United States Patent

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[54] **AUTOMATED SYSTEM FOR PERFORMING SAMPLE MEASUREMENTS, DILUTIONS AND PHOTOMETRIC MEASUREMENTS**
6 Claims, 5 Drawing Figs.

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[51] Int. Cl. **G01n 1/00,** G01n 21/06, G01n 1/10
[50] Field of Search 356/36–40, 181, 186, 208, 246; 250/218; 23/253

[56] **References Cited**

UNITED STATES PATENTS

3,516,752	6/1970	Hrdina	356/246
2,037,213	4/1936	Cannon	356/208 X
3,225,645	12/1965	Baruch et al.	356/246
3,241,432	3/1966	Skeggs et al.	356/181 X
3,418,053	12/1968	Pelavin	356/181
3,448,277	6/1969	Jayko	356/208 X
3,475,128	10/1969	Thiers	356/246 X
3,478,598	11/1969	Nielsen	356/246 X
3,484,170	12/1969	Smythe et al.	356/181

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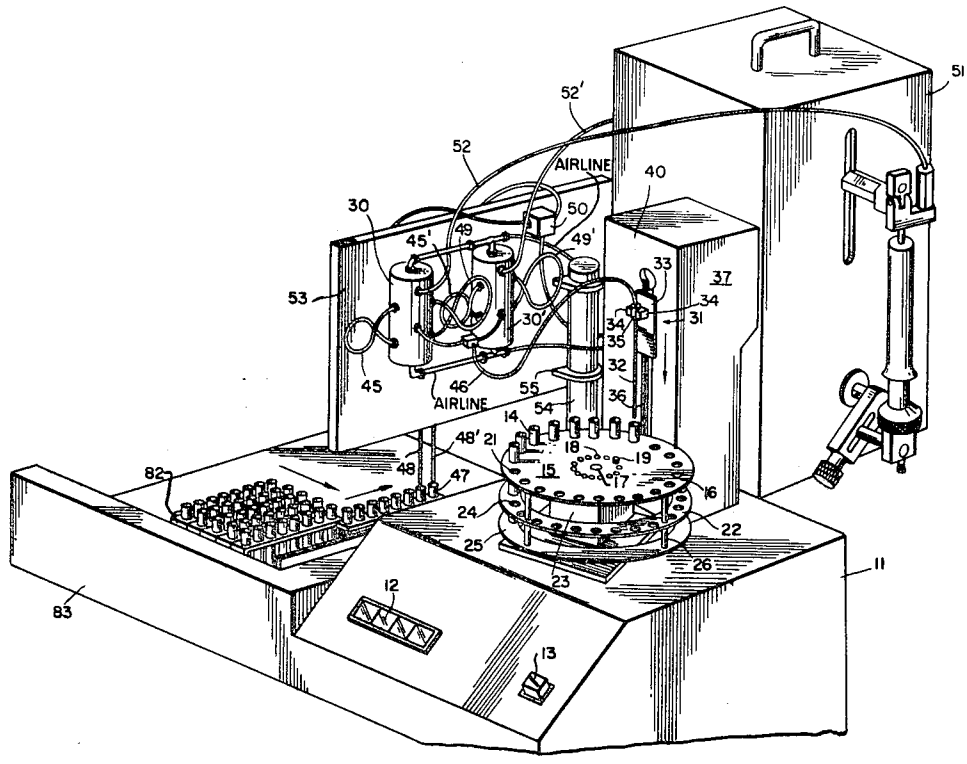
ABSTRACT: An automatic diluter and photometric reader adapted for use in the turbidimetric microbiological assays of antibiotics, vitamins, and the like, as well as in various other analytical assays.



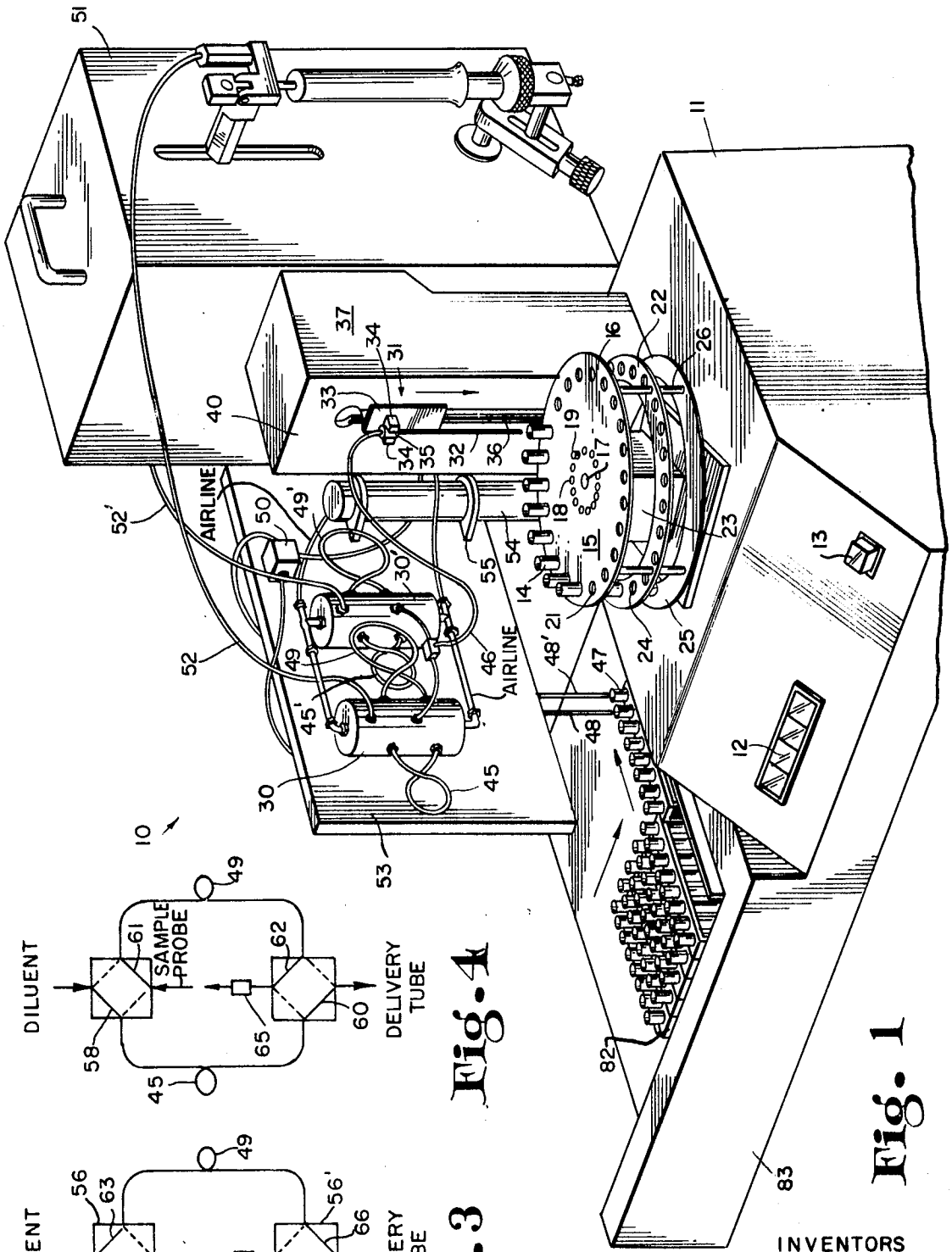


Fig. 1

Fig. 4

Fig. 3

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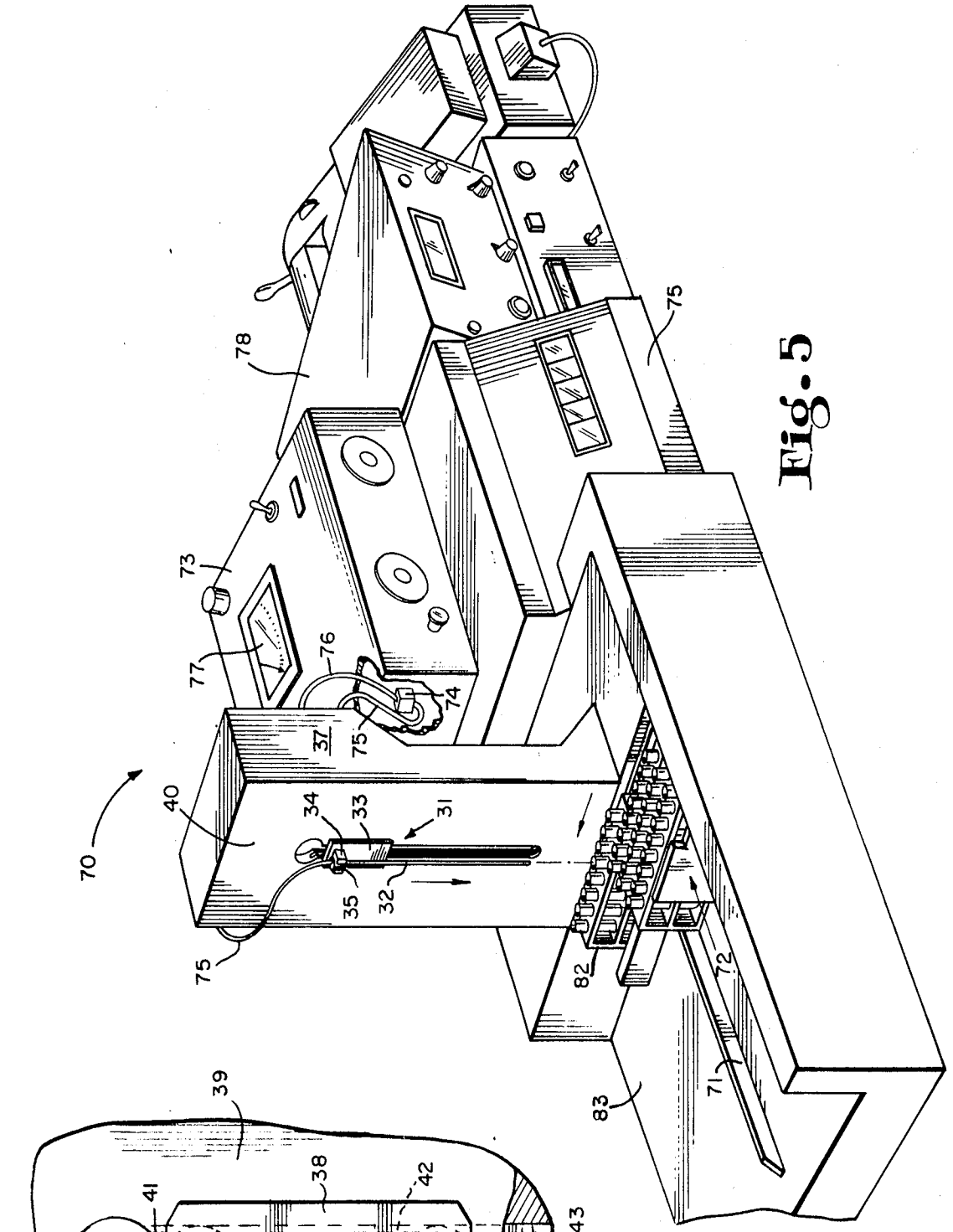

Fig. 5

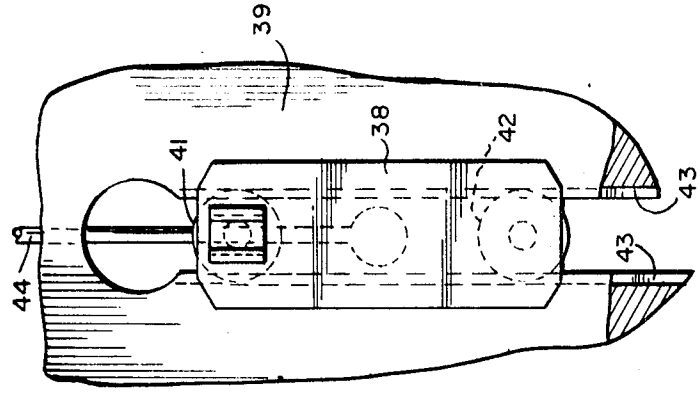

Fig. 2

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AUTOMATED SYSTEM FOR PERFORMING SAMPLE MEASUREMENTS, DILUTIONS AND PHOTOMETRIC MEASUREMENTS

BACKGROUND OF THE INVENTION

This invention relates to a two-component system for performing turbidimetric microbiological assays on substances such as antibiotics, vitamins, and other bacterial growth promoting or inhibiting substances, as well as various other analytical assays, and the like.

Turbidimetric microbiological assays of antibiotics, vitamins, and the like have long been used as a means for identifying and assaying the potency of such substances. The assay procedures are time consuming and usually involve many manual operations, each subject to some degree of error.

In recent years, there have been attempts to provide automated devices for carrying out such assays. While there are currently several such devices, they are plagued with various problems such as cross-contamination between samples, slow rate of analysis, requirements for large volumes of sample, fixed time of incubation or reaction, lack of versatility, need for method changes to accommodate the equipment, etc. Furthermore, because of the cross-contamination problems of some of the currently existing devices, only one antibiotic, vitamin or the like can be analyzed in a single operation. Before a second substance can be assayed, the equipment must be cleaned.

A further disadvantage of currently available equipment is their lack of reproducibility in assays utilizing rod-shaped organisms such as *Klebsiella*, *Lactobacillus*, *Pseudomonas*, etc. because of the problem of flow birefringence (Kavanagh, F.W., *Analytical Microbiology*, Chapter 4, Academic Press, New York, N.Y. 1963).

In addition to the above disadvantages, the cost of many of the currently available devices is prohibitive.

Thus, while attempts have been made to provide automated devices for performing turbidimetric microbiological assays, such attempts have been fraught with problems.

SUMMARY OF THE INVENTION

The two-component assay system of this invention comprises an automatic diluter module and an automatic photometric reader module. The modules perform the same basic operations involved in traditional manual analytical or microbiological turbidimetric methods such as pipetting, diluting, and the like, with far greater accuracy, precision, and efficiency. The modules, for example, can utilize the same organisms, the same media, and can measure the same bacteriological response employed in manual turbidimetric microbiological assays. The system is able to accommodate similar assay designs and standardization procedures as have been used heretofore. It provides for unlimited incubation or reaction times.

Generally speaking, the assay system of this invention consists of two modules, a diluter module, and a photometric reader module.

The diluter module includes a programmed control means, and a sampler tray which is adapted to advance at a predetermined time, making each sample tube available to a first sample probe means. A portion of the sample is withdrawn by a first sample probe means into a first metering means having a zero dead-volume. The combined operations of the probe and metering means performs the pipetting operation. After the sample has been measured, a predetermined amount of diluent is directed through the metering means by a first diluent delivery means, thereby washing out and diluting the sample into a diluted sample receiving means.

The operations are repeated until the desired number of samples have been measured and diluted. So long as the same diluent is used, different substances can be run by different methods without cleaning the equipment between substances. The diluter module is self-cleaning. That is, by carefully choosing the materials of construction, and by providing a sufficiently great dilution factor, the diluent serves to completely flush the sample from the metering means, thereby eliminating cross-contamination without necessitating an intermediate cleaning operation between samples. A variety of dilutions can be obtained by either changing the volume of the diluent delivered or by altering the volume of sample measured by the metering means.

The diluter module can additionally include an automatic means for conveying the diluted sample receiving means to a delivery tube which is cooperatively associated with the metering means.

The diluted samples can be transferred in suitable carriers, after allowing for sufficient incubation or reaction time, to a photometric reader, preferably to the reader module of this invention.

The reader module of this invention includes a photometric reader such as a photometer having a quartz flow cell as the cuvette. The reader module has an automatic sample-conveying means, such as a conventional linear fraction collector or the like, cooperatively associated therewith for conveying each sample to be read to a means for transferring the sample into the flow cell. The sample-conveying means can advantageously be a probe means, or the like, which is adapted to withdraw a sample and convey it to the flow cell. Before each sample reaches the flow cell, the flow is momentarily interrupted to provide an "airhammer" effect which rids the sample flow of air bubbles. Once the sample has been measured, it is drawn through egress means associated with the flow cell to waste. The reading for each sample can be taken from the photometric reader, or the reader module can additionally include a suitable readout means or can be keyed directly into a computer. As to the case with the diluter module, the functions of the reader module are controlled by a programmed control means.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective view of the diluter module.

FIG. **2** is a rear plan view of the probe assembly with the housing therefor broken away.

FIG. **3** is a flow diagram of a metering cell in its first position.

FIG. **4** is a flow diagram of a metering cell in the second position.

FIG. **5** is a perspective view of the reader module with the position of the flow cell designated by dotted lines. C

DESCRIPTION OF PREFERRED EMBODIMENTS

Referring to the drawing, one embodiment of the diluter module is shown generally at **10** in FIG. **1**. The diluter module performs the functions of pipetting and delivering a precise, predetermined amount of sample into a sample holder and of adding a precisely measured amount of diluent or reagent thereto.

The diluter module includes a housing **11** for a programmed control unit and a drive mechanism therefor (not shown). The control unit and drive mechanism are actuated by control switches **12**, and the main power switch **13**. The samples and standards are poured into sample tubes **14** which are then placed into the sample holder **15**. While any suitable sample holder can be utilized, the preferred sample holder **15** includes three circular, spaced-apart plates. The top plate **16** has a centrally located aperture adapted to have a drive shaft **17** removably journaled therein, a plurality of spaced-apart locating apertures **18** forming a concentric circle about said centrally located aperture, said apertures being adapted to receive a positioning pin **19** which is coupled to a mounting and supporting plate (not shown) and which limits the rotational motion of the sample holder so that each sample tube will be aligned with the sampler probe **32**, and a plurality of spaced-apart apertures **21** for receiving sample tubes **14**, the apertures being conveniently spaced apart adjacent the outer

periphery of the plate. The center plate **22** has a central aperture of sufficient size to fit over the housing **23** for the driving motor which controls the indexing of the sample holder. The driving means in turn is controlled by the control unit. Apertures **24** in plate **22** are aligned with apertures **21** of the top plate **16**. The bottom plate **25** similarly has a central aperture of sufficient size to fit over housing **23**. The bottom plate serves as a base for the bottom surface of the sample tubes. The three plates are maintained in their spaced-apart positions by spacing posts **26**.

The samples are drawn into a first metering valve **30** via probe assembly shown generally at **31**. Probe assembly **31** includes a probe **32** which is removably mounted at its upper end to a first mounting plate **33** via bracket means **34**. A retaining means **35** can conveniently be fitted over the top end of the probe to insure a secure fit into the bracket means. The probe is preferably of stainless steel or a like material. The probe is adapted to move vertically along channel **36** in probe assembly housing **37**. As can be seen in FIG. **2**, a second plate **38** is cooperatively associated with plate **33** so that plate **38** is disposed adjacent the interior surface **39** of the front wall **40** of the probe assembly housing. Bearing **41** and **42** are rotatably retained between plates **33** and **38**. As the probe assembly moves along channel **32**, bearings **41** and **42** ride along bearing runners **43**. Drive shaft **44** is coupled to and driven by, for example, a bellcrank and motor (not shown).

When the probe assembly is actuated, the probe moves downwardly and into the sample contained in the particular tube which is presently aligned with the probe. A vacuum system (not shown) is then actuated and sample is drawn through the probe and into a first loop **45** of a first metering valve means **30**, via sample inlet tube **46**. The vacuum system is then shut off whereupon the loop remains full. The size of the sample which is ultimately delivered into sample-receiving means **47** via delivery tube **48** is determined by the volume of the loop **45**.

Once the sample is drawn into a first loop **45**, and the vacuum is shut off via vacuum solenoid (not shown), the sample is delivered via delivery tube **48**, into sample-receiving means **47**, which can be, for example, a test tube, as a predetermined amount of diluent is passed into the valve and through loop **45**, through the valve, and into the sample-retaining means via delivery tube **48**. The presently preferred ratio of diluent to sample is at least 10:1 to insure complete flushing of the valve, thereby eliminating cross-contamination. However, it is only necessary that sufficient diluent be passed through the valve to flush out sample. Thus, for example, when large volumes of diluent are received, part of the diluent can be passed directly into the sample-receiving means. On the next cycle, the same sample is passed through and measured by the second loop **49**, which can differ in volume from the first loop **45** and is again washed out with diluent via delivery tube **48** and into another sample-receiving means.

Any unit capable of adding a specified amount of diluent can be utilized in the practice of this invention, for example, the filling unit manufactured by National Instruments Co. Diluent is pumped from the filling unit **51** into the valve, via diluent inlet tube **52**.

In the preferred embodiment of this invention, two metering valves **30** and **30'** are utilized in order to obtain, for example, two 0.15 ml. samples and two 0.1 ml. samples. The valves are conveniently mounted on a first surface of mounting board **53**, which is, in turn, conveniently coupled to housing **11** via support member **54** and mounting brackets **55**.

The sample injection valve manufactured by Chromatonix Inc. is particularly suitable for providing the sample-metering means of the present invention when modified to operate in accord with the above discussion. However, any other suitable metering means can be utilized.

The operation of the valve **30** can be more fully understood by the diagram of FIG. **3**. The sample is drawn into a first section **56** of valve **30** via sample probe **32**. The sample follows flow passage **57** to loop **45**, and is drawn into a second section **56'** of the valves, through passage **64**, through vacuum valve **65** and to waste. When the vacuum valve is shut off, loop **45** remains filled until sections **56** and **56'** are switched to their alternate positions as shown in FIG. **4**. The diluent then passes through passage **58**, into loop **45**, thereby washing out the sample via passage **60** and through the delivery tube to the awaiting sample-receiving means.

Concurrently with the diluent delivery through loop **45**, loop **49** is connected to the sample probe via passage **61** and **62**, thereby filling loop **49** with the same sample. Return of **56** and **56'** to the first position now connects loop **49** with the diluent via passage **63** and **66**.

The valves are preferably of Teflon and Kel-F, although other suitable materials can be employed. Similarly, the loops are preferably Teflon.

The sample receiving means **47** are conveniently held in racks **82**. The racks are then conveniently advanced automatically into the proper position by an automatic conveyor means **83**, cooperatively associated with housing **11** and mounting board **53** so that for each delivery cycle, a sample-receiving means is aligned beneath a delivery tube.

It can be seen that the diluter module provides a convenient, accurate means for automatically pipetting a given amount of sample, delivering the sample to a sample holder such as a test tube or the like, and adding thereto, a predetermined amount of medium, diluent, reagent, or the like.

Referring now to FIG. **5**, the reader module of this invention is shown generally at **70**. The diluted samples obtained from the diluter module, or any other suitable means, are, after proper incubation or reaction time, transferred in the racks **82** to an automatic conveyor means **83**, or other suitable advancing means. It can be seen that the racks are guided by guide means **71**, and that a retaining plate **72** is positioned behind the last rack. The samples are drawn into a suitable photometer **73** by probe assembly **31**. The samples are passed through flow cell **74** via inlet tube **75** and through outlet tube **76** to a waste-receiving means (not shown). The flow cell is preferably quartz or a like material, and replaces the cuvette of conventional readers. A solenoid valve (not shown) in the control unit **79** controls the sample flow. The control circuit is adapted to momentarily drop out the solenoid and interrupt the sample flow before it reaches the flow cell. This produces an "airhammer" effect which takes care of any air bubbles present in the flow stream. It is presently preferred, when determining the turbidity of rod-shaped organisms, that the flow cell permit a rate of flow of not less than 0.8 ml./sec., and preferably a rate of 1 ml./sec. or higher in order to obtain highly accurate, reproducible results. For other assays, the flow rate is not critical. The readings can be taken from the scale **77** of the photometer **73**, or, as in the preferred embodiment, a suitable "readout" device **78** can be cooperatively associated with the photometer.

The readout is compatible with a variety of data acquisition devices such as printed tape, punched paper tape, punched cards, or can be interfaced directly to a computer.

The reader module of this invention provides a great advance in the art by providing a means for overcoming the problems of measuring the turbidity of rod-shaped organisms by avoiding the flow birefringence problem normally encountered with rod-shaped particles. By producing a sufficiently rapid flow through the flow cell, rod-shaped particles, which would otherwise orient themselves with the slow currents in a static cell, leading to inconsistent readings, are read with the same precision as are spherical particles. Thus the present reader module now makes it possible to assay for substances which affect various rod-shaped organisms such as *Klebsiella*, *Lactobacillus*, *Pseudomonas*, and the like, and to determine them with a precision equivalent to spherical organisms, all other factors being equal.

While the present invention is particularly suited for turbidimetric microbiological assays, it will be apparent to those skilled in the art that the diluter module and the reader module can be used jointly or separately in any number of

analytical procedures, medical laboratory tests, and the like. Therefore, while the following discussion is directed toward turbidimetric microbiological assays, the modules of this invention are applicable to any suitable analytical procedures.

We claim:

1. In a photometric reader for performing a turbidimetric assay of a flowing sample and having a means for receiving a plurality of samples contained in individual sample receptacles, conveying means supporting and transporting the individual receptacles, a sample withdrawal means adjacent the conveying means and in fluid communication with the inlet of a flow cell and pressure differential means connected to an outlet of the flow cell for effecting flow of the sample therethrough, the improvement comprising valve means disposed between the pressure differential means and the flow cell, and valve control means connect to and actuating closure of said valve to momentarily interrupt the flow of a trailing portion of the sample prior to passage through the flow cell whereby gas bubbles in the flowing sample will be dispersed by the valve action and momentary fluid stoppage, thereby allowing for accurate measurement by the photometric reader.

2. In a photometric reader the improvement in accordance with claim 1 in which said sample withdrawal means comprises a reciprocable probe means for successive entry into said sample receptacles.

3. In a photometric reader the improvement in accordance with claim 2 in which the conveying means for said individual receptacles is synchronized with movement of said sample withdrawal means.

4. In a photometric reader the improvement in accordance with claim 3 including a solenoid for actuating the valve means.

5. In a photometric reader the improvement in accordance with claim 4 in which the pressure differential means comprises a vacuum system.

6. In a photometric reader the improvement in accordance with claim 4 in which the pressure differential means comprises means for effecting flow of the sample at a rate of at least 0.8 ml./sec.