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Witz et al.

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[54] **DEVICE FOR PREPARATION OF SAMPLE FOR BIOLOGICAL AGENT DETECTOR**

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[73] Assignes: **Aerojet-General Corporation,** El Monte, Calif. ; by said Witz, Carleton, Anderson and Mayor; **The United States of America as represented by the Secretary of the Army,** Washington, D.C. ; by said Neufeld, part interest to each

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Related U.S. Application Data

[62] Division of Ser. No. 52,606, July 6, 1970, Pat. No. 3,690,837.

[52] **U.S. Cl.** ..... **23/254 R,** 23/253 R, 73/421.5 R, 73/425.4 R, 210/82, 210/198, 210/216

[51] **Int. Cl.** **G01n 1/14,** G01n 1/22

[58] **Field of Search** ..... 23/259, 232, 253, 23/254, 230; 210/82, 275, 198, 216, 387; 195/103.5, 127; 73/421.5 R, 425.4 R

[56] **References Cited**

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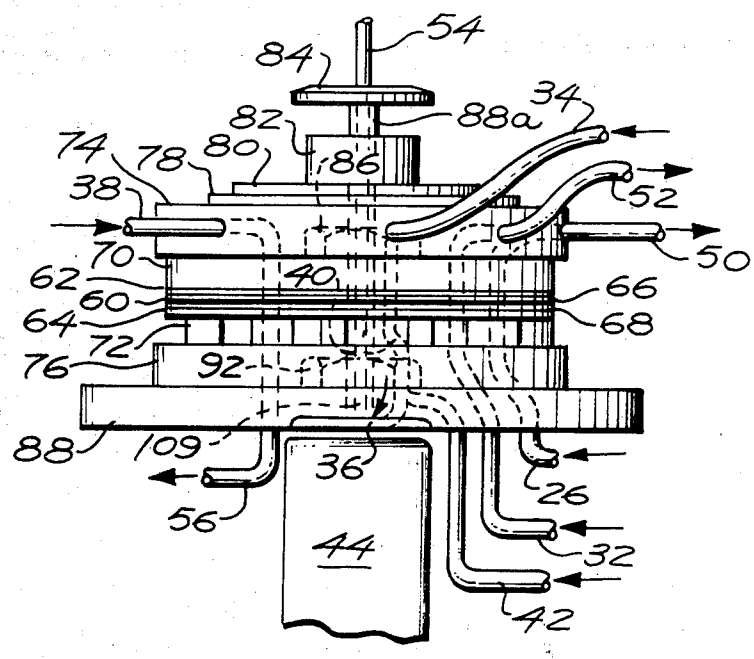
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Primary Examiner—Morris O. Wolk
Assistant Examiner—R. E. Serwin
Attorney—Edward O. Ansell et al.

[57] **ABSTRACT**

Filter means for removing microscopic particles from a fluid stream and delivering said particle to equipment for detecting biological agents such as vegetative bacteria, spores and viruses, capable of operating satisfactorily when supplied with minute samples of material to be tested, even when present in a continuous background of matter similar in nature. The equipment utilizes the phenomenon of chemiluminescence and, more particularly, provides the proper conditions for chemiluminescence of luminol by hydrogen peroxide, operating in an intermittent flow system supplied with the agents by an aerosol particle collector, and in which detection of the chemiluminescence is by a photomultiplier tube the output of which is monitored. Photomultiplier output could be recorded on a chart, magnetic tape or merely designed to set off an alarm when values exceed a prescribed threshold.

3 Claims, 3 Drawing Figures



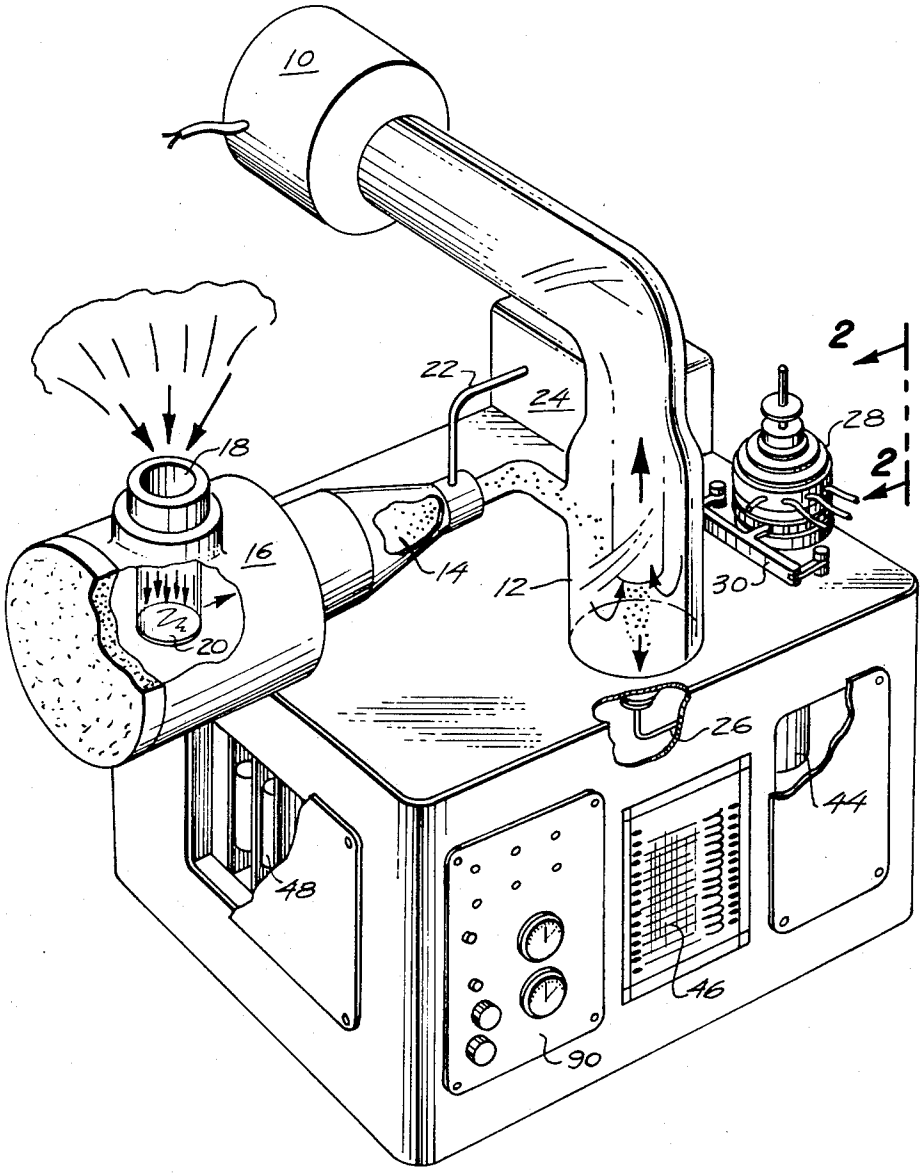


FIG. 1

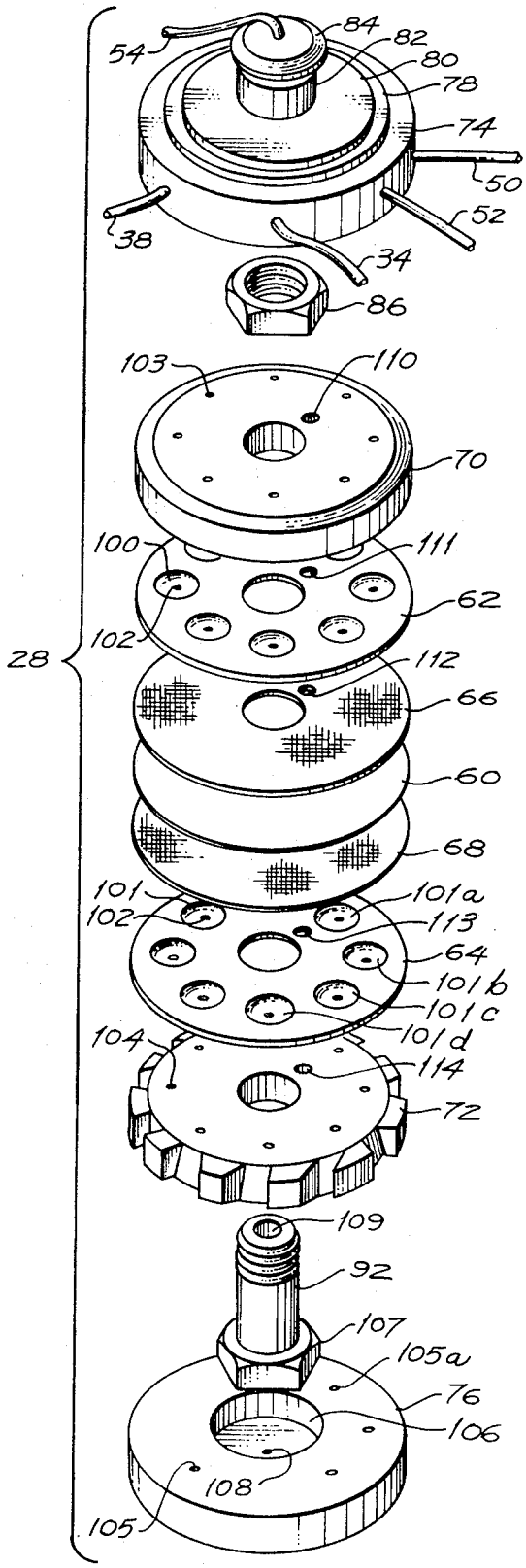

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FIG. 3

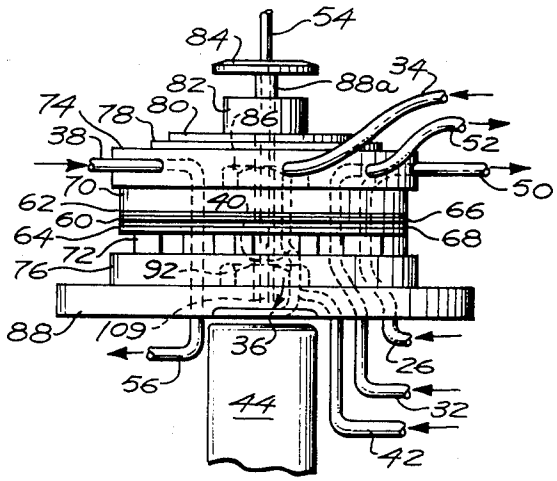

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FIG. 2

DEVICE FOR PREPARATION OF SAMPLE FOR BIOLOGICAL AGENT DETECTOR

This application is a divisional application of copending application Ser. No. 52,606, filed July 6, 1970, now U.S. Pat. No. 3,690,837, filed Sept. 12, 1972.

BACKGROUND OF THE INVENTION

The detection and quantitation of specific biological organisms, especially of small amounts existing in a continuous background of similar matter, has been the subject of considerable attention in recent years by those concerned with air and water pollution, biological warfare, food purification, the efficiency of sterilization methods, etc. From these efforts, there has evolved a technique for such detection which is quite advantageous in several respcts since it is versatile, rapid, inexpensive, easily managed and accurate. This technique involved the phenomenon of chemiluminescence, i.e., the emission of light as a result of a chemical reaction with little or no concurrent production of heat. However, the usual chemiluminescent detectors of micro-organisms which are available are not capable of providing rapid and continuous detection and quantitation of extremely small numbers of organisms against normal background interferences.

BRIEF SUMMARY OF THE INVENTION

The equipment to be described in this specification is free of the above deficiencies to a great extent. It involves the use of luminol (5-amino-2,3-dihydro-1,4-phthalizinedione) a substance which has the unusual property of luminescing, under proper conditions, when activated by hydrogen peroxide, in a reaction catalyzed by the substance hematin, a substance which is almost universally found in living organisms. Hematin catalyzes the chemiluminescence of luminol by peroxide either in its (hematin's) free state or in combination with protein-forming hemoproteins such as catalase or hemoglobin.

Specifically, in one mode of its operation, the equipment monitors the light produced when hydrogen peroxide is injected into a reactor cell containing a mixture of luminol and a hematin source (i.e., in the form of bacteria) to thereby provide a measure of the latter in a test sample. In another of its modes, the apparatus provides for reaction of a premix of luminol and hydrogen peroxide as a single reagent with the hematin source in a reactor and, in still another mode, the apparatus provides for chemiluminescence testing of a biological agent prestained with hemin chloride or hematin so that even vegetative bacteria or spores or virus which may not initiate chemiluminescence (since they may contain little or no hematin), may be detected. In addition to the above, the apparatus includes integral equipment capable of filtering and concentrating the sample bacterial stream operative automatically and under hygienic conditions after being set for desired intervals of effectiveness of its operations.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the automatic chemiluminescent biological agent detector comprising the present invention with certain structural parts broken away to provide some indication of its operation;

FIG. 2 is a side view of the filter-concentrator of the detector as viewed along lines 2—2 of FIG. 1; and

FIG. 3 is an exploded view of the filter-concentrator of FIG. 2.

DESCRIPTION OF THE PREFERRED EMBODIMENT

FIG. 1 shows the preferred embodiment of the detector of the present invention, a device capable of monitoring and measuring the increase in chemiluminescence produced by catalysis by metal porphyrin, occurring in organisms, of an aqueous solution of alkaline luminol and hydrogen peroxide. This reaction is virtually instantaneous and entails in one mode of operation, mixing a stream of alkaline luminol containing hydrogen peroxide with incoming collector fluid containing the porphyrin-carrying agents. The emitted light, which is linearly dependent upon the number of organisms present, is registered by a photomultiplier tube, amplified and recorded. A filtering and concentrating unit provides accumulation and intermittent release of pulses of organisms in the collector fluid, thereby increasing sensitivity and reliability.

Specifically, in FIG. 1, blower **10** provides air flow in the direction indicated by the arrows through collector **12**, aspirator **14** and chamber **16** from the outside atmosphere to be checked for organisms. Air containing the biological particulates enters port **18** in chamber **16** and strikes preimpactor **20**. The latter, consisting of a glass disc coated with an appropriate adhesive film serves to retain particles of size exceeding that of the organisms of interest. The desired airborne particles impinge in apsirator **14** on droplets of collector fluid (which may be water, aqueous sodium borohydride or other salt solution) introduced as a fine mist through tube **22**. The collector fluid is pumped from a reservoir (not shown) within the housing of the apparatus by means of pump **24**. The mist containing the bacteria then coalesces into a liquid stream and is separated from the air stream in the small cyclone of the collector **12**, which can be caused by arranging the conduit carrying the mist to enter the collector tangentially. The organism-carrying stream is then pumped from the collector through tube **26** into filter-concentrator **28**.

It is the function of filter-concentrator **28**, also shown in closeup in FIG. 2, to concentrate and wash the organisms before the reagents are permitted to affect them. In the first position of its indexing mechanism **30**, it accumulates the organisms entering via tube **26** on the surface of a filter through which the stream is passed for a predetermined time interval; in its second position, it provides a wash for the organisms by a fluid such as water, entering through tube **32**; in its third position, it releases the organisms to reactor cell **36** in a coherent pulse through tube **40** by backwashing the filter with fluid such as urea, entering through tube **34**; and, in its fourth position, its filter is cleaned in preparation for the next organism pulse by fluid such as water containing detergent, entering through tube **38**.

The organism-containing fluid in reactor cell **36** is then mixed with reagent entering via tube **42** (connecting to tube **40**) and the resulting illumination captured by photomultiplier **44** and converted to an electrical signal which is amplified in amplifier **48** and recorded by recorder **46**.

It is, of course, clear that fluid flows in the apparatus are accomplished by pumps (not shown) connected to reservoirs and receptacles in its housing, which are energized in proper sequence by, for instance, the cams

of a timing device (also not shown). These devices and their operation and the electronic circuitry which records either the intensity of the light flash or the integrated light output or sets off an alarm when values exceed a prescribed threshold are considered sufficiently well known to experimenters in this and associated arts to require no further elaboration here.

It is further apparent that tubes 50, 52, 54 and 56 are for the waste and wash fluids drained off after the various operations at the corresponding positions of filter-concentrator 28 are completed, as follows: tube 50 for collection fluid after deposit of organism (first position); tube 52 for organism wash fluid (second position); tube 54 for organism sample after test (third position); and, tube 56 for filter cleaning fluid (fourth position) in preparation for succeeding tests.

FIG. 3 provides a disassembled view of filter-concentrator 28 which shows its component parts to better advantage. The preferred structure comprises a single filter 60, which may be an appropriate membrane sandwiched between a pair of cavity discs 62, 64 of an inert material such as teflon, and separated from the latter by a pair of screens 66, 68 of, preferably, stainless steel, which contributes support for filter 60. Cavity discs 62, 64 serve as gaskets and face seals against metal pressure plates 70, 72, to define the port area (8 ports shown) at each position of indexing mechanism (FIG. 1) and also to minimize the liquid volume into which the organisms are backwashed in the third position of filter-concentrator 28 (the sensitivity of the test is directly proportional to the concentration of organisms in the backwashed volume of fluid). Components 60, 62, 64, 66, 68, 70 and 72 are squeezed tightly together into a leakproof assembly by hollow hub bolt 92 and nut 86. Port plates 74, 76 are preferably of teflon and provide for ingrees and egrees of fluids and, of course, are ported correspondingly to pressure plates 70, 72. Mounted about port plate 74 are the following: key plate 78, to insure proper orientation of port plate 74 when filter-concentrator 28 is assembled; disc spring 80, to maintain uniform rotary seal pressure regardless of temperature fluctuations; collar 82, to space and take up the thrust of spring 80; and, hand-operated nut 84, which tightens on the threaded end of spindle plate 88, the plate end of which is secured to the chassis and it is noted that indexing causes rotation of only filter 60, discs 62, 64 screens 66, 68 and plates 70, 72; the other components are stationary.

Operation of the equipment will be clearly understood from the following more detailed description and explanation. Depressions 100 and 101 of the respective discs 62 and 64 are in the form of dished areas juxtaposed to each other and located in pairs at the surfaces of the respective discs, the upper depression 100 of a pair being dished upwardly and the lower depression of the pair being dished downwardly so that together they form a cavity containing the filter 60 and which can retain an amount of fluid sent into them. These cavities do not extend all the way through the thickness of the cavity discs excepting for small ports 102 extending through the disc from each cavity. Since there are eight cavities, spaced equidistant from each other somewhat inside the outer periphery of the respective discs, there will likewise be eight of these ports 102 equi-spaced from each other around each disc. Eight similarly spaced and dimensioned ports 103 and 104 pass through the pressure plates 70 and 72 respectively such that they line up with the respective eight ports 102 of the cavity discs. The lower port plate 76 is provided with four of these similarly dimensioned ports 105 through it which registers with four of the ports 104 of pressure plate 72. The conduits 26, 32, 42 and 56 are connected to the under side of corresponding ports extending through the stationary spindle plate 88, which also register with the respective four ports 105 of stationary port plate 76. The conduits 50, 52, 34 and 38 fixed to the upper port plate connect with four adjacent ones of its ports which register with four adjacent ports 103 of the pressure plate 70, corresponding in position with the above-mentioned four ports 105 of lower port plate 76.

Before completing the final assembly of the stack, the member 64, 68, 60, 66 and 62 will be squeezed between the pressure plates 70 and 72 by passing hollow bolt 92 through the central openings of these members and tightening this group between the pressure plates by tightening nut 86 down on plate 70 with bolt head 107 against the bottom side of plate 72.

The lower port plate 76 is provided with a cylindrical well 106 entering the plate from its upper surface but not passing entirely through the plate. The bottom of this well 106 is provided with an opening 108 through which there can extend the hollow spindle 88*a* fixed to and passing through spindle plate 88. The bolt head 107 is rested in the well 106 of port plate 76 and the spindle 88*a* is passed through the internal hollow of the bolt 92 and up through port plate 74, through the collar 82 to the nut 84 which is threaded on the upper end of the spindle 88*a* to hold this entire assembly between the spindle plate 88 and the nut 84. The tube 54 is inserted into the hollow of the spindle 88*a* at its upper end. The bolt head 107 is free to rotate in well 106 so that the elements 60, 62, 64, 66, 68, 72, 86 and 92 rotate on indexing. Plate 76 is fixed to spindle plate 88 by a pin in plate 88 which enters a matching hole in plate 76. The upper port plate 74 is held in its position on the spindle 88*a* by suitable means (not shown) such as a pin or key in key plate 78 which enters a groove in the side of spindle 88*a* and a registering groove at the central hole of plate 74.

The reactor cell 36 is shaped to fit within a recess in the under side of the spindle plate 88 and at least its lower wall should be of a transparent material such as glass so that the photomultiplier 44 placed in close proximity thereto, will receive light from the cell when it becomes luminescent. The cell 36 may be made by attaching a sheet of transparent material to coincide with the plane of the lower face of the spindle plate, while the walls of the recess of the spindle plate may serve as part of the walls of the reactor cell. The port 109 formed by the hollow of the spindle and its extension through the spindle plate communicates with the reactor cell, and likewise the conduit 42 also communicates with the reactor cell by way of a port through the spindle plate from the conduit 42.

The key plate 78 with its key means prevents relative rotation between it and the port plate 74, thereby maintaining the angular position of port plate 74 so that its ports are positioned to correspond with the positions of the corresponding ports of port plate 76.

The elements 70, 62, 66, 60, 68, 64, and 72, which are rotatable in unison; are each provided with registration holes 110, 111, 112, 113, and 114 respectively such that a registration pin (not shown) fitted through

all these holes maintains the proper alignment of the ports and cavities of this rotary portion of the device, while the remaining elements being stationary are held in their proper aligned positions by the bolt **92.**

In the operation of the system, the timing device (which can be any suitable type of timer) can, for example, conveniently be a cam operated timer, such that cams attached to a timing motor shaft or the like will operate the index mechanism **30** intermittently and at prescribed times established by the timer and its cams. The timer allows the cavity plates to remain in each position for the prescribed time, for example, about half a minute, and then moves them to the next position for its prescribed time. Thus, the timer will operate on the rachet wheel of plate **72** to move the rotary mechanism to its first position, for example, where the particular cavity **101***a* is in communication with the particular port **105***a* of the port plate **76,** at which time material containing the organisms under test, which is continuously under pressure as by a pump, is sent through conduit **26** into cavity **101***a* and onto the underside of the filter, after which the timer will move the rachet wheel of plate **72** to put the same cavity into its second position **101***b* for its allotted time, which will cause flow of the washing fluid through conduit **32** to wash the organisms on the filter for a period of time also determined by the timer. Then, the timer will move the rachet wheel so that the same cavity will occupy the position **101***c*, at which time, the backwash fluid will be pumped through conduit **34** to the upper surface of the filter and through it, to send the organisms down to the reactor cell **26.** The next movement of the timer will then move the same cavity to the position **101***d* at which position the filter cleaning fluid will be pumped through conduit **38,** through the filter, and out of conduit **56.** In this same position and concurrently with this filter cleaning operation the conduit **42** is in communication with the reactor cell so that the pressurized reagent, for example, a mixture of luminol and hydrogen peroxide, is pumped or sent through conduit **42** and into the reactor cell, which at this time already contains the organisms, so that a flash of light occurs in the cell which will be detected by the photomultiplier and indicated or recorded on the indicator. Suitable housing will be provided for the reactor cell and photomultiplier to prevent the photomultiplier from receiving light except from the cell.

The remaining four ports of the eight port system can be connected to conduits and to the timing system and pumped to operate in the same way as the first-mentioned four ports.

It will be observed that, although only four positions of filter-concentrator **28** are required for a test, eight positions are provided; as a result, tests may be repeated or fun in tandem from different input sources or test conditions may be varied.

Thus, with respect to the last-mentioned alternative, if has been discovered that results, particularly test sensitivity, are dependent upon whether or not the reagents are separately added to the organism-containing fluid or pre-mixed and added to it as conglomerate. The detector of the present invention provides for such different modes of operation, i.e., it is not limited to any reagent or mixture or, as a matter of fact, to any prescribed type of unknown organism. For instance, operation has been successful in the following modes: testing for bacteria by adding luminol and hydrogen peroxide separately to the sample fluid in reactor cell **36** or by backwashing (third position of filter-concentrator **28**) with a mixture of urea and luminol and then adding the peroxide; testing for bacteria by adding a premix of luminol and hydrogen peroxide to the sample fluid; testing for spores (low in hematin) after adding hemin, in the form of the chloride, to the incoming collector fluid, since the hemin chloride, which is an effective catalyst for initiation of luminol chemiluminescence, is readily adsorbed on the surface of biological agents. Excess hemin chloride may be removed from the stained agents in filter-concentrator **28** during the concentration and wash cycles.

It is additionally to be observed that the equipment may also be used in the analysis of fluid suspensions of organisms which, of course, do not require the use of a collector as do aerosols. These may be introduced into the system by way of tube **26** for direct feed into filter-concentrator **28.**

From the above description, it is apparent that the detector of the present invention utilizes porphyrin-catalyzed luminescence for detection and assay of a number of biological agents (vegetative bacteria and spores, virus and virus carrier) from the hemin moiety which may be present in the organism or attached to it by staining with hemin chloride or hematin. It thereby comprises an instrument admirably able to accomplish the objects herein stated as desirable in a laboratory analytical tool.

We claim:

1. In a device for preparation of a sample for a biological agent detector, the combination comprising:

a filter for removing microscopic particles from a fluid stream;

means for directing a first fluid stream against one side of said filter at a first station to remove microscopic particles therefrom;

means for directing a second stream of washing fluid against said one side of the filter at a second station to wash the microscopic particles thereon;

means for directing a third stream of backwash fluid against the opposite side of the filter at a third station to effect removal of the microscopic particles therefrom to a collection point; and

means for sequentially placing the filter at the three stations directing the first, second and third fluid streams thereagainst.

2. A device in accordance with claim **1** wherein said filter comprises a membrane.

3. A device in accordance with claim **1** wherein said sequential means may be controlled to provide varying intervals of contact of the first, second and third fluid streams with said filter.

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