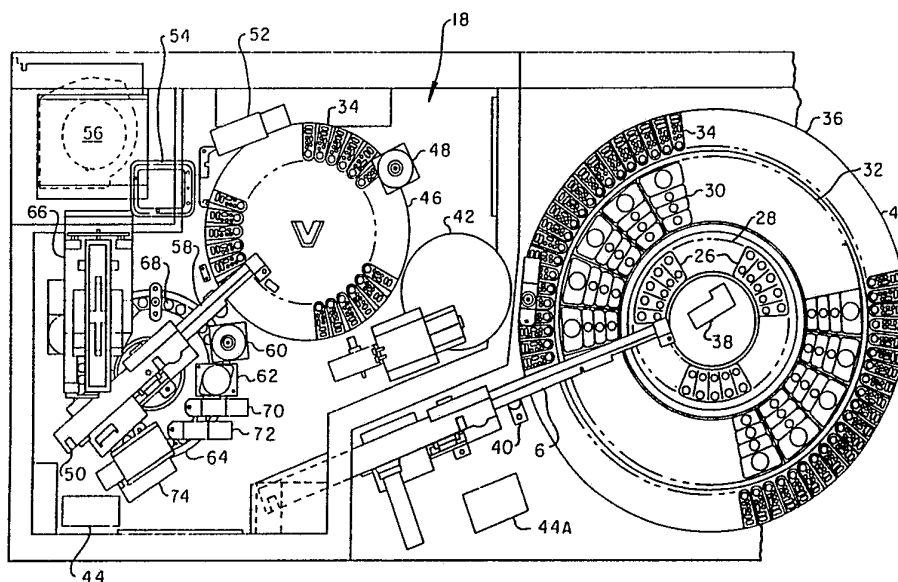


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<b>(21) International Application Number:</b> PCT/US93/02811 <b>(22) International Filing Date:</b> 24 March 1993 (24.03.93) <b>(30) Priority data:</b> 07/859,218                      27 March 1992 (27.03.92)                      US <b>(71) Applicant:</b> ABBOTT LABORATORIES [US/US]; CHADD 0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US). <b>(72) Inventors:</b> CLARK, Frederic, L. ; 2712 Chamberlain Cir- cle, Plano, TX 75023 (US). CLIFT, Gilbert ; 4514 Live Oak, Mesquite, TX 75150 (US). HENDRICK, Kendall, B. ; 1335 Forest Lane, Southlake, TX 76092 (US). LA- GOCKI, Peter, A. ; 225 N. Hamilton Avenue, Park Ridge, IL 60068 (US). MARTIN, Richard, R. ; 8804 Saddlehorn #311, Irving, TX 75063 (US). MITCHELL, James, E. ; 184 River Road, Lake Barrington, IL 60010 (US). MOORE, Larry, W. ; 2713 Hunters Creek, Plano, TX 75075 (US). PENNINGTON, Charles, D. ; 980 Ho- ney Lake Road, Lake Zurich, IL 60047 (US). WALKER, Edna, S. ; 3231 W. Warner, Chicago, IL 60618 (US). SMITH, Jane, B. ; 26 Lindon Lane, Vernon Hills, IL 60061 (US). TAYI, Apparao ; 846 Langley Court, Grays- lake, IL 60030 (US). VAUGHT, James, A. ; 908 Rose- wood Court, Euless, TX 76039 (US). YOST, David, A. ; 19617 Selby Avenue, Poolesville, MD 20837 (US). KA- NEWSKE, William, J., III ; 1502 W. Colorado, Dallas, TX 75208 (US).		<b>(74) Agents:</b> GORMAN, Edward, H., Jr. et al.; Abbott Labo- ratories, CHAD 0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).  <b>(81) Designated States:</b> AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** AUTOMATED CONTINUOUS AND RANDOM ACCESS ANALYTICAL SYSTEM**(57) Abstract**

An automated, continuous and random access analytical system (18), having apparatus and methodology capable of simultaneously performing multiple assays of liquid samples using different assay methodologies, and providing continuous and random access while performing a plurality of different assays on the same or different samples during the same time period, is disclosed. A method is also disclosed of operating an automated continuous and random access analytical system (18) capable of simultaneously effecting multiple assays of a plurality of liquid samples wherein scheduling of various assays of the plurality of liquid samples is followed by creating a unit dose disposable and separately transferring a first liquid sample (26) and reagents (30) to a reaction vessel (34) without initiation of an assay reaction sequence, followed by physical transfer of the unit dose disposable to a processing workstation (52), whereby a mixture of the unit dose disposable reagents and sample (34) are achieved during incubation. The system (18) is capable of performing more than one scheduled assay in any order, and assays where more than such scheduled assays are presented. The automated, continuous and random access analytical system (18) is also capable of analyzing the incubated reaction mixtures independently and individually by at least two assay procedures.



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## AUTOMATED CONTINUOUS AND RANDOM ACCESS ANALYTICAL SYSTEM

5

### Field of the Invention

10 The present invention relates to an automated analytical system and methods for the analysis of liquid test samples. In another aspect, the invention is related to a continuous and random access system which is capable of simultaneously performing a plurality of assays, particularly heterogeneous and/or homogeneous immunoassays.

15

### Background of the Invention

20 Although various known clinical analyzers for chemical, immunochemical and biological testing of samples are available, clinical technology is rapidly changing due to increasing demands in the clinical laboratory to provide new levels of service. These new levels of service must be more cost effective to decrease the operating expenditures such as labor cost and the like, and must provide shorter turnaround  
25 time of test results to reduce the patient's length of stay in the hospital as well as improve efficiency of outpatient treatment. Modernization of analytical apparatus and procedures demands consolidation of work stations to meet the growing challenge placed on clinical laboratories.

30 Generally, analysis of a test sample involves the reaction of test samples with one or more reagents with 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. However, due to the high  
35 demands placed on clinical laboratories regarding not only volume throughput but also the number and frequency of various analyses, there is a need to provide an automated

analysis system which is capable of combining accurate analytical results, high throughput, multiple test menu versatility as well as low reagent consumption.

Typically, analysis of a test sample involves forming a reaction mixture comprising the test sample and one or more reagents, and the reaction mixture is then analyzed by an apparatus for one or more characteristics of the test sample. Reliance on automated clinical analyzers improves the efficiency of the laboratory procedures inasmuch as the technician has fewer tasks to performed. Automated clinical analyzers provide results much more rapidly while frequently avoiding operator or technician error, thus placing emphasis on accuracy and repeatability of a variety of tests. Automated clinical analyzers presently available for routine laboratory tests include a transport or conveyor system designed to transport containers of sample 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. However, such transport systems are not flexible in that transport is in one direction and the reaction tubes or cuvettes, once inserted into the apparatus, must pass through without access before analysis occurs.

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 but typically rely on detection and measurement of optical changes in a reaction mixture during the assay process. For example, a number of well-known techniques using single or multi-wavelength fluorescence include fluorescent polarization immunoassays (FPIA) employing homogeneous immunoassay techniques, microparticle enzyme immunoassays (MEIA) employing heterogeneous immunoassay techniques, and the like. The MEIA technology, such as that used on the Abbott IMx® analyzer, is used 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 in the Abbott IMx® analyzer and Abbott TDx® analyzer by a robotic arm with a pipetting probe and a rotating carousel which positions the samples for processing. These instruments are compact table-top analyzers which offer fully automated, walk-away immunoassay testing capabilities for both routine and specialized immunoassays. These nonisotopic methods eliminate radioactivity disposal problems and increase reagent shelf-life while meeting the diverse requirements of a multitude of different assays.

Instead of loading the test sample into a container and obtaining sequential testing, such as one direction only systems as described above, the Abbott IMx® analyzer and the Abbott TDx® analyzer, often referred to as batch analyzers, permit the analysis of multiple samples and provide for access to the test samples for the formation of subsequent reaction mixtures. However, such batch analyzers permit only one type of analysis at a time. In a random access analyzer, not only can multiple test samples be analyzed, but multiple analytes may be analyzed from each test sample. Another common feature of presently available sequential and random access analyzers is the inclusion of 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. The Abbott IMx®

analyzer automatically performs all the steps required for analysis of test samples and includes numerous checks of the subsystems to insure that the assay can be run to completion and that results are valid. Quantification of the fluorescence  
5 intensity in the MEIA method and polarization in the FPIA method, as well as the final data reduction, are also fully automated on the analyzer. Results are printed by the analyzer and can be accessed through suitable means for automatic data collection by a laboratory computer.

10 Automated analytical apparatus for performing homogeneous assays, the detection of precipitate formed by reaction between antigens and antibodies in a test sample-cell to form light scattering centers, and methods and apparatus for detecting immunological agglutination reactions  
15 are also known in the art. Such apparatus and methods include, for example, the steps of measuring light absorption of the liquid medium 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  
20 this way, the presence or absence of agglutination can be detected based on the fact that the agglutination reaction reduces the concentration of antibody, which affects the light absorption of the liquid medium. As is typical of methods and apparatus for performing homogeneous assays, these  
25 procedures do not require separation of a solid phase from the reaction mixture for further analysis.

Heterogeneous assays are also known through the use of a sample analyzer for quantitating relatively small amounts of clinically significant compounds in a liquid test sample by  
30 focusing a light source onto the sample so that, for example, fluorescent particles in the sample cause fluorescent conditions, the intensity of which is the function of the intensity of the light beam and the concentration of fluorescent particles in the sample. A detector senses photons  
35 forming the fluorescent emissions of the particles when excited by the light beam. The introduction of a solid phase material into the sample requires subsequent separation of

the solid phase from the reaction mixture for further analysis and before the fluorescent emissions can be detected and measured.

5 Recently, 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  
10 utilizing both homogeneous and heterogeneous assay techniques.

Accordingly, since such previously described automated analyzers do not contemplate an automated analytical system for simultaneously performing both homogeneous and  
15 heterogeneous assays in a continuous and random access fashion utilizing a commonality of various process work stations and transfer means, there is a need to provide an automated analytical system having these features and sufficient flexibility to meet the growing needs of the modern  
20 clinical laboratory.

### Summary of the Invention

The automated analytical system of the present invention is capable of simultaneously performing two or more assays on a plurality of test samples in a continuous and random access fashion. In particular, the automated immunoassay analytical system apparatus of the invention can be viewed as a microprocessor-based system of integrated subassemblies with different groups of assays being run through separate and changeable software modules. The microprocessor based system uses robotic arm pipettors with two degrees of freedom and bidirectional rotating carousels to process samples. Critical assay steps such as incubations, washes and specimen dilution are performed automatically by the instrument as scheduled.

According to the invention, automated, continuous and random access analytical system capable of simultaneously effecting multiple assays of a plurality of liquid samples is provided, and enables performing a method wherein various assays are scheduled for a plurality of liquid samples. Through kitting means the present system is capable of creating a unit dose disposable by separately transferring liquid sample and reagents to a reaction vessel without initiation of an assay reaction sequence. From the kitting means multiple, kitted unit dose disposables are transferred to a process area, wherein an aliquot is mixed for each independent sample with one or more liquid reagents at different times in a reaction vessel to form independent reaction mixtures. Independent scheduling of such kitting and mixing is achieved during incubation of the multiple reaction mixtures, simultaneously and independently.

The system of the present invention is capable of performing more than one scheduled assay in any order in which plurality of scheduled assays are presented. The incubated reaction mixtures are analyzed independently and individually by at least two assay procedures which are previously scheduled.



The automated, continuous and random access analytical system apparatus of this invention is comprised of a front end carousel assembly inclusive of a sample cup carousel, a reagent pack carousel and a reaction vessel carousel mounted concentrically and serviced by a transfer pipetting means suitable for kitting and/or mixing reagents with a sample. 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 4 which includes a controlled environment for 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 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.

Additional advantages and novel features of the invention will be set forth in part in the description which follows, and will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the invention. The objects and advantages of the invention may be obtained by means of the exemplary combinations more particularly pointed out in the following specification and appended claims, including all equivalents thereof.

### Brief Description of the Drawings

FIGURE 1 is an isometric view of the automated analytical system 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.

5       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  
10       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.

15       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.

FIGURE 7 is an isometric view of the probe, probe arm  
20       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.

FIGURE 9 is a cross-sectional side elevational view of an automatic bubble flushing syringe apparatus of the  
25       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 toward the bore end portion.

30       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.

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

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

5       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.

10       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  
15       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.

20       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.

25       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.

30       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.

35       FIGURE 22 is a schematic of the FPIA read 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  
5 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.

10 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  
15 analytical system.

### Description of the Invention

#### *Definitions*

20

The following definitions are applicable to the present invention:

The term "test sample", as used herein, refers to a material suspected of containing the analyte. The test sample  
25 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,  
30 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  
35 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.

5       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  
10 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  
15 purposes), virus particles and metabolites of or antibodies to any of the above substances. The term "analyte" also includes any antigenic substances, haptens, antibodies, macromolecules and combinations thereof.

      The term "analyte-analog", as used herein, refers to a  
20 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  
25 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.

30       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  
35 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  
5 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  
10 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  
15 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  
20 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  
25 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.

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

The term "random access", as used herein, refers to the ability of the automated analytical system of the present

invention 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 of the present invention.

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

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

15       The term "quat" refers to a polycationic material solution for assays which use these materials which are not an antibody or antigen to capture the analyte from the sample on the matrix of, for example, MEIA cartridge. In the present inventive system, quat is dispensed to the matrix during test processing, prior to the transfer of the reaction mixture from  
20       the reaction vessel.

      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- and 2-step sandwich and competitive  
25       assay formats; order of activity processing, including the ability to initiate sample processing for both 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  
30       prior art, known random access systems which force all assay protocols to adhere to a strict "lock-step" format, in which assay configuration (i.e. 1- versus 2-step formats), activity order, incubation timing, and other similar protocols are fixed by the instrument.

35

*Scheduler*

According to the present invention, a system scheduler generates and optimizes the workload for 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 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 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 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 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.

In the inventive 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 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 the instrument  
5 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 achieved, the test  
10 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  
15 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  
20 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  
25 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.

### 30 *Stat Procedure*

The inventive system allows special priority handling of specific samples identified by the user as being stat samples. A stat sample, as defined by the inventive system, is a sample  
35 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.

In the inventive 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. 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.

5       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.

10       The automated analytical system of the present invention is capable of performing various assays employing various detection systems known in the art and include, but are not intended to be limited to, spectrophotometric absorbance assay such as end-point reaction analysis and rate of reaction analysis, turbidimetric assays, nephelometric  
15       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 and incorporated herein by reference), ion capture assays, colorimetric assays, fluorometric assays, electrochemical detection systems, potentiometric detection  
20       systems, amperometric detection system 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  
25       moiety employed therein can be measured and correlated to the amount of analyte present in a test sample.

30       Generally, in a spectrophotometric assay, such as those performed on the Abbott Spectrum clinical analyzer and the Abbott Spectrum Series II clinical analyzer (Abbott Laboratories, Abbott Park, IL, USA) the interaction in an assay solution between the analyte to be determined and a reagent system specific for the analyte produces a detectable change in the transmittive properties of the assay solution. The change in the transmittive properties refers to the amount of  
35       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 monochromic 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. 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.

In a colorimetric assay, the change in the transmittive properties of an assay solution 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 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 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 principle of turbidimetric assays is to determine the amount of light scattered or blocked by particulate matter as light passes through an assay solution. In a turbidimetric assay, 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

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 turbidimetric assay refers to the decrease in the intensity of the light transmitted through an assay solution, 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 particles.

A nephelometric assay 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. In a nephelometric assay, the change in the transmittive properties of the assay solution is also related to the amount of incident light scattered or blocked by the suspension of particles, but 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 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. Turbidimetric and nephelometric assays are 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 of the present invention 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 and incorporated herein by reference.

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 performed on the apparatus and by the methods of the present invention.

Typically in a fluorometric assay, an analyte in an assay solution is 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 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 concentration of the analyte. However, the intensity of the fluorescence emitted by the assay solution may be inhibited when the ligand to be determined complexes with nonfluorescent interferences such as protein or phosphates present in the sample, or when the sample containing the 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 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.

Generally, homogeneous and heterogeneous immunoassays depend upon the ability of a first binding member of a binding member pair to specifically bind to a second binding member of a binding member pair wherein a

conjugate, comprising one of such binding members labeled with a detectable moiety, is employed to determine the extent of such binding. For example, where such binding pair members are an analyte and an antibody to such analyte, the extent of binding is determined by the amount of the detectable moiety present in the conjugate, which either has or has not participated in a binding reaction with the analyte, wherein the amount of the detectable moiety detected and measured can be correlated to the amount of analyte present in the test sample.

Homogeneous immunoassays typically are performed in 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 on the automated analytical system of the present invention 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, incorporated herein by reference; triazinylaminofluoresceins, such as described in U.S. Patent No. 4,420,568 and U.S. Patent No. 4,593,089, incorporated herein by reference; carboxyfluoresceins, such as described in U.S. Patent No. 4,668,640, incorporated herein by reference; and the like.

Heterogenous immunoassays typically involve 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 as described above 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, a heterogeneous immunoassay which can be performed by the automated analytical system of the present invention, in either a competitive or sandwich immunoassay format, is 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.

In addition, the use of sucrose in microparticle diluent has been found to achieve neutral density of the microparticles. The methodology entails the determination of the optimum sucrose concentration which will eliminate the settling of microparticles. The sucrose concentration required to achieve neutral density is assay specific and microparticle lot specific. The principal involves dissolving sucrose in solution to increase the density of the diluent. When the density of the diluent and microparticles are equivalent, the microparticles will be in a suspended state. Density neutralization can also be achieved by using other materials such as metrizamide and/or metrizoic acid.

Separation of the bound and free species is 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  
5 microparticles during the optical quantification phase of the assay protocol as described herein.

When performing a sandwich immunoassay, microparticles coated with antibody to the analyte in the test sample are incubated with the test sample containing the  
10 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  
15 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  
20 unbound conjugate is accomplished with the glass fiber matrix of the 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  
25 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.

30 The MEIA cartridge which is employed by the automated analytical system of the present invention 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  
35 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  
5 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 predominantly lie in a plane perpendicular to the lower surface of the fibrous matrix. The  
10 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 well, therefore, is generally sized or contains positioning means to maintain the fluid  
15 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 the fibrous matrix. Additionally, it is preferable to vent to the atmosphere the  
20 gases displaced in the absorbant material by the liquids absorbed therein during the performance of an immunoassay.

According to the immunoassay methodologies described above, standard solutions of the analyte of known concentrations covering the clinical concentration range are  
25 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  
30 concentration value through interpretation from the blank or standard curve.

Automated analytical methodology for effecting analysis of a plurality of test samples according to the present invention is achieved by introducing reagent packs, test  
35 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  
5 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  
10 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  
15 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-  
20 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  
25 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  
30 has the capability of aligning the reagent pack carousel and test sample container carousel and a reaction vessel for 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  
35 until the reaction vessel, a reagent pack and a test sample 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 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 immunoassay (FPIA) with the automated analytical system of the present invention, 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, FPIA reagents, is at the read station of the FPIA processing stations and the FPIA determination 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 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) performed with the automated analytical system of the present invention, after the various pipetting activities for the MEIA, 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 mixture is transferred from the reaction vessel to a matrix of an MEIA cartridge 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  
5 described above, FPIA and MEIA, are incorporated into the automated analytical system of the present invention; however, more than two distinct analytical technologies can be incorporated into the inventive system. These methods are complimentary and share a commonality of apparatus and  
10 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  
15 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.

20 The FPIA optic systems (such as described in U.S. Patent No. 4,269,511 and incorporated herein by reference) 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  
25 performing FPIA assays utilizing the automated analytical system of the present invention, 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  
30 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  
35 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  
5 like.

In another embodiment, the FPIA format utilizes a unique, round, plastic, reaction cuvette suitable for fluorescence polarization and absorbance assay technologies which require no orientation other than top-to-bottom. This  
10 plastic reaction cuvette has physical characteristics of low birefringence throughout the optical read region as well as stringent dimensional tolerances which allow reproducible absorbance readings. Bifringence is defined as the degree of retardation of the extraordinary ray as it passes through a  
15 material. The greater the degree of retardation, the greater will be the level of birefringence. Retardation of the extraordinary ray is dependent on the magnitude and direction of the induced stress. Therefore, passing a ray of linearly polarized light through a material with induced stress will  
20 result in depolarization of the ray. In order for a cuvette to be utilized for fluorescence polarization measurements, it is important that the cuvette be prepared under conditions which yield minimum stress. The geometry of the cuvette has been designed to utilize the inherent fluidics of automated medical  
25 diagnostic instrumentation to minimize the hydrophobic effect of plastic.

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  
30 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  
35 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. A system of lenses and optical filters focus filtered light (wavelength = 365) from a mercury arc lamp on to the surface of the matrix and focus emitted fluorescence from 4-MU (wavelength = 448) 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 microparticle reagent, an alkaline phosphatase conjugate and, in some cases, a dilute buffer specific for the assay being performed. Because the microparticles tend not to settle out of suspension during the course of the assay, they can readily be pipetted. 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

- 5 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.

10

### *Detailed Description of the Drawings*

- Preferred embodiments of the automated immunoassay analytical system according to the present invention are
- 15 presented only with those components of primary interest with respect to the inventive system apparatus and processes 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
- 20 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
- 25 samples and determining analytical results.

- Referring to the drawings, FIGURES 1 and 2 present isometric views of the automatic immunoassay analytical system apparatus of the present invention. The system apparatus as it appears in FIGURE 1 presents the system
- 30 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 of the present invention is identified generally by the reference numeral 2 in FIGURE 1. The system apparatus 2 has an exposed
- 35 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

- 5 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.

10 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  
15 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.

- 20 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  
25 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  
30 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  
35 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.

- 5 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
- 10 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
- 15 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
- 20 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
- 25 FPIA test schedule reaction vessel 34 for FPIA processing. 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
- 30 mixed sample to the MEIA cartridge 68 is by the function of 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
- 35 cartridges 68 onto the cartridge wheel 64. The process area 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 cartridge ejector 62 after the MEIA read has been completed.

5        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 thereby prevent false negative results in the event there are  
10 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 elevational view in isolation and partial section of elements  
15 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.

20        FIGURE 5 provides a top view in isolation and partial 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  
25 is also shown with a mounting spring 82. The reaction vessel 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  
30 spring 90. The reagent pack carousel is provided with roller 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  
35 when motivated by the individual stepper motors.

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 tubes, or 90 sample cups which are injection molded as one  
5 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. The reaction vessel carousel 36 provides 90 reaction vessels  
10 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 vessel 34 is in position for FPIA read. The process carousel 46  
15 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  
20 reaction vessels 34.

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  
25 probe arm 104, probe adjustment means 106 and probe tip 108 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  
30 used to wash the interior and exterior surfaces of the first 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,  
35 thus avoiding damage to the system apparatus. For example, 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.

5 The first transfer pipette mechanism 6 and the second transfer pipette mechanism 50 are closely related in general 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  
10 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  
15 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  
20 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  
25 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  
30 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  
35 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  
5 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  
10 130 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.

15 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  
20 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.

25 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 wherein cuvette 140 is illustrated in both the top plan view, FIGURE 10, and the side view, FIGURE 10A. S  
30 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  
35 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.

5 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  
10 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  
15 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  
20 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  
25 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,  
30 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  
35 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.

5 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  
10 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  
15 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  
20 to 5.0 inches.

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  
25 mechanism pipette point, the MUP dispense station 72, the 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  
30 insertion point, the second wheel at the pipette point, and the 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  
35 which feeds the MEIA cartridges 68 into the auxiliary carousel 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 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.

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 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.

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.

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 optic@ 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  
5 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  
10 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  
15 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  
20 mixture. Light is emitted from the cuvette through lens means 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  
25 splits out part of the light from the original source through 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  
30 preread time 316 divided into carousel move time 318 and 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  
35 converter settle time 350. Liquid crystal relaxation time is 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 activities where scheduling windows 332 as exemplified by  
5 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 optical assembly 364. An MEIA light source is provided by  
10 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 tube 374 after passing through a wide band-pass emission  
15 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  
20 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  
25 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  
30 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.

35 Multiple automated assay analytical systems are feasible through use of the apparatus, software, hardware and process technology of the present invention and include, but

are not intended to be limited to, the following menus:  
ferritin, creatinine kinase MIB (CK-MB), digoxin, phenytoin,  
phenobarbital, carbamazepine, vancomycin, valproic acid,  
quinidine, leutinizing hormone (LH), follicle stimulating  
5 hormone (FSH), estradiol, progesterone, IgE, vitamin B2 micro-  
globulin, glycated hemoglobin (Gly. Hb), cortisol, digitoxin, N-  
acetylprocainamide (NAPA), procainamide, rubella-IgG,  
rubella-IgM, toxoplasmosis IgG (Toxo-IgG), toxoplasmosis IgM  
(Toxo-IgM), testosterone, salicylates, acetaminophen,  
10 hepatitis B surface antigen (HBsAg), anti-hepatitis B core  
antigen IgG 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 envelope antigen (HBeAg), anti-hepatitis B  
envelope antigen (Anti-HBe), thyroid stimulating hormone  
15 (TSH), thyroxine (T4), total triiodothyronine (Total T3), free  
triiodothyronine (Free T3), carcinoembryonic antigen (CEA), and  
alpha feta protein (AFP).

In order to insure consistent, rapid resuspension and  
continued mixing of reagents with minimal operator  
20 involvement, the reagents are mixed automatically each time  
a new reagent pack is added to the reagent carousel, and  
periodically during instrument operation. This automated  
mixing can be accomplished by a back and forth motion of the  
reagent carousel with asymmetric pauses and is complete  
25 within approximately 1-2 minutes. The carousel acceleration,  
velocity, distance moved, and pause-asymmetry are optimized  
to yield the most rapid reagent resuspension without foaming  
or bubble formation for the range of fill volumes used on the  
instrument.

30 Automated reagent mixing provides the following  
benefits. The operator need not manually mix (e.g. by inversion  
or shaking) reagents which have been stored prior to their  
placement on the instrument. This allows the reagents to be  
loaded onto the instrument in less time and with less  
35 involvement of the operator. There is less tendency for  
reagents to foam or form bubbles with automatic mixing than  
with manual mixing such as inversion. Foam and bubble

formations are detrimental to instrument function and can negatively impact assay performance. Automated mixing insures that reagents are always mixed sufficiently and that they are mixed consistently. Occasional automatic mixing during instrument operation keeps reagents in a consistent suspension, and makes it unnecessary for the operator to periodically remove reagent packs in order to mix the reagents. In some circumstances, automated mixing can dissipate bubbles present at the start of mixing. A detailed description of kitting and process activities according to the invention are presented in the following for FPIA procedures; system description of process activities for a phenobarbital assay; and MEIA procedures for a CEA assay.

It is to be appreciated that the following description comprises an outline of the various functions and steps involved in preferred methods of the automated analytical system of the invention, which functions and methods as also will be appreciated by those skilled in the art, are conducted under computer control using various types of mathematical algorithms and associated computer software, depending on the particular menu of assays being performed on the instrument.

## *DESCRIPTION OF KITTING AND PROCESS AREA ACTIVITIES FOR FPIA*

### SYSTEM DESCRIPTION OF KITTING AREA FOR PHENOBARBITAL 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.)

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.

5

#### B. PREPARATION STEPS

1. User loads empty Reaction Vessel (RV) 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 cover, 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 (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.

25 (c) If the barcode is unreadable, the system will request a barcode override.

(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 to use.

30

#### C. REQUESTING A TEST

35 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.

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

5       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 position in segment.

10       (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 samples have been loaded.

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

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

(h) Instrument reads segment identification.

20       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.

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

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

30

#### D. SCHEDULING A TEST

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

- (b) The System checks for adequate inventory (reagent packs, cartridges, buffer, MUP), system resources, sample time to complete the test.
- 5 (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.
- 10 (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  
15 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.

20

#### 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  
25 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  
30 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  
35 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.
- 10 (ii) Pipette R-axis is moved over sample cup.
- (iii) Pipette Z-axis is moved down to the Z-above position.
- 15 (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)
- 20 (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 insufficient volume is present, the test is aborted and the test request moved to the exception list. The exception list provides notice to an operator of tests which cannot be completed).
- 25 (vii) The following occur simultaneously until the total volume of sample required is aspirated:
- 30
- 35

- 5
- (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.
- (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.
- 10
- (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.
- 15
- (6) Syringe dispenses "X" uL of sample at a rate of "X" ul/sec.
- (7) Pipette Z-axis is moved up to Z-clear position.

(b) Probe Post-Wash

20

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

25

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.

- 30
- (i) The inside of the probe is cleaned first.
- (1) Pipette R-axis is moved over waste area.
- (2) Pipette Z-axis is moved down to appropriate position within the waste area.
- 35

- 5 (3) The wash valve is opened for the amount of time specified in the assay protocol.
- (4) Wash valve is closed.
- 5 (5) Pipette Z-axis is moved up to the Z-clear position.
- (ii) The outside of the probe is cleaned next.
- 10 (1) Pipette R-axis is moved over wash cup.
- (2) Pipette Z-axis is moved down to wash position within the wash cup.
- 15 (3) The wash valve is opened for the amount of time specified in the assay protocol.
- (4) Wash valve is closed.
- (iii) Pipette is returned to "HOME" position.
- 20 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 and hereby incorporated by reference.)
- (a) Popper aspirate.
- 25 (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.
- 30 (iii) Pipette Z-axis is moved down to the Z-above position.
- (iv) LLS is enabled to ensure no liquid currently detected.
- 35 (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).

- 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 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
- 15 (vii) The following occur simultaneously until the total volume of popper required is aspirated:
- 20 (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.
- 25 (5) Pipette Z-axis is moved up to Z-clear position.
- (6) Pipette R-axis is moved over the RV reagent 1 well.
- (7) Pipette Z-axis is moved down to the dispense position within the RV reagent 1 well.
- 30 (8) Syringe dispenses "X" uL of popper at a rate of "X" ul/sec.
- (9) Pipette Z-axis is moved up to Z-clear position.
- 35

(b) Probe post-wash.

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

5           8. Antiserum kitting

(a) Antiserum aspirate

- 10           (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- (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.
- 15           (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).
- 20           (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).
- 25           (vii) The following occur simultaneously until the total volume of antiserum required is aspirated:
- 30               (1) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
- 35

- 5 (2) Syringe aspirates "X" micro liter (uL) at a rate of "X" ul/sec. LLS is checked to ensure probe still in liquid.
- (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.
- 10 (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.
- 15 (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).
- 20 9. Tracer kitting.
- (a) Tracer aspirate.
- (i) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
- 25 (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.
- 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 not is  
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 tracer  
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.
- (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 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.

20

25

30

(b) Probe post-wash.

35

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

## 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  
5 position is aligned with the transfer station.
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.
- 10 5. Transfer mechanism O-axis is rotated so that RV is aligned with the empty position on the process carousel.
6. RV is loaded onto process carousel.

15                    SYSTEM DESCRIPTION OF FPIA PROCESS AREA FOR  
   PHENOBARBITAL

- A. Wait for temperature equilibration time and evaporation window to expire.
- 20 B. FIRST PIPETTE ACTIVITY (preparation of sample blank comprising diluted sample and popper).
1. Incubation timer is set according to assay file specifications.
  2. Precision diluent aspirate. The following  
25 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.
    - 30 (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  
35 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).
- 5 (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
- 10 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:
- 15 (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.
- 20 (iv) LLS is disabled.
- (v) Pipette Z-axis is moved up to Z-above position.
4. Diluent/sample dispensed to the RV predilute well.
- 25 (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.
- 30 (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
- 35 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.
- 5 (b) Wash valve is opened.
- (c) Wait "n" seconds.
- (d) Wash valve is closed.

7. Popper aspirate.

- 10 (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).
- 15 (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).
- 20 (e) The following occur simultaneously until the total volume of popper required is aspirated:
  - (i) Pipette Z-axis motor is moved down at a rate of "X" steps/sec.
  - 25 (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.
  - 30 (v) Pipette Z-axis is moved up to the Z-above position.
  - 35

8. 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:
- (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.
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.

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

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

10

C. BLANK READ PREPARATION

When incubation timer expired, the following activities are started:

- 15 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.

D. BLANK READ (BACKGROUND)

20

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

2. Process carousel is rotated so that the RV is at the read station.

25 3. Horizontal intensity is read for "X.XX" seconds.

4. The crystal is flipped for the vertical read.

5. Wait "n" seconds until the crystal settles.

6. Vertical intensity is read for "X.XX" seconds.

30 7. The raw reads are converted to normalized reads (light intensity hitting detector/lamp intensity) by the optics microprocessor.

8. Background reads are stored.

9. System calculates BLANK I to complete blank read.

35 10. Next activity started when incubation timer expires.

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

1. Incubation timer is set according to assay file specifications.
2. Precision diluent aspirate.
  - (a) The following activities are performed simultaneously:
    - (i) Syringe aspirates "X" uL at a rate of "X" 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 2 (antiserum) well.
  - (ii) LS is enabled to ensure no liquid currently detected.
  - (iii) 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).
  - (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 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.)
  - (v) The following occur simultaneously until the total volume of antiserum required is aspirated:
    - (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 the Z-above position.
4. Tracer aspirate.
- 10
- (a) Syringe aspirates "X" uL of air at a rate of "X" ul/sec.
  - (b) Pipette R-axis is moved over the RV Reagent 3 (tracer) well.
  - (c) LLS is enabled to ensure no liquid currently detected.
- 15
- (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).
- 20
- (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).
- 25
- (f) The following occur simultaneously until the total volume of tracer required is aspirated:
- 30
- (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.
- 35
- (v) LLS is disabled.



- (vi) Pipette Z-axis is moved up to the Z-above position.
5. Diluted sample aspirate.
- 5 (a) Pipette R-axis is moved over the RV predilute well.
- 10 (b) LLS is enabled to ensure no liquid currently detected.
- 10 (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).
- 15 (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.)
- 20 (e) The following occur simultaneously until the total volume of diluted 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.
- (3) LLS is checked to ensure probe still in liquid.
- 30 (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.
- 35 (a) Pipette R-axis is moved over to the RV cuvette position.

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

7. Probe post-wash.

10 The probe is again washed to ensure that it is free from contamination as described in section 6 (Sample kitting) to complete the second pipette activity.

8. Next activity started when incubation timer expires.

15 E FINAL READ PREPARATION

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.

20

F. FINAL READ

1. Process carousel is rotated so that the RV is at the read station.
- 25 2. Horizontal intensity is read for "X.XX" seconds.
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.
- 30 6. The raw reads are converted to normalized reads (light intensity hitting detector/lamp intensity) by the optics microprocessor.
7. Reads are stored.
8. System calculates NET intensity (I) and
- 35 milipolarization (mP).
9. mP value is fitted to calibration curve to yield a concentration result.

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 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.
4. RV is pushed into the waste container.

*DESCRIPTION OF KITTING AND PROCESS AREA ACTIVITIES FOR MEIA*

SYSTEM DESCRIPTION OF KITTING AREA FOR CEA ASSAY

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).
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.

B. PREPARATION STEPS

1. User loads empty RVs into RV carousel.
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, 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.

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.

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

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.
- (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.
- (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.
- (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.

(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.).

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

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

#### 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 scheduled separately.

(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.

(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.

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.

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

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

- 5 (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).
- 10 (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
- 15 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.
- (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.
- 30 (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.
- 35

- 5
- (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.
- 10
- (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 .
- 15
- (i) The inside of the probe is cleaned first.
- 20
- (1) Pipette R-axis is moved over waste area.
- (2) Pipette Z-axis is moved down to appropriate position within the waste area.
- 25
- (3) The wash valve is opened for the amount of time specified in the assay protocol.
- (4) Wash valve is closed.
- (ii) Pipette Z-axis is moved up to the Z-clear position.
- 30
- (iii) The outside of the probe is cleaned next.
- (1) Pipette R-axis is moved over wash cup.
- 35
- (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

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).

10

(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.

15

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

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

20

(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)

25

(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).

30

35

- (viii) The following occur simultaneously until the total volume of microparticles required is aspirated:
- 5 (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.
  - 10 (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.
  - 15 (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.
  - 20 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
- 25 section 6 (Sample kitting).
8. Conjugate kitting.
- (a) Conjugate aspirate (conjugate, special wash fluid, and/or specimen diluent are pipetted into either RV reagent wells or RV
- 30 predilution well, depending on volume requirements).
- (i) Syringe aspirates "X": uL of air at a rate of "X" ul/sec.
  - (ii) Pipette R-Axis is moved over the
  - 35 conjugate reagent bottle in the Reagent Pack.

- 5
- 10
- 15
- 20
- 25
- 30
- 35
- (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 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).
  - (viii) The following occur simultaneously until the total volume of conjugate 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.
    - (3) LLS is checked to ensure probe still in liquid.
  - (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.

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

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 0-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 0-axis is rotated so that RV is aligned with the empty position on the process carousel.
6. RV is loaded onto process carousel.

#### SYSTEM DESCRIPTION OF MEIA PROCESS AREA FOR CEA

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

B. FIRST PIPETTE ACTIVITY (microparticle/sample reaction)

1. Incubation timer is set according to assay file specifications.
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.
  - (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.
  - (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 MEIA 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.
- 5 (l) Pipette Z-axis is moved up to Z-above position.
3. Sample aspirate
- (a) Pipette R-axis is moved over the RV sample well.
- 10 (b) Pipette Z-axis is moved down to the Z-LLS position.
- (c) LLS is enabled to ensure no liquid currently detected.
- 15 (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).
- 20 (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).
- 25 (f) The following occur simultaneously until the total volume of sample required is aspirated:
- 30 (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.
- (g) LLS is checked to ensure probe still in liquid.
- (h) LLS is disabled.
- 35 (i) Pipette Z-axis is moved up to Z-above position.

4. MEIA buffer and sample are added to microparticles in incubation well.

- (a) Pipette Z-axis is moved down to the dispense position within the RV incubation well.
- 5 (b) Syringe dispenses "X" uL of MEIA buffer and sample at a rate of "X" ul/sec.
- (c) Pipette Z-axis is moved up to Z-clear position.

5. Probe post-wash.

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

C. CARTRIDGE LOAD (This activity occurs when resources are not in use)

15

- 1. Move the auxiliary carousel so that reserved position is under feeder.
- 2. Cycle trap-door mechanism to load flashlight into carousel.

20 3. Cycle shuttle mechanism to place another MEIA cartridge on trap door (for next tab load).

- 4. Check incubation timer. When expires start next pipetting.

25

D. SECOND PIPETTE ACTIVITY (transfer of reaction mixture to matrix)

30 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.
- 35 (c) Pipette R-axis is moved over the RV buffer well.

- 5 (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.
- 10 (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).
- 15 (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).
- 20 (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.
- 25 (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.
- 30 3. Reaction mixture aspirate.
- (a) Pipette R-axis is moved over the RV incubation well.
- (b) Pipette Z-axis is moved down to the Z-LLS position.
- 35 (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).
- 5 (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
- 10 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 (f) The following occur simultaneously until the total volume of reaction mixture 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.
- 20 (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.
- 25 4. Reaction mixture dispense onto matrix.
- (a) The following are performed simultaneously and concurrently with the reaction mixture aspirate (above):
- (i) The auxiliary carousel is moved so that the cartridge is at the pipetting
- 30 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.
- 35 (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.

- 5 (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.

- 10 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.

15 E THIRD PIPETTE ACTIVITY (conjugate addition)

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

2. Conjugate aspirate.

- 20 (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.
- 25 (d) Pipette Z-axis is moved down to the Z-above position.
- (e) LLS is enabled to ensure no liquid currently detected.
- 30 (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).
- 35 (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

- 5 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).
- (h) The following occur simultaneously until the total volume of conjugate required is aspirated:
- 10 (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.
- 15 (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.
- 20 (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.
- 25 (e) Pipette Z-axis is moved up to the Z-clear position.
- (f) Wait "X" seconds until reaction mixture has been absorbed by matrix.
- 30 4. Probe post-wash.
- 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)
- 35

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.
- 5           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.
- 10           5.   Check incubation timer. When expires start next activity.

#### G   MEIA READ PREPARATION

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

#### 20   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
- 25   buffer specified in the assay file for cartridge wash has been dispensed.
- (a)   "X" uL of heated MEIA buffer are dispensed in 50uL cycles at a rate of "X" ul/sec onto the matrix.
- 30           (b)   Wait "n" seconds.

#### I.   MUP DISPENSE

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

3. Wait "n" seconds.

#### J. MEIA READ

- 5           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 (usually 8)
- 10           (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.
- (b) PMT gain is set.
- 15           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 normalized reads vs time.
- 20           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 is positive or negative (or reactive or nonreactive).

25

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

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

          Schematic reaction sequences are presented in FIGURES-

35          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 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 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 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.

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 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 fluorescent. The rate of MU production is measured.

In accordance with the embodiments of the present invention, 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  
5 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  
10 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. It will also be appreciated that although particular embodiments of  
15 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.

20

## CLAIMS:

1. A method of operating an automated, continuous  
5 and random access analytical system capable of  
simultaneously effecting multiple assays of a plurality of  
liquid samples, comprising:
  - a. scheduling various assays of a  
plurality of liquid samples;
  - 10 b. creating one or more unit dose  
disposables by separately transferring a first of said liquid  
samples and reagents to a reaction vessel without initiation  
of an assay reaction sequence;
  - c. transferring one or more of said unit dose  
15 disposables to a processing workstation;
  - d. mixing an aliquot of said first liquid sample  
with one or more of said reagents at different times in said  
reaction vessel to form a first reaction mixture;
  - e. mixing aliquots of the same or different one or  
20 more samples with one or more of said reagents at different  
times in different reaction vessels to form multiple,  
independently scheduled reaction mixtures;
  - f. incubating said multiple reaction mixtures  
simultaneously and independently;
  - 25 g. Performing more than one scheduled assay on  
said reaction mixtures in any order in which more than  
scheduled assays are presented; and
  - h. analyzing said incubated reaction mixtures  
independently and individually by at least two assay  
30 procedures.



2. A method according to claim 1 wherein at least two different assays are scheduled to be performed on the system for a plurality of liquid samples, said method provides scheduling of said assays in advance of performance thereof,  
5 each assay test definition containing several timing parameters with each activity of the assay test containing time values which scheduling uses to determine which resources of the system and activity required by each of said assays and the time period that said resources need.

10

3. A method according to claim 1 wherein the scheduling process includes scheduling of each activity to be performed in an assay before the assay is kitted, and scheduling performance of each assay activity prior to its originally  
15 scheduled execution time, thus minimizing resource idle time.

4. A method according to claim 3 wherein assay throughput is increased in the system.

20

5. A method according to claim 3 wherein operating the automated continuous and random access analytical system includes kitting a unit dose disposable by separately transferring assay samples and reagents to a reaction vessel without initiation of an assay reaction  
25 sequence.

6. A method according to claim 2 wherein the system is capable of allowing special priority handling through stat procedure scheduling of specific samples, and wherein said  
30 stat procedure scheduling interrupts prior scheduling, allowing the system to finish preparing assays on a current sample and then prepare to perform an assay on the sample through a modification of the scheduling.

35

7. A method according to claim 2 wherein scheduling for performing assays maximizes the number of assays the system is capable of processing per unit time by allowing

sufficient time gaps between assay protocol steps to enable other assay protocol steps to be performed within such time gaps.

5           8. A method according to claim 6 wherein calibration procedures scheduling is scheduled as a stat procedure.

          9. A method according to claim 1 wherein the assay performed=ed on said reaction mixture in said reaction vessel  
10 is a homogeneous assay.

          10. A method according to claim 1 wherein the assay performed on said reaction mixture in said reaction vessel is a heterogeneous assay.  
15

          11. A method according to claim 1 wherein at least two assays are immunoassays.

          12. A method according to claim 11 wherein said  
20 immunoassays are comprised of MEIA and FPIA assays.

          13. A method according to claim 1 wherein said analyzing step includes optically monitoring said reaction mixtures.  
25

          14. A method according to claim 1 wherein said reaction mixtures are monitored by turbidimetric, colorimetric, fluorometric or luminescent means.

30           15. A method according to claim 1 wherein partial initiation of an assay reaction sequence is achieved simultaneously with the creating of the unit dose disposable.

          16. A method according to claim 1 wherein the system  
35 achieves simultaneous creation of unit dose disposables, transferring of a unit dose disposable reaction vessel and mixing of a reaction mixture while incubating multiple

reaction mixtures and performing at least one scheduled assay and analysis simultaneously.

17. A method of operating an automated, continuous and random access analytical system capable of simultaneously effecting multiple assays of a plurality liquid samples, comprising:
- a. introducing sample cups, reagent packs, and reaction vessels for performing said assays onto concentric carousels of a front end carousel, the reaction vessels being introduced to an outer carousel;
  - b. identifying the reagent packs and sample cups;
  - c. scheduling the assays;
  - d. aligning the sample cups and reagent packs with a reaction vessel at a kitting station by rotating the respective carousels;
  - e. kitting a unit dose disposable in a reaction vessel having multiple independent open chambers in accordance with the scheduled assay by transfer of the sample from the sample cup to a reaction vessel chamber and transfer of specific reagents to separate reaction vessel chambers from the reagent pack;
  - f. transferring the kitted reaction vessel to a process carousel which is maintained under controlled environment conditions;
  - g. pipetting the sample and various reagents into a reaction well of the reaction vessel, the amounts of reagent, sequencing of transfer and time spacing there between being predetermined by assay scheduling;
  - h. incubating the pipetted sample and reagent mix;
  - i. identifying and transferring the incubated mixture in the reaction well to one of at least two assay analytical stations;

- j. performing an analysis by reading the prepared reaction mixture and calibrating the reading; and
- k. recording the resulting assay reading analysis.

5

18. A method according to claim 17 wherein the front-end carousel and the concentric carousels of the front end carousel as well as the process carousel are rotatably disposed for bidirectional rotational motion about a vertical axis.

10

19. A method according to claim 18 wherein the front end carousel being capable of bidirectional motion provides a bidirectional shaking motion for stirring or agitating reagent pack reagents after a period of inactivity of the front end carousel.

15

20. A method according to claim 17 wherein both kitting and partial initiation of an assay reaction sequence is achieved simultaneously creating a unit dose disposable within the reaction vessel.

20

21. A method according to claim 17 wherein said assay being performed on said reaction mixture in said reaction vessel is a heterogeneous assay.

25

22. A method according to claim 17 wherein said assay being performed on said reaction mixture in said reaction vessel is a homogenous assay.

30

23. A method according to claim 17 wherein at least two assays are immunoassays.

24. A method according to claim 23 wherein said immunoassays are comprised of a fluorescent polarization immunoassay and a microparticle immunoassay.

35

25. A method according to claim 24 wherein settling of the microparticles is substantially eliminated by providing a sufficient sucrose concentration to microparticle diluent ratio to achieve neutral density.

5

26. A method according to claim 24 wherein the kitted sample and reagents are pipetted directly from the reaction vessel on the process carousel to a microparticle immunoassay matrix for optically monitoring said reaction mixtures.

10

27. A method according to claim 17 wherein the reagent packs are provided with closure elements for avoiding reagent evaporation.

15

28. A method according to claim 27 wherein covering is provided to the reagent packs when not in use to avoid evaporation of the reagents.

20

29. A method according to claim 17 wherein pipetting functions on the front end carousel and pipetting functions on the process carousel are achieved by aspirating-dispensing, driven by an airless syringe pump.

25

30. A method according to claim 24 wherein a FPIA reading sequence includes lamp simmer and full burn modes.

31. A method of operating an automated, continuous and random access analytical system capable of

30 simultaneously effecting multiple assays to determine the presence or amount of a plurality of analyte of interest in a plurality of liquid samples, comprising:

a. scheduling various assays of a plurality of liquid samples;

35

b. creating one or more unit dose disposables by separately transferring a first of said liquid samples and

reagents to a reaction vessel without initiation of an assay reaction sequence;

c. transferring one or more of said unit dose disposables to a processing workstation;

5 d. mixing an aliquot of said first sample with one or more of said reagents at different times in said reaction vessel to form a first reaction mixture;

e. mixing aliquots of the same or different ones of said samples with one or more of said reagents at different  
10 times in different reaction vessels to form multiple, independently scheduled reaction mixtures;

f. incubating said multiple reaction mixtures simultaneously and independently;

g. performing more than one scheduled assay on  
15 said reaction mixtures in any order in which said scheduled assays are presented; and

h. analyzing said incubated reaction mixtures independently and individually by at least two assay procedures to determine the presence or amount of one or  
20 more analyte of interests in said samples.

32. An automated, continuous and random access analytical system apparatus capable of simultaneously  
25 effecting multiple assays of a plurality of liquid samples, comprising:

a. a front end carousel assembly inclusive of a sample cup carousel, a reagent pack carousel and a reaction vessel carousel mounted concentrically and serviced by a  
30 transfer pipetting means suitable for kitting a reaction vessel;

b. a transfer station providing means for transferring a kitted reaction vessel to a process carousel, the process carousel maintained within a controlled  
35 environment;

c. a process carousel transfer pipetting means suitable for mixing reagents with the sample in a reaction well of the reaction vessel;

5 d. means for transferring the resulting reaction mixture to one of at least two assay reader means;

e. means for transferring a reaction vessels from the assay reader to a transfer station; and

10 f. means associated with said transfer station for removing the disposable reaction vessel from the system.

33. An apparatus according to claim 32 wherein one  
15 assay reader means is comprised of a cartridge wheel carousel containing multiple disposable cartridges, the apparatus providing means for supplying said cartridges to the cartridge wheel carousel and disposing of the cartridges from the cartridge wheel carousel.

20

34. The apparatus according to claim 32 wherein the assay reader means include means for optically monitoring said assay reaction.

25 35. The apparatus according to claim 32 wherein the assay reader means provides calibration and reader means as well as recording means for the resulting assay data.

30 36. The apparatus according to claim 32 wherein the transfer station transfer means is comprised of a carousel rotatable about an axis, an arm with a pick for mating with a reaction vessel transfer projection means and means for pulling the reaction vessel from the front end carousel, rotating and placing the reaction vessel onto the process  
35 carousel through rotation and rack and pinion movement of the pick arm.

37. The apparatus according to claim 32 wherein the sample handling means and reagent handling means include means for identifying said liquid samples and liquid reagents from coded information associated with the sample cups and  
5 the reagent packs.

38. The apparatus according to claim 32 which further includes means to store the output readings of the assay reader.  
10

39. The apparatus according to claim 32 which further includes means to calculate the concentration of an analyte from the output readings of said assay reader.

15 40. The apparatus according to claim 32 wherein the reaction vessel contains a reaction cuvette having a physical characteristic of low birefringence through an optical read region.

20 41. An automated, continuous and random access analytical system capable of simultaneously effecting multiple assays of a plurality of liquid samples, comprising:  
a. a front end carousel assembly inclusive of sample cup carousel, reagent pack carousel and reaction  
25 vessel carousel, the reaction vessel carousel being concentrically mounted exterior of the reagent pack carousel and the reagent pack carousel being concentrically mounted exterior of the sample cup carousel;

30 b. means for rotating the respective carousels to align with a kitting pipettor means for kitting reaction vessels;

c. means for transferring kitted reaction vessels from the reaction vessel carousel to a transfer station, the transfer station providing means for transferring  
35 the reaction vessel to a process carousel, the process carousel having environmental means for maintaining temperature control and timing of reaction incubation;



d. a transfer pipettor means for servicing the process carousel and a cartridge wheel carousel offset from the process carousel, the cartridge wheel carousel having means for receiving pipetted reaction mixtures from the process carousel and; means for supplying cartridges to the process carousel;

e. the process carousel having integrated therewith a microparticle enzyme immunoassay reader and processing station;

f. a fluorescent polarization immunoassay reader and processing station integrated with the process carousel;

g. means for removing the reaction vessel from the process carousel by operation of the transfer station and means for removing the cartridges from the cartridge wheel carousel; and.

h. means for analyzing the reaction mixture either by fluorescent polarization immunoassay or microparticle immunoassay.

42. The system according to claim 41 wherein assay-reader means include means for optically monitoring said assay reaction.

43. The system according to claim 41 wherein the assay reader means provides calibration and reader means as well as recording means for the resulting assay data.

44. The system according to claim 41 wherein the transfer station transfer means is comprised of a carousel rotatable about an axis, an arm with a pick for mating with a reaction vessel transfer projection means and means for pulling the reaction vessel from the front end carousel, rotating and placing the reaction vessel onto the process carousel through rotation and rack and pinion movement of the pick arm.

45. The system according to claim 41 wherein the sample handling means and reagent handling means include means for identifying said liquid samples and liquid reagents from coded information associated with the sample cups and the reagent packs.

46. The system according to claim 41 which includes means to store the output readings of the assay reader.

47. The system according to claim 41 which includes a means to calculate the concentration of an analyte from the output readings of said assay reader.

48. The system according to claim 41 wherein the sample cup is free standing on its base when separated from the sample cup carousel.

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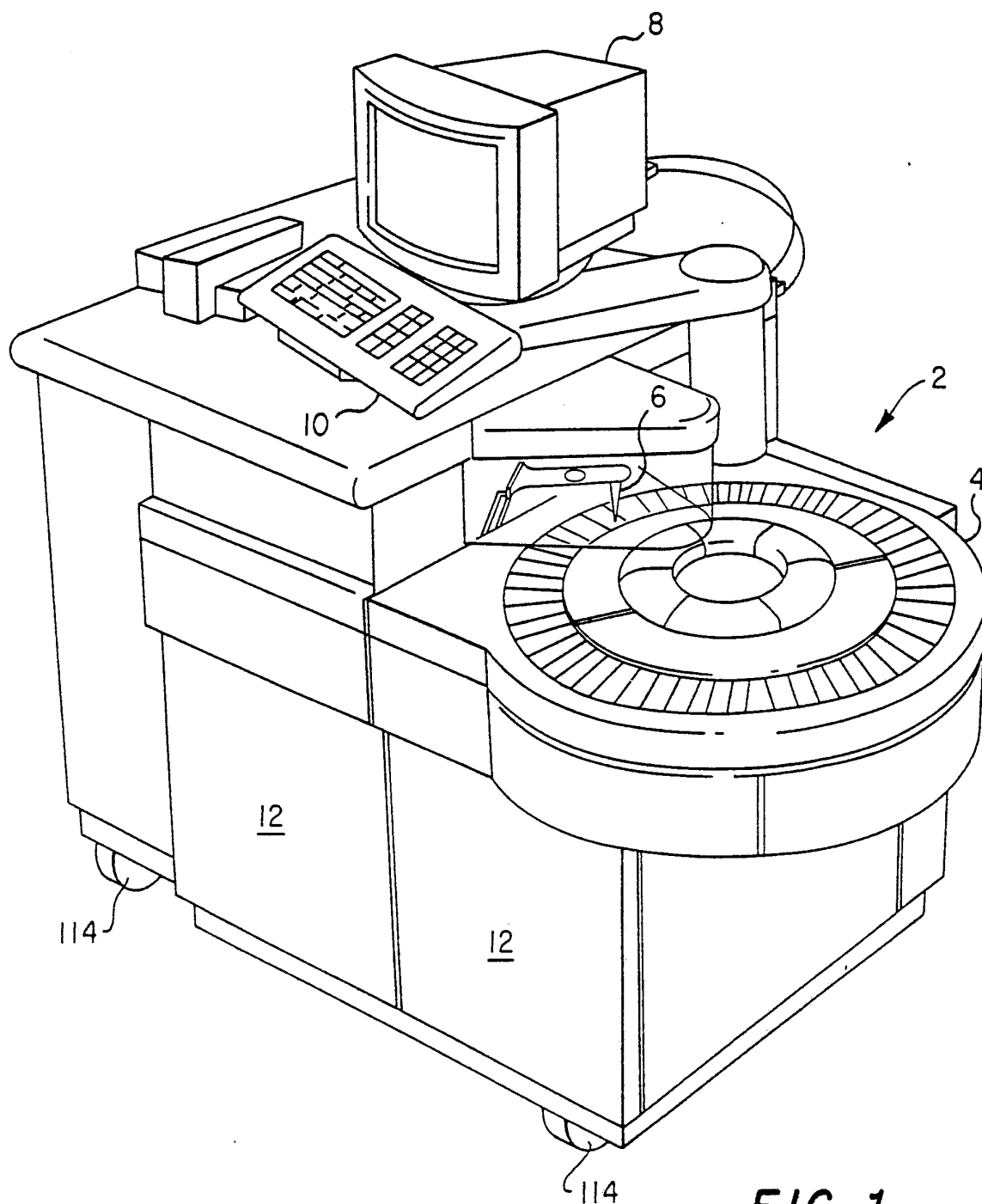
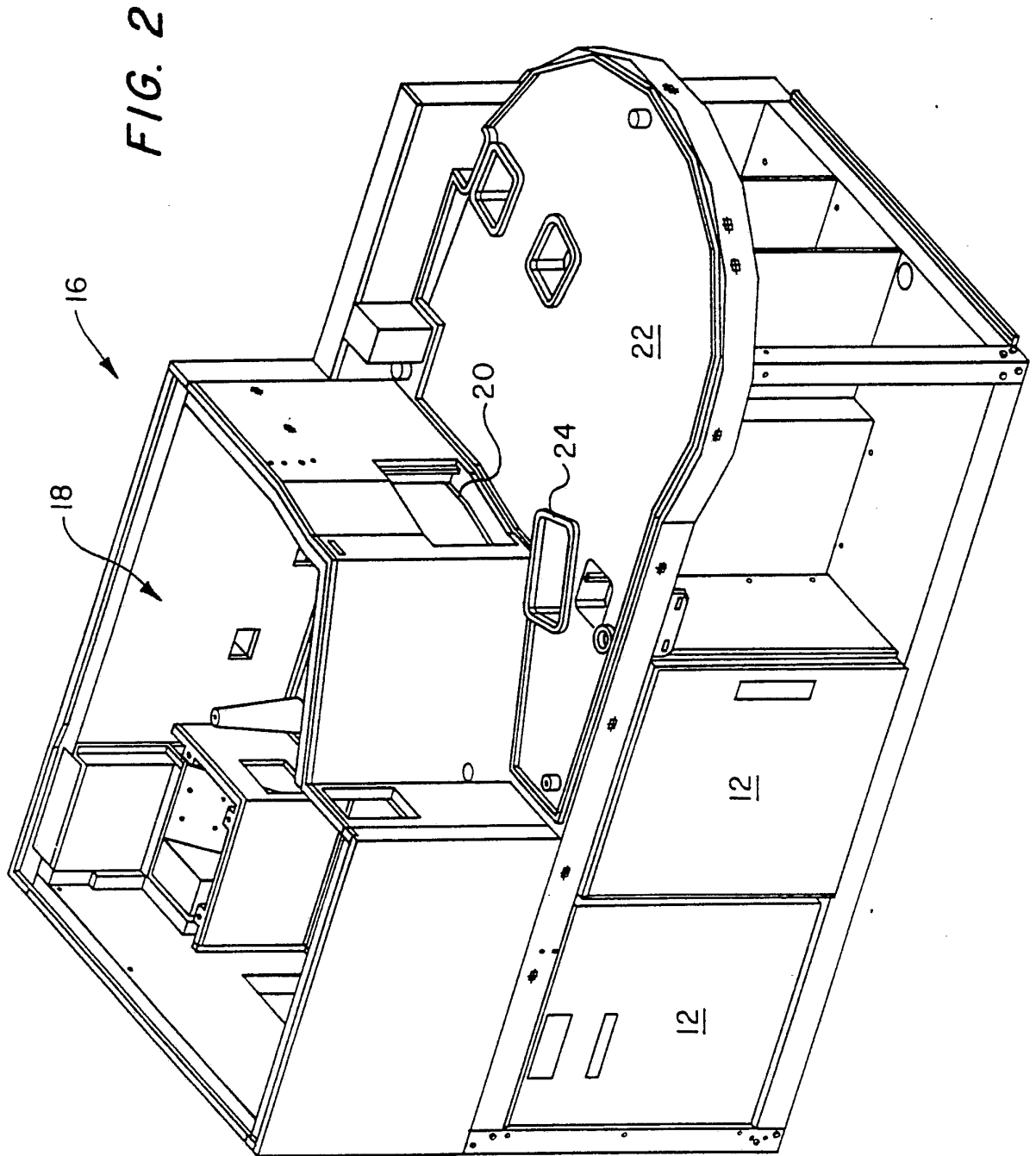


FIG. 1

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**SUBSTITUTE SHEET**

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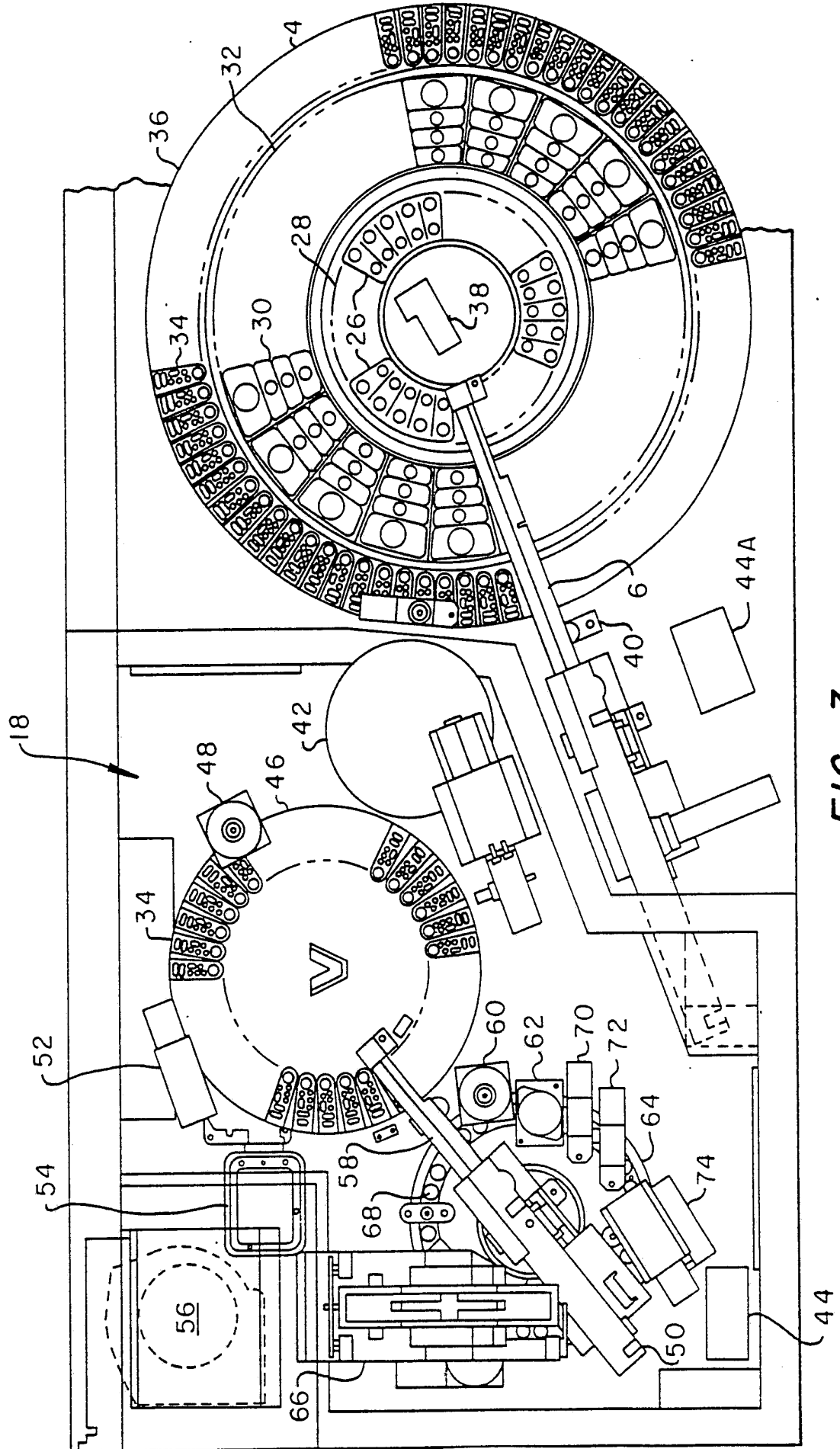


FIG. 3

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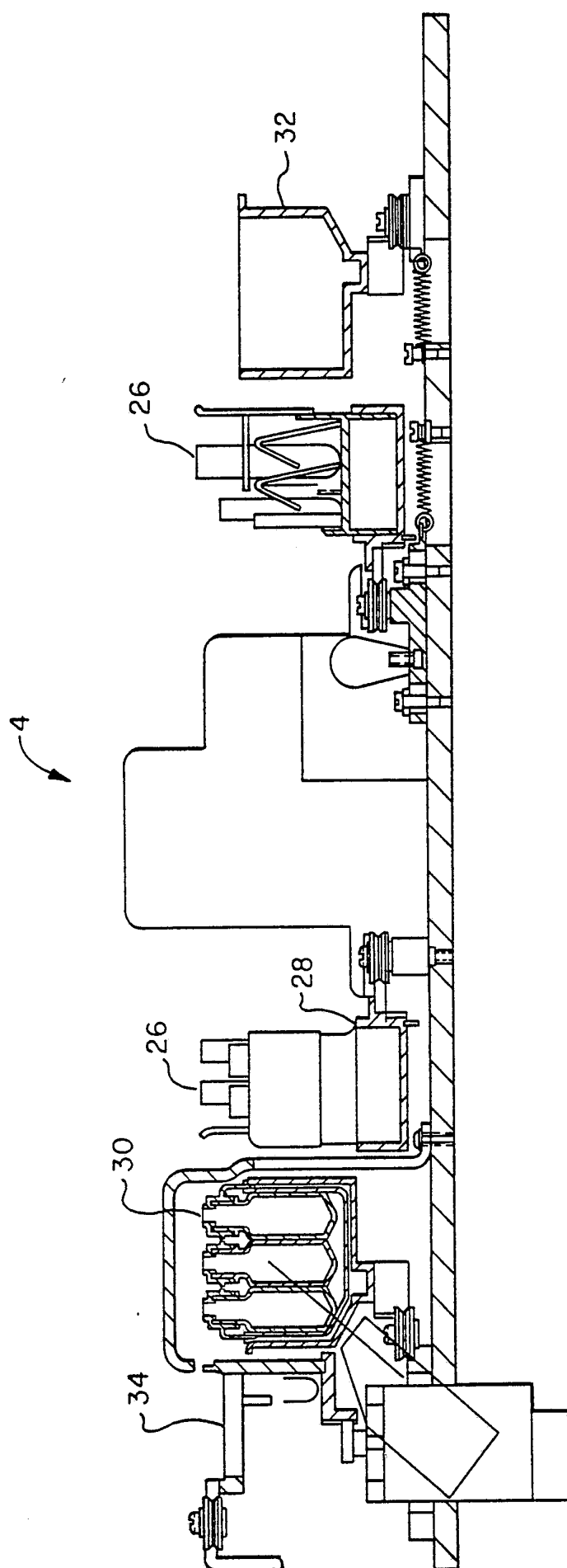
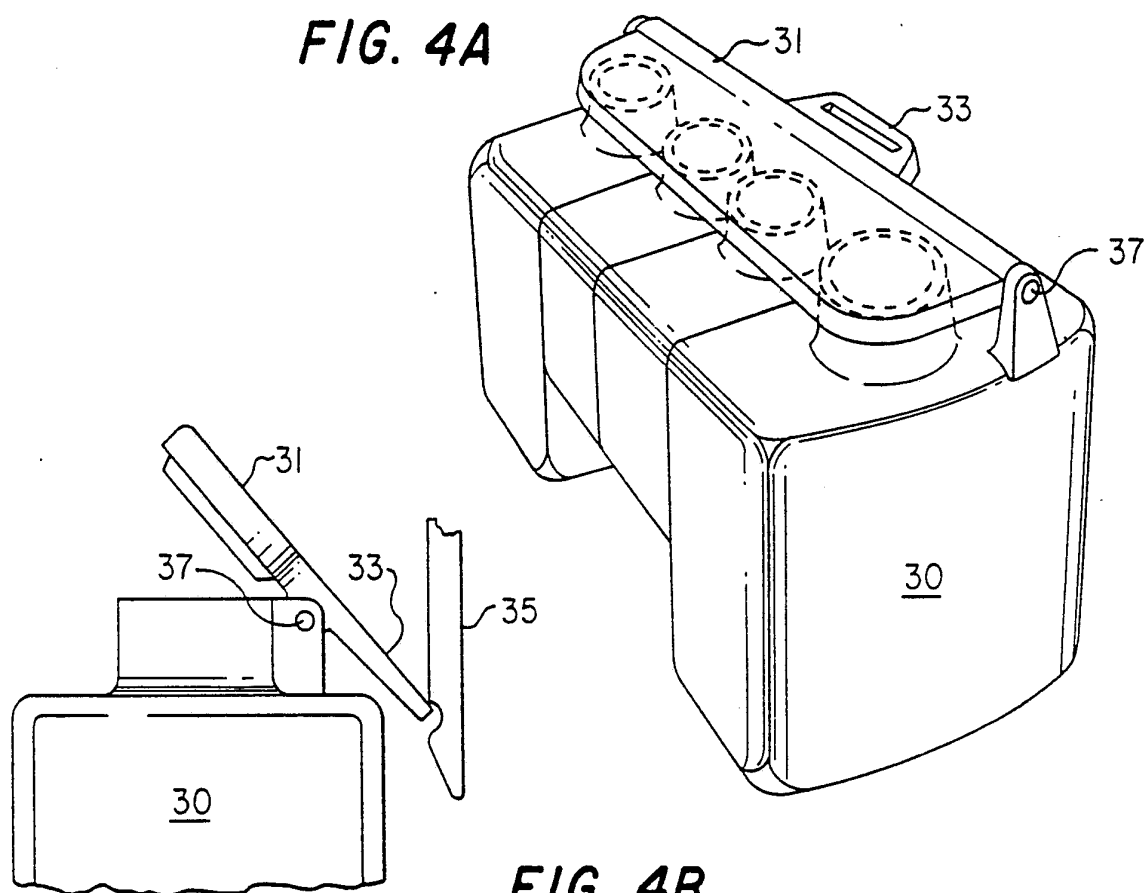
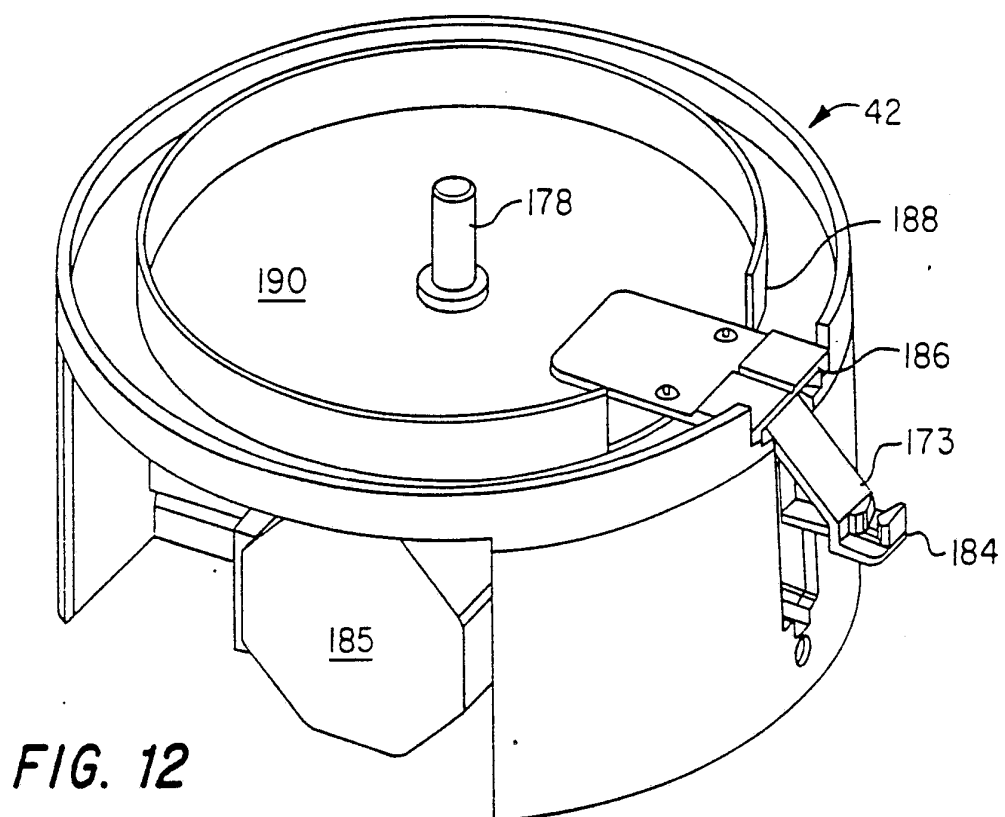


FIG. 4

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**FIG. 4A****FIG. 4B****FIG. 12****SUBSTITUTE SHEET**

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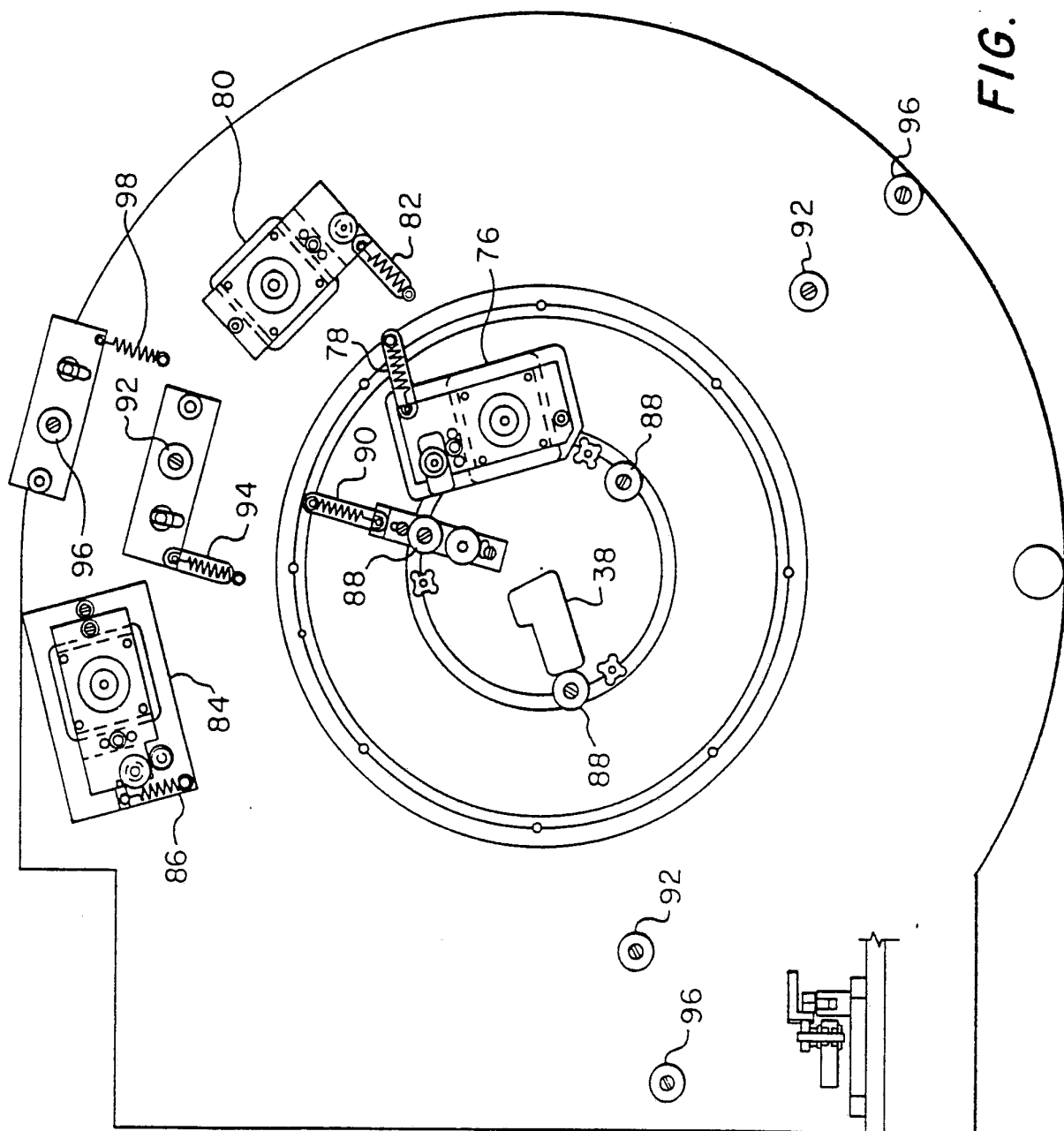


FIG. 5



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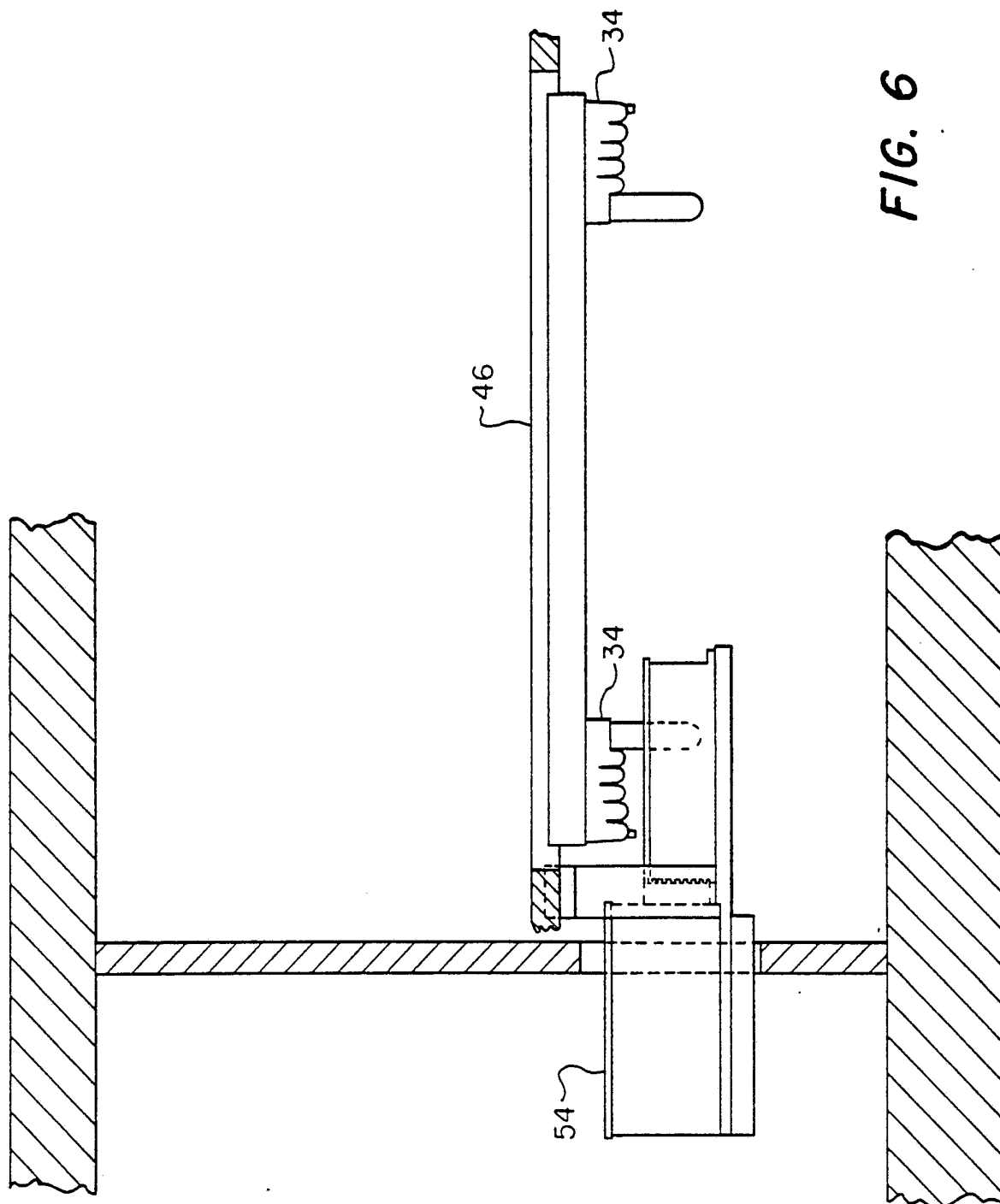
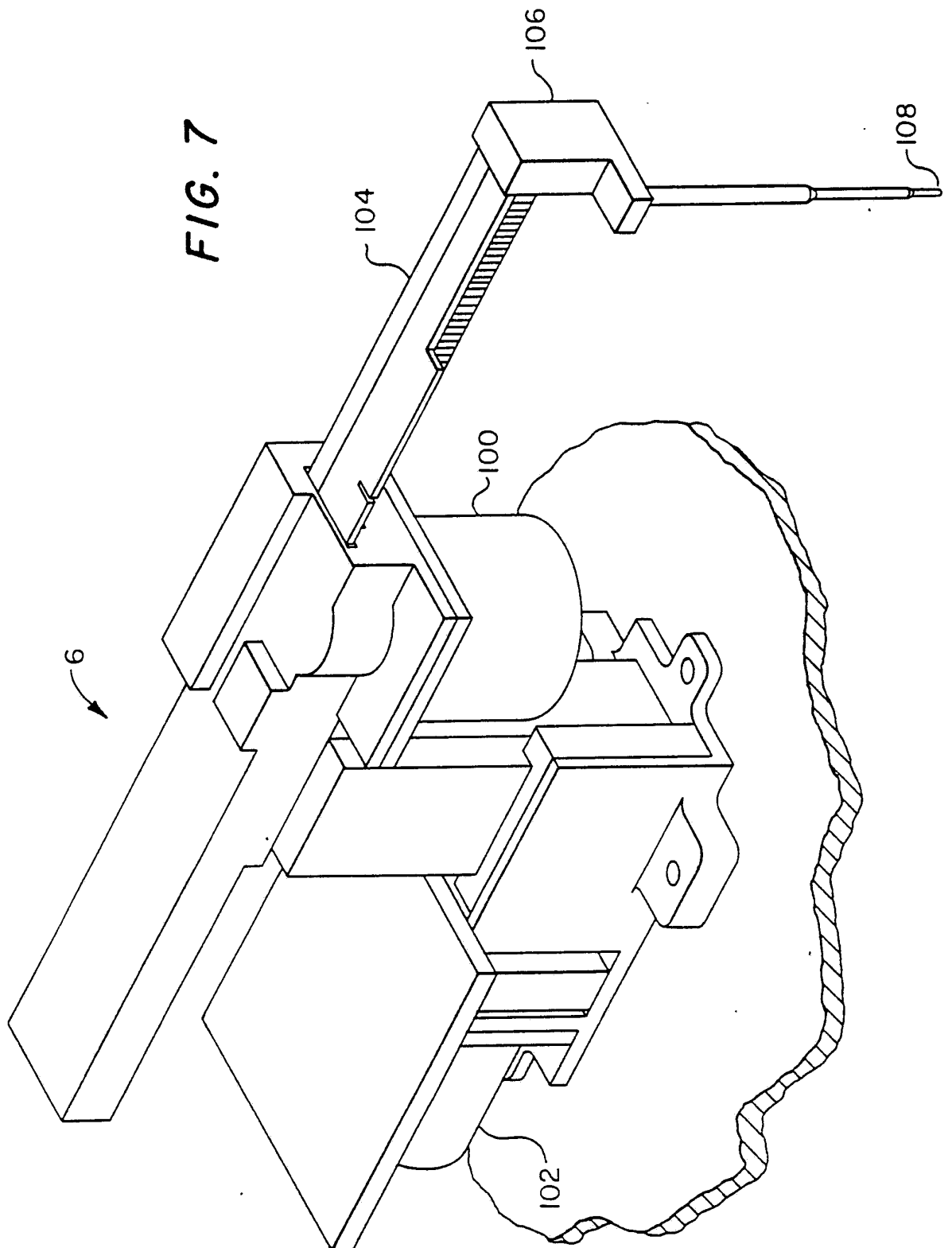


FIG. 6

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**SUBSTITUTE SHEET**

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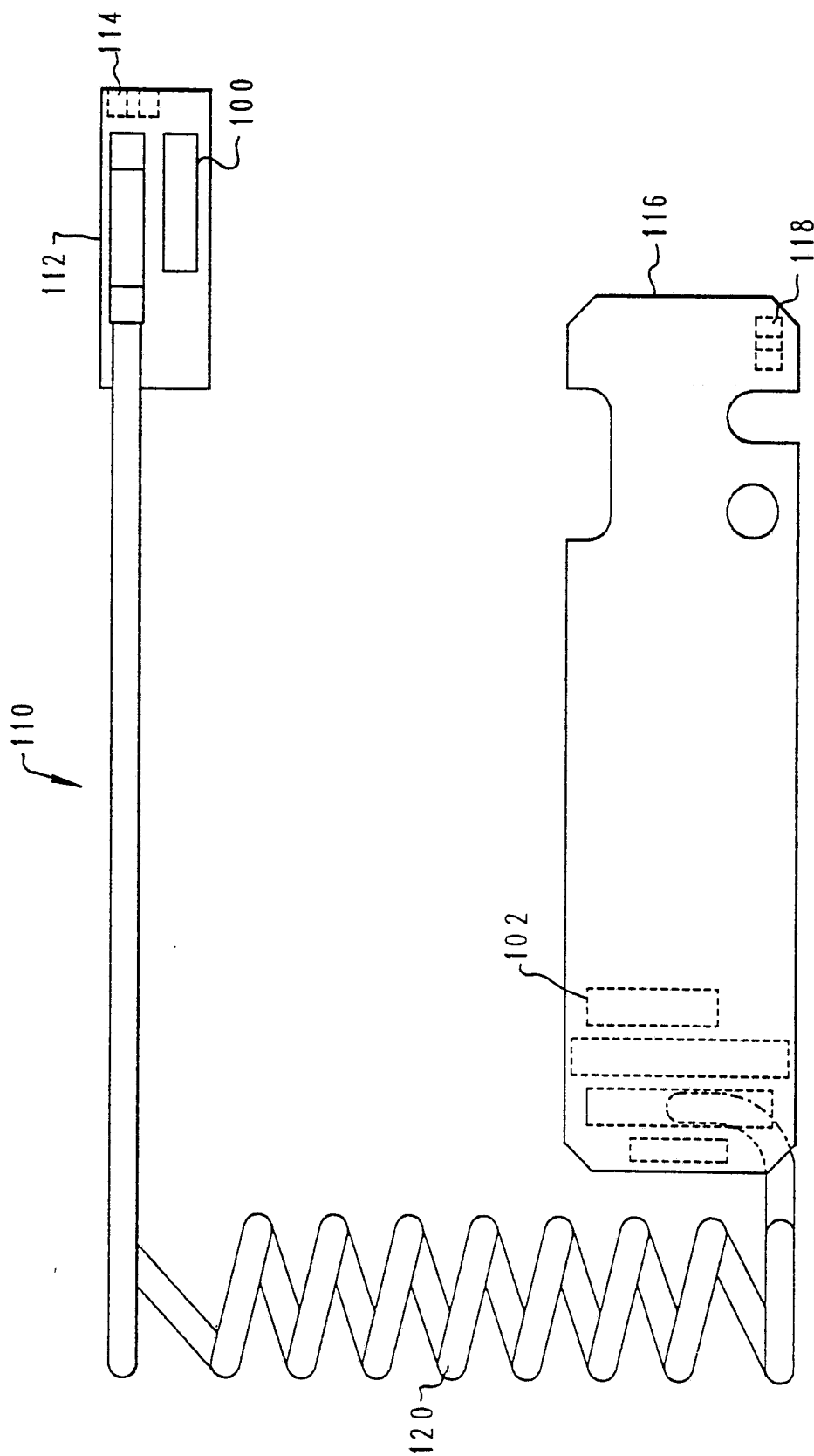


Fig. 8

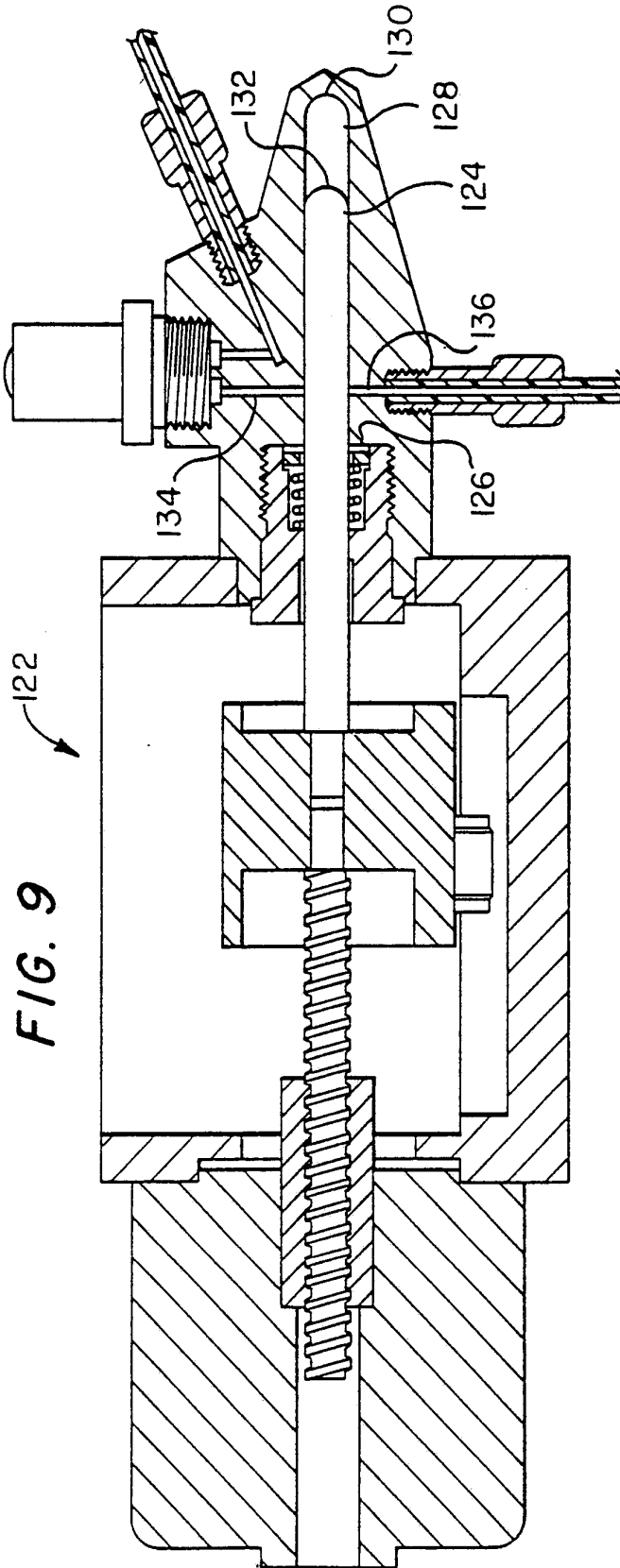


FIG. 9

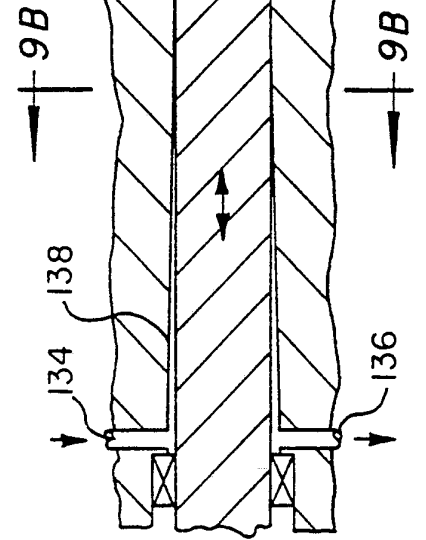


FIG. 9A

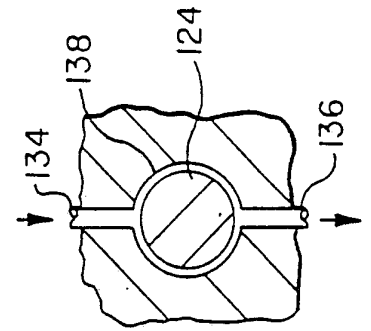
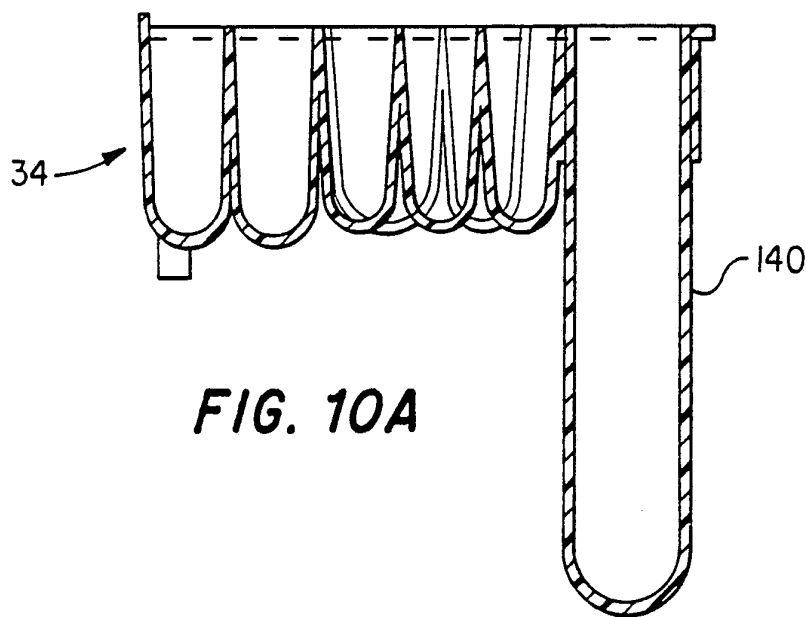
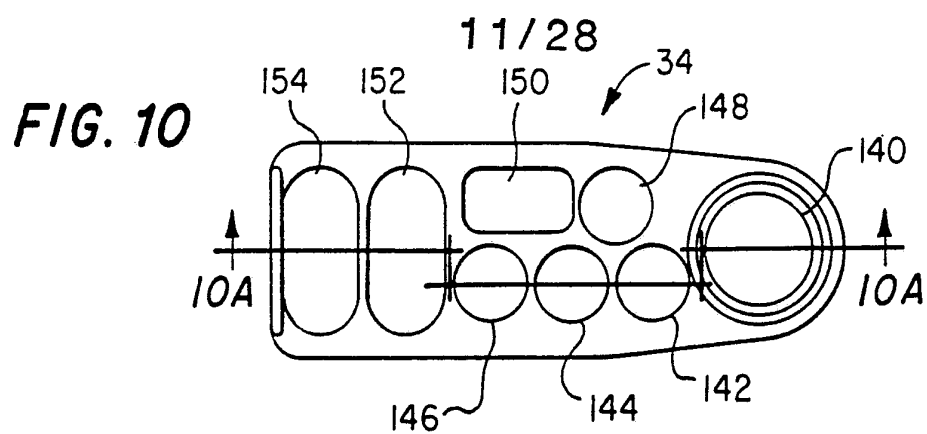
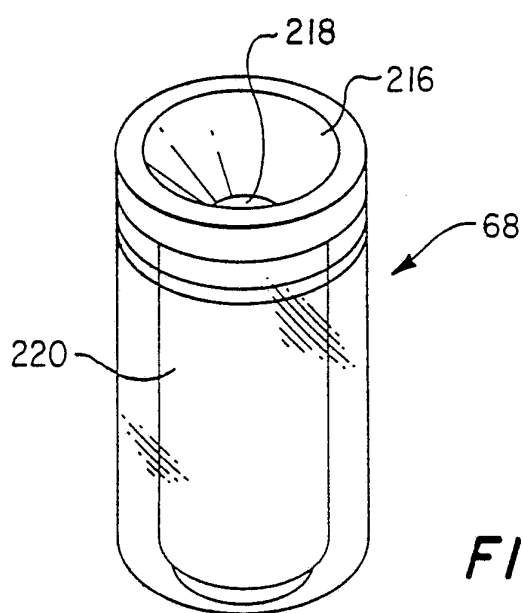


FIG. 9B

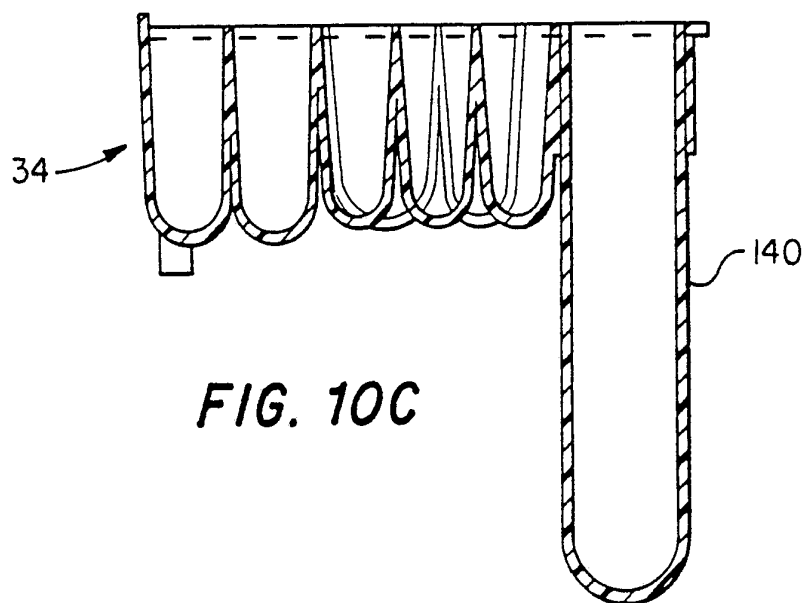
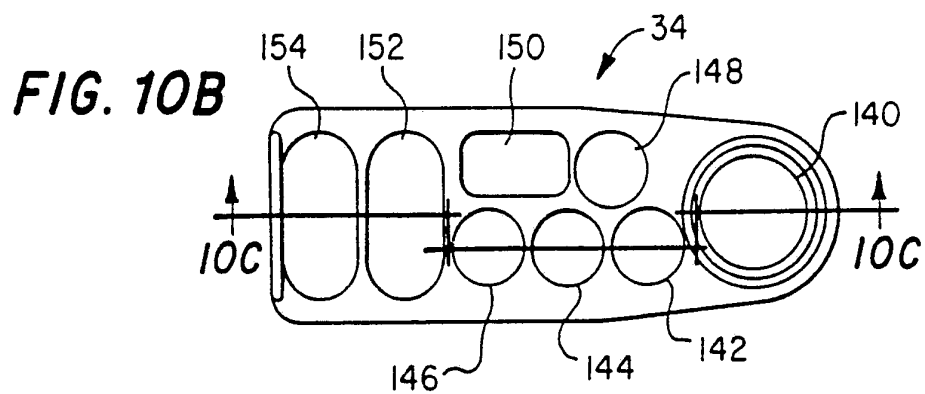


**FIG. 10A**



**FIG. 16**

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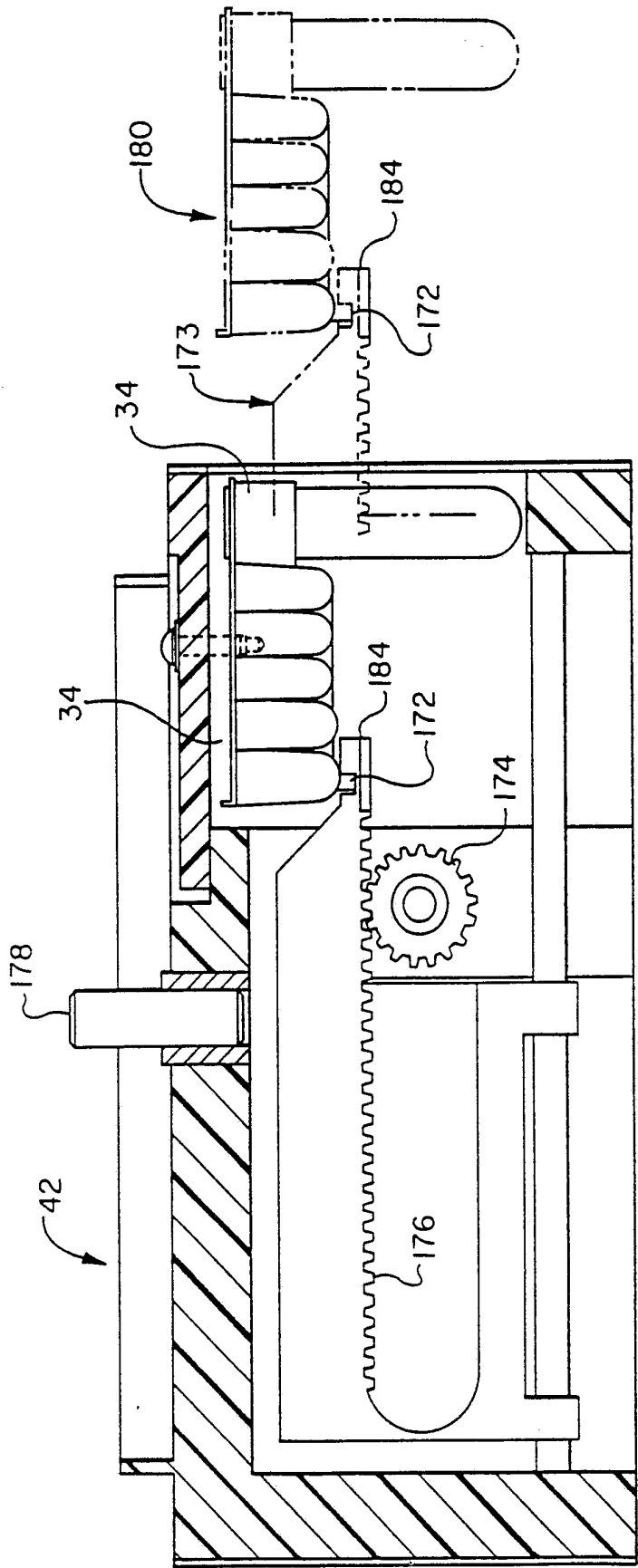
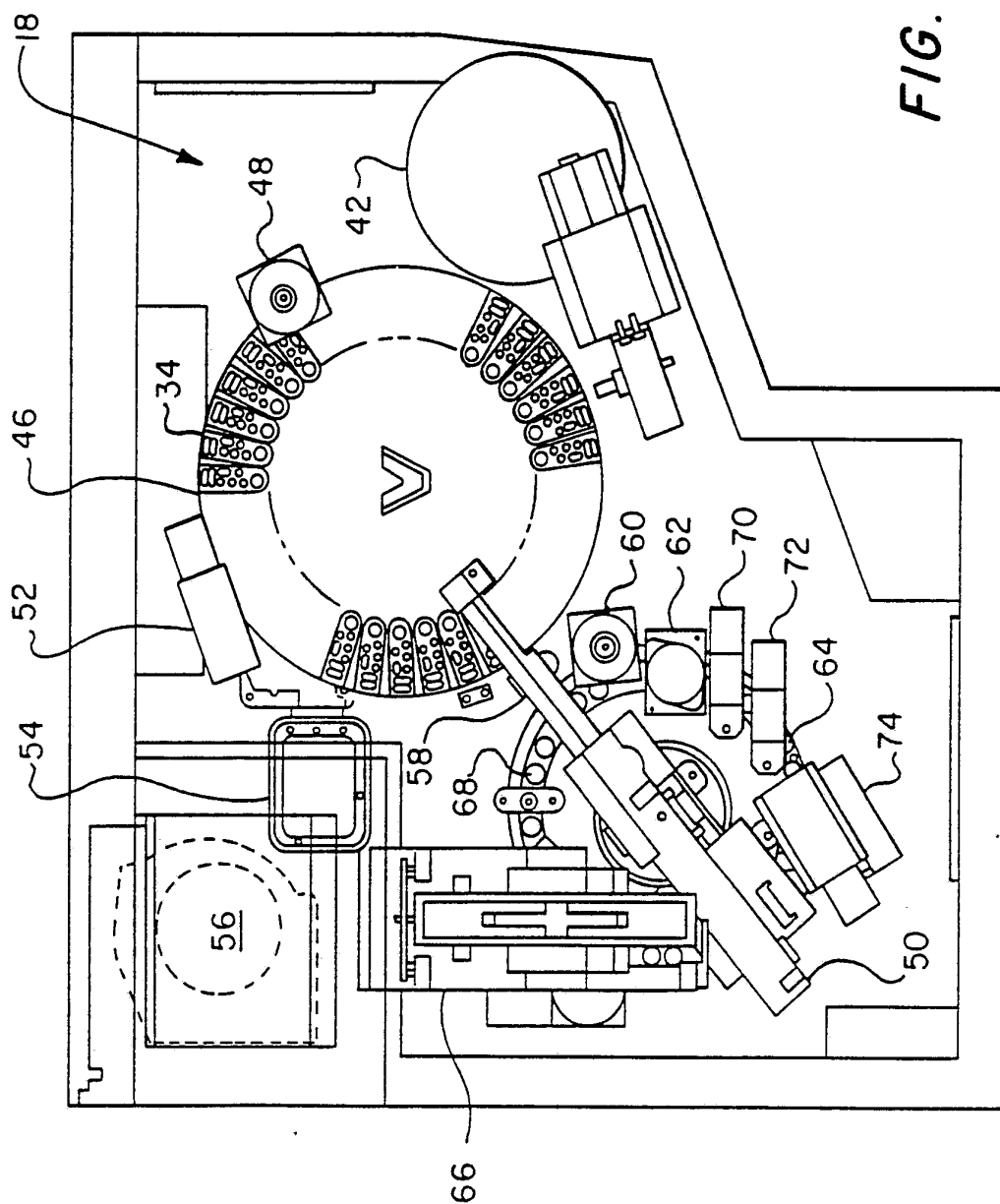


FIG. 11

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FIG. 13





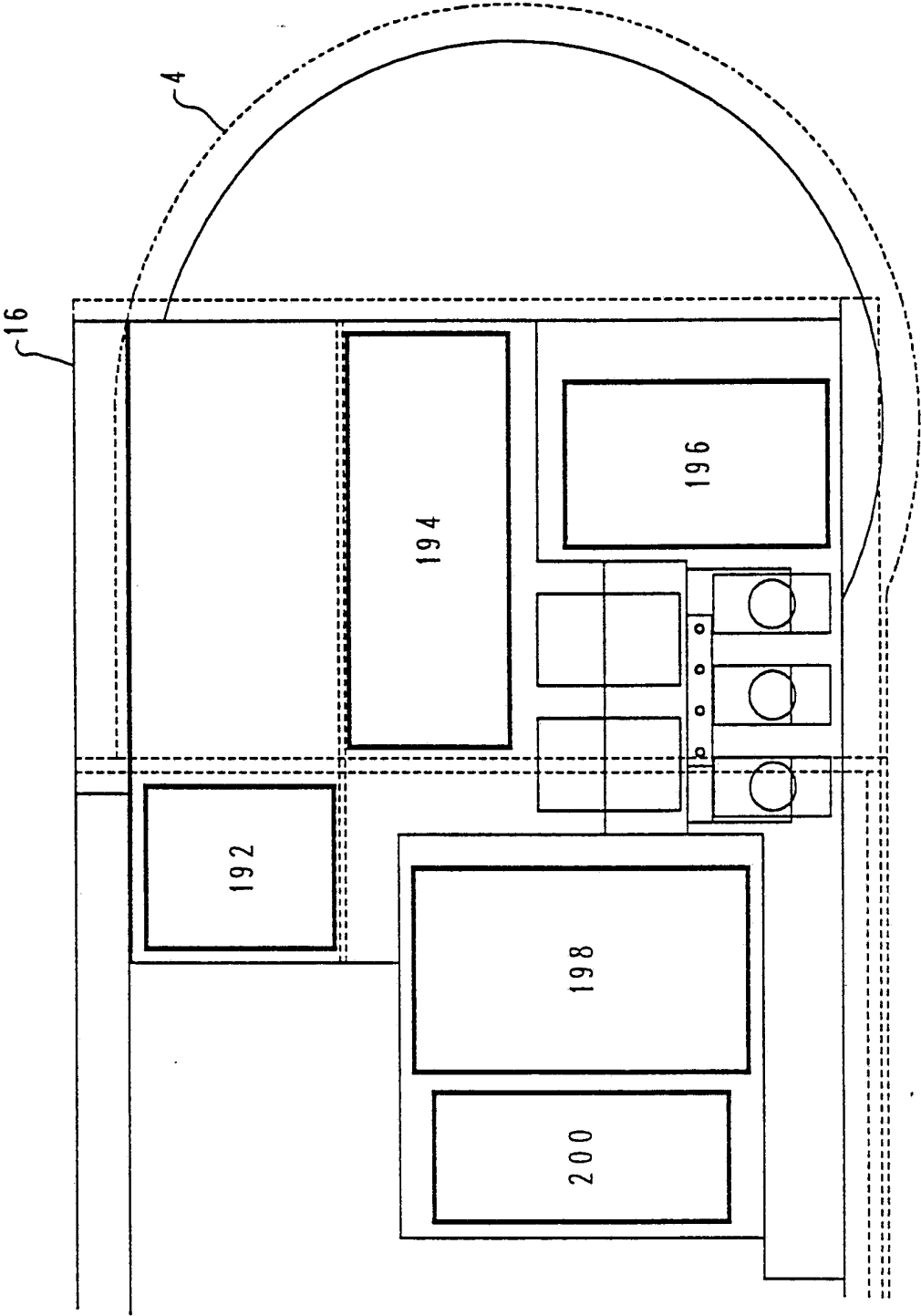


Fig. 14

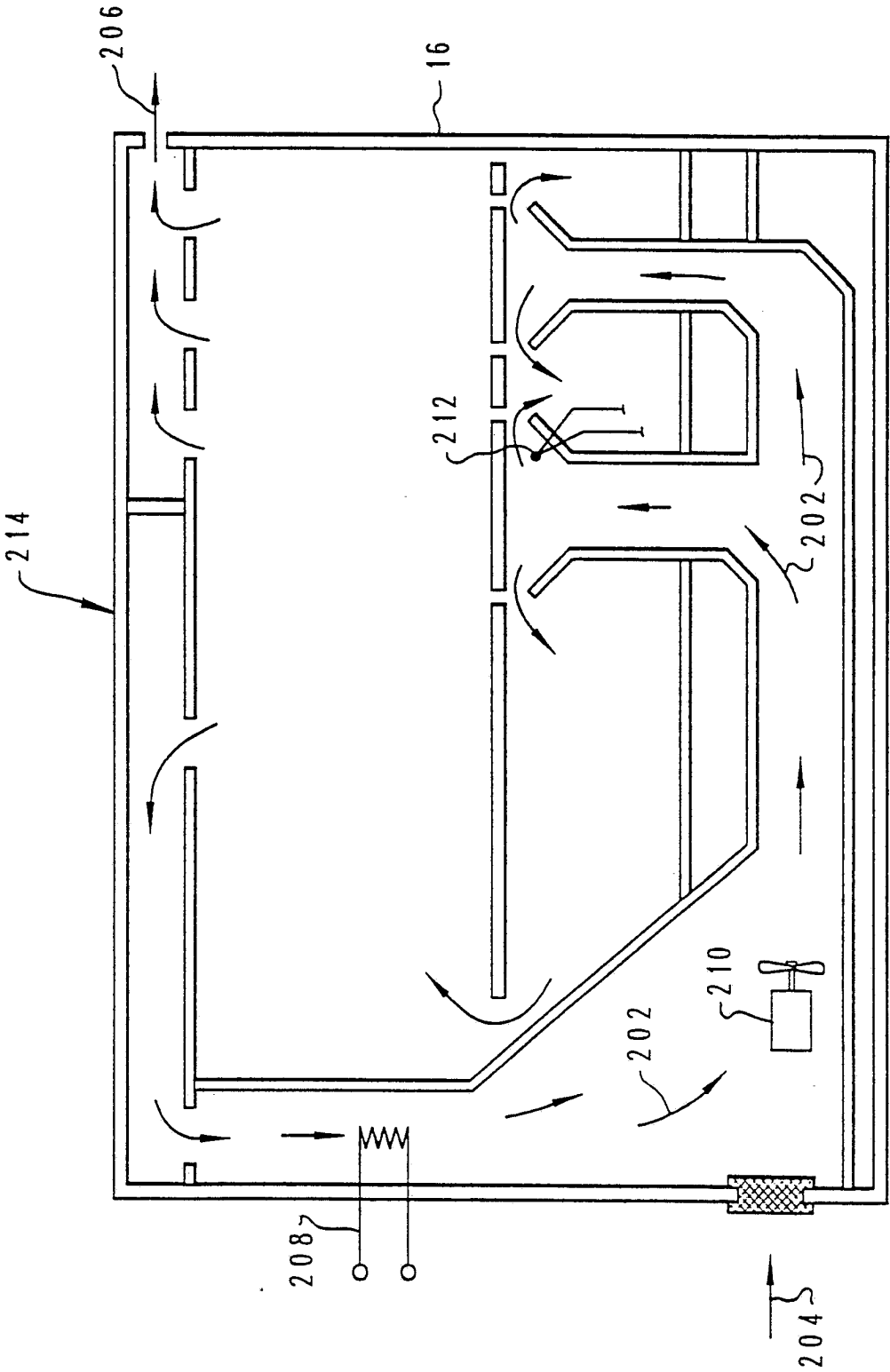
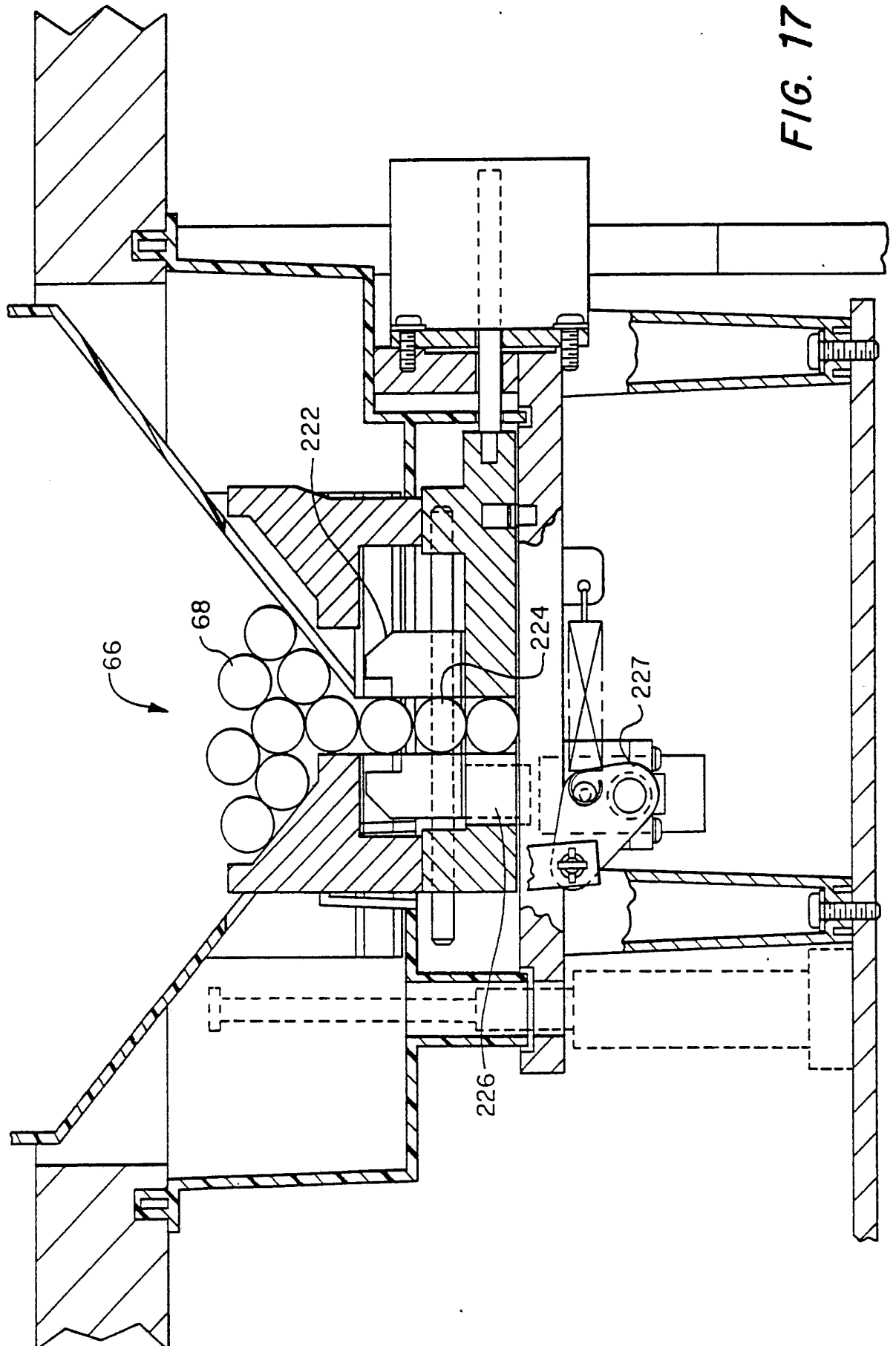


Fig. 15

FIG. 17



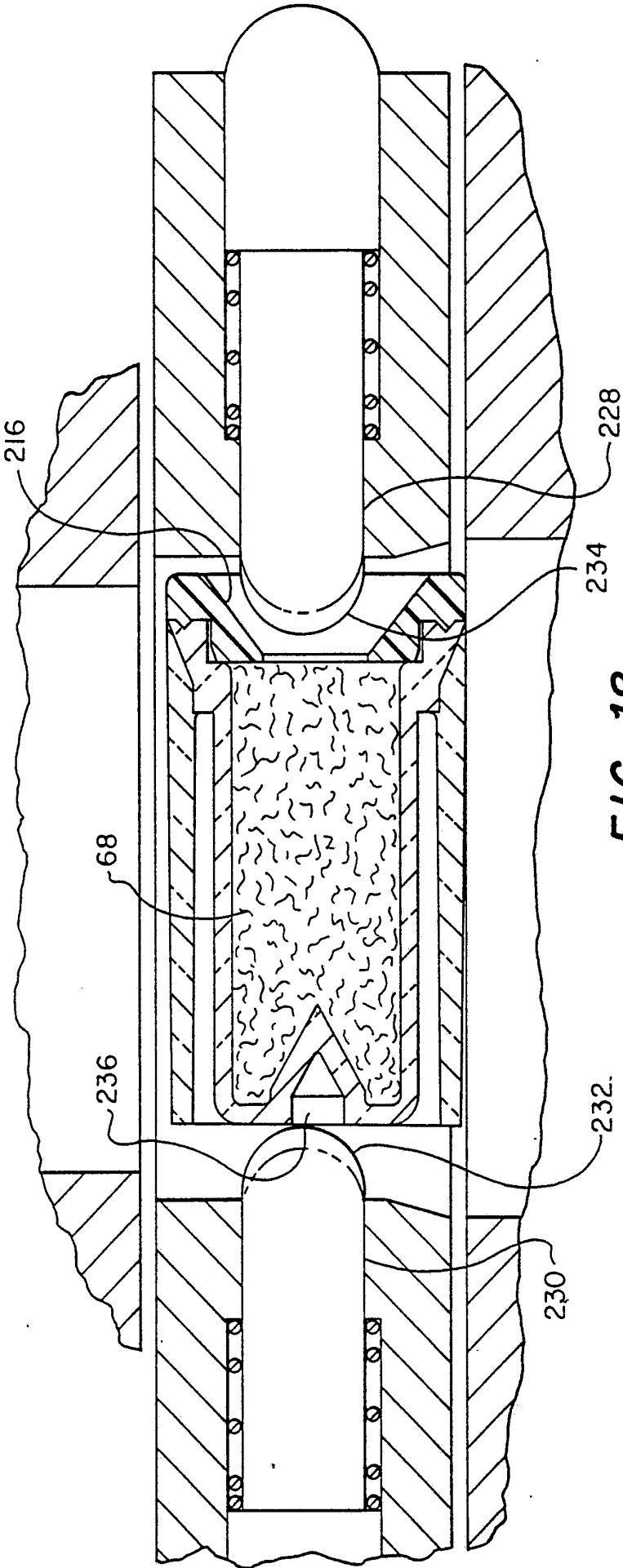
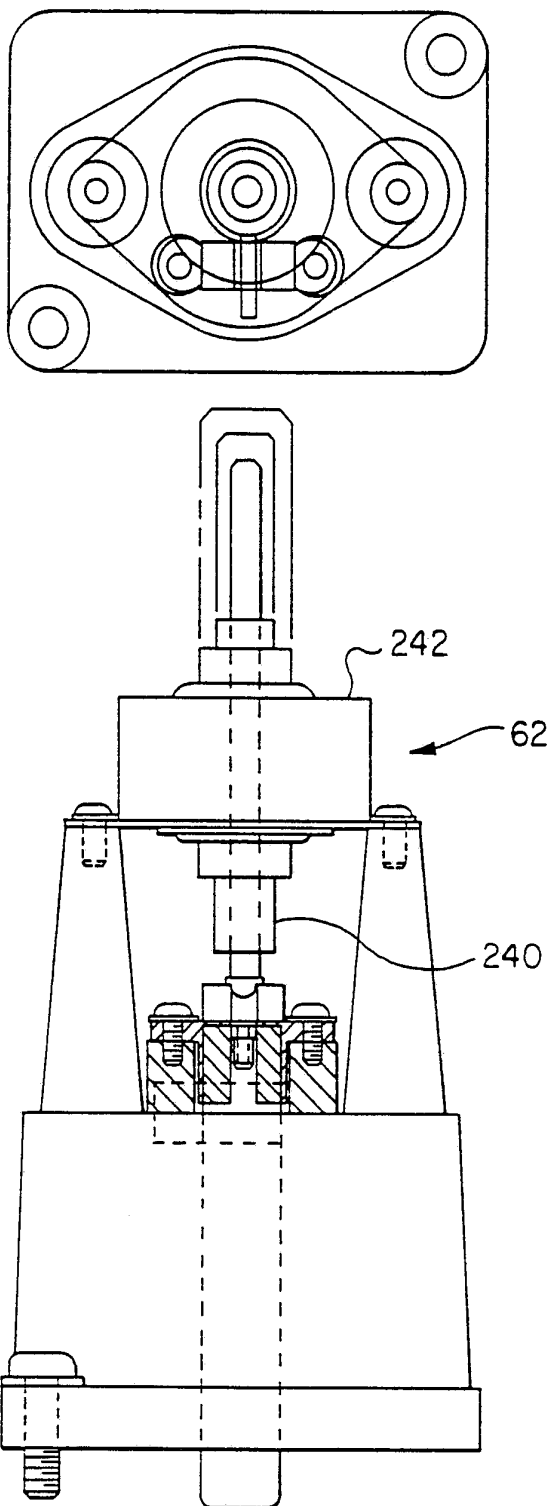


FIG. 18

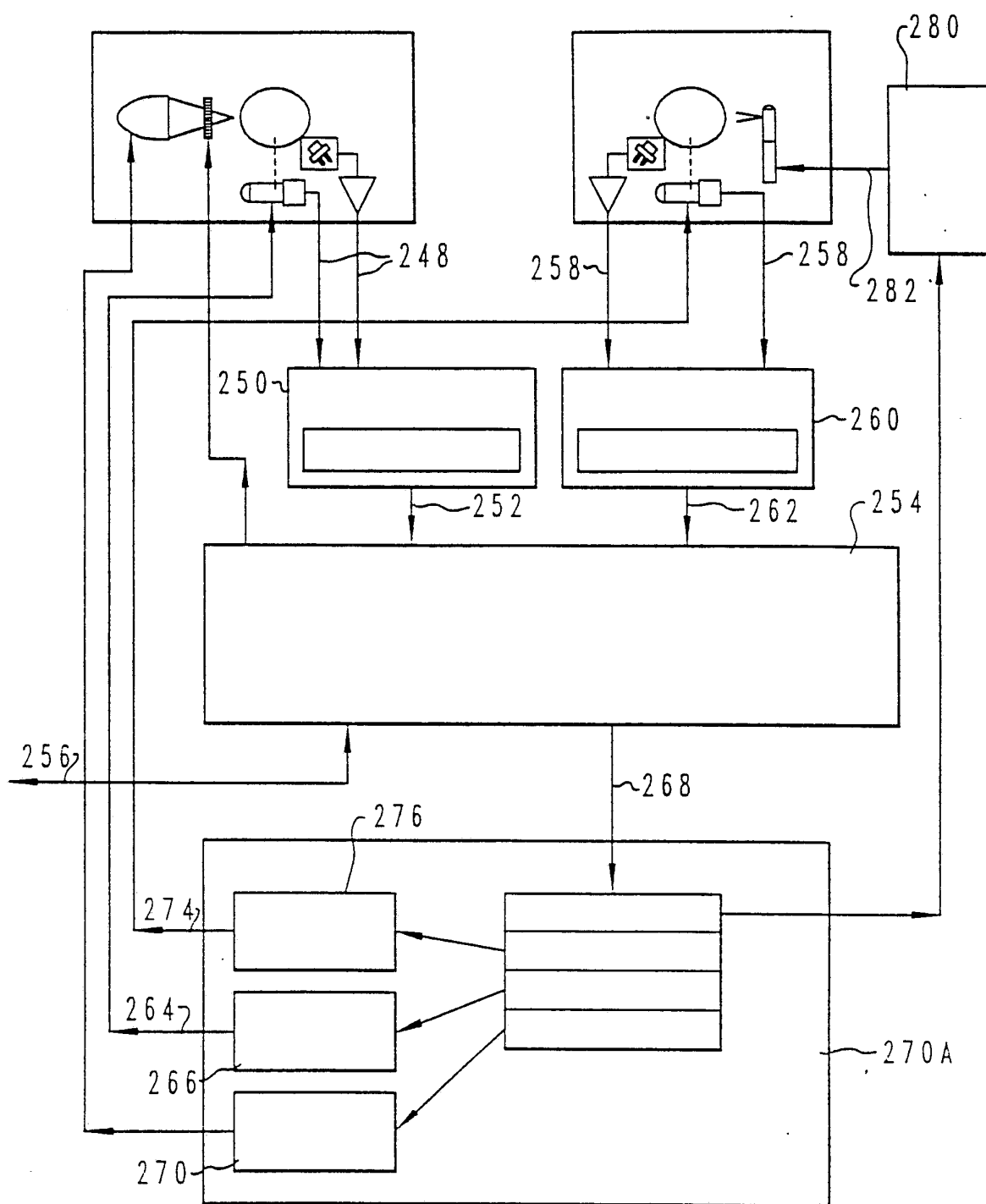
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*FIG. 19*

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*Fig. 20*

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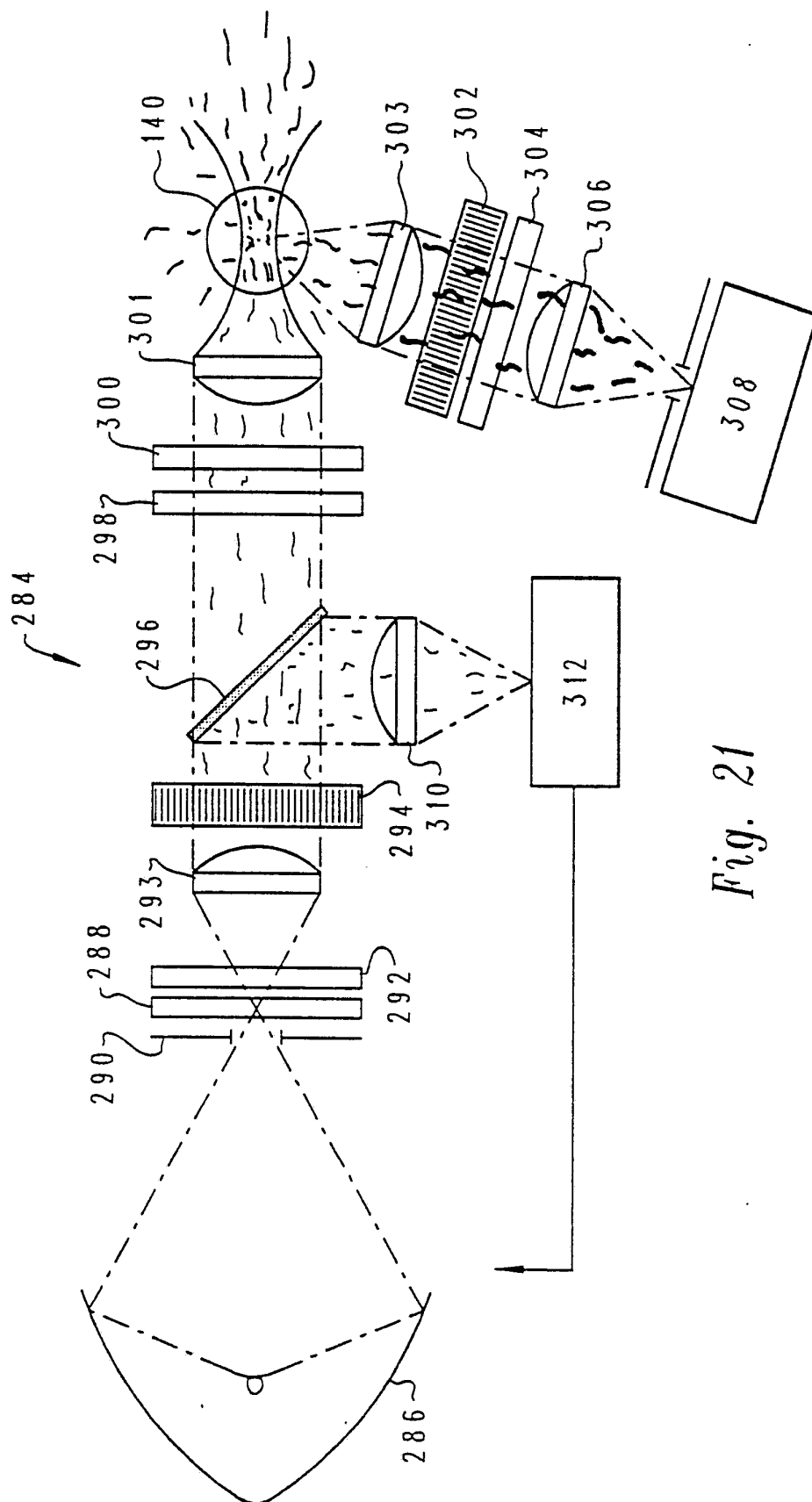


Fig. 21

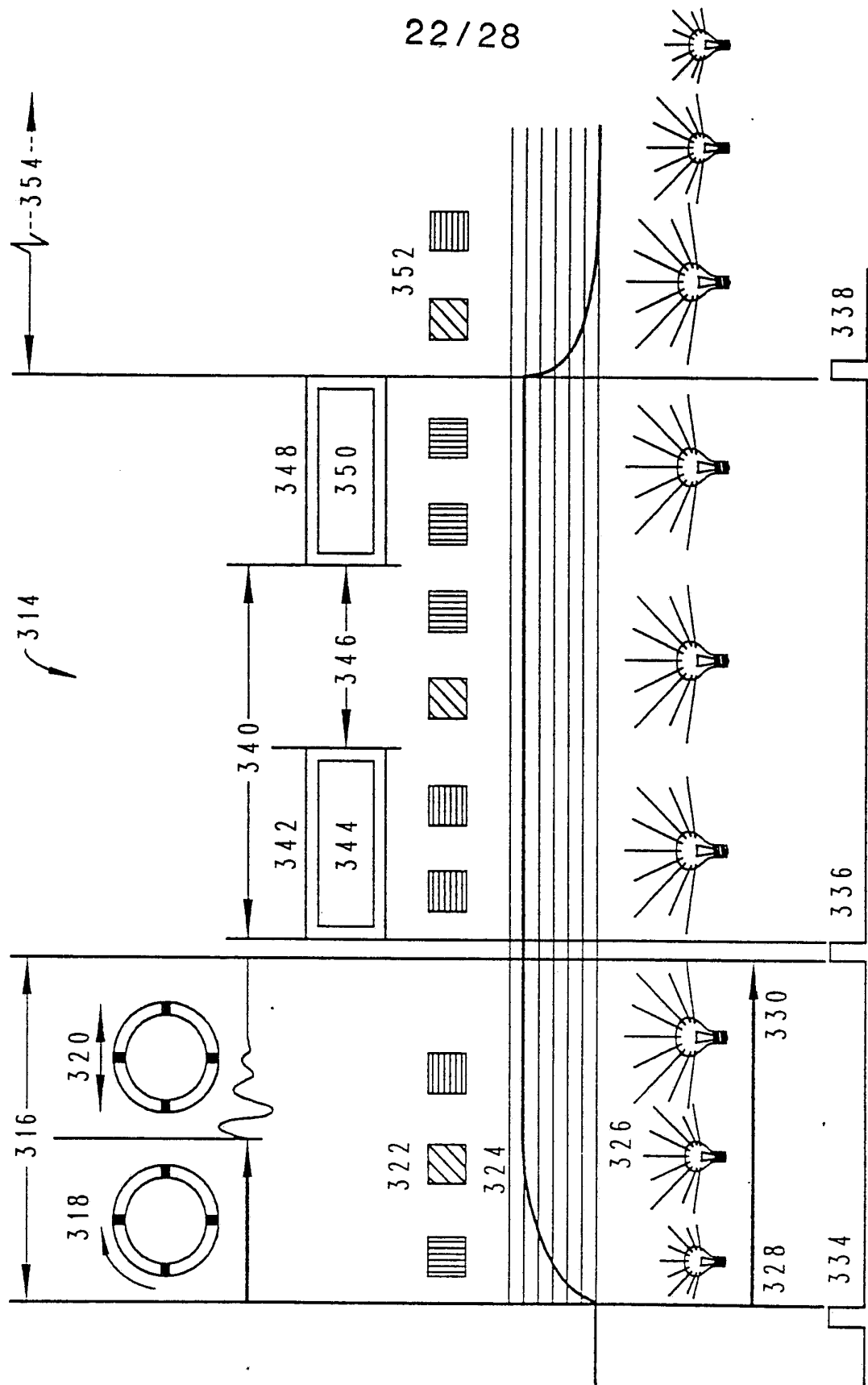


Fig. 22



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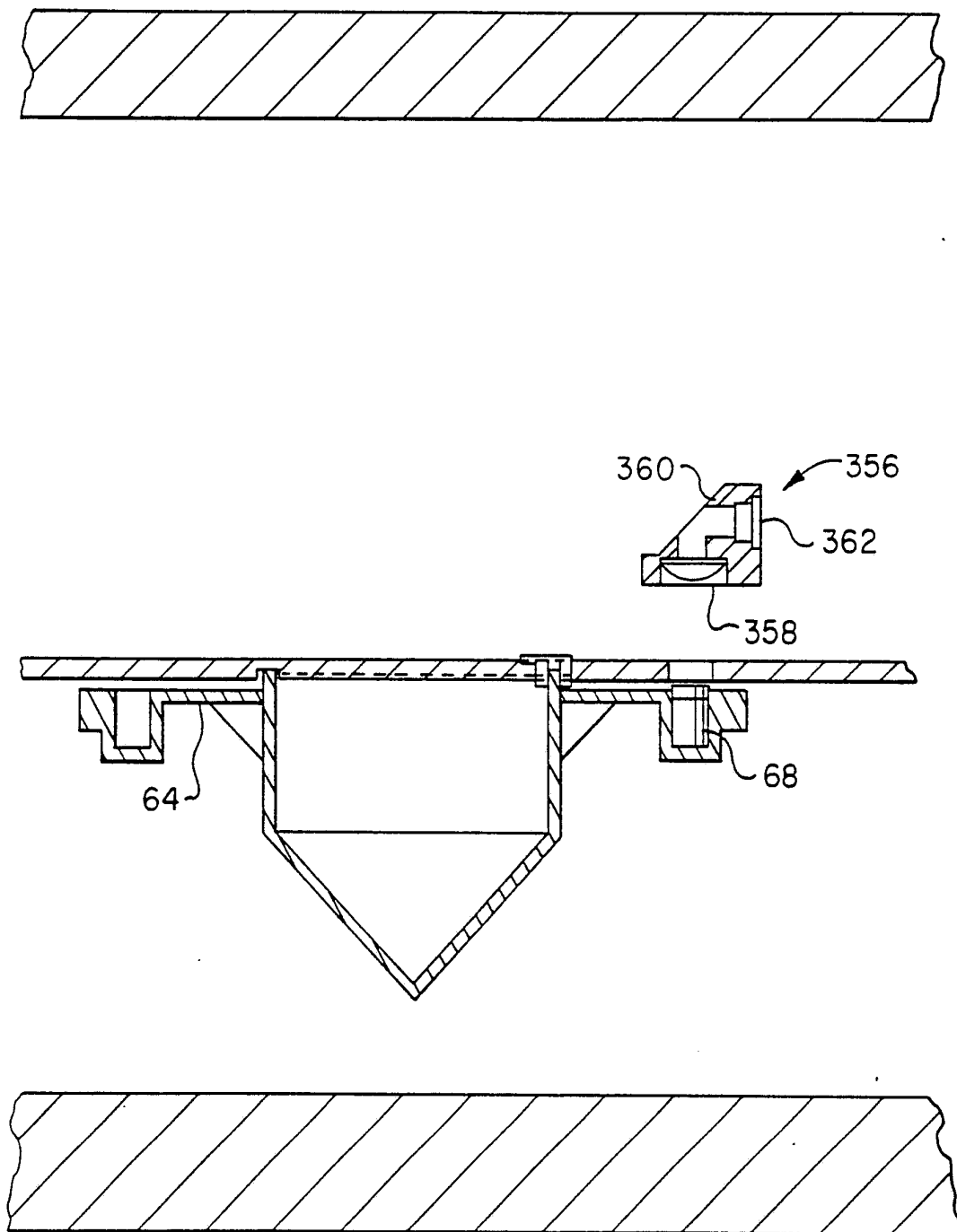
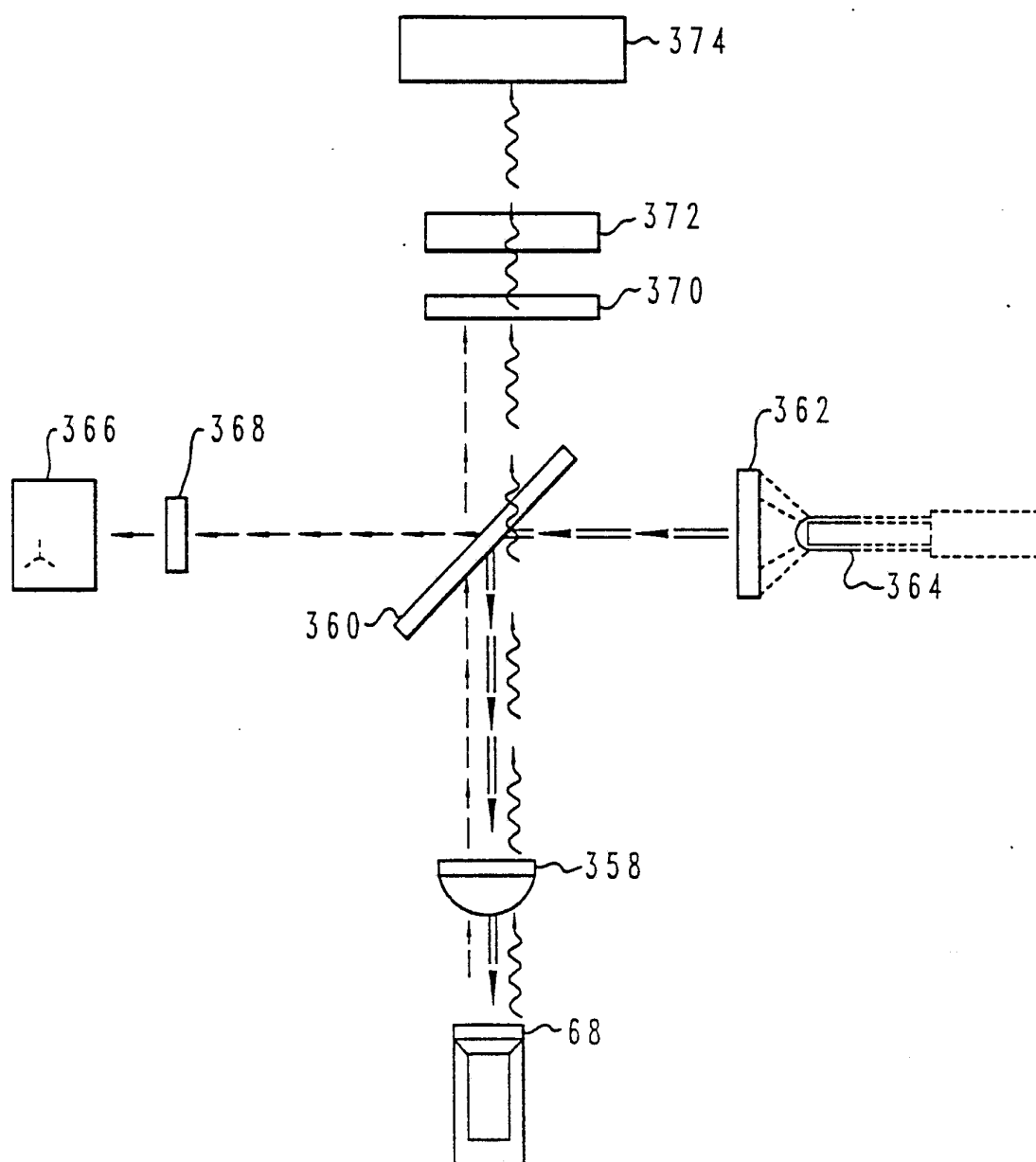


FIG. 23

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*Fig. 24*

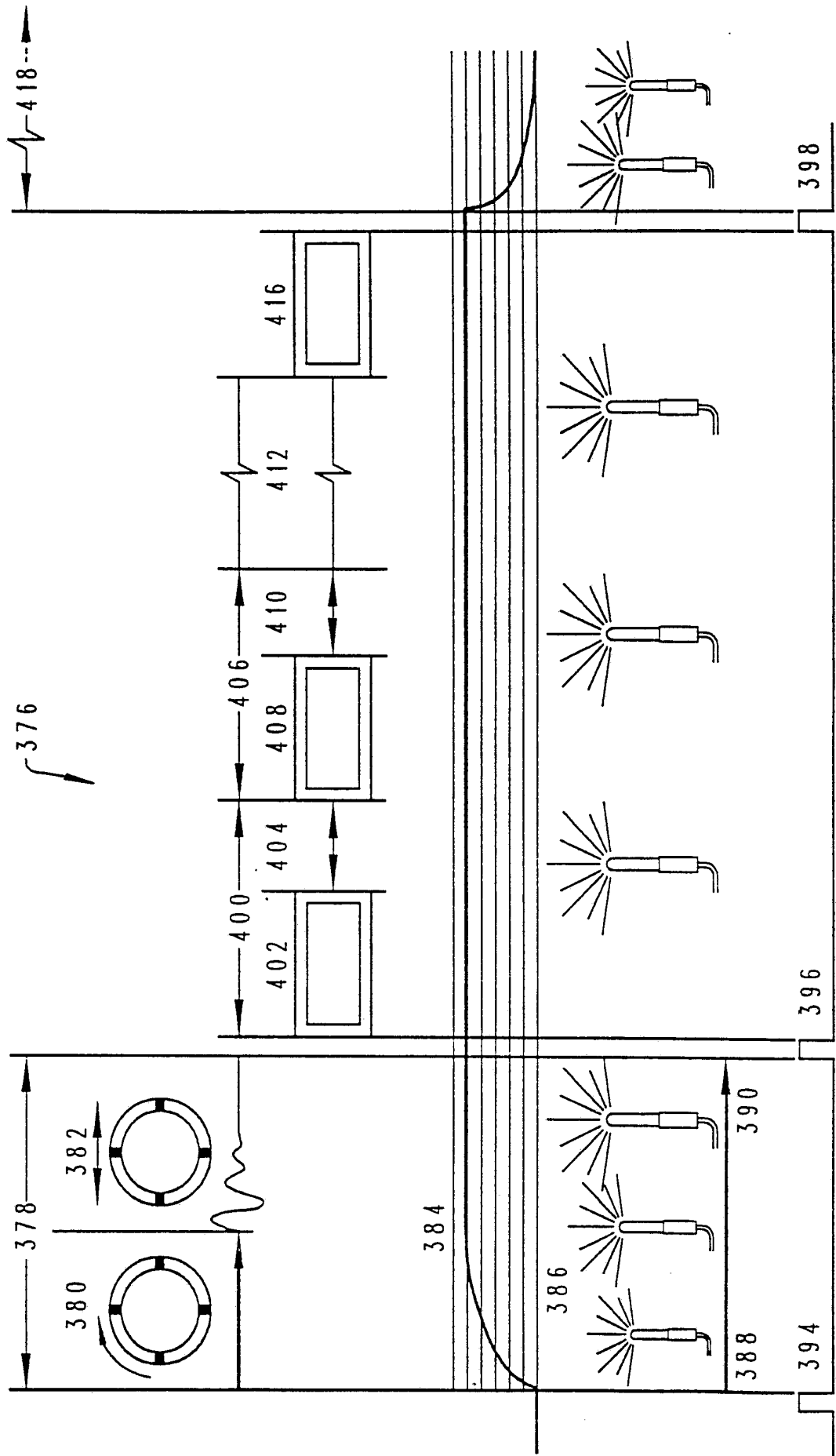


Fig. 25

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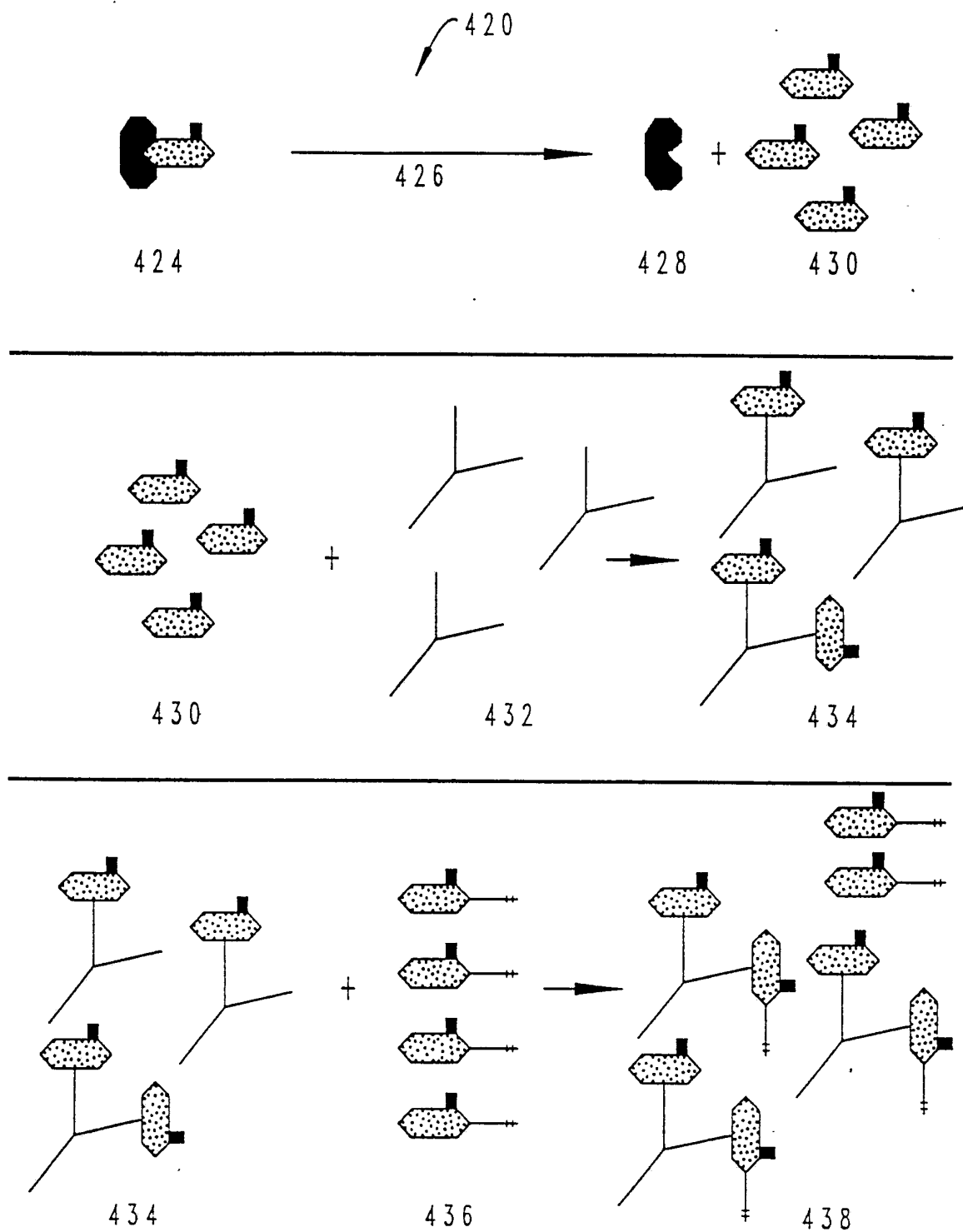


Fig. 26

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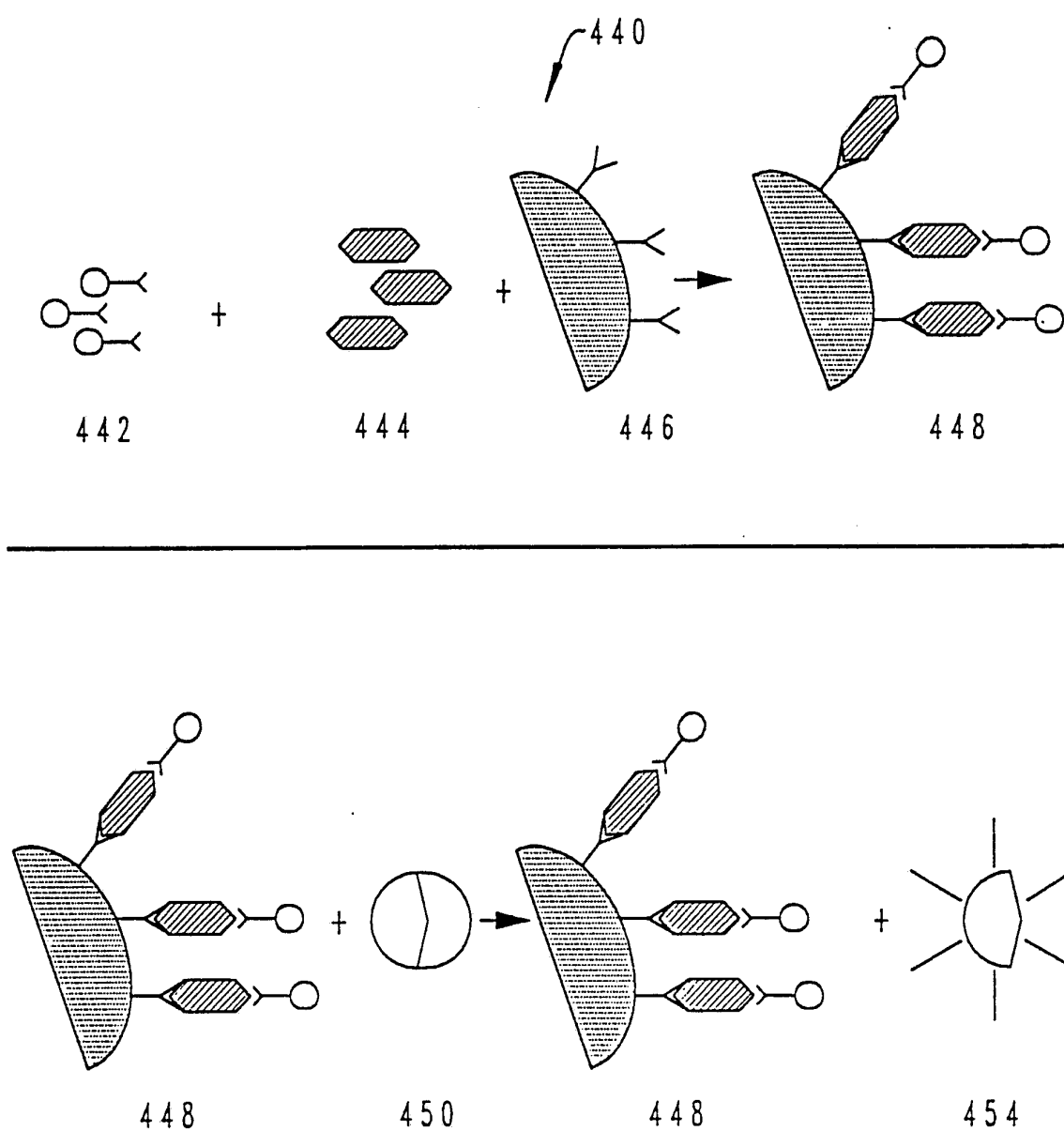


Fig. 27

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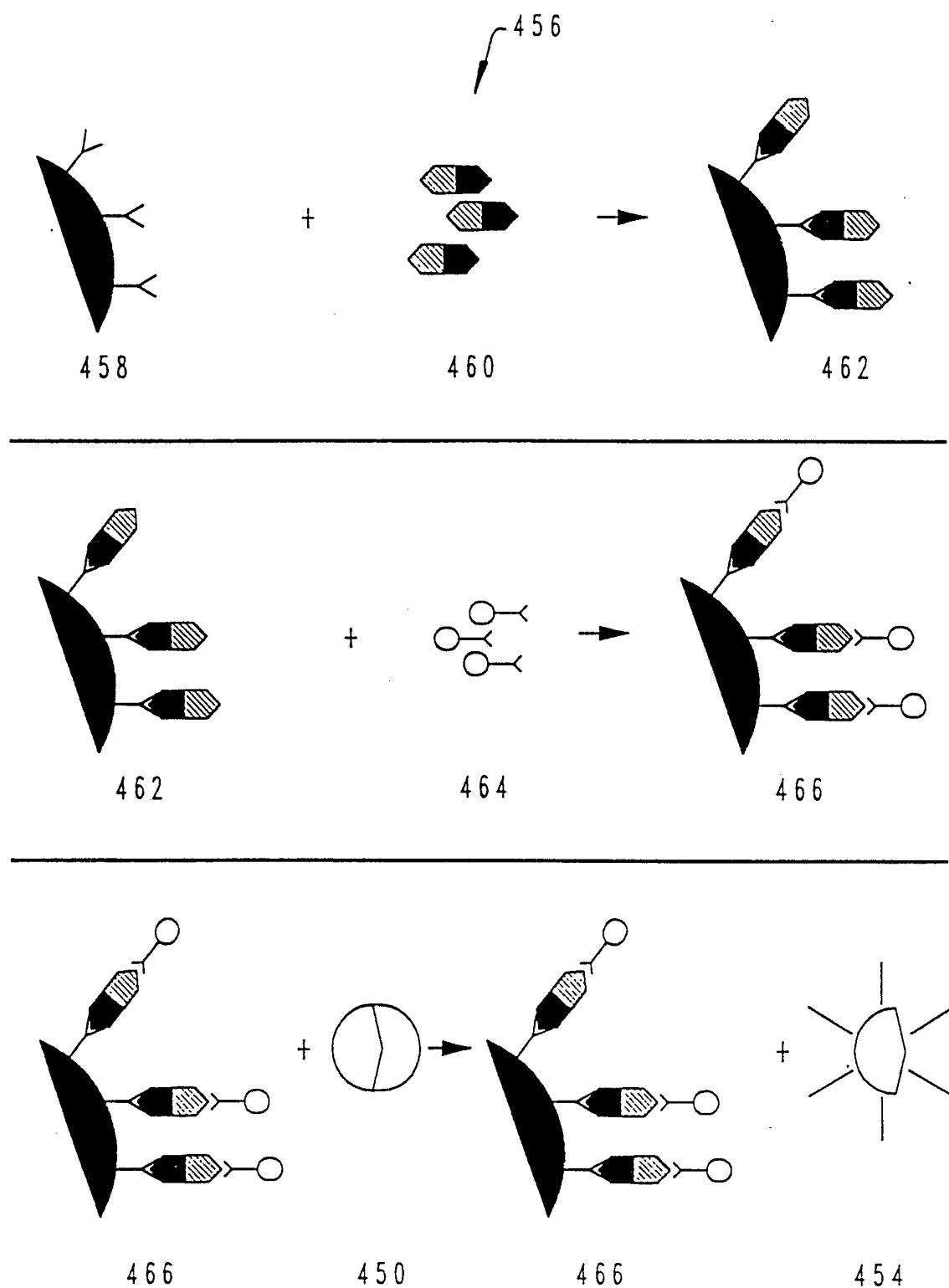


Fig. 28

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/02811

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : G01N 35/02, 35/06, 33/53, 21/64

US CL : 422/63, 64, 67, 82.05, 82.08; 436/43, 47, 48, 54, 164, 172, 180

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63, 64, 67, 82.05, 82.08; 436/43, 47, 48, 54, 164, 172, 180

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

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

APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A, 0,410,645 (COHEN ET AL) 19 JULY 1990, see entire document.	1-48
A	US, A, 4,774,055 (Wakatake et al.) 27 September 1988, see entire document.	1-48
A	US, A, 5,051,238 (Umetsu et al.) 24 September 1991, see entire document.	1-48
A	US, A, 4,647,432 (Wakatake) 03 March 1987, see entire document.	1-48
A	US, A, 4,781,891 (Galle et al.) 01 November 1988, see entire document.	1-48

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 May 1993

Date of mailing of the international search report

18 JUN 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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Authorized officer

LONG LE

Telephone No. (703) 308-0196