



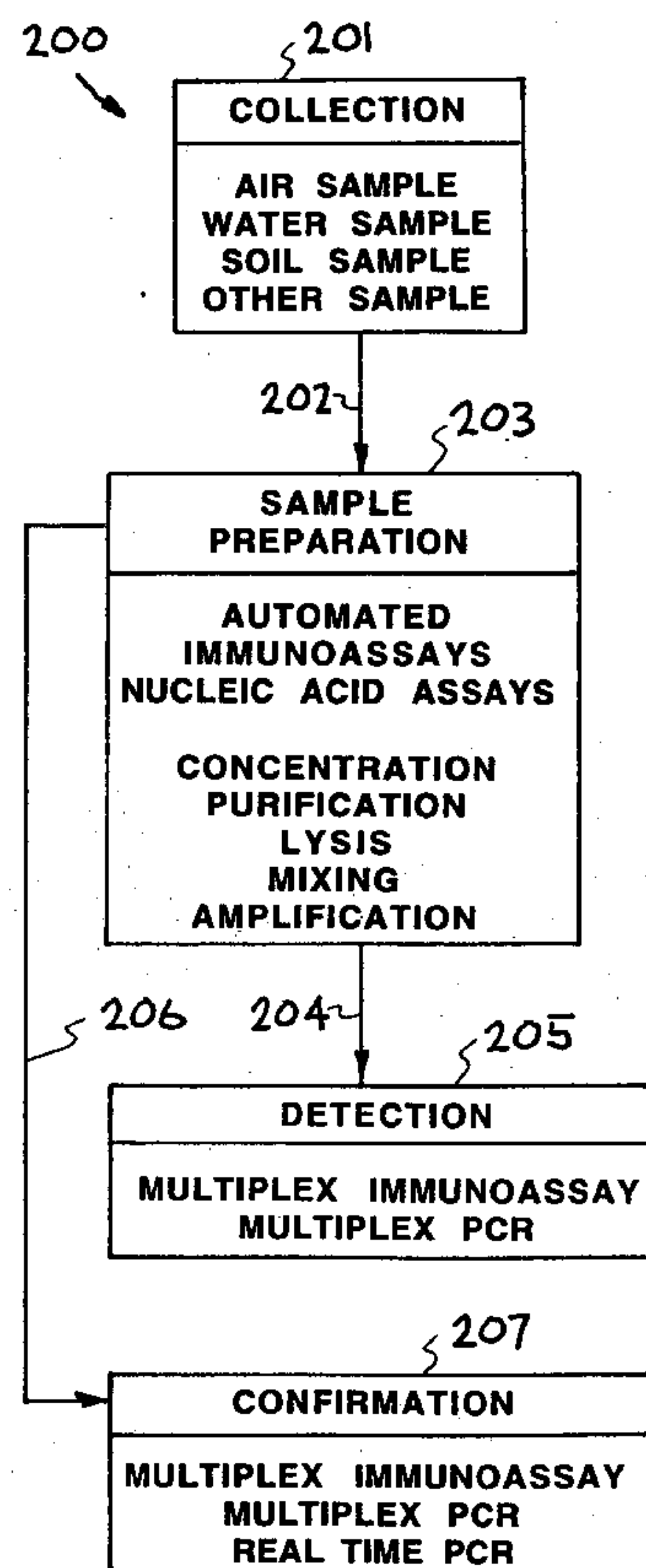
US 20060057599A1

(19) **United States**(12) **Patent Application Publication**
Dzenitis et al.(10) **Pub. No.: US 2006/0057599 A1**(43) **Pub. Date: Mar. 16, 2006**(54) **SYSTEM FOR AUTONOMOUS
MONITORING OF BIOAGENTS**(73) **Assignee: The Regents of the University of Cali-
fornia**(75) **Inventors: John M. Dzenitis, Danville, CA (US);
Steve B. Brown, Livermore, CA (US);
Billy W. Colston JR., San Ramon, CA
(US); Dora M. Gutierrez, Livermore,
CA (US); Bruce D. Henderer,
Livermore, CA (US); Benjamin J.
Hindson, Belmont (AU); Ramakrishna
S. Madabhushi, Fremont, CA (US);
Anthony J. Makarewicz, Livermore,
CA (US); Don A. Masquelier, Tracy,
CA (US); Mary T. McBride,
Brentwood, CA (US); Thomas R.
Metz, Tracy, CA (US); Shanavaz L.
Nasarabadi, Livermore, CA (US);
Ujwal S. Setlur, Livermore, CA (US);
Sally M. Smith, Salida, CA (US);
Kodumudi S. Venkateswaran,
Livermore, CA (US)**(21) **Appl. No.: 11/000,491**(22) **Filed: Nov. 29, 2004****Related U.S. Application Data**(63) **Continuation-in-part of application No. 10/643,797,
filed on Aug. 19, 2003.**(60) **Provisional application No. 60/406,159, filed on Aug.
26, 2002.****Publication Classification**(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C12M 1/34 (2006.01)(52) **U.S. Cl. 435/6; 435/30; 435/287.2;
435/288.5**(57) **ABSTRACT**

Correspondence Address:

Eddie E. Scott**Assistant Laboratory Counsel****Lawrence Livermore National Laboratory****P.O. Box 808, L-703****Livermore, CA 94551 (US)**

An autonomous monitoring apparatus for monitoring air, water, soil, or other substance for bioagents. A collector gathers a quantity of the air, water, soil, or other substance being monitored. A sample preparation system prepares a sample of the selected potential bioagent particles. The sample is analyzed by a system for detecting said bioagents.



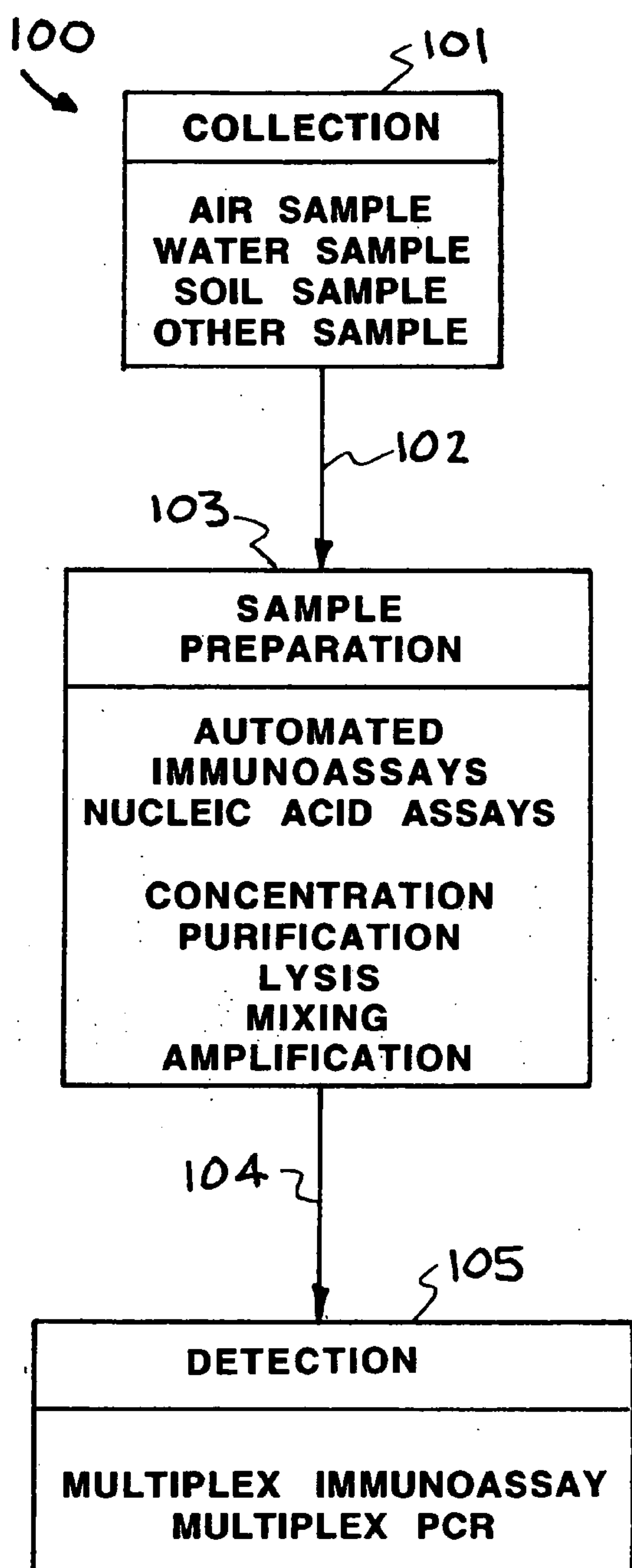


FIG. 1

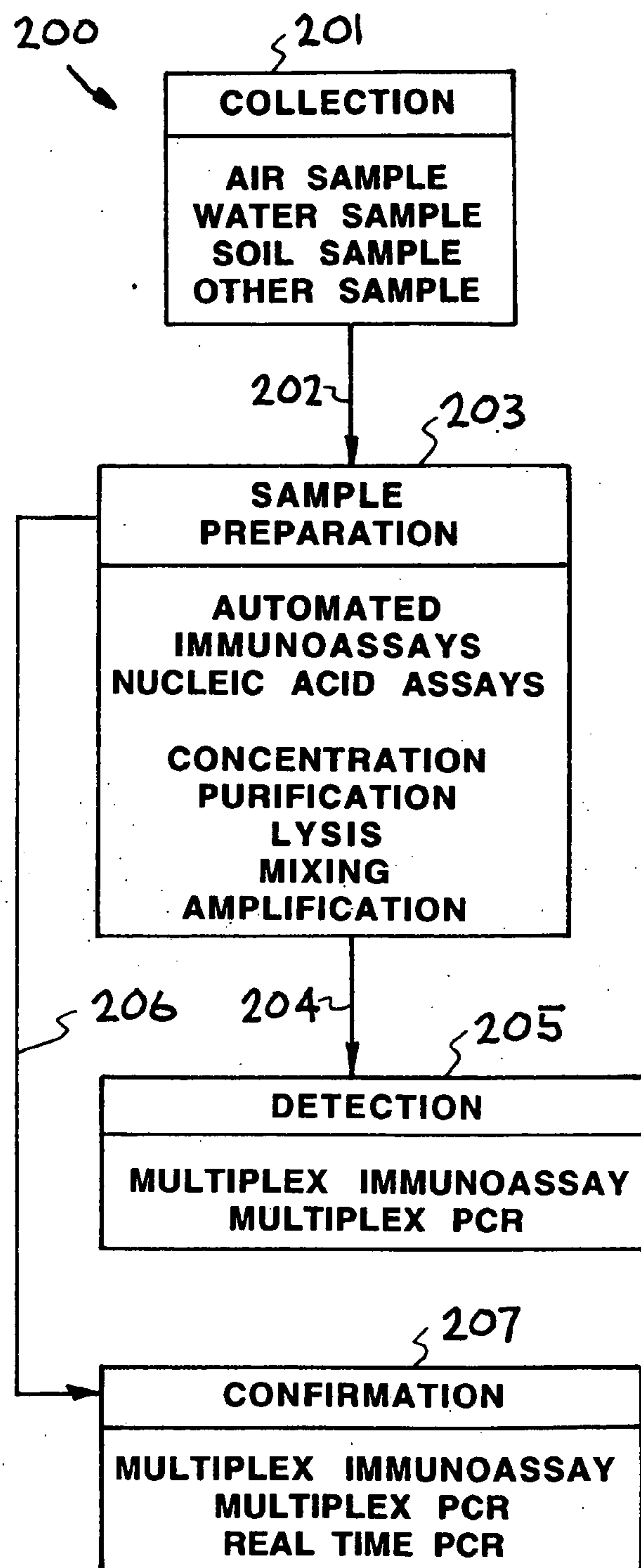


FIG. 2

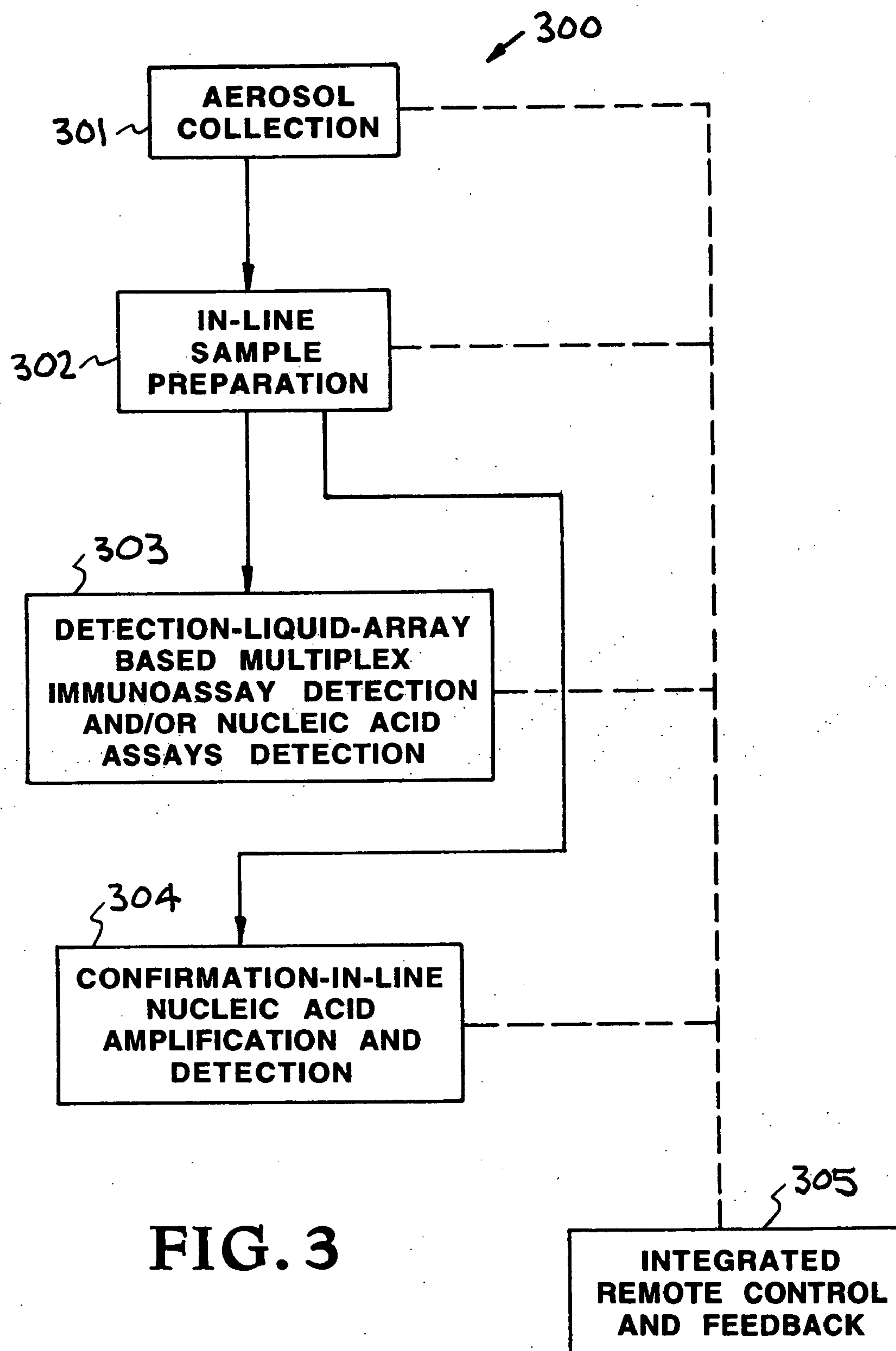


FIG. 3

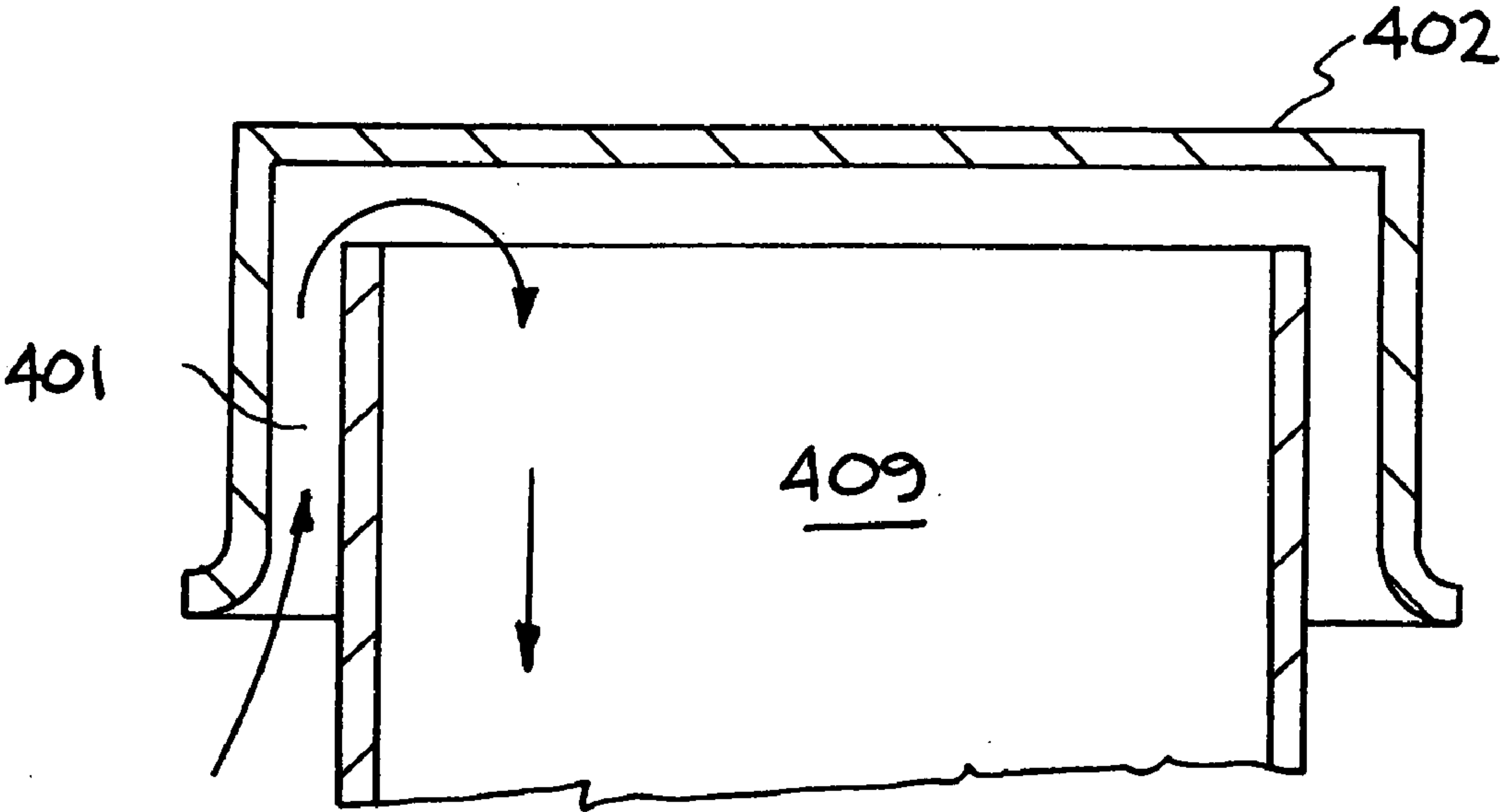
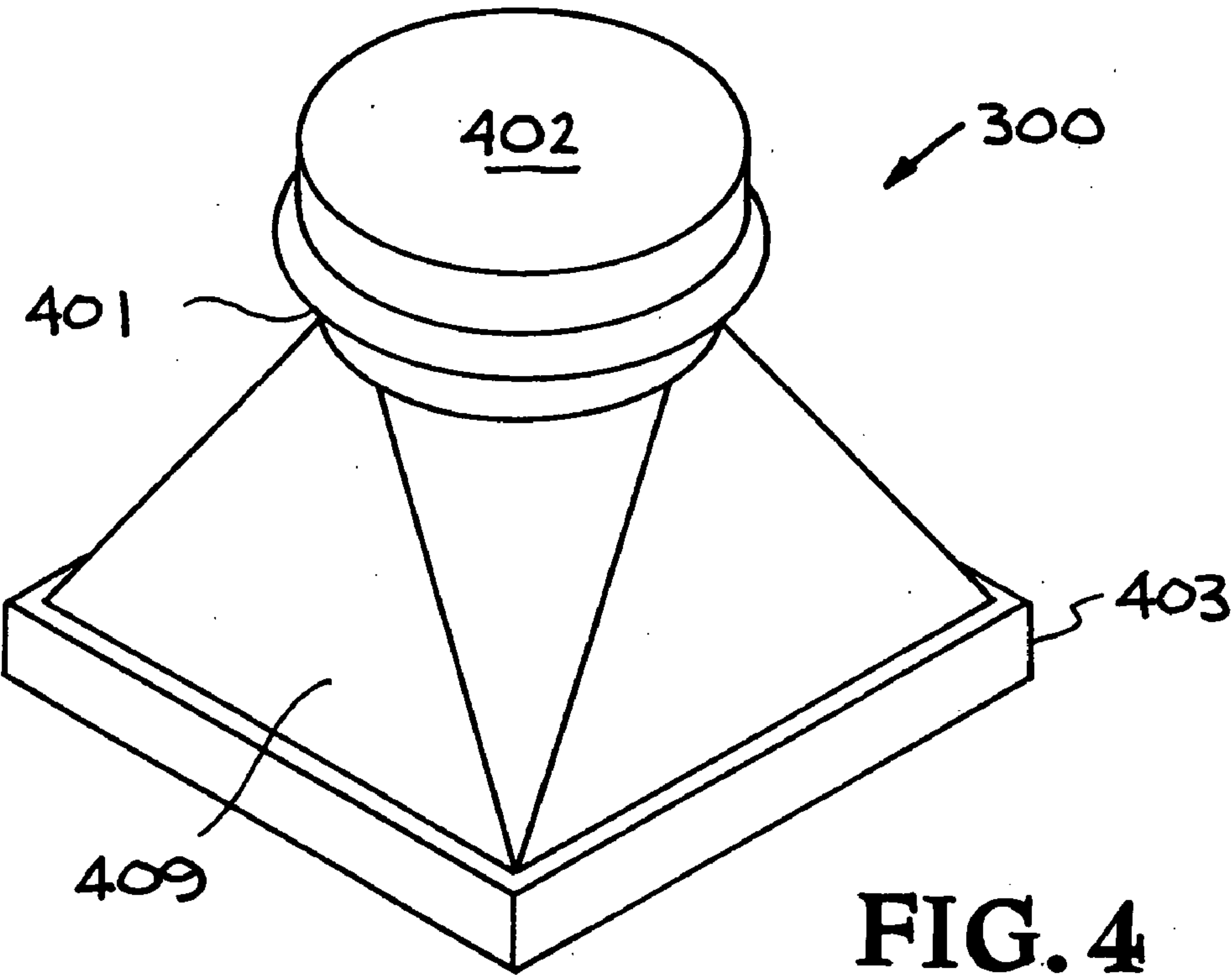


FIG. 5

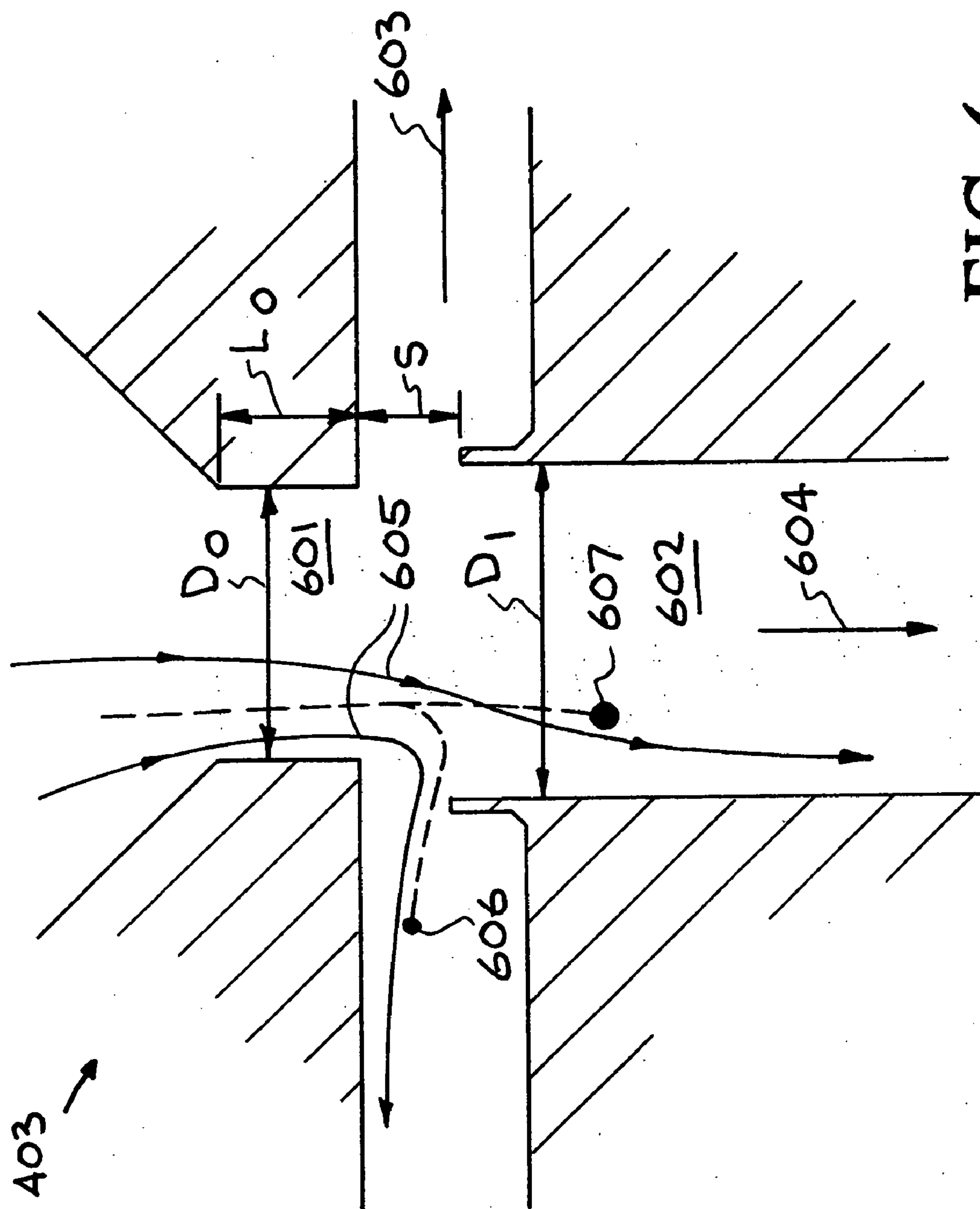


FIG. 6

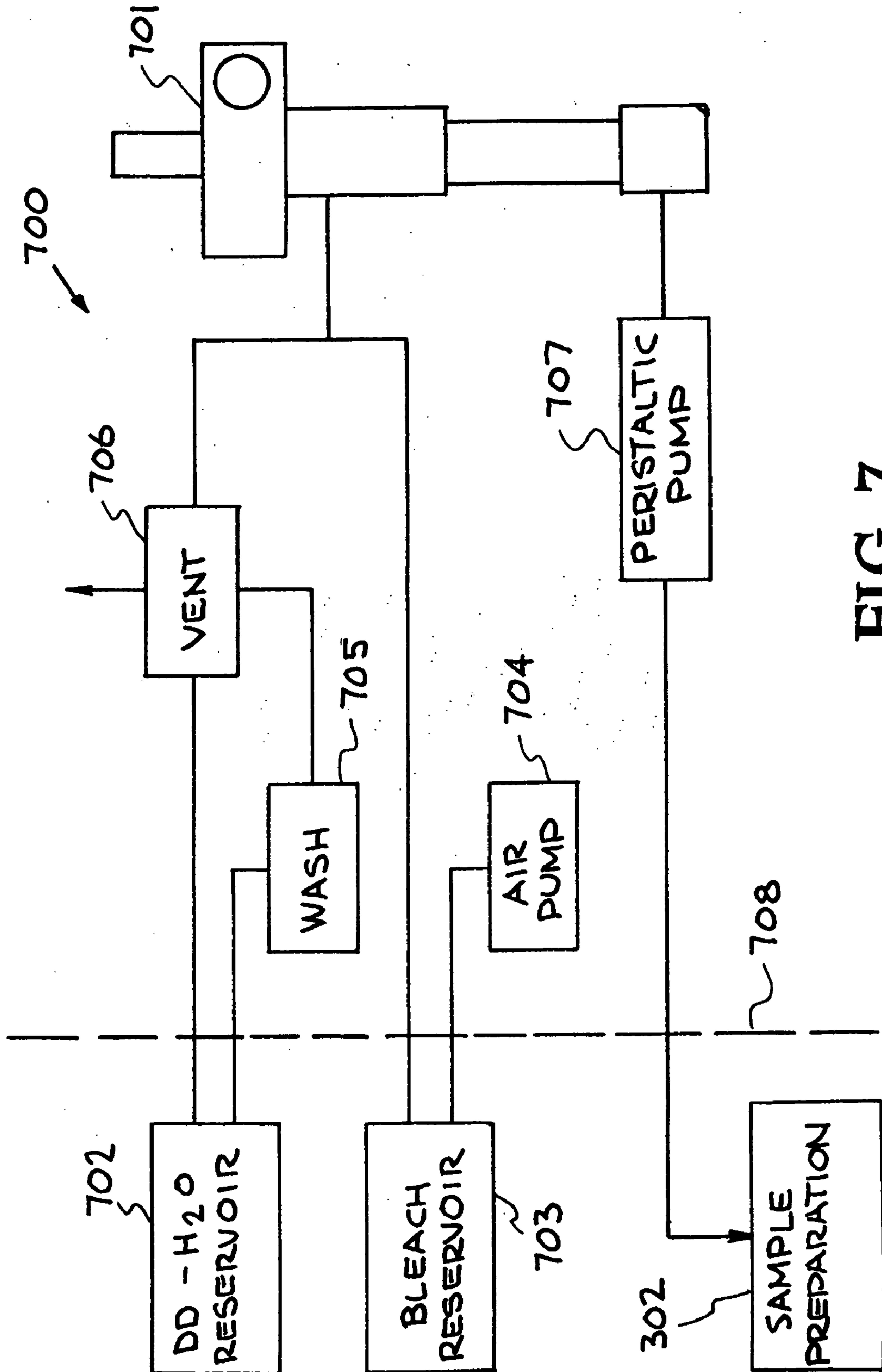


FIG. 7

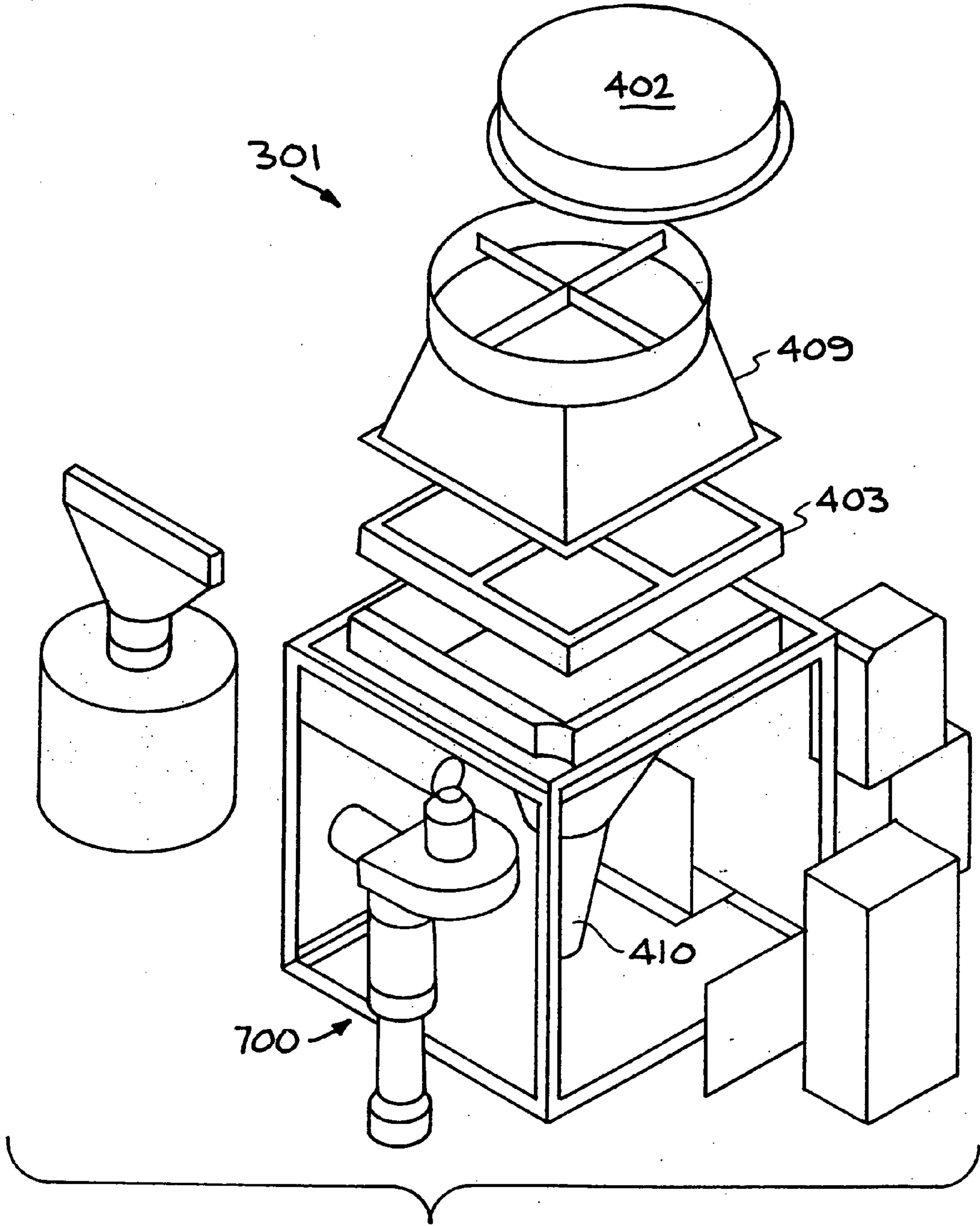


FIG. 8A

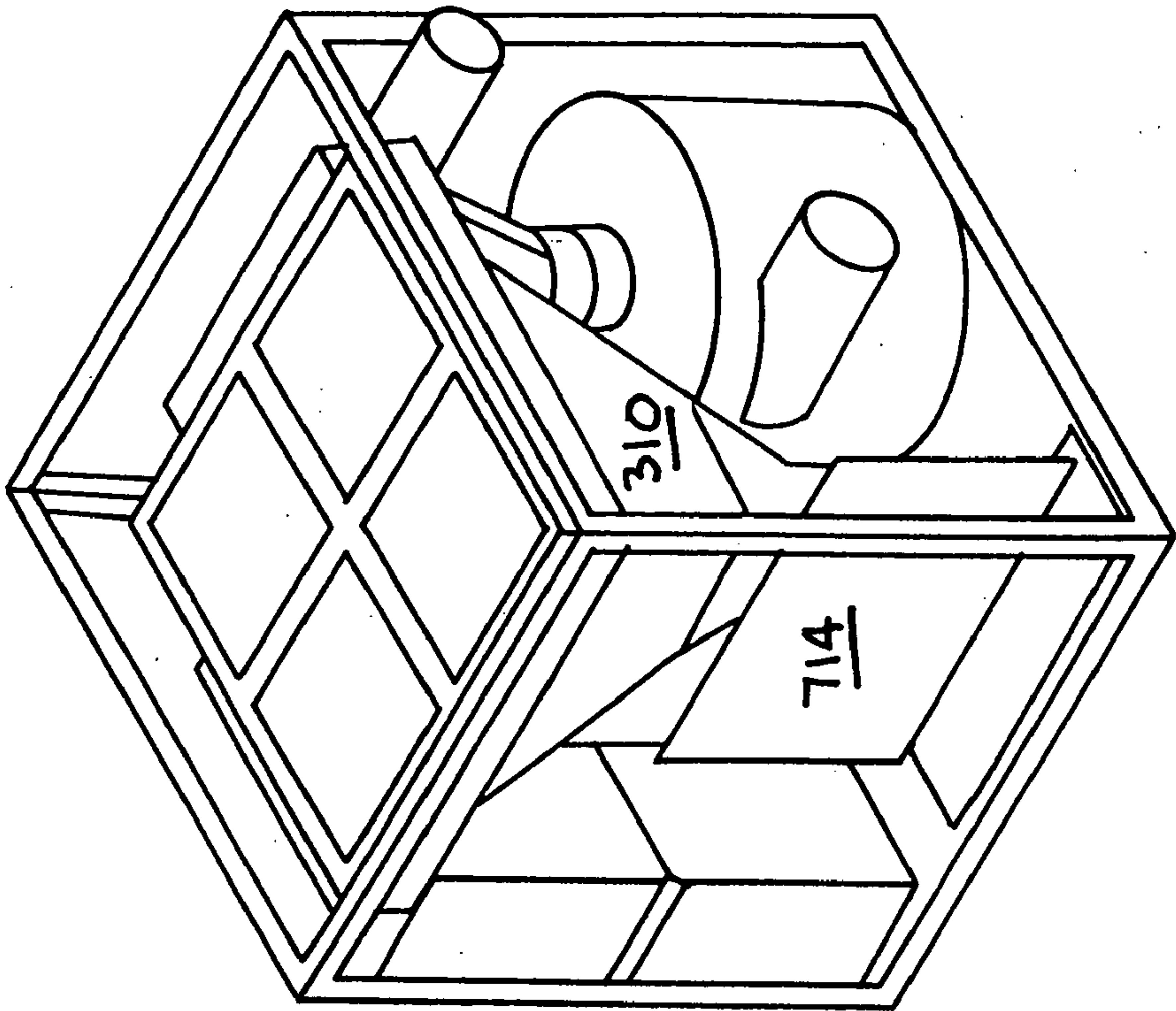


FIG. 8C

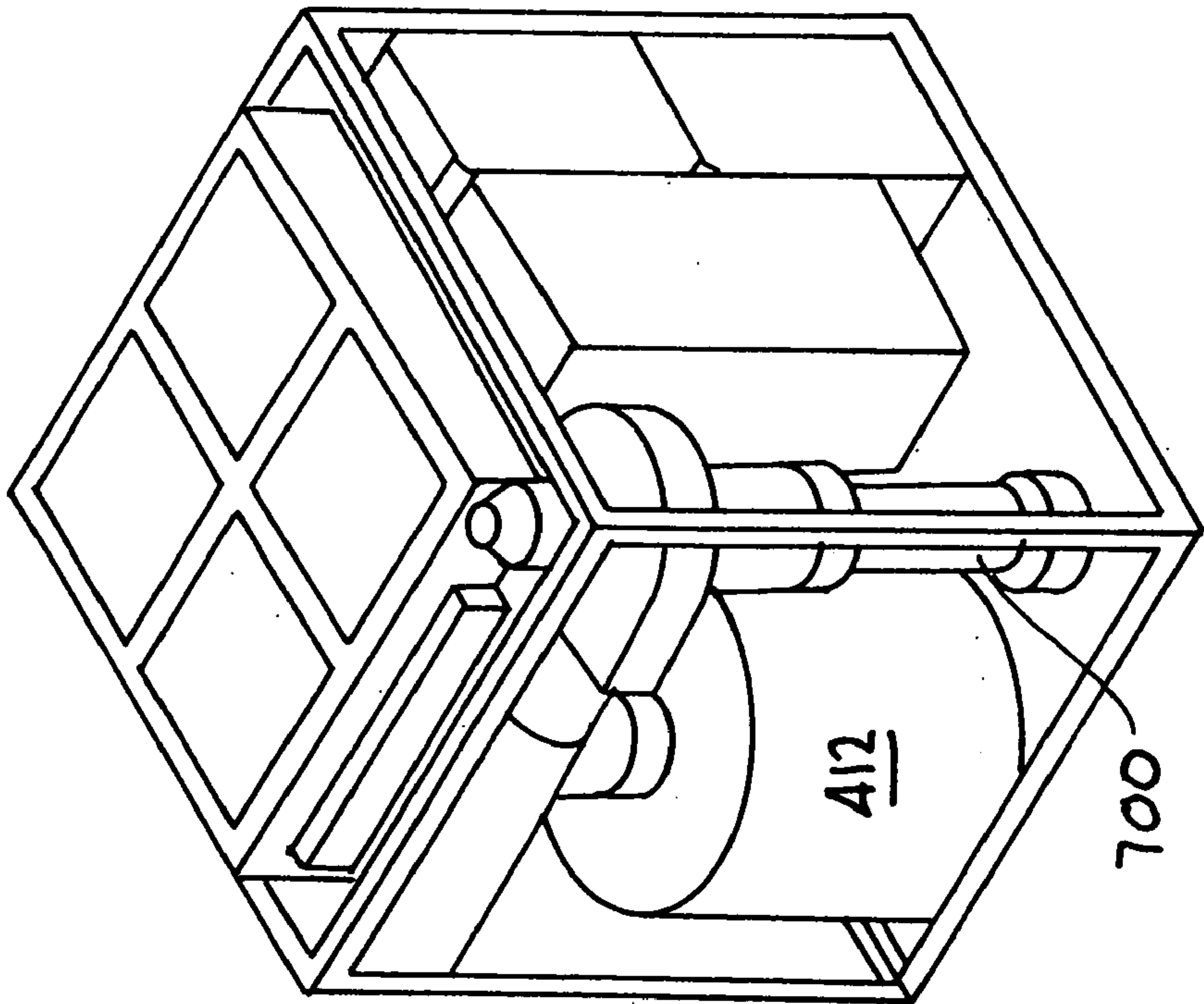


FIG. 8B

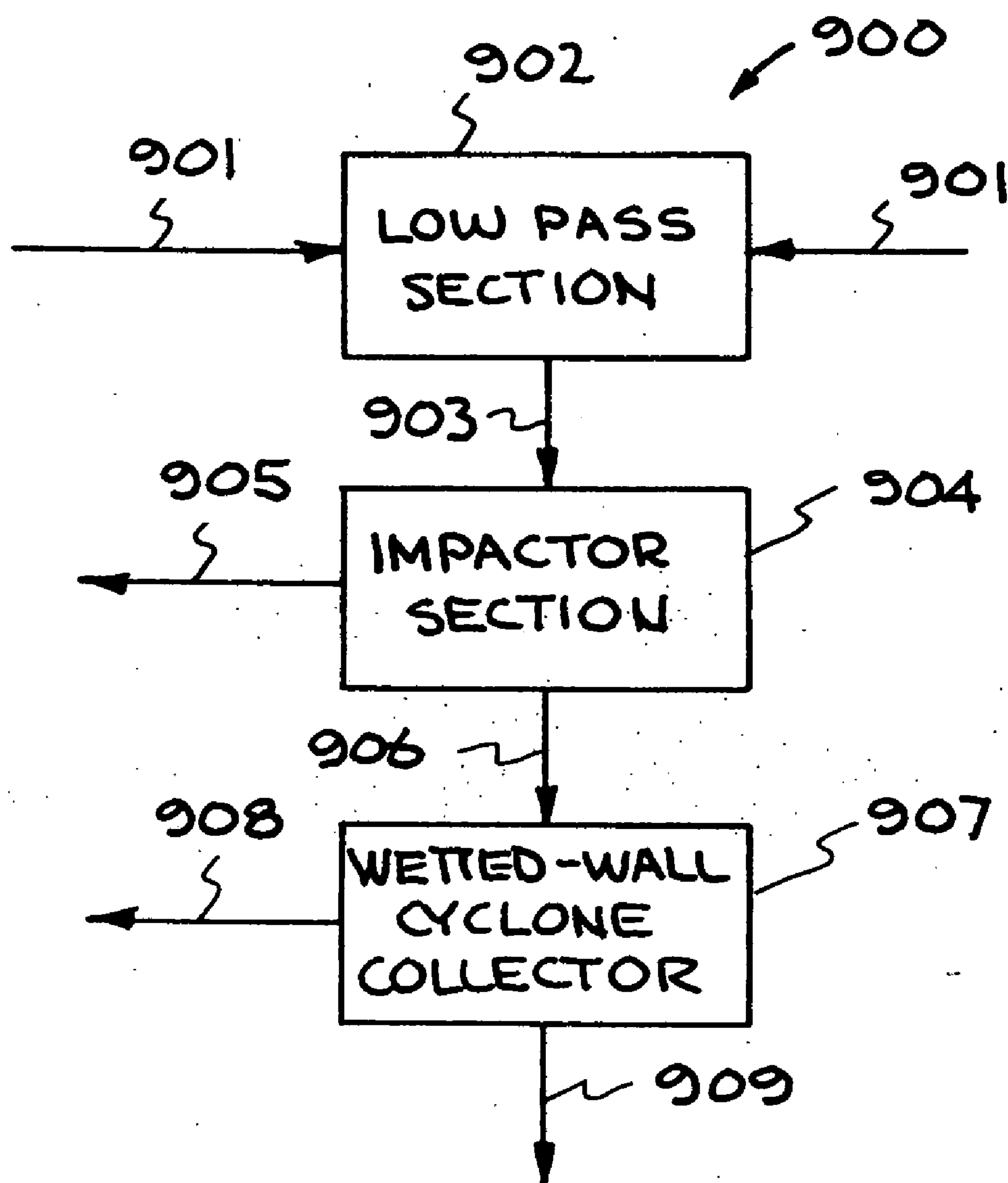


FIG. 9

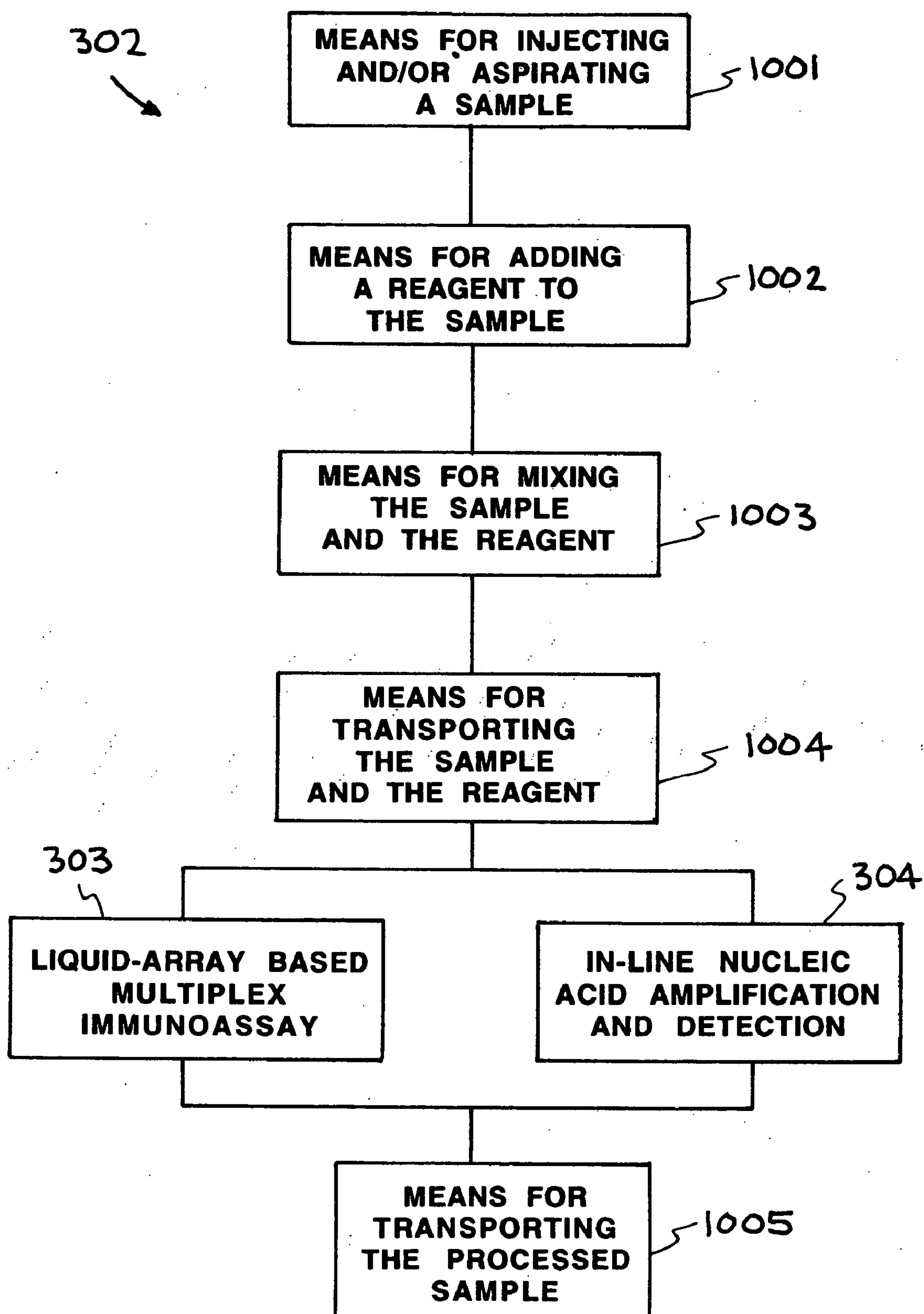


FIG. 10

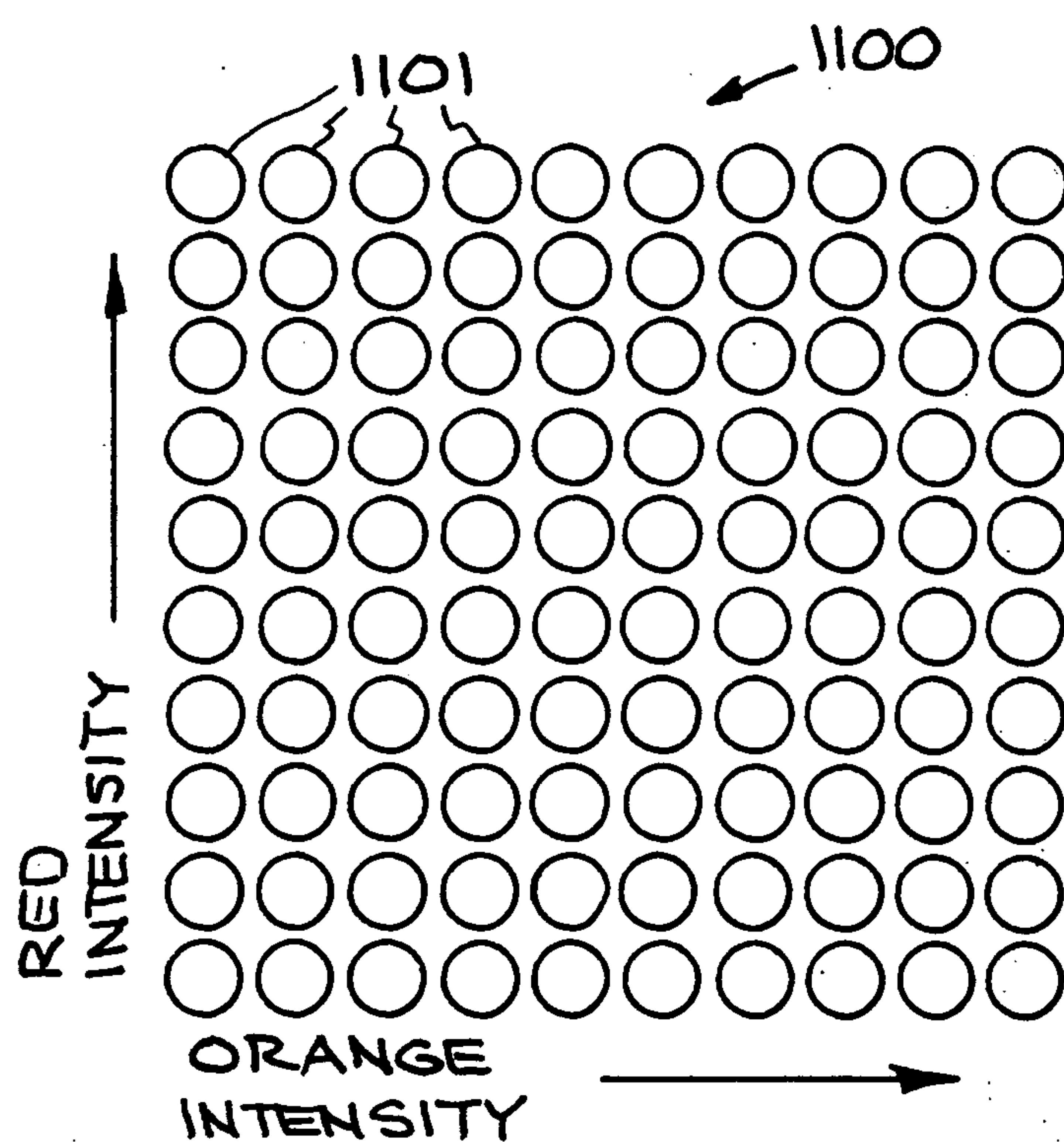


FIG. 11

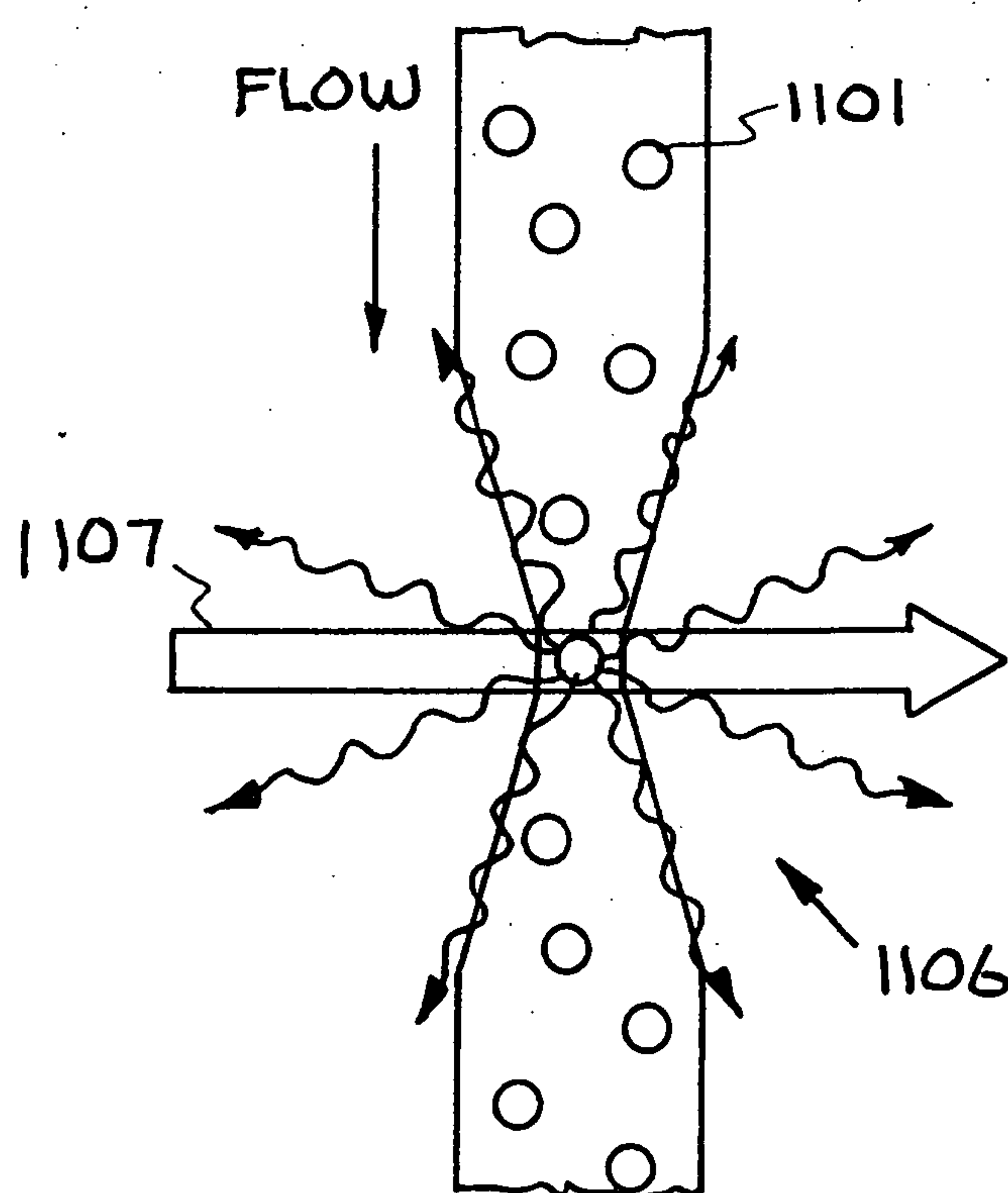


FIG. 13

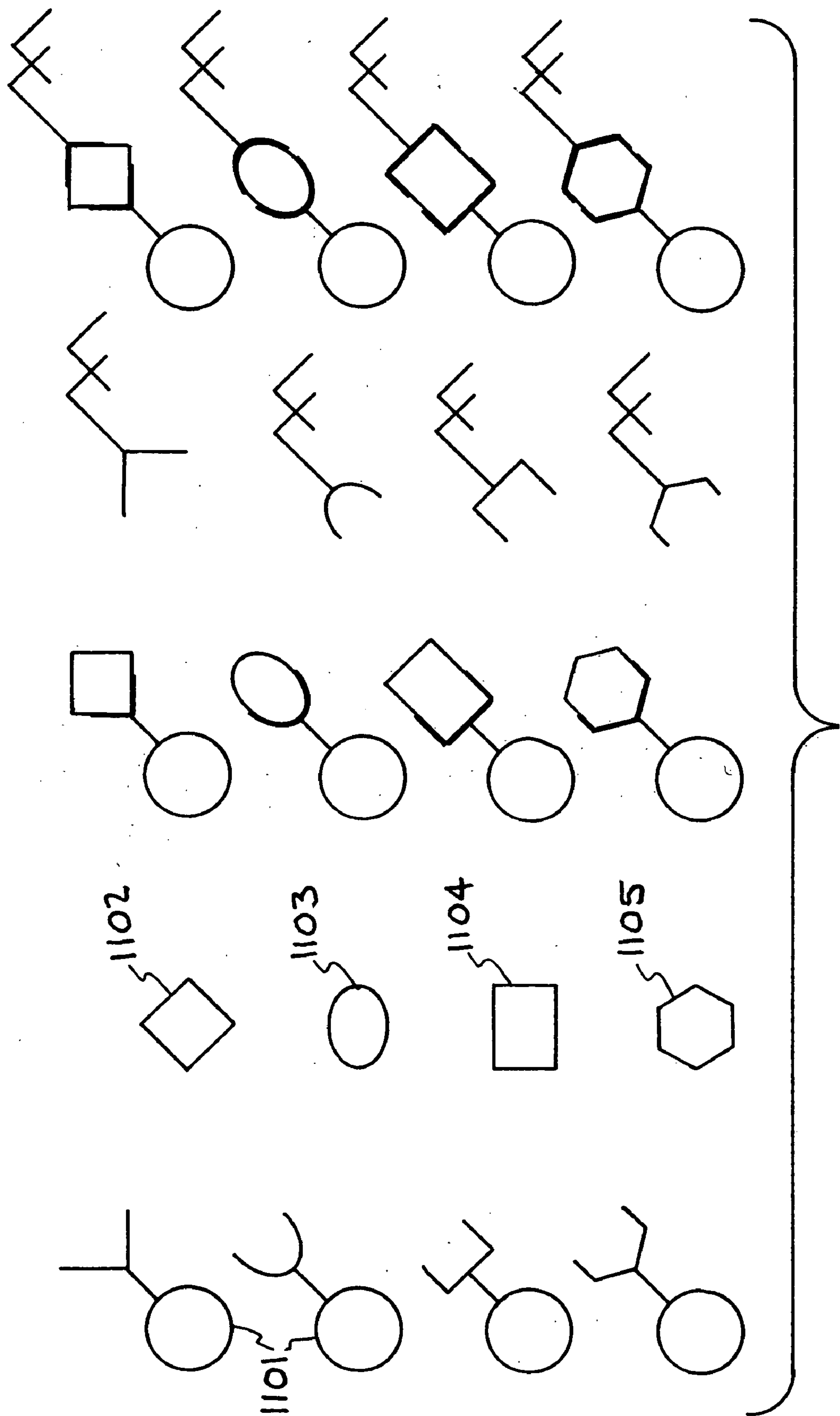


FIG. 12

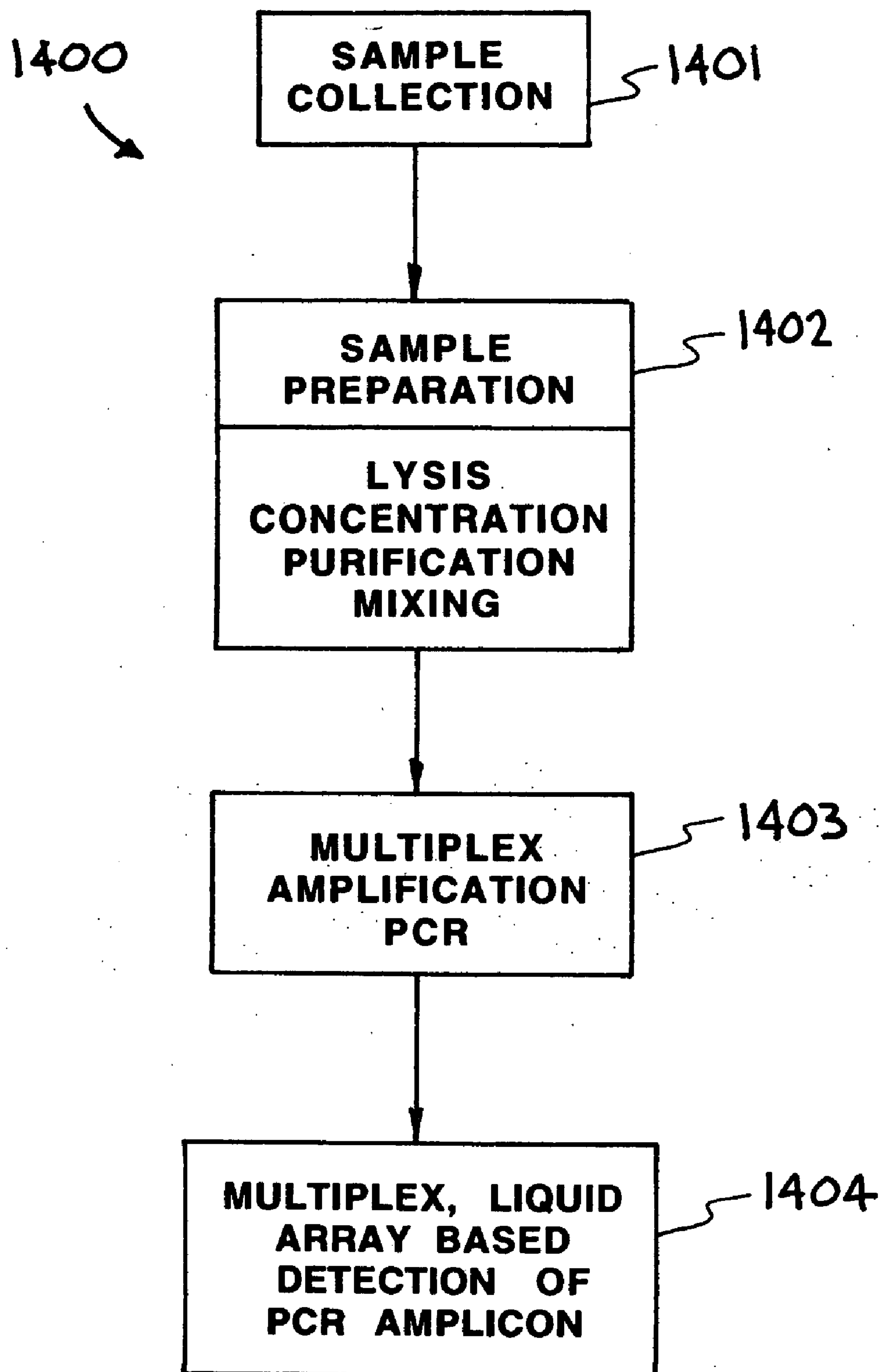


FIG. 14

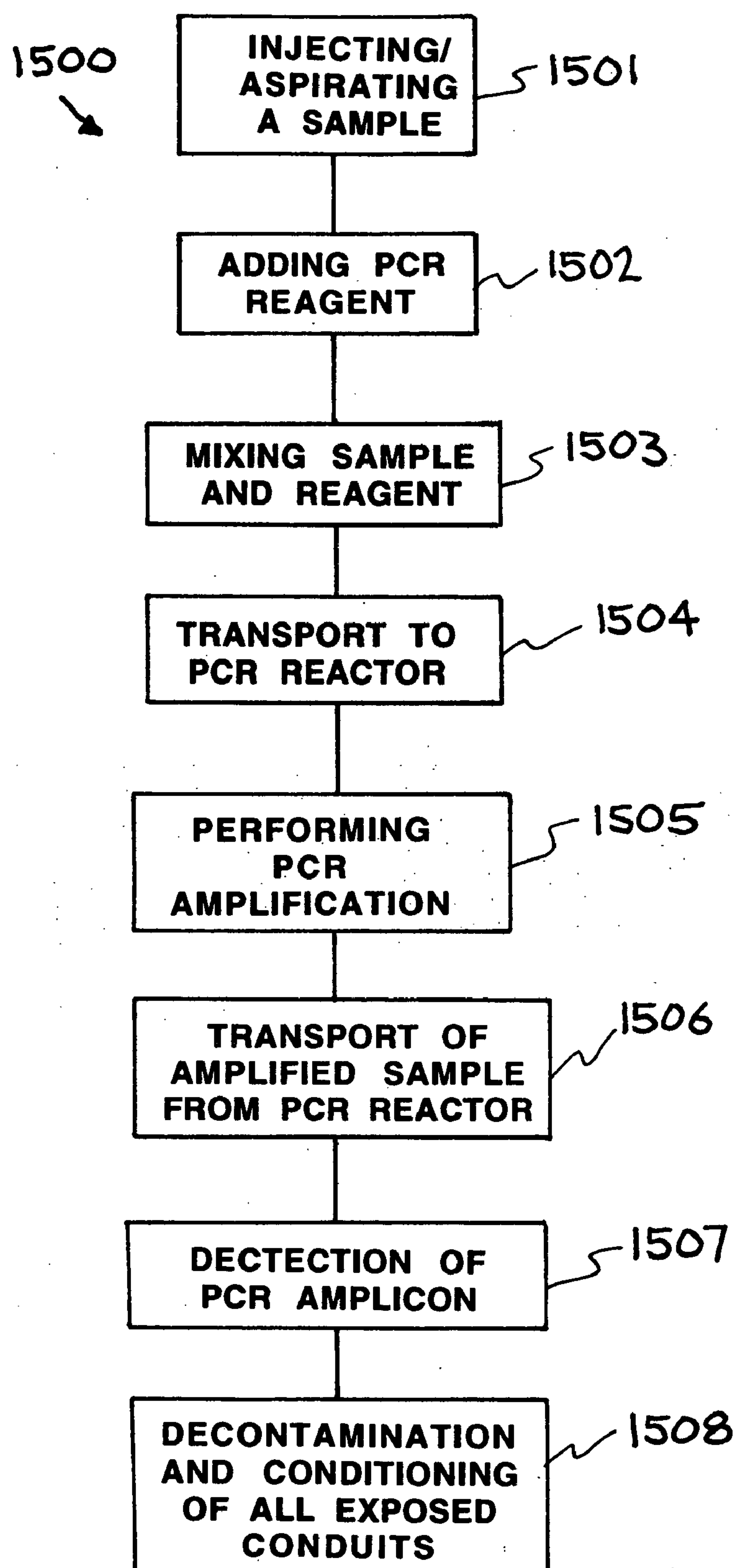


FIG. 15

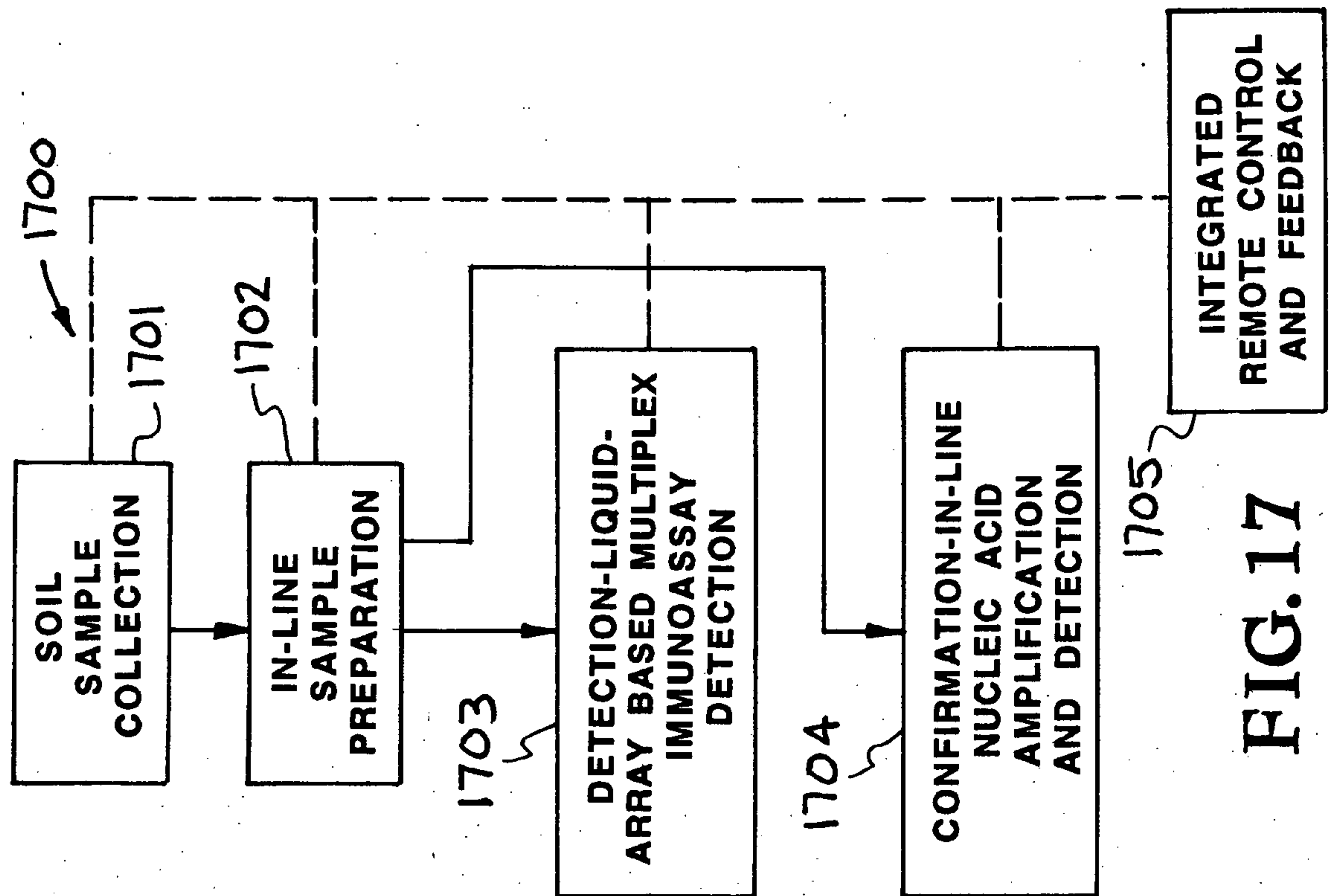


FIG. 16

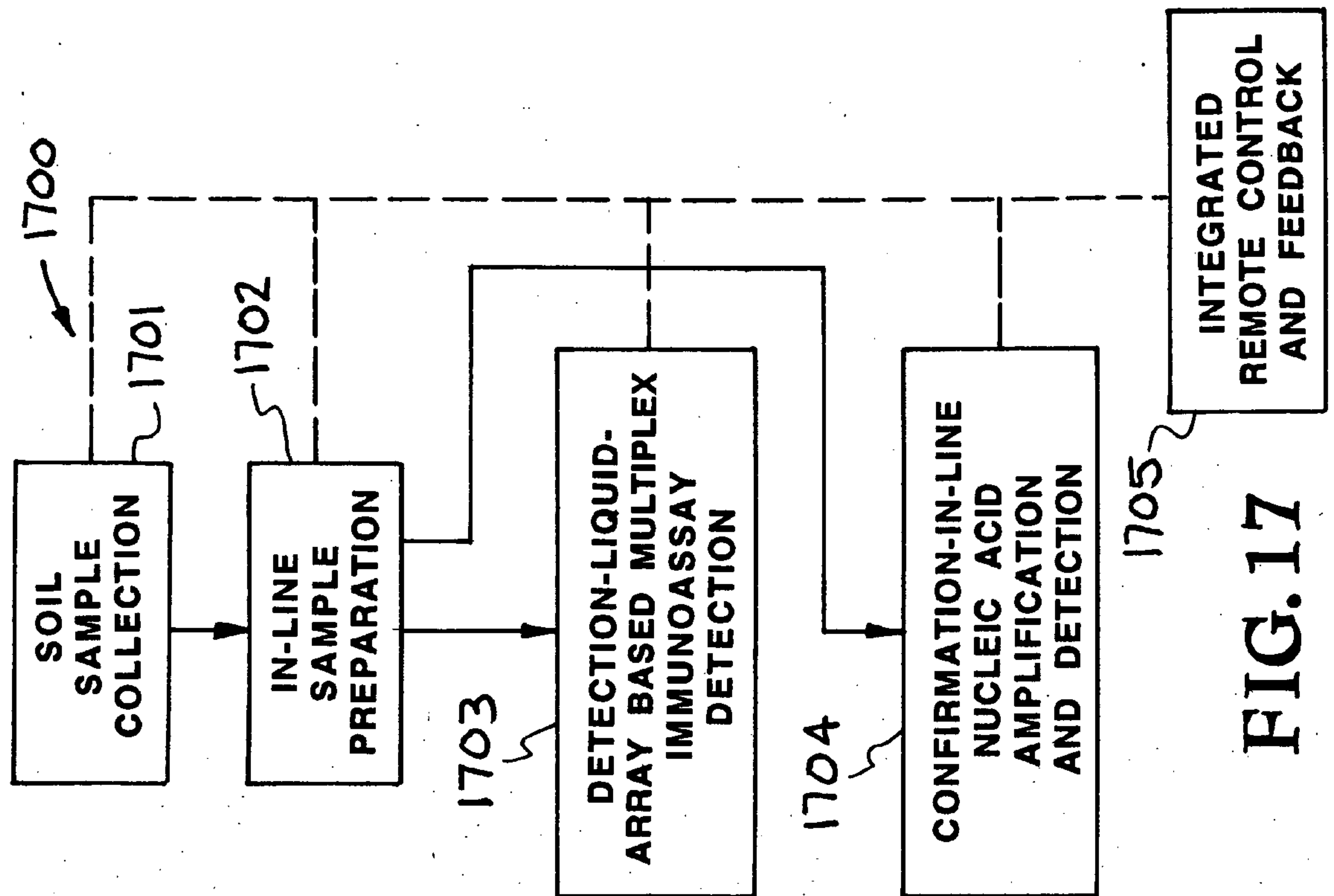


FIG. 17

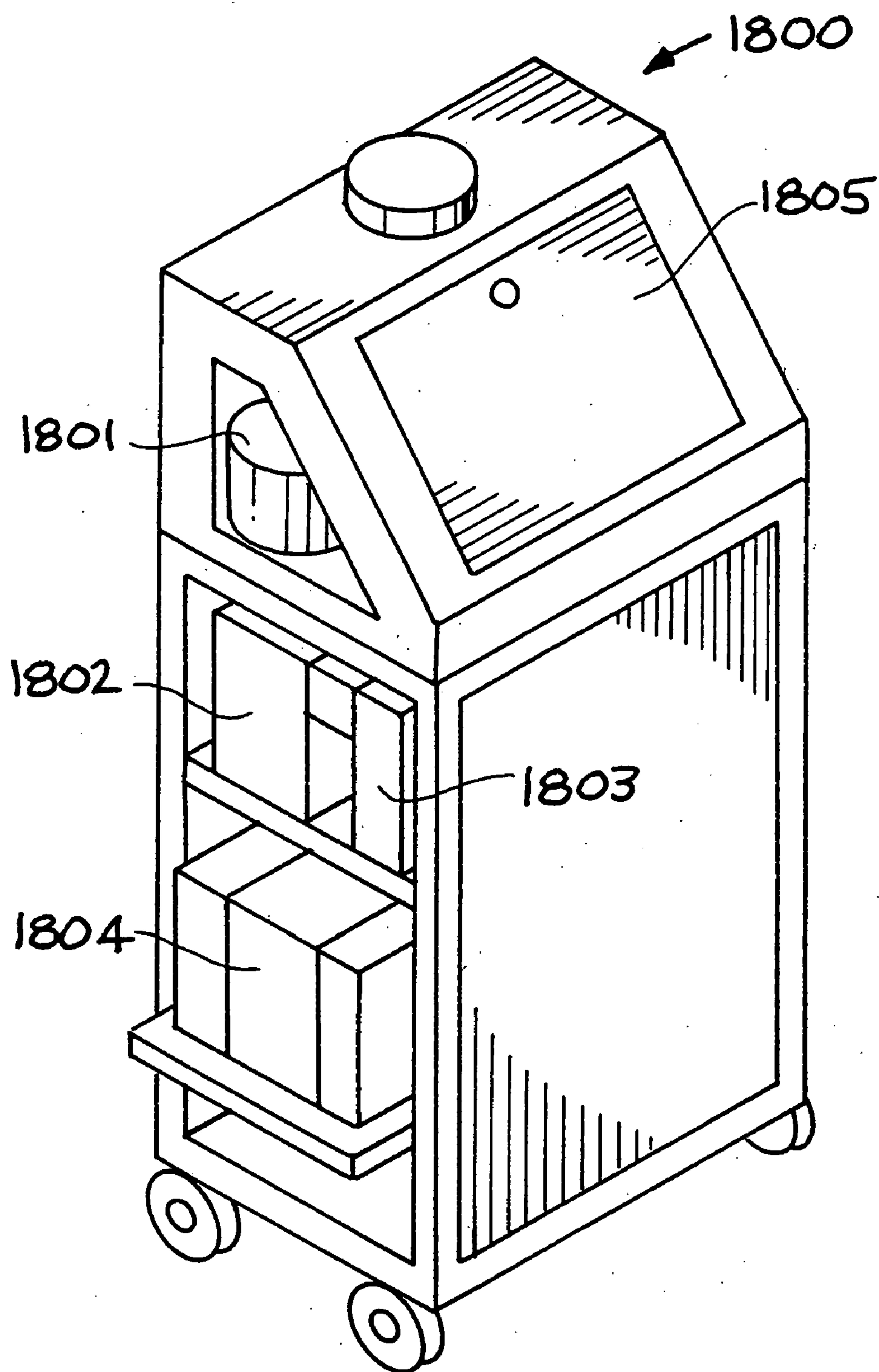


FIG. 18

SYSTEM FOR AUTONOMOUS MONITORING OF BIOAGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation-in-Part of U.S. patent application Ser. No. 10/643797 filed Aug. 19, 2003 and titled, "System for Autonomous Monitoring of Bioagents" which claims the benefit of U.S. Provisional Patent Application No. 60/406159 filed Aug. 26, 2002 titled "System for Autonomous Monitoring of Bioagents." U.S. patent application Ser. No. 10/643797 filed Aug. 19, 2003 and U.S. Provisional Patent Application No. 60/406159 filed Aug. 26, 2002, both titled, "System for Autonomous Monitoring of Bioagents," are incorporated herein by this reference.

[0002] The United States Government has rights in this invention pursuant to Contract No. W-7405-ENG-48 between the United States Department of Energy and the University of California for the operation of Lawrence Livermore National Laboratory.

BACKGROUND

[0003] 1. Field of Endeavor

[0004] The present invention relates to bioagents and more particularly to monitoring bioagents.

[0005] 2. State of Technology

[0006] There exists a critical need to develop distributed biothreat agent sensor networks that can operate in civilian applications. To operate in "Detect to Protect/Warn" type detection architectures, these platforms need to have several key properties. They need to be capable of detecting pathogens within a 1-2 hour time window, allowing for enough time to respond to an event. They need to be extremely low cost to maintain, since continuous monitoring is essential for many applications. These platforms need to have sufficient sensitivity to cover a broad geographical area (limiting the necessary number of sensors) and have sufficient selectivity to virtually eliminate false positives. Currently available bioweapons detection systems are designed primarily for military use on the battlefield. These systems are often expensive to deploy and ultimately unsuited for civilian protection.

[0007] In an article titled, "U.S. Is Deploying a Monitor System for Germ Attacks," by Judith Miller in The New York Times on Jan. 22, 2003, it was reported, "To help protect against the threat of bioterrorism, the Bush administration on Wednesday will start deploying a national system of environmental monitors that is intended to tell within 24 hours whether anthrax, smallpox and other deadly germs have been released into the air, senior administration officials said today. The system uses advanced data analysis that officials said had been quietly adapted since the September 11 attacks and tested over the past nine months. It will adapt many of the Environmental Protection Agency's 3,000 air quality monitoring stations throughout the country to register unusual quantities of a wide range of pathogens that cause diseases that incapacitate and kill The new environmental surveillance system uses monitoring technology and methods developed in part by the Department of Energy's national laboratories. Samples of DNA are analyzed using polymerase chain reaction techniques, which

examine the genetic signatures of the organisms in a sample, and make rapid and accurate evaluations of that organism Officials who helped develop the system said that tests performed at Dugway Proving Ground in Utah and national laboratories showed that the system would almost certainly detect the deliberate release of several of the most dangerous pathogens. 'Obviously, the larger the release, the greater the probability that the agent will be detected,' an official said. 'But given the coverage provided by the E.P.A. system, even a small release, depending on which way the wind was blowing and other meteorological conditions, is likely to be picked up.'"

[0008] In an article titled, "Biodetectors Evolving, Monitoring U.S. Cities," by Sally Cole in the May 2003 issue of Homeland Security Solutions, it was reported, "The anthrax letter attacks of 2001, and subsequent deaths of five people, brought home the reality of bioterrorism to Americans and provided a wake-up call for the U.S. government about the need for a method to detect and mitigate the impact of any such future attacks. Long before the anthrax letter attacks, scientists at two of the U.S. Department of Energy's national laboratories, Lawrence Livermore National Laboratory (LLNL) and Los Alamos National Laboratory (LANL), were busy pioneering a "biodetector" akin to a smoke detector to rapidly detect the criminal use of biological agents. This technology is now expected to play a large role in the U.S. government's recently unveiled homeland security counter-terrorism initiative, Bio-Watch, which is designed to detect airborne bioterrorist attacks on major U.S. cities within hours. Announced back in January, Bio-Watch is a multi-faceted, multi-agency program that involves the U.S. Department of Energy, the Environmental Protection Agency (EPA), and the U.S. Department of Health and Human Services' Centers for Disease Control and Prevention (CDC). Many of the EPA's 3,000 air-quality monitoring stations throughout the country are being adapted with biodetectors to register unusual quantities of a wide range of pathogens that cause diseases that incapacitate and kill, according to the EPA. The nationwide network of environmental monitors and biodetectors, which reportedly will eventually monitor more than 120 U.S. cities, is expected to detect and report a biological attack within 24 hours. Citing security reasons, the EPA declined to disclose further details about the program at this time The Autonomous Pathogen Detection System (APDS) is a file-cabinet-sized machine that sucks in air, runs tests, and reports the results itself. APDS integrates a flow cytometer and real-time PCR detector with sample collection, sample preparation, and fluidics to provide a compact, autonomously operating instrument capable of simultaneously detecting multiple pathogens and/or toxins. The system is designed for fixed locations, says Langlois, where it continuously monitors air samples and automatically reports the presence of specific biological agents. APDS is targeted for domestic applications in which the public is at high risk of exposure to covert releases of bioagents—subway systems, transportation terminals, large office complexes, and convention centers APDS provides the ability to measure up to 100 different agents and controls in a single sample,' Langlois says. 'It's being used in public buildings right now.' The latest evolution of the biodetector, APDS-II, uses bead-capture immunoassays and a compact flow cytometer for the simultaneous identification of multiple biological simulants. Laboratory

tests have demonstrated the fully autonomous operation of APDS-II for as long as 24 hours.”

SUMMARY

[0009] Features and advantages of the present invention will become apparent from the following description. Applicants are providing this description, which includes drawings and examples of specific embodiments, to give a broad representation of the invention. Various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this description and by practice of the invention. The scope of the invention is not intended to be limited to the particular forms disclosed and the invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims. (0008) The present invention provides an autonomous monitoring apparatus for monitoring air, water, soil, or other substance for bioagents. A collector gathers a quantity of the air, water, soil, or other substance being monitored. The collector separates selected potential bioagent particles from the air, water, soil, or other substance that is being collected. A sample preparation system prepares a sample of the selected potential bioagent particles. The sample is analyzed by a system for detecting said bioagents.

[0010] One embodiment includes a wash assay sample preparation system for preparing a sample of the selected potential bioagent particles. The wash assay sample preparation system is operatively connected to the collector and prepares the sample from the air, water, soil, or other substance gathered by the collector. The wash assay sample preparation system includes optically-encoded beads, a number of reagents, a washing buffer, and a detector for detecting any bioagents in the sample.

[0011] Another embodiment includes a no-wash assay sample preparation system for preparing a sample of the selected potential bioagent particles. The no-wash assay sample preparation system is operatively connected to the collector for preparing the sample from the air, water, soil, or other substance gathered by the collector. The no-wash assay sample preparation system includes optically-encoded beads, a number of reagents, and a detector for detecting any bioagents in the sample.

[0012] Another embodiment of the present invention provides an apparatus for sampling air and collecting sample particles of a predetermined particle size range from the air for autonomous monitoring air, water, soil, or other substance for bioagents. A low pass section has an opening for gathering said air. A prescreen unit is positioned in the opening that prevents large particles from blocking the opening.

[0013] The invention is susceptible to modifications and alternative forms. Specific embodiments are shown by way of example. It is to be understood that the invention is not limited to the particular forms disclosed. The invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The accompanying drawings, which are incorporated into and constitute a part of the specification, illustrate

specific embodiments of the invention and, together with the general description of the invention given above, and the detailed description of the specific embodiments, serve to explain the principles of the invention.

[0015] FIG. 1 is a block diagram illustrating an embodiment of an autonomous pathogen detection system constructed in accordance with the present invention.

[0016] FIG. 2 is a block diagram illustrating another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention.

[0017] FIG. 3 is a block diagram illustrating a specific embodiment of the invention designated as an AUTONOMOUS PATHOGEN DETECTION SYSTEM (APDS).

[0018] FIGS. 4A and 4B are illustrations that show the aerosol collection system.

[0019] FIGS. 5A and 5B are illustrations that show the cap section limiting the larger particulate size range entering the collector.

[0020] FIG. 6 is an illustration that shows the virtual impactor section.

[0021] FIG. 7 shows the multistage, wetted-wall cyclone collector section.

[0022] FIGS. 8A, 8B, and 8C show details of a specific embodiment of the aerosol collection system.

[0023] FIG. 9 is an illustration that shows another embodiment of the aerosol collection system.

[0024] FIG. 10 illustrates a system for sample preparation and detection.

[0025] FIGS. 11, 12, and 13 illustrate the liquid-array based multiplex immunoassay detection system.

[0026] FIG. 14 is a block diagram illustrating the multiplex amplification and detection system.

[0027] FIG. 15 illustrates one specific embodiment of the in-line nucleic acid amplification and detection system.

[0028] FIG. 16 is a block diagram illustrating another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention.

[0029] FIG. 17 is a block diagram illustrating another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention.

[0030] FIG. 18 illustrates another embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Referring now to the drawings, to the following detailed description, and to incorporated materials, detailed information about the present invention is provided including the description of specific embodiments. The detailed description and the specific embodiments serve to explain the principles of the invention. The invention is susceptible to modifications and alternative forms. The invention is not limited to the particular forms disclosed. The invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims.

[0032] Terrorists sending anthrax-contaminated packages. Militant organizations obtaining potassium cyanide. Religious cult members poisoning local residents to fix an election. Sadly, these scenarios are not the plots of the three latest bestsellers, but rather, very real incidents with a very real danger. By the mid-1990s, the U.S. Congress began to assess the vulnerability of the U.S. civilian population to biological terrorism and found us considerably lacking in our ability to cope with even a small-scale biological event. Initial thinking was that Department of Defense technology could be readily transferred to the civilian arena. However, upon further reflection, it was concluded that although there was overlap between military and civilian defense needs, in the case of a biological threat, there are marked differences: (1) the soldier is trained and equipped with protective gear so he may respond to a threat quickly enough to prevent a lethal dose; (2) military intelligence usually reduces the potential threat to a relatively small number of biological agents; and, (3) military battlefield tactics are designed to minimize the density of soldiers. The civilian population, however, is neither trained nor equipped, is vulnerable to any conceivable pathogen and often gathers in large crowds (special events, sporting venues, etc.) where a small release could potentially infect thousands. In response to these differences, federal agencies, including Department of Energy, have recently begun funding directed research efforts to reduce civilian biological terrorist vulnerabilities.

[0033] At present there are more than 30 pathogens and toxins on various agency threat lists. Public health personnel rarely see most, of the pathogens so they have difficulty identifying them quickly. In addition, many pathogenic infections aren't immediately symptomatic, with delays as long as several days, limiting options to control the disease and treat the patients. The lack of a practical monitoring network capable of rapidly detecting and identifying multiple pathogens or toxins on current threat lists translates into a major deficiency in the United States ability to counter biological terrorism.

[0034] Referring now to **FIG. 1**, an embodiment of an autonomous pathogen detection system constructed in accordance with the present invention is illustrated by a block diagram. The autonomous pathogen detection system is designated generally by the reference numeral **100**. The autonomous pathogen detection system **100** provides collection **101**, sample preparation **103**, and detection **105**. The collection **101** includes gathering air, water, soil or other substance to provide an air sample, water sample, soil sample or a sample of other substances.

[0035] After the collection **101**, the sample is transferred as shown by arrow **102** for sample preparation **103**. The sample preparation **103** provides an automated sample, an immunoassay sample, and/or a nucleic acid assay sample. In sample preparation **103** the sample may be concentrated, purified, lysed, pulverized or otherwise made to have smaller particulates, mixed, and/or amplified.

[0036] After sample preparation **103**, the sample is transferred as shown by arrow **104** for detection. In one embodiment of the autonomous pathogen detection system **100**, the detection is by a multiplex immunoassay detector. In another embodiment of the autonomous pathogen detection system **100**, the detection is by a multiplex PCR detector. The

detection may also be performed in the same location as the sample preparation, in which case the transfer arrow **104** is not required.

[0037] The autonomous pathogen detection system **100** provides an apparatus and method for monitoring air, water, soil, or other substance for particles containing bioagents. The autonomous pathogen detection system **100** comprises a collector for gathering the air, water, soil, or other substance being monitored; sample preparation system for preparing a sample from the air, water, soil, or other substance gathered by the collector; and a detector for detecting any bioagents in the sample. In one embodiment the collector is an aerosol collector. In other embodiments the collector gathers water, soil, or other substances. The collector in one embodiment includes separator system for separating the particles of interest from other particles. The particles of interest are of a predetermined size range.

[0038] In one embodiment the collector is an aerosol collector that collects air and includes a system for separating the air into a bypass air flow that does not contain the particles of a predetermined particle size range and a product air flow that does contain the sample particles of a predetermined particle size range. A wetted-wall cyclone collector receives the product air flow and traps and concentrates the particles of a predetermined particle size range in a liquid.

[0039] In one embodiment the sample preparation system is automated. In one embodiment the sample preparation system provides an immunoassay sample. In another embodiment the sample preparation system provides a nucleic acid assay sample. In another embodiment the sample preparation system includes concentration of the air, water, soil, or other substance. In another embodiment the sample preparation system includes purification of the air, water, soil, or other substance. In another embodiment the sample preparation system includes lysis of the air, water, soil, or other substance. In another embodiment the sample preparation system includes mixing of the air, water, soil, or other substance. In another embodiment the sample preparation system includes amplification.

[0040] In one embodiment of the autonomous pathogen detection system **100**, the detector is a multiplex immunoassay detector. In one embodiment of the autonomous pathogen detection system **100**, the detector is a multiplex PCR detector.

[0041] The primary focus of the autonomous pathogen detection system **100** is the protection of civilians from terrorist attacks; however, the system also has a role in protecting military personnel from biological warfare attacks. The autonomous pathogen detection system **100** also has uses in medical facilities and research and development facilities. The autonomous pathogen detection system **100** has uses in medical monitoring. There are a variety of medical applications where monitoring for biological pathogens would be useful. A good example of this is monitoring in hospitals and clinics for highly infectious agents such as tuberculosis or nosocomial diseases that can threaten the well being of patients and health care professionals. The autonomous pathogen detection system **100** also has uses in environmental monitoring, that is, any application that would benefit from environmental monitoring of biological species. One example is continuous aerosol monitoring of bacterial and other pathogens that could affect

the health of livestock (such as the recent hoof and mouth disease outbreak). Another example is continuous aerosol monitoring of viruses that could affect the health of large portions of the population (such as the recent SARS outbreak).

[0042] Referring now to **FIG. 2**, another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention is illustrated by a block diagram. This embodiment of the autonomous pathogen detection system is designated generally by the reference numeral **200**. The autonomous pathogen detection system **200** provides collection **201**, sample preparation **203**, detection **205**, and confirmation **207**. The collection **201** includes gathering air, water, soil or other substance to provide an air sample, water sample, soil sample or a sample of other substances.

[0043] After the collection **201**, the sample is transferred as illustrated by arrow **202** for sample preparation **203**. The sample preparation **203** provides an automated sample, an immunoassay sample, and/or a nucleic acid assay sample. In the sample preparation **203** the sample may be concentrated, purified, lysed, pulverized or otherwise made to have smaller particulates, mixed, and/or amplified.

[0044] After sample preparation **203**, the sample is transferred as illustrated by arrow **204** for detection. In one embodiment of the autonomous pathogen detection system **200**, the detection is by a multiplex immunoassay detector. In another embodiment of the autonomous pathogen detection system **200**, the detection is by a multiplex PCR detector.

[0045] After sample preparation **203** and detection **205** when a pathogen has been detected, a sample is transferred from sample preparation **203** to the confirmation module **207**. This is illustrated by arrow **206** in **FIG. 2**. In one embodiment, the system for confirmation of a bioagent in the sample is a multiplex immunoassay detector. In one embodiment of the autonomous pathogen detection system **200**, the system for confirmation of a bioagent in the sample is a multiplex PCR detector. In one embodiment of the autonomous pathogen detection system **200**, the system for confirmation of a bioagent in the sample is a real-time PCR detector. The detection may also be performed in the same location as the sample preparation, in which case the transfer arrows **204** and/or **206** are not required.

[0046] The autonomous pathogen detection system **200** provides an apparatus and method for monitoring air, water, soil, or other substance for particles containing bioagents. The autonomous pathogen detection system **200** comprises a collector for gathering the air, water, soil, or other substance being monitored; sample preparation system for preparing a sample from the air, water, soil, or other substance gathered by the collector; a detector for detecting a bioagents in the sample; and a system for confirmation of a bioagent in the sample. In one embodiment the collector is an aerosol collector. In other embodiments the collector gathers water, soil, or other substances. The collector in one embodiment includes separator system for separating the particles of interest from other particles. The particles of interest are of a predetermined size range.

[0047] In one embodiment the collector is an aerosol collector that collects air and includes system for separating

the air into a bypass air flow that does not contain the particles of a predetermined particle size range and a product air flow that does contain the sample particles of a predetermined particle size range. A wetted-wall cyclone collector receives the product air flow and traps and concentrates the particles of a predetermined particle size range in a liquid.

[0048] In one embodiment the sample preparation system is automated. In one embodiment the sample preparation system provides an immunoassay sample. In another embodiment the sample preparation system provides a nucleic acid assay sample. In another embodiment the sample preparation system includes concentration of the air, water, soil, or other substance. In another embodiment the sample preparation system includes purification of the air, water, soil, or other substance. In another embodiment the sample preparation system includes lysis of the air, water, soil, or other substance. In another embodiment the sample preparation system includes mixing of the air, water, soil, or other substance. In another embodiment the sample preparation system includes amplification of the sample.

[0049] In one embodiment of the autonomous pathogen detection system **200**, the detector **205** is a multiplex immunoassay detector. In one embodiment of the autonomous pathogen detection system **200**, the detector **205** is a multiplex PCR detector.

[0050] In one embodiment of the autonomous pathogen detection system **200**, the system **207** for confirmation of a bioagent in the sample is a multiplex immunoassay detector. In one embodiment of the autonomous pathogen detection system **200**, the system **207** for confirmation of a bioagent in the sample is a multiplex PCR detector. In one embodiment of the autonomous pathogen detection system **200**, the system **207** for confirmation of a bioagent in the sample is a real-time PCR detector.

[0051] Referring now to **FIG. 3** through **FIG. 12** a specific embodiment of the invention designated as an AUTONOMOUS PATHOGEN DETECTION SYSTEM (APDS) is shown. The APDS is designated generally by the reference numeral **300**. The APDS **300** integrates a flow cytometer and PCR detector with sample collection, sample preparation, and fluidics to provide a compact, autonomously operating instrument capable of simultaneously detecting multiple pathogens and/or toxins. The APDS **300** is designed for locations where it continuously monitors air samples and automatically reports the presence of specific biological agents. Plague and anthrax are two of the pathogens the APDS **300** identifies, along with a host of others. The APDS **300** includes the potential to measure up to **100** different agents and controls in a single sample.

[0052] The APDS **300** provides a stand-alone pathogen detection system capable of rapid, continuous, low cost environmental monitoring of multiple airborne biological threat agents. The system **300** provides a "Detect to Protect/Warn" system with a number of key properties. The system **300** is capable of detecting pathogens within a 1-2 hour time window, allowing for enough time to respond to an event. The system **300** is extremely low cost to maintain, since continuous monitoring is essential for many applications. The system **300** has sufficient sensitivity to cover a broad geographical area (limiting the necessary number of sensors) and has sufficient selectivity to virtually eliminate false positives.

[0053] Multiplexed assays are used to reduce reagent costs, making long term monitoring operations possible. An orthogonal detection section combines antibody-based and nucleic acid-based assays and reduces false positives to a very low level. Antibody assays allow the detector to respond to all types of bioagents, including those without nucleic acids such as protein toxins. Nucleic acid assays allow much more sensitive detection, reducing the number of sensors needed to protect a given area. The fully autonomous aerosol collection and sample preparation capabilities limit maintenance requirements and makes integration into a central security or monitoring network possible.

[0054] Referring again to **FIG. 3**, a block diagram illustrates the APDS **300**. In operation, an aerosol collector system continuously samples the air and traps particles in a swirling liquid solution. Particles of a given size distribution are selected by varying the flow rate across a virtual impactor unit. The in-line sample preparation system provides all sample preparation steps (i.e., mix, wash, incubation, etc.), and performs multiplex detection using a Luminex flow cytometer.

[0055] In the “detection” sub-system, a collected sample is mixed with optically encoded microbeads. Each color of microbead contains a capture assay that is specific for a given bioagent. Fluorescent labels are added to identify the presence of each agent on the bound bead. Each optically encoded and fluorescently labeled microbead is individually read in a flow cytometer, and fluorescent intensities are then correlated with bioagent concentrations.

[0056] In the “confirmation” sub-system, PCR amplification and detection of nucleic acids confirms the presence of the bioagent. An archived sample is mixed with the TaqMan real-time PCR reagent, and then introduced by a SIA system into a flow-through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified and detected using fluorescence generated from nucleic acid replication from the TaqMan probes.

[0057] In the “Integrated Remote Control and Feedback” sub-system, a central computer uses a simple serial based LabVIEW control system to control all instrument functions. A software system provides data acquisition, real-time data analysis, and result reporting via a graphical user interface.

[0058] The APDS **300** is integrated into a self-contained “ATM” style chassis. All fluids and reagents are contained in the instrument. The ADPS **300** includes the following sub-systems: Aerosol Collection **301**, In-Line Sample Preparation **302**, Detection—Liquid-Array Based Multiplex Immunoassay Detection and/or Nucleic Assays Detection **303**, Confirmation—In-Line Nucleic Acid Amplification and Detection **304**, and Integrated Remote Control and Feedback **305**. The subsystem will be described in greater detail.

APDS Aerosol Collection—**301**

[0059] The first stage of the APADS **300** is “aerosol collection” that provides collection of airborne particles that could contain targeted bioagents. Aerosol release of bioagents is considered one of the possible scenarios of a terrorist organization. One of the methods of rapidly exposing a large population to a biowarfare agent is through use of an aerosol (witness the effect of the recent, relatively small-scale

anthrax mailroom releases). The aerosol collection system **301** continuously samples the air and traps particles in a swirling liquid solution. Particles of a given size distribution are selected by varying the flow rate across a virtual impactor unit.

[0060] The aerosol collection system **301** is a multi-stage aerosol collector that utilizes a low pass aerosol section and a virtual impactor preconcentration that delivers the particles of interest to a wetted wall cyclone collector. The virtual impactor preferentially captures particles 1-10 micrometer (micron) which is the size of particles most likely to be captured in the human lung. In the wetted wall cyclone collector, the particles are collected in a fluid, making downstream processing much easier. The fans and inputs to the obtain high collection rates, up to 3000 liters of air per minute flow through the detection system, allowing many particles to be collected over a short period. The aerosol collection system provides improved sensitivity and reduced collection times. An on-board computer controls air flow rates and the size range of particles collected.

[0061] As shown by **FIGS. 4A and 5A**, a very high volume flow of aerosol particles is drawn into an annular slot **401A** formed in a cap **402A** that is designed to preferentially allow the passage of particles smaller than a pre-set size. The pre-set size can be selected as desired. A very high volume flow of aerosol particles (e.g., up to 3313 Lpm) can be drawn into the annular slot **401A** formed in the cap **402A** that is designed to preferentially allow the passage of particles smaller than 10 microns. The accepted particles continue on into a dichotomous virtual impaction section **403A** that preferentially returns the aerosol particles smaller than 1-micron back into the environment. The remaining particles, (1-10 microns) are known as the product flow. The product flow continues into the next section.

[0062] As best illustrated by **FIG. 5A**, a high volume flow of aerosol particles is drawn into the annular slot **401A** formed in the cap **402A**. The annular slot **401A** is designed to limit the upper or larger particulate size range as they enter the collector. To efficiently pass the smaller particulate, the cap **402A** is a “passive” device in that it has no moving parts and uses the fact that particulate with a finite mass and moving in a flow stream (in this case air) will not follow the streamlines exactly due to their inertia. If the curvature of a streamline is sufficiently large and the mass of the particulate is correspondingly high, the particle deviates far enough from the streamline to impact with a surface. The particles are drawn into the annular slot **401A** and directed into the transition section **409A**.

[0063] Referring now to **FIGS. 4B and 5B**, another embodiment of an aerosol collection system is illustrated. This embodiment is designated generally by the reference numeral **300B**. In the embodiment **300B**, a very high volume flow of aerosol particles **405B** is drawn into an annular slot **401B** formed in a cap **402B** that is designed to only allow the passage of particles smaller than a pre-set size. The pre-set size can be selected as desired. A very high volume flow of aerosol particles **405B** can be drawn into the annular slot **401B** formed in the cap **402B** that is designed to only allow the passage of particles smaller than 10 microns. A pre-screen **404B** prevents large particles from blocking small flow paths such as the flow path **401B**. The accepted particles continue on into a dichotomous virtual impaction

section **403B** that returns all the aerosol particles smaller than 1-micron back into the environment. The remaining particles, (1-10 microns) are known as the product flow. The product flow continues into the next section. **(0047)** The pre-screen unit **404B** in one embodiment is a grill positioned in the opening **401B**. The pre-screen unit in another embodiment is a screen positioned in the opening **401B**. In another embodiment, the opening **401B** in the low pass section is an annular slot and the pre-screen unit **404B** is an annular screen positioned in the opening **401B**. In another embodiment, the pre-screen **404B** is located inside the cap **402B** covering the top of the inner cylinder shown as **409B** in **FIGS. 5A and 5B**.

[0064] As best illustrated by **FIG. 5**, in the embodiment **300B** a high volume flow of aerosol particles **405B** is drawn into the annular slot **401B** formed in the cap **402B**. The annular slot **401B** is designed to limit the upper or larger particulate size range as they enter the collector. The embodiment **300B** is particularly suited for operating in dirty environments such as subway stations. A prescreen unit **404B** is positioned at the entrance to the annular slot **401B** to prevent large particles from blocking the small flow paths of the virtual impactor.

[0065] To efficiently pass the smaller particulate, the cap **402B** is a “passive” device in that it has no moving parts and uses the fact that particulate with a finite mass and moving in a flow stream (in this case air) will not follow the streamlines exactly due to their inertia. If the curvature of a streamline is sufficiently large and the mass of the particulate is correspondingly high, the particle deviates far enough from the streamline to impact with a surface. The particles are drawn through the pre-screen **404B** into the annular slot **401B** and directed into the transition section **409B**. In operation of the embodiment **300B**, nightly clean-in-place protocols are implemented to keep the walls of the wetted-wall-cyclone clean using reagents such as dilute bleach and surfactant solutions. Also, the optical bubble counter is cleaned periodically with a pipe-cleaner, catheter, or other such tool.

[0066] The APDS **300A** has the capability to measure particle sizes in the sampling environment via a built in particle counter with four size ranges, and can store and display the results in real-time. The system is entirely self-contained requiring only a 110 V ac power connection. The on-board computer has high-speed communications capability allowing networks of these sampling systems to be remotely operated.

[0067] The APDS **300A** is useful for most environmental sampling. It is particularly useful with biological material collection, but can be used for collecting any airborne matter. The APDS **300A** can be used to sample air quality in public buildings such as convention centers and sports arenas, for sampling in food processing facilities, sampling animal pens (such as poultry houses), or for use in monitoring orchards or agricultural areas for the presence of pollens or pesticides. Because of its relatively compact size and weight it can be used to sample in confined spaces such as found in aircraft or subway systems.

[0068] Referring now to **FIG. 6**, the virtual impactor section **403** is shown in greater detail. In the virtual impactor section **403**, the separation efficiency is determined by the ratio of the major and minor flows (or Bypass to Product)

and the physical dimensions of the nozzle and collection probe. The key is particulate larger than the cut size become concentrated in the minor flow. The concentration factor is the ratio of the total flow to the minor flow. (If the minor flow is 25% of the total flow, then the concentration factor is 4.) The aerosol passes through an acceleration nozzle **601**. The acceleration nozzle **601** has a diameter D_0 . The aerosol is directed toward a collection probe **602**. The collection probe has a diameter D_1 . Between the acceleration nozzle **601** and the collection probe **602**, a major portion of the flow **603** is diverted 90° away. The minor or “product” flow **604** continues axially.

[0069] The flow forms streamlines **605**. Small particles with low inertia **606** follow the flow streamlines and are carried away radially with the major flow **603**. Large particles with greater inertia **607** deviate from the flowlines but they continue moving axially in their forward path down the collection probe **602** with the minor or “product” flow **604**. The separation efficiency is determined by the ratio of the major and minor flows (or Bypass to Product) and the physical dimensions of the nozzle D_0 and collection probe D_1 . The key is particulate larger than the cut size become concentrated in the minor flow. The concentration factor is the ratio of the total flow to the minor flow.

[0070] Referring now to **FIG. 7**, additional details of the sample collection operation are shown. The particles, (1-10 microns) known as the product flow are directed into a multi-stage, wetted-wall cyclone collection section. In this stage of the sampling system the product particles are trapped and concentrated into a liquid, typically water, in a volume between 2 and 7 cc. An on-board computer monitors and controls the flow of air through the system using built in hot wire anemometers, as well as controlling the liquid level in the cyclone. At a selected time the computer will stop the flow of air and turn on a built-in peristaltic pump to deliver the sample via an external liquid sample port.

[0071] The product flow particles enter a stainless steel funnel section into the input of a multistage, wetted-wall cyclone collector section **700**. The system includes a cyclone collector **701**, peristaltic pump **707**, an air pump **704**, a vent **706**, wash **705**, 8 liter DD-H₂O (double deionized water) reservoir **702**, and 1 liter bleach reservoir **703**. The reservoirs **702** and **703** are provided as external tanks outside of the front panel interface **708**. The multistage, wetted-wall cyclone collector section **700** directs the particles of interest to the sample preparation system **302**.

[0072] The on-board computer monitors and controls the flow of air through the system using built in hot wire anemometers that have been mounted in the two exhaust ports of the sampler. The computer and control software also act to control the liquid level in the cyclone, and monitor all status indicators of the sampling system. At a selected time the computer will stop the flow of air and turn on a built-in peristaltic pump to deliver the collected liquid sample via an external sample port. The system also has the capability to measure particle sizes in the background environment via a built in particle counter such as particle counter Biotest APC-1000, with four size ranges, and can store and display the results in real-time.

[0073] The system **300** is entirely self-contained requiring only a 110 vac power connection. The on-board computer has high-speed communications capability allowing net-

working of multiple sampling systems to be remotely operated. The computer has extra RS-232 or RS-485 serial ports that can be used to control other instrumentation. A keyboard, mouse, printer, displays, and other peripherals can be “plugged” in at the rear of the system, or it can be started “headless” (headless=Without a display, mouse, etc.)

[0074] Referring now to **FIGS. 8A, 8B, and 8C**, the APDS Aerosol Collection **301** and APDS In-Line Sample Preparation **302** sub systems are shown in greater detail. The aerosol collection system **301** is designated “High Collection Rate Aerosol Sampling System” (HiCRASS). The HiCRASS comprises: Low Pass “Cap” **402**; Transition Section **409**; Virtual Impactor **403**; Funnel Section **410**; Multi-stage, Wetted-wall Cyclone Collector **700**; Bypass Fan **412**; and Control Computer **714**.

[0075] The HiCRASS system provides a very high volume flow of aerosol particles (e.g., up to 3313 Lpm) that are drawn into the annular slot **401** formed in the cap **402** that is designed to limit the upper or larger particulate size range as they enter the collector. The annular slot **401** allows the passage of particles smaller than 10 microns. To efficiently pass the smaller particulate, the cap **402** is a “passive” device in that it has no moving parts and uses the fact that particulate with a finite mass and moving in a flowstream (in this case air) will not follow the streamlines exactly due to their inertia. The curvature of the streamline is sufficiently large and the mass of the particulate is correspondingly high that the particle deviates far enough from the streamline to impact with a surface. The accepted particles continue around the corner and onto the dichotomous virtual impaction section **403** that returns substantially all the aerosol particles smaller than 1-micron back into the environment.

[0076] The virtual impactor **403** works as the aerosol passes through an accelerating nozzle **601** and is directed toward a collection probe **602** where a major portion of the flow **603** is diverted 90° away from it. The flow forms streamlines **605**. Small particles with low inertia **606** follow the flow streamlines and are carried away radially with the major flow **603**. Large particles with greater inertia **607** deviate from the flowlines but they continue moving axially in their forward path down the collection probe **602** with the minor or “product” flow **604**. The separation efficiency is determined by the ratio of the major and minor flows (or Bypass to Product) and the physical dimensions of the nozzle D_0 and collection probe D_1 . Particulate larger than the cut size become concentrated in the minor flow. The concentration factor is the ratio of the total flow to the minor flow. (If the minor flow is 25% of the total flow, then the concentration factor is 4).

[0077] The remaining particles (1-10 microns) now known as the product **604**, flow down a stainless steel funnel section into the input of the multistage, wetted-wall cyclone collector section **700**. In this stage of the system **301** the product particles are trapped and concentrated into a liquid, typically water, in a volume between 2 and 7 cc. The wetted-wall cyclone collector section **700** is a system that causes the product flow particles **604** to be collected by a liquid. The wetted-wall cyclone collector section **700** operates by forcing the air stream tangentially into a cylinder causing the air stream to circulate around the inside of the cylinder. Particles in the air stream having sufficient inertia will collide with the interior wall where they are collected by the liquid that circulates along the interior wall.

[0078] The on-board computer **714** monitors and controls the flow of air through the system using built-in hot wire anemometers, as well as controlling the liquid level in the cyclone **700**. At a selected time the computer **714** will stop the flow of air and turn on a built-in peristaltic pump to deliver the sample via an external sample port. The on-board computer **714** monitors and controls the flow of air through the system using built in hot wire anemometers that have been mounted in the two exhaust ports of the sampler. The computer and control software also act to control the liquid level in the cyclone, and monitor all status indicators of the sampling system. At a selected time the computer will stop the flow of air and turn on a built-in peristaltic pump to deliver the collected liquid sample via an external sample port.

[0079] The system also has the capability to measure particle sizes in the sampling environment via a built in particle counter such as particle counter Biotest APC-1000, with four size ranges, and can store and display the results in real-time. The system is entirely self-contained requiring only a 110 vac power connection. The on-board computer has high-speed communications capability allowing networks of these sampling systems to be remotely operated.

[0080] Referring now to **FIG. 9**, another embodiment of the collection section of the present invention is illustrated. This collection section system is designated generally by the reference numeral **900**. The system **900** samples the air **901** and collects sample particles of a predetermined particle size range from the air. The system **900** is particularly useful with the latest generation of Biological Warfare agent detection systems. An air sampling system is a critical component in integrated biological warfare detection system. The system **900** also has use in medical facilities and research and development facilities.

[0081] A low pass section **902** has an opening of a preselected size for gathering the air **901** but excluding particles larger than the sample particles. In one embodiment, the opening of a preselected size is an annular slot that only allows the passage of particles smaller than 10 microns. The low pass section **902** produces a total air flow **903** that contains the sample particles of a predetermined particle size range. The low pass section **902** allows a very high volume flow of air to be drawn through the preselected size opening. In one embodiment, the very high volume flow of air is 3313 Lpm or less.

[0082] An impactor section **904** is connected to the low pass section **902** and receives the total air flow **903**. The impactor section **904** separating the total air flow **903** into a bypass air flow **905** that does not contain the sample particles and a product air flow **906** that does contain the sample particles. An accelerating nozzle and a collection probe in the impactor section **904** diverts the bypass air flow 90° from the product air flow thereby separating the bypass air flow and the product air flow. In one embodiment, the bypass air flow and the product air flow separation is determined by the ratio of the bypass air flow and the product air flow. In one embodiment, the bypass air flow and the product air flow separation is determined by the physical dimensions of the accelerating nozzle and the collection probe. In one embodiment, the bypass air flow and the product air flow separation is determined by the ratio of the

bypass air flow and the product air flow and the physical dimensions of the accelerating nozzle and the collection probe.

[0083] A wetted-wall cyclone collector section **907** is connected to the impactor section **904**. The wetted-wall cyclone collector section **907** receives the product air flow **906** and traps the sample particles in a liquid. The sample particles of a predetermined particle size range are concentrated in the liquid. In one embodiment, the wetted-wall cyclone collector section **907** traps and concentrates the sample particles into a liquid in a volume between 2 and 7 cc. In one embodiment, the liquid is water.

[0084] The system **900** is useful for most environmental sampling. It is particularly useful with biological material collection, but can be used for collecting any airborne matter. The system **900** can be used to sample air quality in public buildings such as convention centers and sports arenas, for sampling in food processing facilities, sampling animal pens (such as poultry houses), or for use in monitoring orchards or agricultural areas for the presence of pollens or pesticides. Because of its relatively compact size and weight it can be used to sample in confined spaces such as found in aircraft or subway systems.

APDS In-Line Sample Preparation—**302**

[0085] As best illustrated in **FIG. 3**, the in-line sample preparation module **302** moves the sample from the aerosol collection module **301** to appropriate modules within the APDS **300** and provides sample preparation. In one mode, the sample preparation module **302** prepares the sample (mixing, filtering, incubation, etc.) and delivers the sample reaction volume to the liquid-array based multiplex immunoassay detection system **303**. In another mode, the sample preparation module **302** prepares the sample (mixing, filtering, incubation, etc.) and delivers the sample reaction volume to the in-line nucleic acid detection system **304**.

[0086] The prior art sample preparation instrumentation uses robotic manipulation of micropipettes coupled to disposable filter wells. Robotics are inherently complex and difficult to scale. The sample preparation module **302** uses Zone fluidics. Zone fluidics is the precisely controlled physical, chemical, and fluid-dynamic manipulation of zones of miscible and immiscible fluids in narrow bore conduits to accomplish sample conditioning and chemical analysis. A zone is a volume region within a flow conduit containing at least one unique characteristic. A unit operation in zone fluidics comprises of a set of fluid handling steps intended to contribute to the transformation of the sample into a detectable species or prepare it for manipulation in subsequent unit operations. Examples of unit operations include sample filtering, dilution, enrichment, medium exchange, headspace sampling, solvent extraction, matrix elimination, de-bubbling, amplifying, hybridizing, and reacting. In current analytical practice many of these steps are handled manually or in isolated pieces of equipment. Integration is scant at best, and there is a high degree of analyst involvement. In zone fluidics, sample and reagent zones are subjected to these unit operations in a sequential manner being transported from one unit operation to the next under fluidic control.

[0087] Samples in zone fluidics are not limited to liquids. Rather, gases, and suspensions containing solids or cells are also included. Where solid samples are used, particles are

limited to a size that ensures no blockages. In most cases, reagents are prepared and then coupled to the zone fluidics manifold. The metering capability of the pump and mixing unit operations allow for reagents and standards to be prepared in situ. Reagents can therefore be presented to the zone fluidics manifold in an appropriately designed cartridge as ready-made, reagent concentrates, lyophilized, or crystalline form. Standards can be plumbed to the multi-position valve as discrete reservoirs providing the required range of concentrations. As for reagents though, standards can also be prepared in situ or diluted to cover a larger dynamic range.

[0088] The sample preparation module **302** uses a powerful, highly flexible technique called sequential injection analysis (SIA). Automation is achieved through the manipulation of small solution zones under conditions of controlled dispersion in narrow bore tubing. Zone fluidics makes use of a multi-position selection valve and a high precision, bi-directional pump to construct a stack of well-defined sample and reagent zones in a holding coil of narrow bore tubing. By appropriate manipulation of this zone stack, a wide range of sample handling unit operations can be accommodated. The pump is used to move the sample from one device to the next achieving the required sample manipulation in the process. Once a detectable species has been formed, the zone stack is transported to the immunoassay detector **303** and to the nucleic acid detector **304**.

[0089] Various embodiments of system for sample preparation and detection have been described in connection with **FIGS. 10-13**. Applicants will now describe additional embodiments of the immunoassay system of the present invention. These embodiments of the immunoassay system can use either “wash assay” system or “no-wash assay” system. The “wash assay” system and the “no-wash assay” system use optically-encoded beads. The beads are kept in a small (~15 mL) stirred tank. Bead loss from agglomeration is reduced by using dispersing agents such as ethanol in the bead slurry. This reduces reagent cost.

[0090] The wash assay system embodiment uses a number of reagents in addition to the liquid sample. The reagents include detector antibody, and fluorescent reporter (streptavidin-phycoerythrin). In the wash assay system, a bed of beads is deposited on a filter then exposed to the sample, washing buffer, detector antibody, washing buffer, fluorescent reporter, washing buffer, and then the beads are released to the detector. For the wash assay system, the bead filter performance in releasing beads is improved by implementing frequent clean-in-place protocols using reagents such as bleach for cleaning and morpholine propane sulfonic acid (MOPS) citrate buffer for reconditioning the filter. This extends the time between servicing the instrument and thus reduces operating cost.

[0091] In the no-wash assay embodiment, the reagents are sequentially mixed but the embodiment does not include the filter or the wash steps. It has been shown that the wash assays are more sensitive and selective, providing better signal-to-noise ratios in response to bioagents.

[0092] Referring now to **FIG. 14**, another embodiment of a system constructed in accordance with the present invention is illustrated. The system is designated generally by the reference numeral **1400**. The system **1400** comprises the following: Sample Collection **1401**, Sample Preparation **1402**, Multiplex Amplification PCR **1403**, and Multiplex, Liquid Array Based Detection of PCR Amplicons **1404**.

[0093] The first stage of the system **1400** is “sample collection **1401**” that provides collection of particles that could contain targeted bioagents. The sample collection **1401** gathers air, water, soil, or other substance being monitored. The sample collection **1401** separates selected potential bioagent particles from the air, water, soil, or other substance.

[0094] The “sample preparation **1402**” moves the sample from the sample collection to appropriate modules within the system **1400** and provides sample preparation. In one mode, the sample preparation **1402** prepares the sample (Lysis, Concentration, Purification, Mixing, etc.) and delivers the sample to “Multiplex Amplification PCR **1403**.” One mode provides “Multiplex, Liquid Array Based Detection of PCR Amplicons **1404**.” An example of a flow cytometric detection method for DNA samples is shown in U.S. patent application 2002/0155482 by Shanavaz Nasarabadi, Richard G. Langlois, and Kodumudi Venkateswaran published Oct. 24, 2002. The disclosure of U.S. patent application 2002/0155482 is incorporated herein by reference.

[0095] Referring now to **FIG. 15**, one specific embodiment of the in-line nucleic acid amplification and detection system **1500** is illustrated. The system **1500** is capable of performing, singly or in combination, nucleic acid amplification, and nucleic acid detection functions. The nucleic acid assay system **1500** includes a number of components including system for injecting/aspirating a sample, **1501**, system for adding PCR reagent **1502**, system for mixing sample and reagent **1503**, system for transport to PCR reactor **1504**, system for performing PCR amplification **1505**, system for transport of amplified sample from PCR reactor **1506**, system for detection of PCR amplicons **1507**, and system for decontamination and conditioning of all exposed conduits **1508**.

[0096] The system **1501** for injecting and or aspirating a sample provides injection and/or aspiration of the sample. In one embodiment the injecting/aspirating system **1501** consists of a zone fluidics system. In another embodiment the injecting/aspirating system **1501** consists of an FIA system. The system **1501** for injecting and or aspirating a sample can be, for example, a injecting/aspirating device available under the trademark milliGAT™ pump, Global FIA, Inc., Fox Island, Wash.

[0097] The system **1502** for adding PCR reagent to the sample is operatively connected to the system **1501** for injecting and or aspirating a sample. The system **1502** for adding PCR reagent to the sample can be, for example, a unit for adding PCR reagent to the sample such as an injection or multi position selection valve, available from VICI, Houston, Tex.

[0098] The system **1503** for mixing the sample and the reagent is operatively connected to the system **1502** for adding PCR reagent to the sample. The mixing system **1503** mixes the sample with a PCR reagent. In one embodiment the PCR reagent includes primers. In another embodiment the PCR reagent includes oligos. The system **1503** for mixing the sample and the reagent can be, for example, a super serpentine reactor, available from Global FIA, Inc., Fox Island, Wash.

[0099] The system **1504** for transporting the sample and the reagent to a PCR reactor is operatively connected to the

system **1503** for mixing the sample and the reagent. The system **1504** for transporting the sample and the reagent to a PCR reactor consists of a fluidics system. The system **1504** for transporting the sample and the reagent to a PCR reactor can be, for example, FEP tubing available from Cole-Parmer, Vernon Hills, Ill.

[0100] The system **1505** for performing PCR amplification is operatively connected to the system **1504** for transporting the sample and the reagent to a PCR reactor. This results in an amplified sample. In one embodiment the PCR amplification system **1505** includes an embedded thermocouple calibration conduit. PCR amplification devices are described in publications such as U.S. Pat. No. 5,589,136 for silicon-based sleeve devices for chemical reactions, assigned to the Regents of the University of California, inventors: M. Allen Northrup, Raymond P. Mariella, Jr., Anthony V. Carrano, and Joseph W. Balch, patented Dec. 31, 1996 and many are commercially available such as ABI PRISM® 7700 Sequence Detection System by Applied Biosystems; iCycler iQ Real-Time PCR Detection System by Bio-Rad; and Smart Cycler® System by Cepheid.

[0101] The system **1506** for transporting the amplified sample from the PCR reactor is operatively connected to the system **1205** for performing PCR amplification. The system **1506** for transporting the amplified sample from the PCR reactor can be, for example, FEP tubing available from Cole-Parmer, Vernon Hills, Ill.

[0102] The system **1507** for detection of PCR amplicons is operatively connected to the system **1506** for transporting the amplified sample from the PCR reactor. The system **1507** for detection of PCR amplicons can be, for example, a detection system described in publications and products produced by Cepheid and Baltimore-based Environmental Technologies Group, Inc. (ETG), a part of London-based Smiths Aerospace.

[0103] Conduits are included within the system **1501** for injecting and or aspirating a sample, system **1502** for adding PCR reagent to the sample, system **1503** for mixing the sample and the reagent, system **1504** for transporting the sample and the reagent to a PCR reactor, system **1505** for performing PCR amplification, system **1506** for transporting the amplified sample from the PCR reactor, and system **1507** for detection of PCR amplicons. A system **1508** for decontamination and conditioning the conduits is directly connected to the system **1507** for detection of PCR amplicons. The system **1508** for decontamination and conditioning the conduits is operatively connected to the system **1501** for injecting and or aspirating a sample, system **1502** for adding PCR reagent to the sample, system **1503** for mixing the sample and the reagent, system **1504** for transporting the sample and the reagent to a PCR reactor, system **1505** for performing PCR amplification, system **1506** for transporting the amplified sample from the PCR reactor, and system **1507** for detection of PCR amplicons. The decontamination and conditioning of all exposed conduits can be, for example, be performed by using a decontaminant, such as bleach, which is pumped through the exposed conduits and then washed from the system with a suitable wash solution.

[0104] Referring now to **FIG. 16**, a block diagram illustrates another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention. This embodiment of an autonomous pathogen

detection system is designated generally by the reference numeral **1600**. The autonomous pathogen detection system **1600** provides water sample collection **1601**, sample preparation **1602**, and detection **1603** and **1604**.

[0105] In operation, a water sample collection unit **1601** continuously samples a water source. Water sampling systems are known in the art. For example, a water sampling system is shown in U.S. Pat. No. 6,306,350 issued Oct. 23, 2001 titled “Water Sampling Method and Apparatus With Analyte Integration.” The disclosure of U.S. Pat. No. 6,306,350 is incorporated herein by reference.

[0106] The in-line sample preparation unit **1602** concentrates the sample in a swirling buffer solution. Particles of a given size distribution are selected by varying the flow rate across a separator unit. The in-line sample preparation system **1602** provides all sample preparation steps (i.e., mix, wash, incubation, etc.), and performing multiplex detection using a Luminex flow cytometer.

[0107] In the “detection” sub-system **1603**, a collected sample is mixed with optically encoded microbeads. Each color of microbead contains a capture assay that is specific for a given bioagent. Fluorescent labels are added to identify the presence of each agent on the bound bead. Each optically encoded and fluorescently labeled microbead is individually read in a flow cytometer, and fluorescent intensities are then correlated with bioagent concentrations.

[0108] In the “confirmation” sub-system **1604**, PCR (nucleic acid) amplification and detection confirms the presence of the bioagent. An archived sample is mixed with the Taqman reagent, and then introduced by a SIA system into a flow through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified and detected using fluorescence generated from nucleic acid replication from the Taqman probes. In the “Integrated Remote Control and Feedback” sub-system **1605**, a central computer uses a simple serial based LabVIEW control system to control all instrument functions. A software system provides data acquisition, real-time data analysis, and result reporting via a graphical user interface.

[0109] The first stage of the system **1600** is “water sample collection **1601**” that provides collection of particles from a source of water that could contain bioagents. The water sample collection system **1601** and in-line sample preparation **1602** provide preconcentration and delivery of the particles of interest to a wetted wall cyclone collector. The separator system captures particles of interest.

[0110] In the wetted wall cyclone collector, the particles are collected in a fluid, making downstream processing much easier. An on board computer controls water flow rates and the size range of particles collected. A particle counter provides real-time feedback on the size and quantity of particles collected.

[0111] Particles are drawn into the system that is designed to only allow the collection of particles of a pre-set size. The pre-set size can be selected as desired. The system is designed to only collect particles that are desired. The accepted particles continue on into a separator section that returns all the particles that are not of the desired size back into the environment. The remaining particles are known as the product flow. The product flow continues into the detection sections.

[0112] The system **1600** has the capability to measure particle sizes in the sampling environment via a built in particle counter with four size ranges, and can store and display the results in real-time. The system is entirely self-contained requiring only a power connection. The on-board computer has high-speed communications capability allowing networks of these sampling systems to be remotely operated.

[0113] The **1600** is useful for many application of water sampling. The system **1600** can be used to sample water quality in public buildings, for sampling in food processing facilities, for use in monitoring agricultural areas for the presence of pollens or pesticides and other water sampling uses.

[0114] Referring now to **FIG. 17**, a block diagram illustrates another embodiment of an autonomous pathogen detection system constructed in accordance with the present invention. This embodiment of an autonomous pathogen detection system is designated generally by the reference numeral **1700**. The autonomous pathogen detection system **1700** provides soil sample collection **1701**, sample preparation **1702**, and detection **1703** and **1704**.

[0115] In operation, a soil sample collection unit **1701** continuously samples a soil source. Soil sampling systems are known in the art. For example, a soil sampling system is shown in U.S. Pat. No. 6,363,803 titled “Vehicle Mounted Soil Sampler,” invented by Elmer Hubers, patented Apr. 2, 2002. The disclosure of U.S. Pat. No. 6,363,803 is incorporated herein by reference.

[0116] The in-line sample preparation unit **1702** concentrates the sample in a swirling buffer solution. Particles of a given size distribution are selected by varying the flow rate across a separator unit. The in-line sample preparation system **1702** provides all sample preparation steps (i.e., mix, wash, incubation, etc.), and performing multiplex detection using a Luminex flow cytometer.

[0117] In the “detection” sub-system **1703**, a collected sample is mixed with optically encoded microbeads. Each color of microbead contains a capture assay that is specific for a given bioagent. Fluorescent labels are added to identify the presence of each agent on the bound bead. Each optically encoded and fluorescently labeled microbead is individually read in a flow cytometer, and fluorescent intensities are then correlated with bioagent concentrations.

[0118] In the “confirmation” sub-system **1704**, PCR (nucleic acid) amplification and detection confirms the presence of the bioagent. An archived sample is mixed with the TaqMan reagent, and then introduced by a SIA system into a flow through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified and detected using fluorescence generated from nucleic acid replication from the TaqMan probes. In the “Integrated Remote Control and Feedback” sub-system **1705**, a central computer uses a simple serial based LabVIEW control system to control all instrument functions. A software system provides data acquisition, real-time data analysis, and result reporting via a graphical user interface.

[0119] The first stage of the system **1700** is “soil sample collection **1701**” that provides collection of particles from a source of soil that could contain bioagents. The soil sample

collection system **1701** and in-line sample preparation **1702** provide preconcentration and delivery of the particles of interest to a wetted wall cyclone collector. The separator system captures particles of interest.

[0120] In the wetted wall cyclone collector, the particles are collected in a fluid, making downstream processing much easier. An on board computer controls soil flow rates and the size range of particles collected. A particle counter provides real-time feedback on the size and quantity of particles collected.

[0121] Particles are drawn into the system that is designed to only allow the collection of particles of a pre-set size. The pre-set size can be selected as desired. The system is designed to only collect particles that are desired. The accepted particles continue on into a separator section that returns all the particles that are not of the desired size back into the environment. The remaining particles are known as the product flow. The product flow continues into the detection sections.

[0122] The system **1700** has the capability to measure particle sizes in the sampling environment via a built in particle counter with four size ranges, and can store and display the results in real-time. The system is entirely self-contained requiring only a power connection. The on-board computer has high-speed communications capability allowing networks of these sampling systems to be remotely operated. The **1700** is useful for many application of soil sampling. The system **1700** can be used to sample soil quality in monitoring agricultural areas for the presence of pollens or pesticides and other soil sampling uses.

[0123] Referring now to **FIG. 18**, another embodiment of a system for sample preparation and detection is illustrated. The system is generally designated by the reference numeral **1800**. The system **1800** is capable of performing, singly or in combination, liquid-array based multiplex immunoassay detection and/or in-line nucleic acid amplification and detection. In operation of the system **1800**, the aerosol collector system **1801** samples the air, particles of a given size distribution are trapped in a liquid, and a sample of interest is prepared. The next step is detection of any pathogens in the sample particles. This is accomplished by a liquid-array based multiplex immunoassay detection system **1802** and an in-line nucleic acid amplification and detection system **1803**.

[0124] The liquid-array based immunoassay detection system **1802** measures multiple pathogen targets in the sample. The immunoassay system **1802** can use either “wash assay” system or “no-wash assay” system. The “wash assay” system and the “no-wash assay” system use optically-encoded beads. The beads are kept in a small (~15 mL) stirred tank. Bead loss from agglomeration is reduced by using dispersing agents such as ethanol in the bead slurry. This reduces reagent cost.

[0125] The wash assay system embodiment uses a number of reagents in addition to the liquid sample. The reagents include detector antibody, and fluorescent reporter (streptavidin-phycoerythrin). In the wash assay system, a bed of beads is deposited on a filter then exposed to the sample, washing buffer, detector antibody, washing buffer, fluorescent reporter, washing buffer, and then the beads are released to the detector. For the wash assay system, the bead filter performance in releasing beads is improved by implement-

ing frequent clean-in-place protocols using reagents such as bleach for cleaning and morpholine propane sulfonic acid (MOPS) citrate buffer for reconditioning the filter. This extends the time between servicing the instrument and thus reduces operating cost.

[0126] In the no-wash assay embodiment, the reagents are sequentially mixed but the embodiment does not include the filter or the wash steps. It has been shown that the wash assays are more sensitive and selective, providing better signal-to-noise ratios in response to bioagents.

[0127] The PCR (nucleic acid) amplification and detection system **1803** confirms the presence of any bioagent. An archived sample is mixed with the TaqMan reagent, and then introduced by a system into a flow through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified and detected using fluorescence generated from nucleic acid replication from the TaqMan probes.

[0128] The PCR assays for confirming immunoassay positives are implemented using real-time PCR and the TaqMan process. Both FAM and TAMRA dyes are used as TaqMan reporters and TAMRA and BHQ (Black Hole Quencher) dyes are used as TaqMan quenchers. In one embodiment the FAM-BHQ is used for the bioagent probe and TAMRA-BHQ for an internal control probe. This allows an internal control to be used on a two-color detector. Internal controls are critical in PCR due to the delicacy of the reaction; otherwise, negative results are not definitive. In one embodiment, two color excitation is used to give strong signals for duplexed TaqMan PCR (agent plus internal control).

[0129] In some embodiments, the PCR reagents for TaqMan PCR are stored on the system together as MasterMix (enzyme, buffer, dNTPs) mixed with primers and probes. In another embodiment it was found that storing the MasterMix in one reservoir and primers plus probes in another reservoir made the reagents more stable, potentially eliminating the need for cooling in the instrument. In one embodiment, extraction of DNA onto microfabricated silica pillars is used as a means of purifying DNA from PCR inhibitors.

[0130] Sample preparation moves the sample from the sample collection **1801** to appropriate modules within the system. The nucleic acid assay system **1803** includes a number of components including system for injecting/aspirating a sample, system for adding PCR reagent, system for mixing sample and reagent, system for transport to PCR reactor, system for performing PCR amplification, system for transport of amplified sample from PCR reactor for detection of PCR amplicons. A central computer **1805** uses a simple serial based LabVIEW control system to control all instrument functions. A software system provides data acquisition, real-time data analysis, and result reporting via a graphical user interface.

[0131] In operation of the pathogen detection system, the in-line nucleic acid amplification and detection system provides nucleic acid assay methods. The methods include a number of steps. One step consists of automatically injecting and or aspirating a sample. Another step consists of automatically adding PCR reagent to the sample. Another step consists of automatically mixing the sample and the reagent. Another step consists of automatically transporting the sample and the reagent to a PCR reactor. The PCR reactor

consists of a fluidics system. Another step consists of automatically performing PCR amplification resulting in an amplified sample. Another step consists of automatically transporting the amplified sample from the PCR reactor. Another step consists of automatically detecting PCR amplifications. The method is performed in a nucleic acid assay system and the nucleic acid assay system is decontaminated and conditioned before a new sample is analyzed.

[0132] The system includes both real-time and post-PCR detection. The system is ideal for monitoring type systems, such as those currently being developed to detect terrorist releases of aerosolized bioagents. On-site detection systems for infectious diseases under development will need to incorporate sample preparation and analysis functions. The system allows relatively unskilled personnel, such as early responders, to perform real-time field or point-of-care nucleic acid assays. In various other embodiments of the autonomous pathogen detection system, the confirmation of bioagent(s) in the sample is provided by a multiplex immunoassay detector, a multiplex PCR detector, and a real-time PCR detector.

[0133] The present invention provides an Autonomous Pathogen Detection System (APDS) for monitoring the environment to protect the public from the release of hazardous biological agents. The Autonomous Pathogen Detection System is a countermeasure to bioterrorism, one of the most serious threats to the safety of United States citizens, citizens of other countries, and the military.

[0134] The APDS program was initiated to fill the requirement of a distributed environmental monitoring system for civilian applications. Multiplexed assays are used to reduce reagent costs, making long term monitoring operations possible (e.g., U.S. Postal Service mail screening). A unique, orthogonal detection approach that combines antibody-based and nucleic acid-based assays reduces false positives to a very low level. Antibody assays allow the detector to respond to all types of bioagents, including those without nucleic acids such as protein toxins. Nucleic acid assays allow much more sensitive detection, reducing the number of sensors needed to protect a given area. The fully autonomous aerosol collection and sample preparation capabilities limit maintenance requirements and makes integration into a central security or monitoring network possible.

[0135] There are other environmental or clinical pathogen detection system needs. Mobile units could be transported to suspected "sick buildings" to test for mold or fungal spores that might be causing tenant illnesses. Units with reagents for animal diseases could be placed in livestock transport centers or feedlots to rapidly detect airborne pathogens and protect against disease outbreaks. Monitors in hospitals could be used to test for airborne spread of contagious materials among patients. The system could be used at high profile events such as the Olympics for short-term, intensive monitoring or more permanent installation in major public buildings or transportation nodes. All of the individual units can be networked to a single command center so that a small group of technical experts can maintain and respond to alarms at any of the units. The system is capable of meeting all of these needs.

[0136] The primary needs describe above are directed to protection of civilians from terrorist attacks. The system also has uses in protecting military personnel from biological

warfare attacks. The military continues to evaluate options to their current biowarfare detection systems and the system meets many of the needs of the military.

[0137] While the invention may be susceptible to various modifications and alternative forms, specific embodiments have been shown by way of example in the drawings and have been described in detail herein. However, it should be understood that the invention is not intended to be limited to the particular forms disclosed. Rather, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the following appended claims.

The invention claimed is:

1. An autonomous monitoring apparatus for monitoring air, water, soil, or other substance for bioagents that could be in potential bioagent particles, comprising:

collector means for gathering said air, water, soil, or other substance being monitored, said collector separating the potential bioagent particles from said air, water, soil, or other substance;

sample preparation means for preparing a sample of the potential bioagent particles, said sample preparation means operatively connected to said collector means for preparing said sample from said air, water, soil, or other substance gathered by said collector;

detector means for detecting said bioagents in said sample, said detector means comprising a liquid-array based multiplex immunoassay detector and a multiplex PCR detector.

2. The apparatus of claim 1 wherein said sample preparation means is a means for providing an immunoassay sample.

3. The apparatus of claim 2 wherein said means for providing an immunoassay sample is a multiplex immunoassay detector that utilizes optically encoded microbeads.

4. The apparatus of claim 1 wherein said sample preparation means is a means for providing a nucleic acid assay sample.

5. The apparatus of claim 1 wherein said multiplex PCR detector is a real time PCR detector.

6. The apparatus of claim 1 wherein said multiplex PCR detector includes means for performing PCR amplification.

7. An autonomous monitoring apparatus for monitoring air, water, soil, or other substance for bioagents, the bioagents potentially being in potential bioagent particles within the air, water, soil, or other substance, comprising:

a collector for gathering said air, water, soil, or other substance being monitored, said collector separating selected potential bioagent particles from said air, water, soil, or other substance;

a wash assay sample preparation system for preparing a sample of said selected potential bioagent particles, said wash assay sample preparation system operatively connected to said collector for preparing said sample from said air, water, soil, or other substance gathered by said collector; said wash assay sample preparation system including optically-encoded beads, a number of reagents, and a washing buffer; and

a detector for detecting the bioagents in said sample, said detector operatively connected to said sample preparation system.

8. The apparatus of claim 7 wherein said reagents include detector antibodies.

9. The apparatus of claim 7 wherein said reagents include fluorescent reporters.

10. The apparatus of claim 7 wherein said wash assay sample preparation system includes a filter.

11. The apparatus of claim 7 wherein said wash assay sample preparation system includes a filter and optically-encoded beads.

12. An autonomous monitoring apparatus for monitoring air, water, soil, or other substance for bioagents, the bioagents potentially being in potential bioagent particles within the air, water, soil, or other substance, comprising:

a collector for gathering said air, water, soil, or other substance being monitored, said collector separating selected potential bioagent particles from said air, water, soil, or other substance;

a no-wash assay sample preparation system for preparing a sample of said selected potential bioagent particles, said no-wash assay sample preparation system operatively connected to said collector for preparing said sample from said air, water, soil, or other substance gathered by said collector; said no-wash assay sample preparation system including optically-encoded beads and a number of reagents; and

a detector for detecting the bioagents in said sample, said detector operatively connected to said sample preparation system.

13. An apparatus for sampling air and collecting sample particles of a predetermined particle size range from said air for monitoring air, water, soil, or other substance for bioagents, the bioagents potentially being in potential bioagent particles within the air, water, soil, or other substance, comprising:

a low pass section having an opening for gathering said air,

a pre-screen unit positioned in said opening that prevents large particles from blocking said opening,

an impactor section operatively connected to said low pass section, said impactor section receiving said air and separating said air into a bypass air flow that does not contain said sample particles and a product air flow that contains said sample particles, and

a sample preparation system for preparing a sample of the potential bioagent particles, said sample preparation system operatively connected to said impactor section for preparing said sample; and

a detector for detecting the bioagents in said sample, said detector operatively connected to said sample preparation system.

14. The apparatus of claim 13 wherein said opening in said low pass section size is a slot and said pre-screen unit is a grill positioned in said opening.

15. The apparatus of claim 1 wherein said opening in said low pass section size is a slot and said pre-screen unit is a screen positioned in said opening.

16. The apparatus of claim 13 wherein said opening in said low pass section size is an annular slot and said pre-screen unit is an annular screen positioned in said opening.

17. A method of monitoring air, water, soil, or other substance for bioagents, said air, water, soil, or other substance containing potential bioagent particles of various sizes, comprising the steps of:

gathering said air, water, soil, or other substance containing potential bioagent particles of various sizes;

separating said potential bioagent particles by size and collecting said potential bioagent particles of a size range that are likely to contain said bioagents; and

detecting said bioagents in said potential bioagent particles of a size range that are likely to contain said bioagents, said step of detecting said bioagents comprising mixing optically encoded microbeads with said potential bioagent particles and detecting said bioagents with said optically encoded microbeads.

18. The method of monitoring air, water, soil, or other substance for bioagents of claim 17 wherein said step of detecting said bioagents comprises exposing said microbeads to said air, water, soil, or other substance; depositing said microbeads on a filter; exposing said microbeads to a washing buffer, and releasing said microbeads to a detector.

19. The method of monitoring air, water, soil, or other substance for bioagents of claim 17 wherein said washing buffer comprises buffer solutions.

20. The method of monitoring air, water, soil, or other substance for bioagents of claim 18 wherein steps following releasing said microbeads to a detector includes using cleaning reagents for reconditioning said filter.

21. The method of monitoring air, water, soil, or other substance for bioagents of claim 20 wherein said cleaning reagents include bleach.

22. The method of monitoring air, water, soil, or other substance for bioagents of claim 20 wherein said cleaning reagents include morpholine propane sulfonic acid (MOPS) citrate buffer.

* * * * *