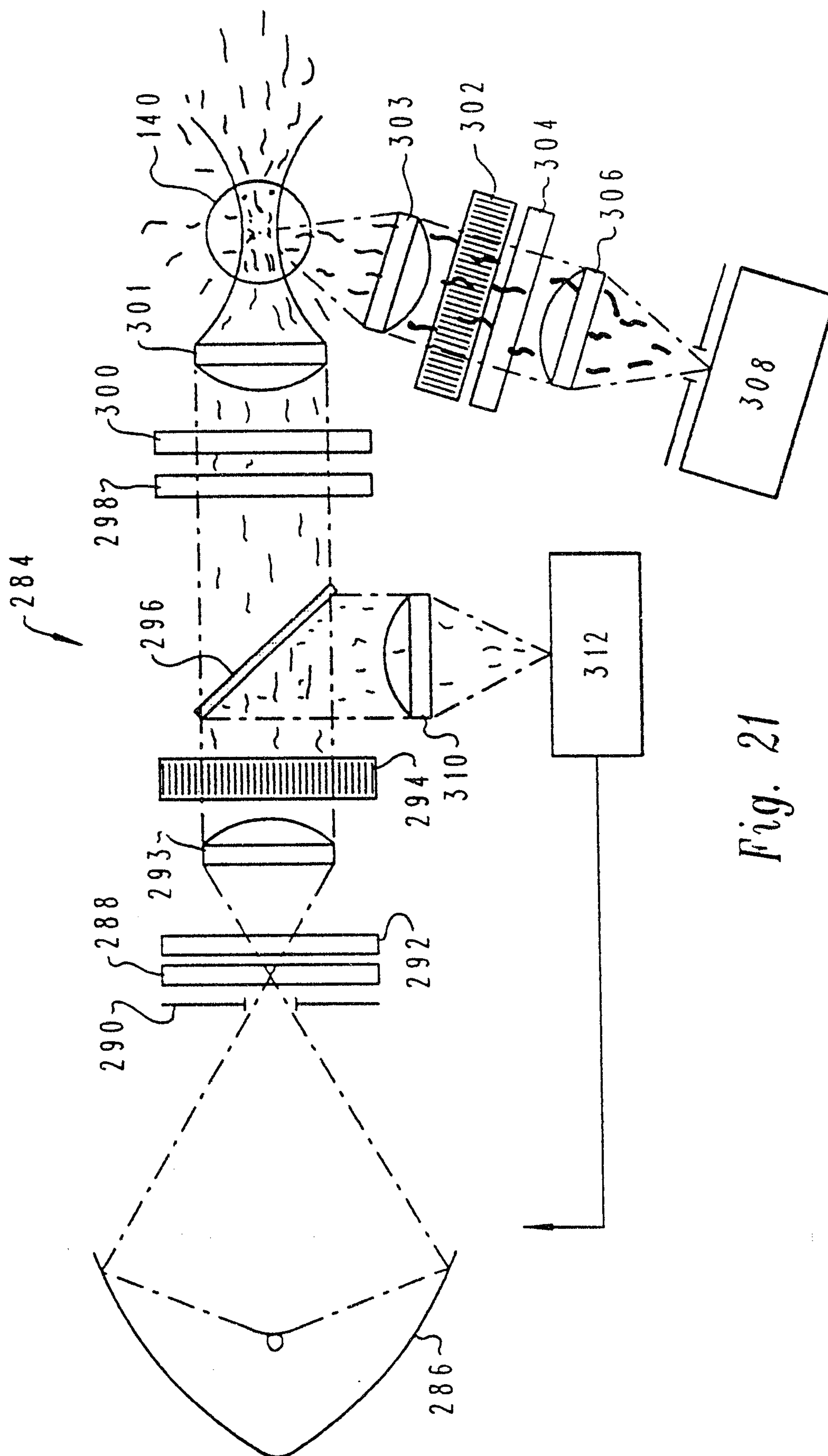


Fig. 21

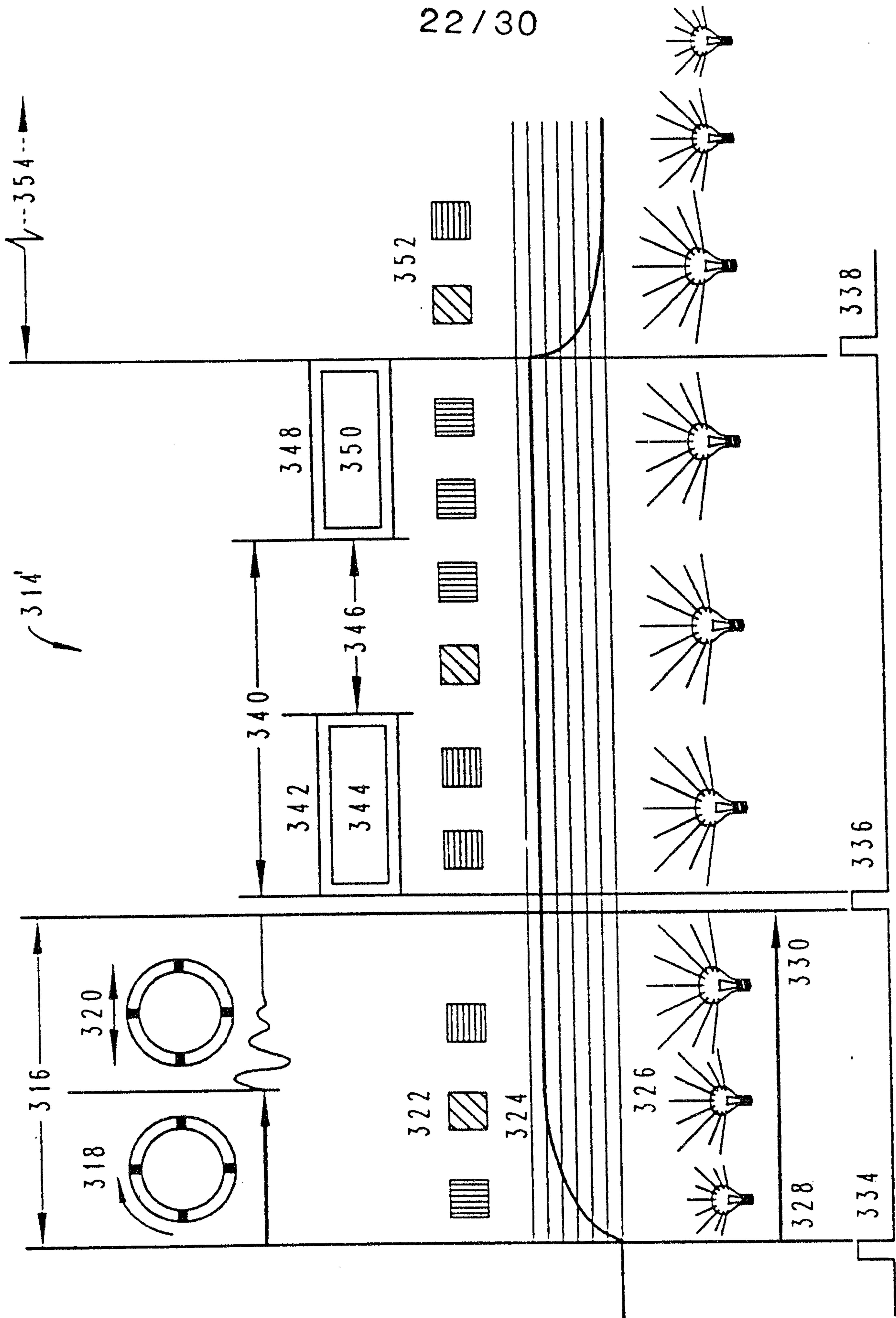


Fig. 22

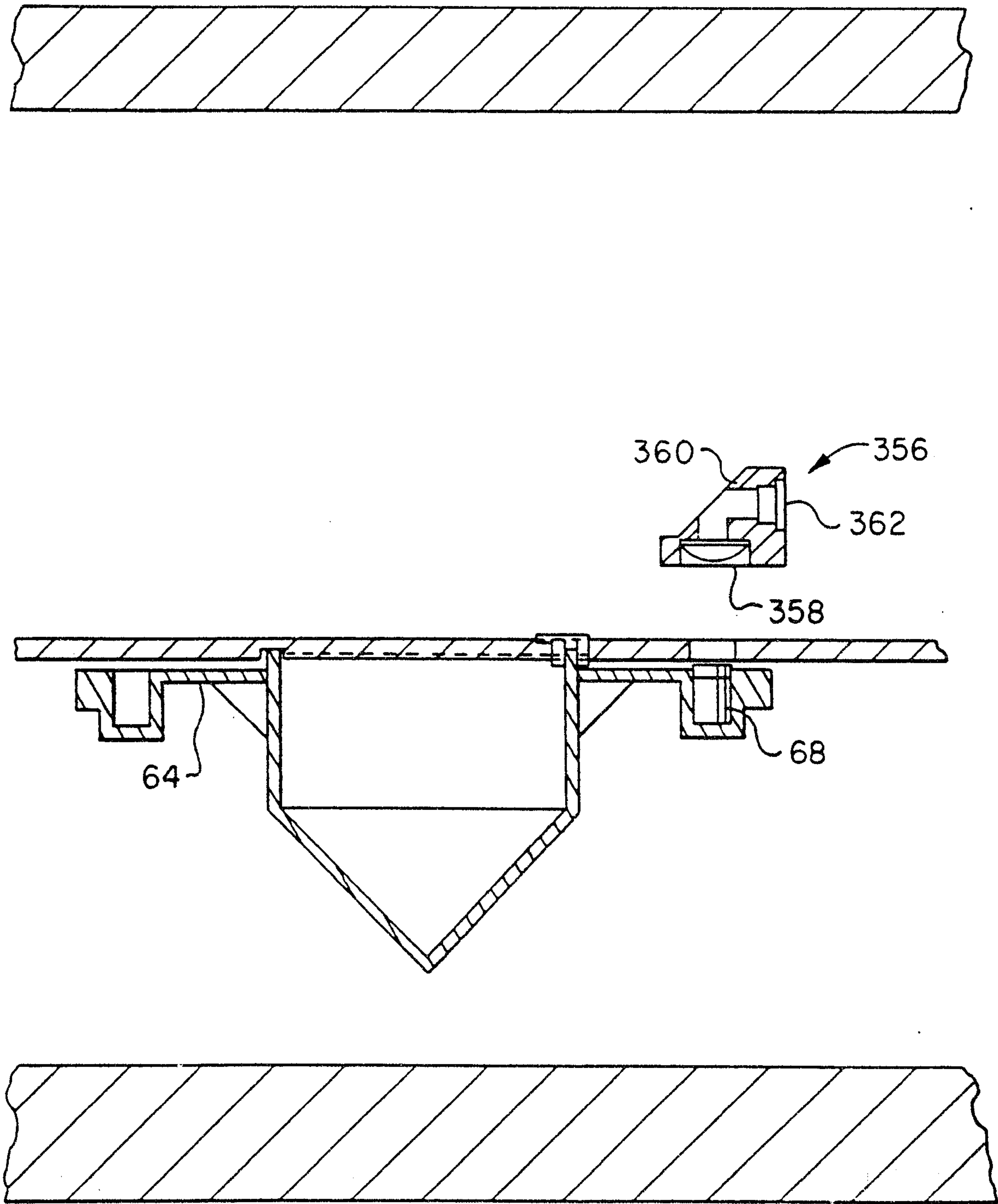


FIG. 23

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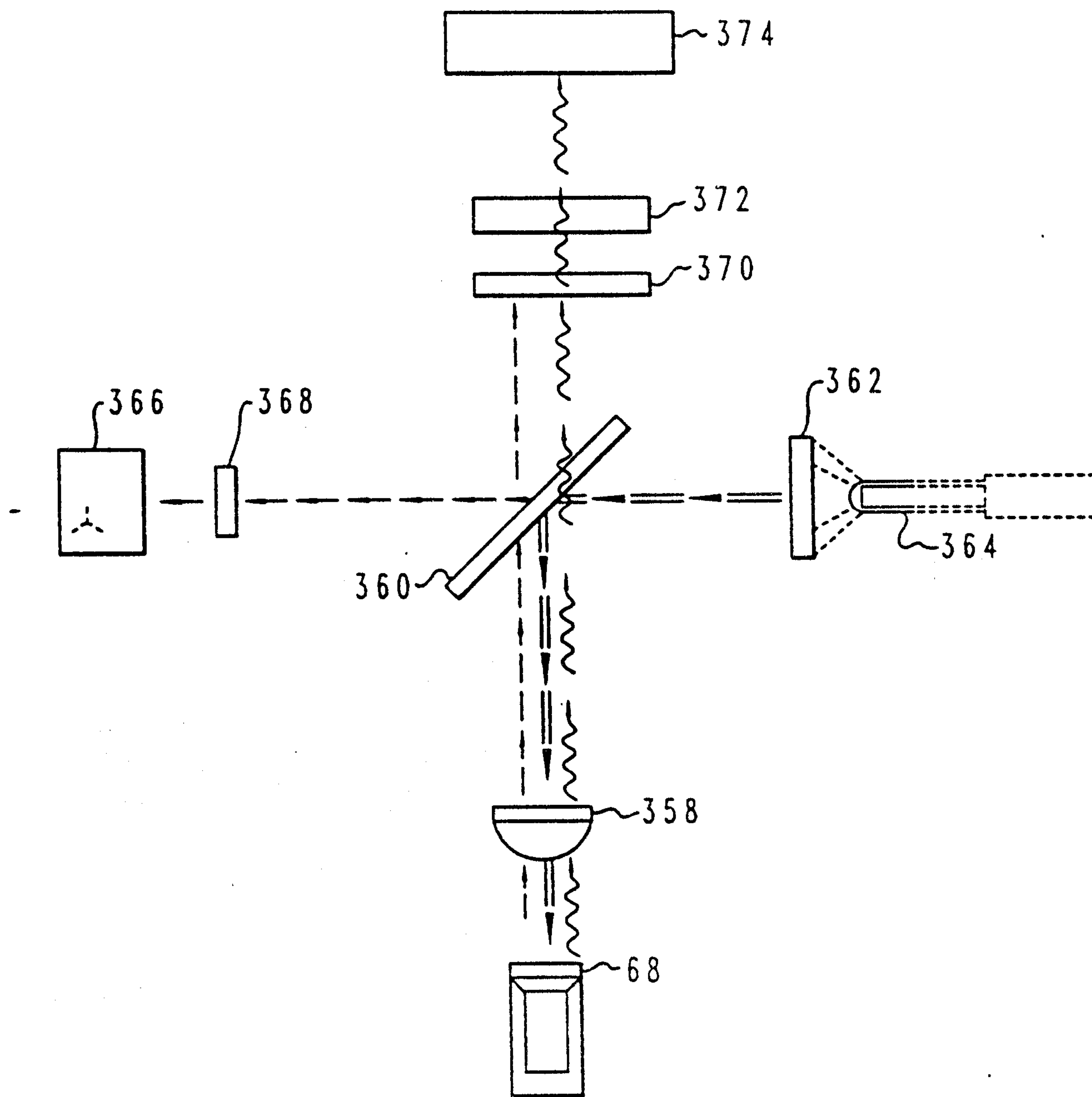


Fig. 24

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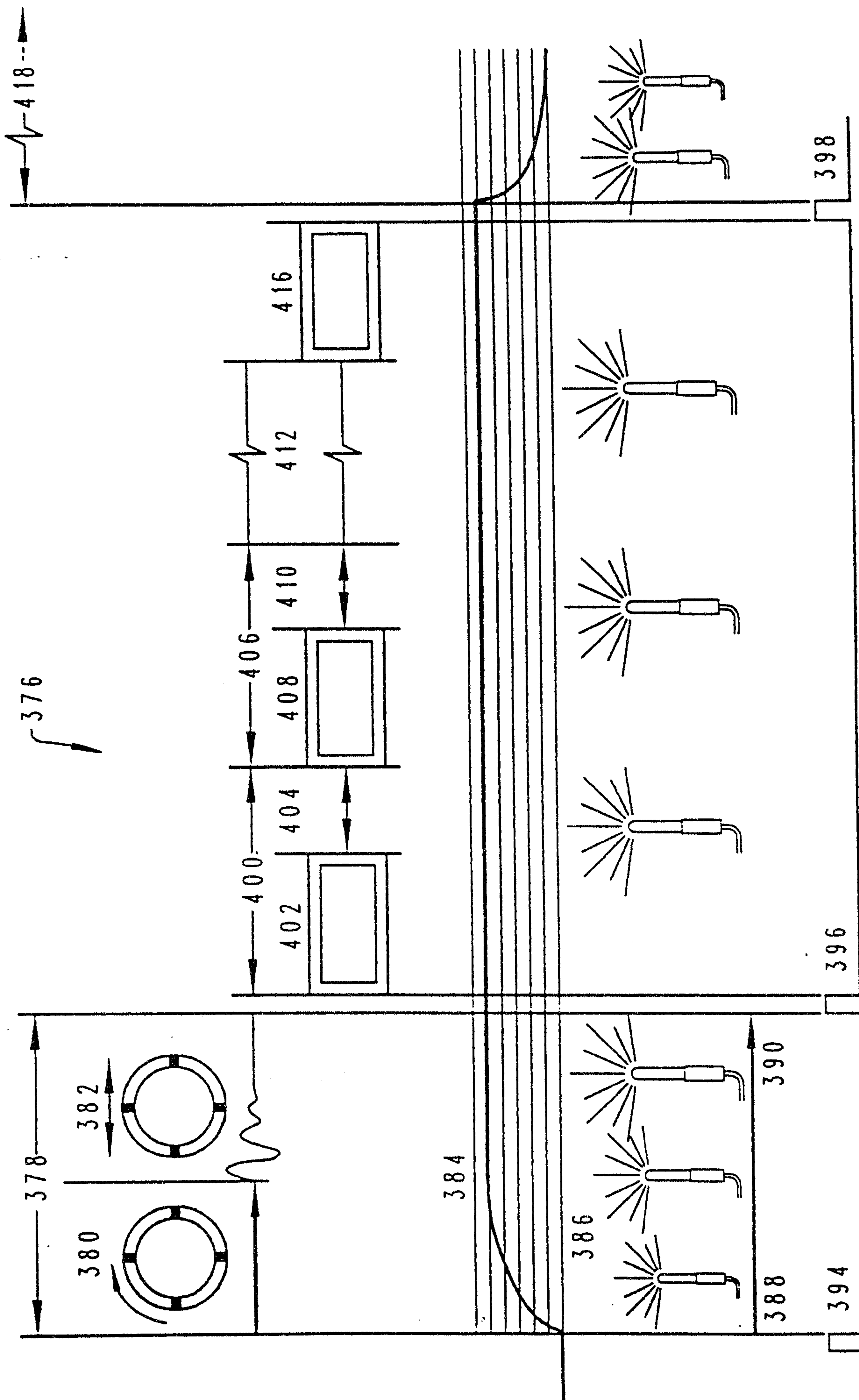


Fig. 25

SUBSTITUTE SHEET

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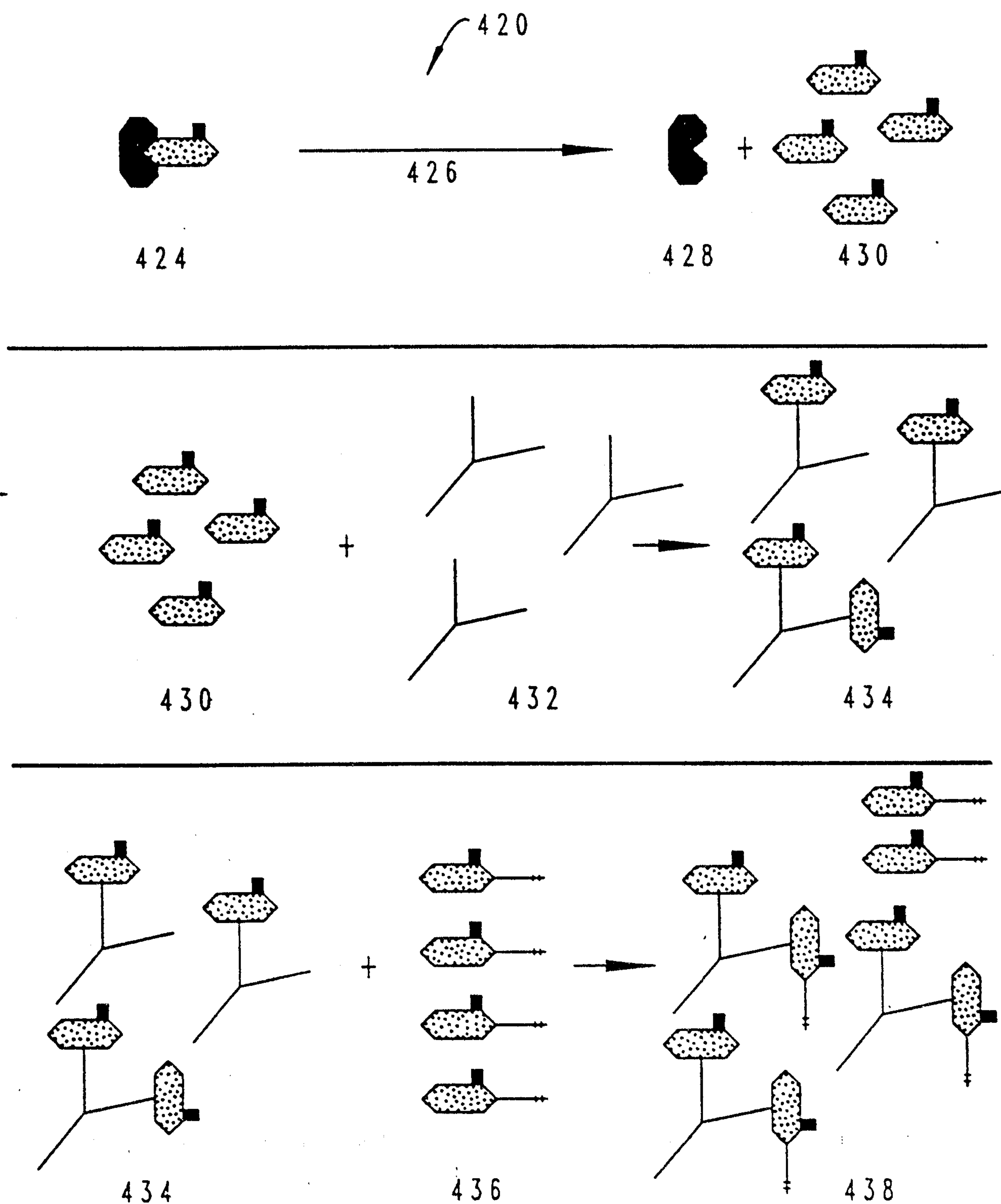


Fig. 26

SUBSTITUTE SHEET

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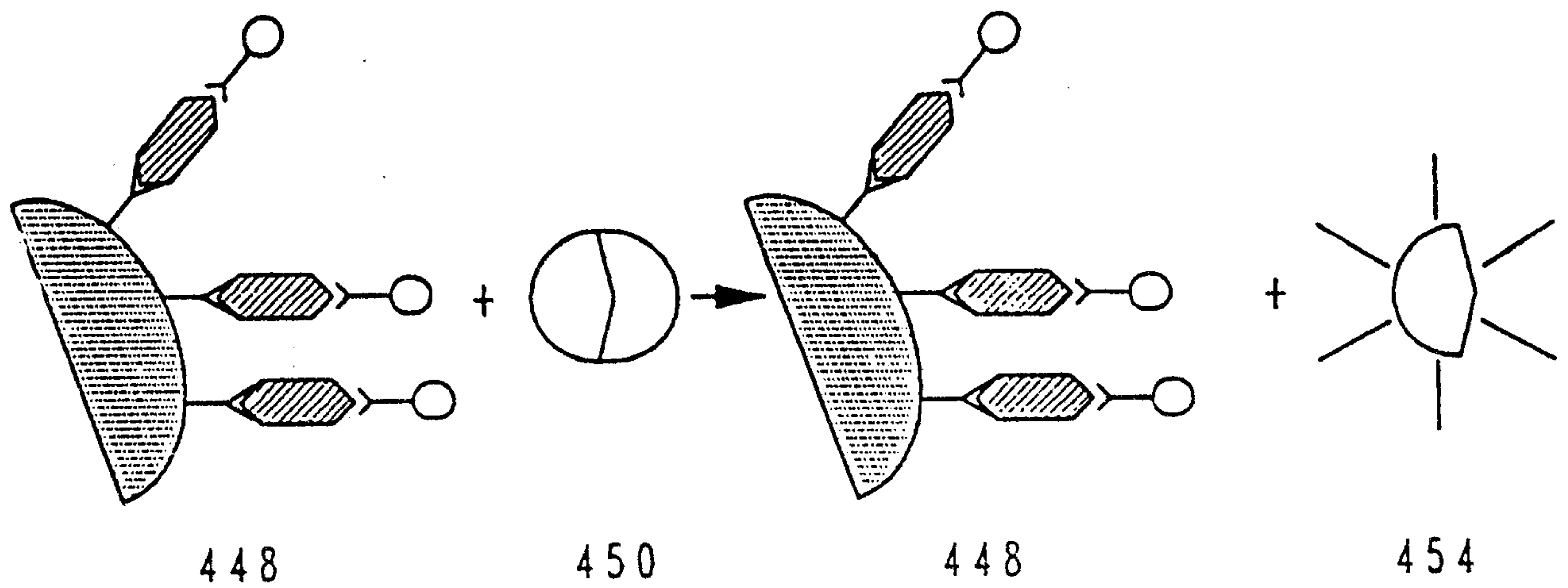
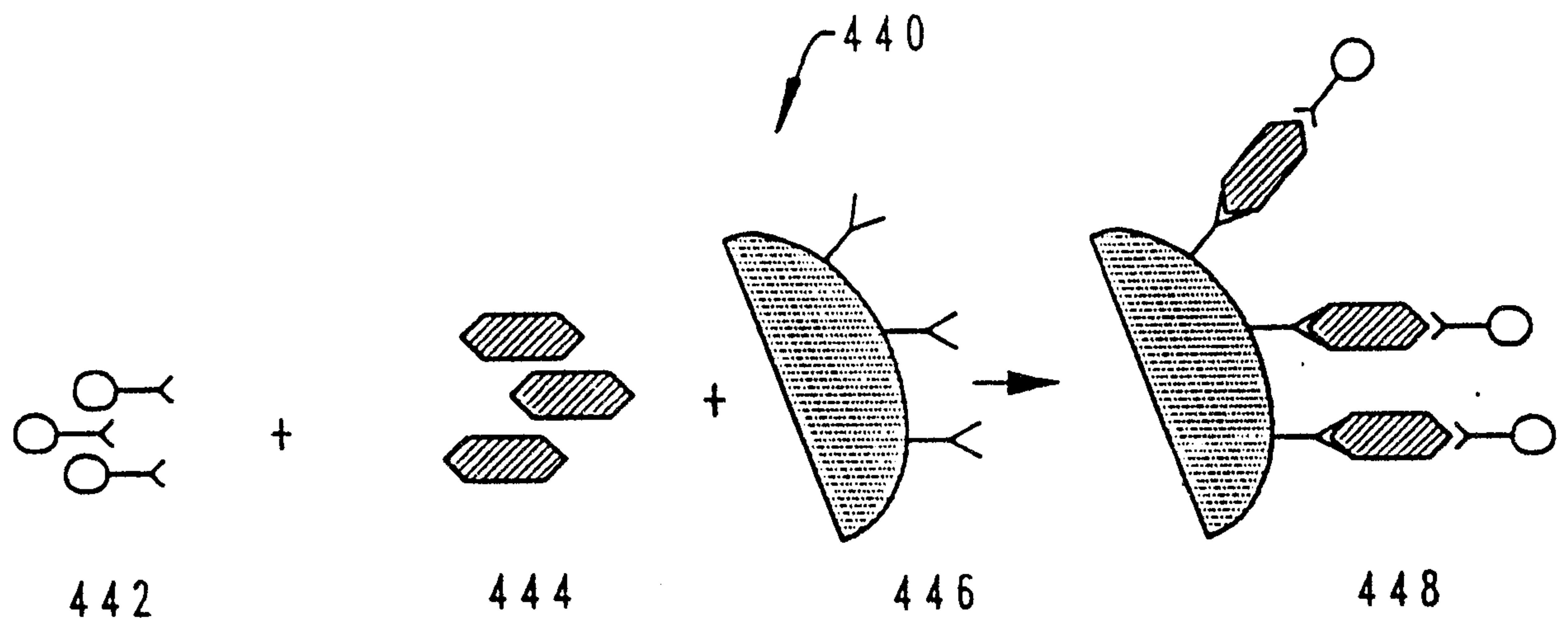


Fig. 27

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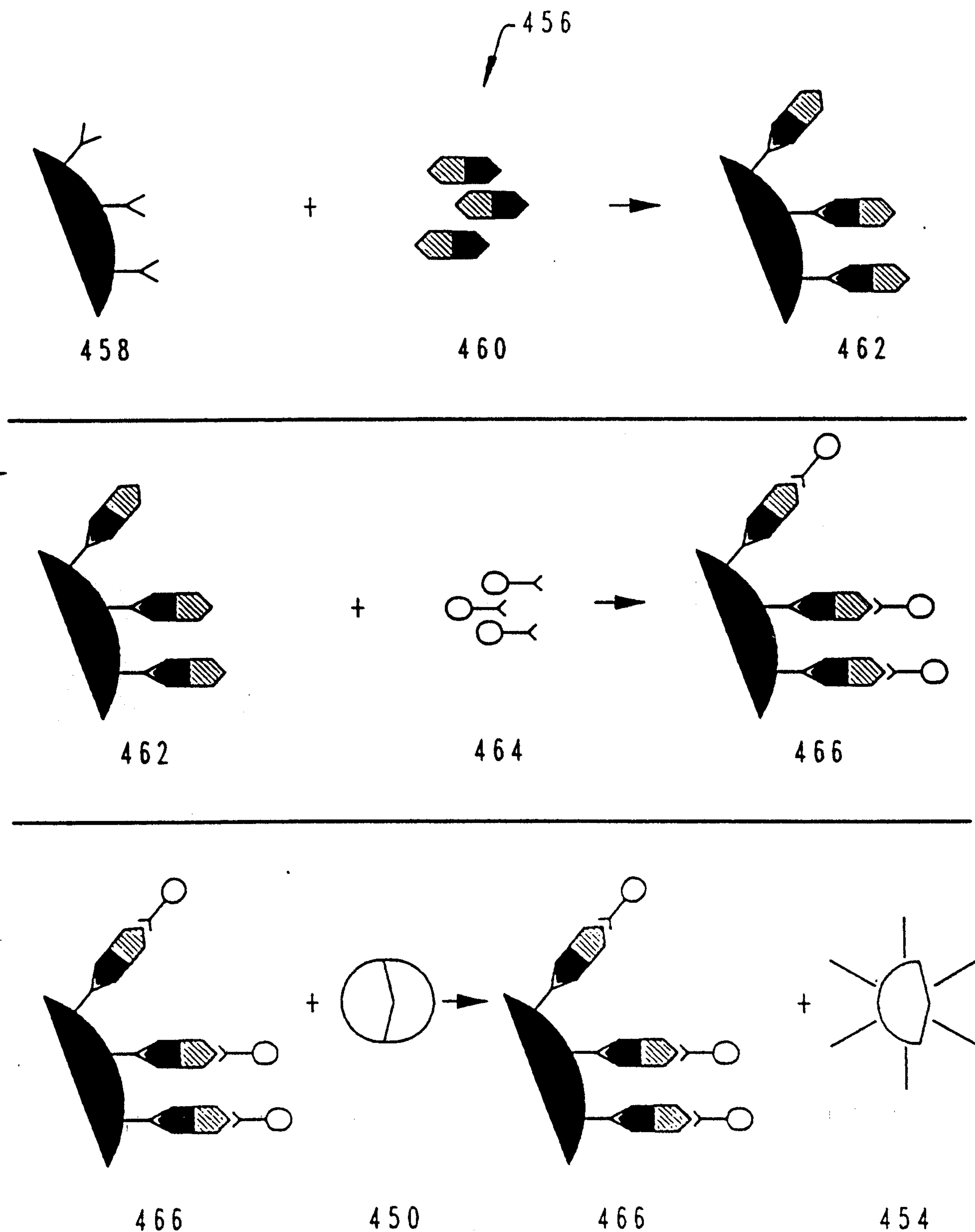


Fig. 28

SUBSTITUTE SHEET

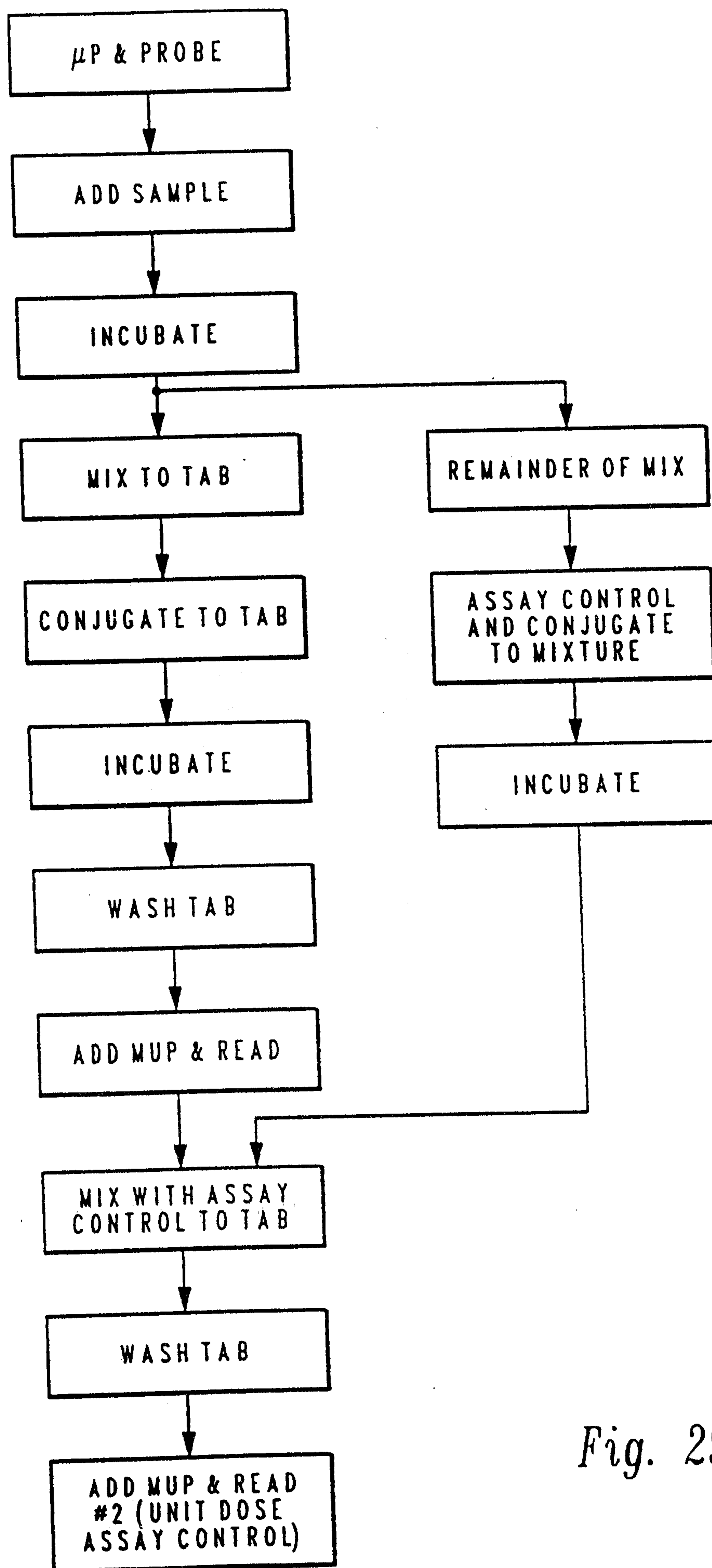


Fig. 29

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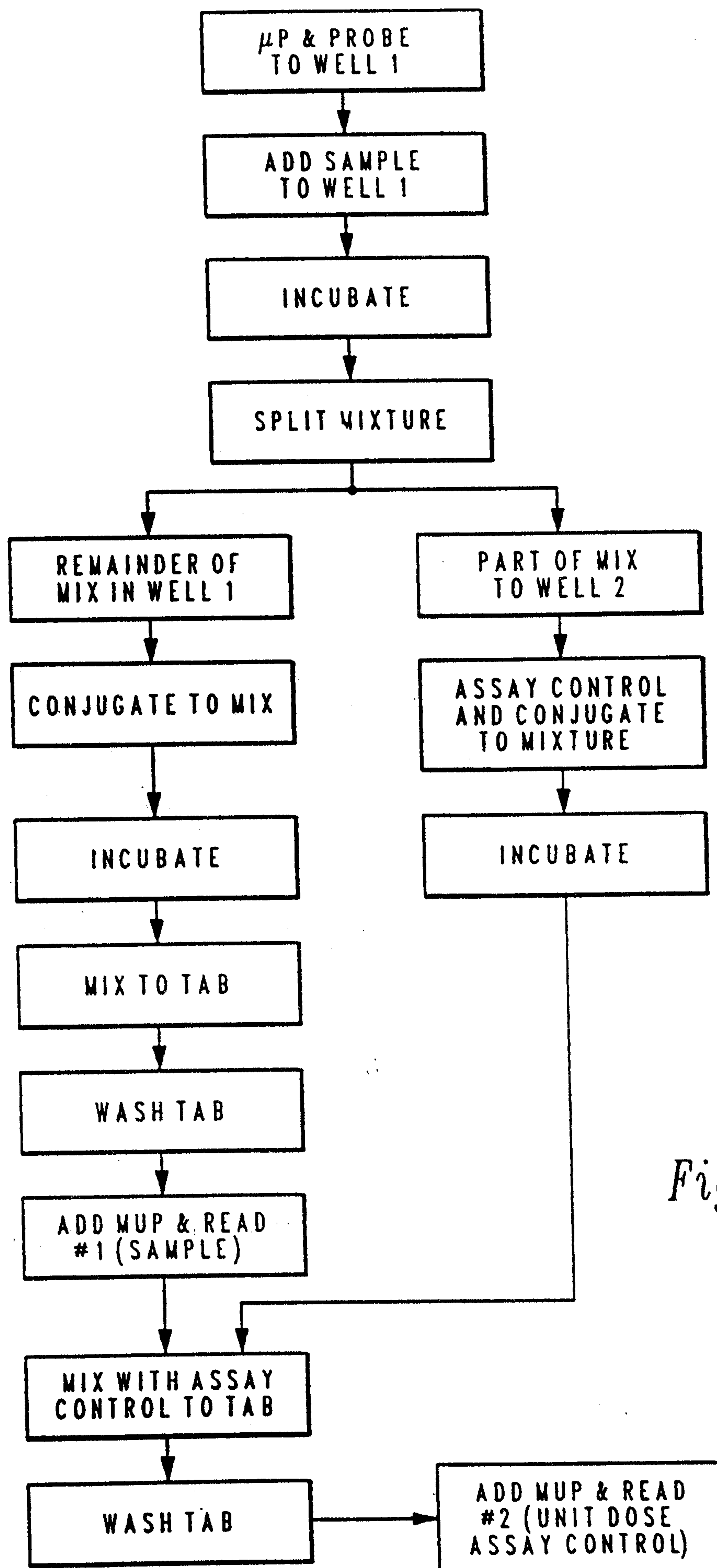


Fig. 30

SUBSTITUTE SHEET