NASA CASE NO. GSC 11533-1

PRINT FIG. 4

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NASA Case GSC 11,533-1

BACTERIA DETECTION INSTRUMENT AND METHOD

The instant invention is directed to method and apparatus for screening a sample of fluid for bacterial presence by monitoring a change in the concentration of dissolved oxygen in the fluid due to bacterial activity. The monitoring is performed by measuring the potential difference between a pair of electrodes which are inserted into the fluid.

The invention is useful for rapidly detecting the presence of bacteria in biological fluids such as urine and cerebrospinal fluid and in other fluids characterized as foods, e.g. water and milk. The invention is capable of detecting the presence of bacteria in initial concentrations of 1000 per milliliter in under $1\frac{1}{2}$ hours. This rapid time of detection as well as the simplicity and inexpensiveness of the instrument represents an important advancement in the art.

The basic instrument is termed a "redox-monitoring" cell, since it measures the reduction of oxygen concentration. It is shown in Figure 1. Solution 4 contains the fluid sample as well as a culture media if required. Electrode 6 is a test electrode having a noble metal wire 10 which reacts with the oxygen dissolved in the fluid to give a half cell potential which is a function of oxygen concentration. Reference electrode 8 provides a constant half cell reaction and is similar to a silver-silver chloride electrode.

The novelty of the invention appears to lie in the steps of monitoring the reduction of oxygen concentration in a fluid by electrode means over a period of time and comparing the amount of said reduction to a detection criterion to indicate whether bacteria are present.

INVENTORS: William Renner and Robert D. Fealey

EMPLOYER: George Washington University

~~EVALUATOR:~~ ~~Wayne Chen~~

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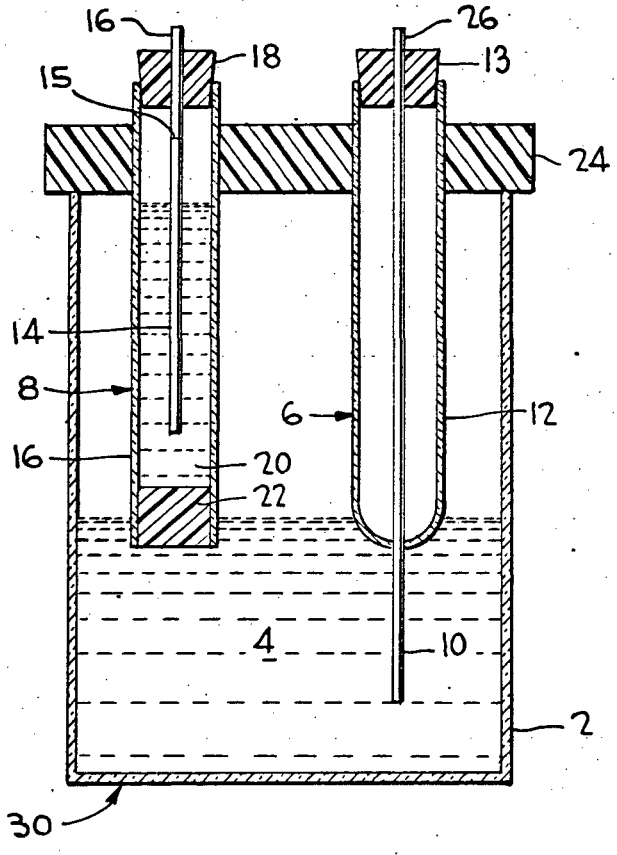
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FIG. 1

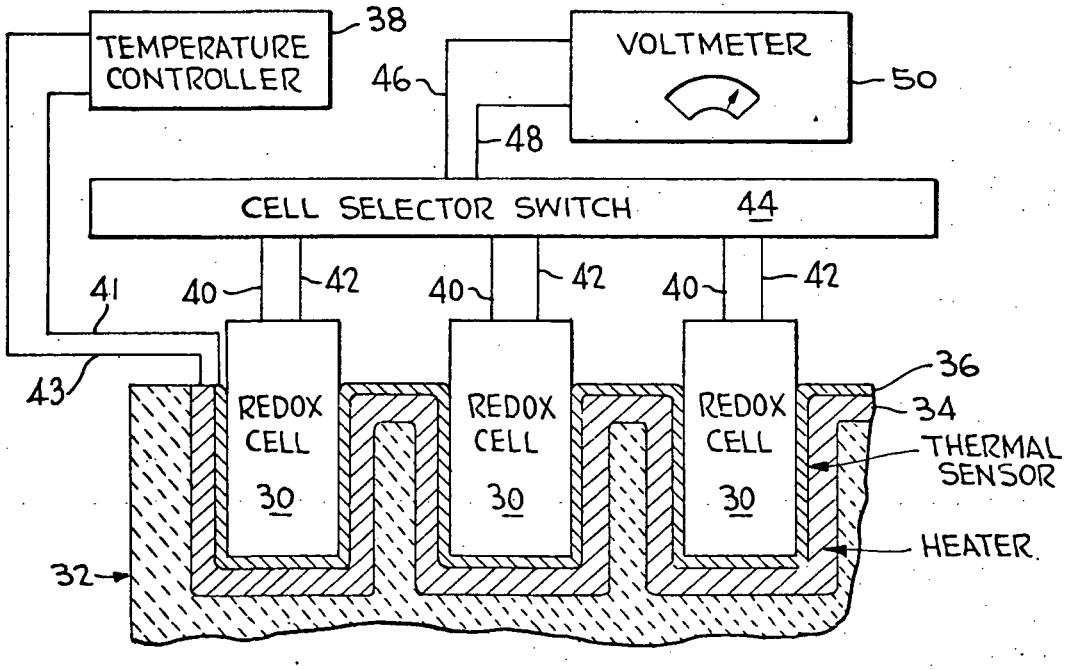
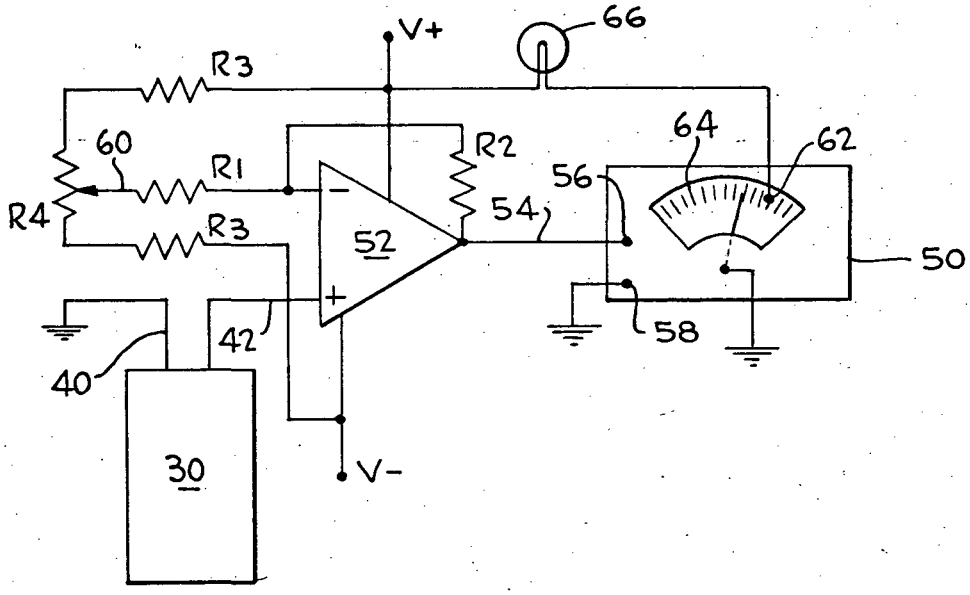

TEMPERATURE CONTROLLER
38
VOLTMETER
46
50
48
CELL SELECTOR SWITCH 44
41
40
42
40
42
40
42
43
REDOX CELL 30
REDOX CELL 30
REDOX CELL 30
36
34
THERMAL SENSOR
HEATER
32

FIG. 2

FIG. 3



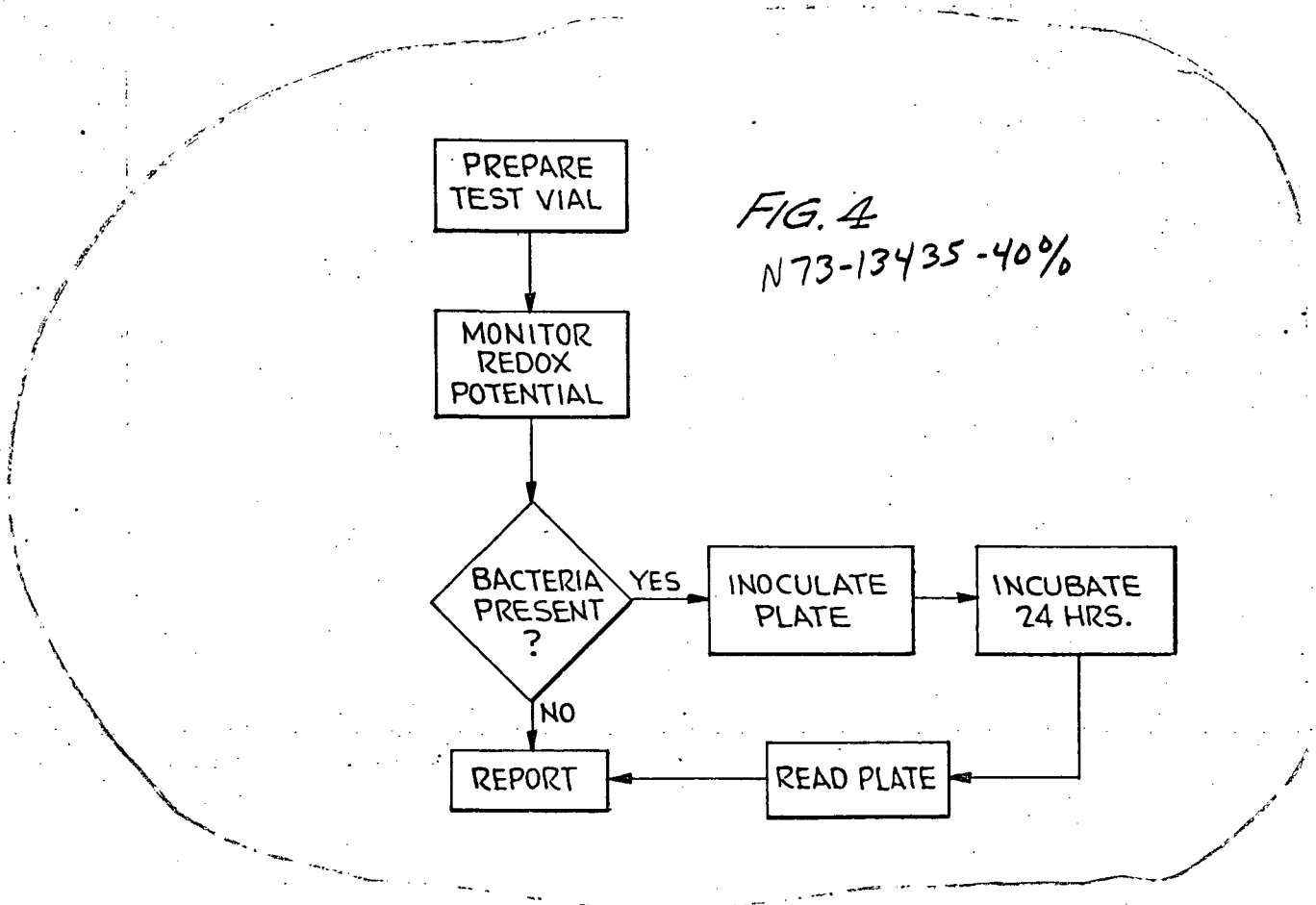


FIG. 4

NASA Case GSC 11,533-1

APPLICATION FOR LETTERS PATENT OF THE UNITED STATES

TO ALL WHOM IT MAY CONCERN

BE IT KNOWN THAT WE, ROBERT D. FEALEY AND WILLIAM RENNER, both citizens of the United States of America and residents of the States of New York and Ohio, respectively, have invented certain new and useful improvements in BACTERIA DETECTION INSTRUMENT AND METHOD of which the following is a specification.

ABSTRACT OF THE DISCLOSURE

Method and apparatus for screening a sample fluid for bacterial presence are disclosed wherein the fluid sample is mixed with culture media of sufficient quantity to permit bacterial growth in order to obtain a test solution. The concentration of oxygen dissolved in the test solution is then monitored using the potential difference between a reference electrode and a noble metal electrode which are in contact with the test solution. The change in oxygen concentration which occurs during a period of time as indicated by the electrode potential difference is compared with a detection criterion which exceeds the change which would occur absent bacteria.

ORIGIN OF THE INVENTION

The invention described herein was made in the performance of work under a NASA contract and is subject to the provisions of Section 305 of the National Aeronautics and Space Act of 1958, Public Law 85-568 (72 Stat. 435; 42 U.S.C. 2957).

BACKGROUND OF THE INVENTION

The invention relates to techniques for the detection of bacterial presence in fluids and more particularly to bacterial detection in biological fluids and foods.

The incidence of bacterial infections in human biological fluid is enormous. Commonly sampled biological fluids include the blood, urine, cerebrospinal fluid and serious effusions of the chest, abdomen and joints.

Laboratory testing for bacterial infections may be logically divided into two distinct determinations. The first is a screening step involving the determination of whether an abnormally large number of bacteria are present in a sampled biological fluid. If it is found that in fact an abnormal amount of bacteria are present, then a second step of determining the type and perhaps the quantity of bacteria is normally required.

In bacteriological laboratories today, the method of "culture growth and observation" commonly provides both determinations. Results from this method are available no sooner than 18 to 24 hours. A method of rapidly performing the first determination by screening for the presence of bacteria in a sample of biological fluid, could make valuable information available to the physician earlier than in the "culture growth and observation" technique. Such a method has a potential of substantially reducing the costs of bacteriological testing

since the overwhelming majority of samples prove to be negative. Only in the minority of instances where screening shows a sample to be positive would it be necessary to determine the type or types of bacteria present, typically by "culture growth and observation".

Various techniques exist for the screening of biological fluid for bacterial presence. These may be divided broadly into chemical and non-chemical techniques. Some of the available chemical techniques have been automated. Thus, a radioisotope apparatus exists wherein labelled C^{14} in glucose is added to a biological fluid sample in an incubation media. The bacteria oxidize the C^{14} to CO_2 and this gas is detected. This apparatus, gives results in four hours.

Another automated chemical reaction device utilizes a bioluminescence assay for Adenosine Trisphospate (ATP). This device is described in a copending application of the assignee, Serial # 137,094, filed April 30, 1971 and invented by Burton N. Kelbaugh, et al.

Some chemical reaction techniques are not suitable for all types of bacteria. For example, another widely used technique, the nitrite-nitrate method, fails in some cases because certain bacteria do not have the proper metabolite in their cell wall to initiate the characteristic reaction.

Non-chemical techniques, involve the use of various types of automated particle counters. Such techniques, at present, cannot thoroughly discriminate between bacteria and other particles. In addition, particle counters are quite expensive.

The need exists for a simple, reliable and inexpensive technique for rapidly screening a sample of biological fluid for the presence of bacteria.

The danger of bacterial contamination of food products, particularly in packaged food, continually is present in our nation. Given a rapid, inexpensive and reliable technique for the detection of an inordinate amount of bacteria, it would be economically feasible to test significantly more food samples than at present. The result would be an even higher level of confidence in the purity of our food. Thus a further need exists for a simple, reliable and inexpensive technique for the screening of other fluids, such as food products, for the presence of bacteria as well as for the screening of biological fluid samples.

It is an object of the present invention to satisfy both the need for screening biological fluid samples as well as for screening other fluids. In particular, it is an object of the invention to provide apparatus for rapidly screening a plurality of samples of fluid for bacterial presence.

That the above objects and other inherent objects of the invention are satisfied will become apparent from the following brief summary of the invention and the subsequent detailed description in conjunction with the accompanying drawings.

According to this invention, a vial containing a test solution is prepared wherein a sample of biological fluid, or other fluid to be tested, is placed in the vial along with a culture medium. A sterilized noble metal sensing electrode and a sterilized reference electrode are then placed in relation to the test vial so as to form a type of electrolytic cell comprising the electrodes and the test solution. The potential difference between the electrodes is then monitored.

The noble metal electrode exhibits a half cell potential which varies with the concentration of dissolved oxygen as is

taught by patent #3,272,725, while the reference electrode merely provides a half cell reaction of stable potential. If bacteria are present in the biological fluid they reduce the concentration of oxygen dissolved in the test fluid as a consequence of both their metabolic activity and their multiplication. Thus the potential difference between the test and reference electrodes will continually change if bacterial are present in the sample of biological fluid, but will remain essentially constant if they are not present. The term "redox-monitoring" has been coined by the inventors to describe this technique of determining whether bacteria are present in a test solution by the existence of activity leading to reduction of oxygen.

The invention will be further understood by those skilled in the art from the following detailed description of the annexed drawings, which by way of preferred example only, illustrate several embodiments of the invention.

FIGURE 1 is a cross-sectional view of a vial containing a test solution in which electrodes are placed so as to form a "redox-monitoring" cell.

FIGURE 2 is a schematic embodiment of apparatus for monitoring a plurality of the redox cells of Figure 1.

FIGURE 3 shows schematically apparatus for comparing the change of potential of the "redox-monitoring" cell of Figure 1 with a detection criterion.

FIGURE 4 is a flow chart indicating the processing of a sample of biological fluid by a bacteriological laboratory using screening by "redox-monitoring".

In Figure 1 is shown a "redox-monitoring" cell. It comprises a test vial 2 of a type commonly available in chemical

laboratories, in which is placed a test solution 4. A test tube of 5/8 of an inch diameter is very suitable for the vial 2. The solution may contain, for example, a sample of biological fluid such as urine, cerebrospinal fluid or a serious effusion of the chest, abdomen, or joints. At the present time, the presence of hemoglobin in blood cells makes somewhat more difficult the technique of determining that bacteria are present by their reduction of oxygen. However, the blood may be centrifuged to obtain plasma which may then be used as the biological fluid sample. The difficulty of applying the technique to blood is the exception rather than the rule and in fact the technique of "redox-monitoring" may be applied advantageously to most common fluids. For example the fluid to be tested may belong to the general class of food products. Using the invention, water or milk may be tested for bacterial presence.

The test solution must ordinarily contain a culture medium along with the fluid to be tested unless the fluid is itself an adequate culture medium, as may be the case for food products. Suitable culture media are those which are commonly available in microbiology laboratories, for example, Bacto Nutrient Broth, Bacto Eugon Broth or trypticase soy broth.

In the case of biological fluid testing, the fluid sample should be in the range of 5% to 30% by volume of the test solution, the remainder being the culture medium. While in the case of food product testing, the amount of culture medium, if any, to be added for optimum results depends on the characteristics of the food product. Thus, in the case of food products, the amount of culture media to be added must be determined with reference to a criterion as to the sufficiency of nutrient to allow bacterial growth.

A sensing electrode 6 and a reference electrode 8 are inserted into the test vial 2 so that they communicate with the test solution 4, generally in the manner shown in Figure 1. The test electrode comprises a wire 10 formed from a noble metal such as platinum, gold or palladium or other similar metals which are inert and compatible with the test solution. This electrode provides a half cell reaction whose potential is proportional to the oxygen content in the fluid. Care must be taken to control the exposure to oxygen of that part of the wire 10 which is not in the test solution 4. Thus an envelope 12, preferably of glass, in combination with a resiliant plug 13, seals the upper part of the wire 10 in a constant environment.

The reference electrode 8, may be any well known cell designed to yield a constant half cell potential, for example, a colomel or a silver-silver chloride electrode. The reference electrode preferably used comprises a silver wire 14 the lower part of which (below the line 15) has been electrolyzed in one molar HCL for 45 minutes at 10 milliamperes, later placed in .05 molar HCL for 10 hours and then placed in distilled water for 24 hours whereby a silver chloride coating if formed. The silver wire 14, is inserted into a tube 8 which is plugged at both ends. The upper end 16 of the silver wire, which has no silver chloride coating, passes through the top plug 18. This reference electrode contains a solution 20 which is a source of Chloride ions, for example potassium chloride. The optimum electrode solution has been found to be a saturated solution of potassium chloride and silver chloride in ethylene glycol containing 5 per cent water. The bottom plug 22, which is porous, provides a bridge for ionic flow between the solution 20 in the

reference electrode and the test solution 4. The porosity of the bottom plug must be such that ions can pass through it while complex molecules can not. The optimum porosity of the bottom plus 22 should be approximately 40 angstroms.

In order to monitor a test solution for bacterial presence the "redox-monitoring" cell should be sealed. Although it has been found that the lack of sealing doesn't affect its monitoring of oxygen concentration, aerobic bacteria would enter the cell if it were not sealed. Thus, resilient cover 24, through which the upper ends of the electrodes pass seals off the "redox-monitoring" cell from the external atmosphere. The upper ends of the wires of each of the electrodes, 16 and 26, protrude from the electrodes so that shielded cables (not shown) for connecting them to a voltmeter suitable for monitoring their potential difference may be there attached.

Instruments for measuring the potential difference between the electrodes are commercially available but must be carefully chosen. The electrodes described have impedances of somewhat less than 10^5 ohms, while the test solution typically has an impedance of 5×10^5 ohms. It has been found in further testing that a 1 millivolt sensitivity is required of the measuring instrument. Therefore, a suitable voltmeter must have an input impedance of at least 10 to 50 megohms and be sensitive to a 1 millivolt change. Most commercially available electrometers are suitable devices. Furthermore, some integrated circuit operational amplifiers of the field effect transistor type also are suitable for interfacing the "redox-monitoring" cell to most voltmeters.

It has also been found that to assure optimum and repeatable results, the "redox-monitoring" cell must be placed in a

nearly constant temperature environment, typically at human body temperature. This must be done since the half cell reaction potentials of the electrodes are sensitive to temperature and also that the maintenance of body temperature is critical to the growth of certain bacteria.

Best results are obtained within a three degree centigrade deviation from 37 degrees centigrade. For this reason the cell should be placed in a 37 degree centigrade heating block or other temperature controlled device.

Various tests have been performed which indicate the workability of the invention for the detection of bacteria in biological fluids. In one test a control solution and a contaminated solution were prepared. Each solution was a mixture of 14 ml. trypticase soy broth and 4 ml. urine. The urine in the control solution was sterilized while the urine in the contaminated solution contained approximately 1/2 million bacteria. Each solution was placed in its own "redox-monitoring" cell and the two were placed in an oven at 40°C. It was found that the potential difference of the control "redox-monitoring" cell dropped .05 millivolts in 5 minutes while that of the "redox" in which bacteria were present linearly dropped 40 millivolts in 5 minutes. Thus, in approximately 15 seconds it would have been possible to ascertain the presence of the bacterial if the voltage drop of the contaminated "redox" cell were periodically compared with a detection criterion of 2 millivolts. It should be noted that the above measurements were made after an incubation period of 45 minutes at 40 degrees centigrade.

There are ten different strains of bacteria commonly found in urine samples. Of these a representative group have been tested against controls in the above manner. It has been

found that after an incubation period of 45 minutes to 1 hour, E. Coli (10^5/ml) produced a drop of 8 millivolts in 5 minutes, Entercoccus produced a drop of 6 millivolts in less than 8 minutes and Pseudomonas produced a drop of 4 millivolts in 95 minutes while in all cases the control remained constant within .05 millivolts. Thus, with a detection criterion of a 2 millivolt drop in "redox" potential, detection occurs for most strains in a matter of minutes in addition to the incubation time. A pre-warming or incubation period is particularly necessary in the case of Pseudomonas, which appear to exhibit an initial growth lag.

A similar test was done with cerebrospinal fluid which was mixed with trypticase soy broth medium, the total having a volume of 10 ml. Staphylococcus bacteria in the range of 400 to 800 organisms per milliliter were injected into the solution. The result was that the "redox" potential dropped 8 millivolts in 30 minutes. In that test, the sample was pre-warmed, prior to testing, for a period of one hour giving a total detection time of under one and one half hours.

In view of the testing done, the presence of any bacterial strain in initial concentrations of 500 to 1000 organisms per milliliter or greater should be detectable in a biological fluid within one and a half hours after the fluid sample has been obtained.

Figure 2 shows schematically apparatus for screening a plurality of samples of fluid for bacterial presence. It comprises a base 32 which has been drilled for holding a plurality of the "redox-monitoring" cells 30 of Figure 1. The base 32 is preferably aluminum. The upper part of the base has a heater 34 and thermal sensor 36 whereby the redox cells may be heated

and their temperature may be sensed. The thermal sensor which is preferrably implemented as a temperature sensitive resistor placed into an electrical bridge configuration, provides a signal 41 to the temperature controller 38 which in turn controls the power 43 in the heater in order to maintain the temperature of the "redox" cells at approximately body temperature. The heater 34 may be implemented as a sheet or sheets of resistive material or as coils of resistance wire to which alternating current is applied. The temperature controller 38 may control this current using well known switching techniques incorporating silicon control rectifiers, triacs and the like as should be clear to those skilled in the art. A commercially available item, a 37 degree centigrade heating block, may used in lieu of a base specially adapted to hold the test vials. Shielded cables 40 and 42 are attached to each electrode of the "redox-monitoring" cells and to shielded cell selector switch 44. This switch selects which cell's output is carried by the cell selector switch output shielded cables 46 and 48. The switch outputs are connected to a voltmeter 50 whose input impedance and sensitivity are as previously discussed. An integrated circuit operational amplifier may also be used to interface the "redox-monitoring" cells to a voltmeter.

The use of this embodiment by a bacteriological laboratory would proceed as follows. As samples of biological or other fluid are obtained they would be mixed in a vial with culture media of sufficient quantity to allow growth of bacteria. Vials containing the mixture would be placed for a period of about 20 minutes to 1 hour in a heating block or other device for stabilizing their temperature at approximately body temperature. The stabilized vials would then be loaded into the base

32 and the test and reference electrodes after being sterilized would be lowered into the vials. Alternatively, electrodes could be placed in the vials initially and the entire redox cell could be temperature stabilized prior to being loaded into the base 32. As still another alternative, the vials could initially be temperture stabilized right in the base 32 prior to "redox-monitoring". In either event, cables 40 and 42 would then be connected to the "redox-monitoring" cells 30. The temperature controller would maintain the temperature of the redox cells while the selector switch is either manually or automatically, as for example if the selector switch were a motor driven commutator, caused to sample each cell. The voltmeter indication for each "redox" cell would then be in some manner recorded either by an operator or automatically in an associated recording instrument or memory system. The recorded data for each "redox-monitoring" cell would then be examined to determine if the change of potential of any cell exceeded an amount equal to a detection criterion. This comparison between recorded data and a detection criterion could be carried out in hardware, software or by an operator as should be apparent to those skilled in the art of instrumentation.

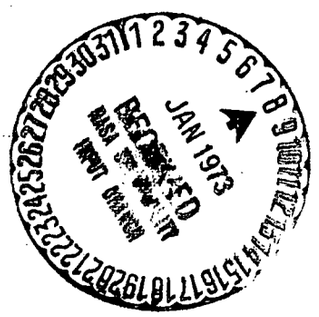
In Figure 3 an illustrative embodiment of hardware is schematically shown for carrying out the comparison of "redox" cell potential change with a detection criterion. The output 40 of the "redox-monitoring" cell 30 is grounded while output 42 is connected to the non-inverting input of differential amplifier 52. Resistors R_2 and R_1 set the gain of the amplifier which equals $1+R_2/R_1$. The output 54 of the differential amplifier is applied to voltmeter 50 at voltmeter terminal 56.

The other voltmeter terminal 58 is grounded. The meter thus measures an amplified "redox" cell potential difference. Potentiometer R_4 in series with resistors R_3 provides a means for initially zeroing the voltage at the output of the differential amplifier as indicated by the voltmeter. The resistance of R_3 and R_4 should be selected to be small compared to R_1. The voltage 60 appearing on the arm of the potentiometer R_4 is reflected to the output 54 of the amplifier through a gain factor of $-R_2/R_1$. The range of the potentiometer should be chosen so that the output may be biased from -.5 to .5 volts to allow an adequate range for zeroing the initial reading. Indicating needle 61 of the voltmeter comprises a part of a meter relay. That is, it is capable of electrically contacting pin 62 which is adjustably set on the meter scale 64. The location of pin 62 with respect to the zero location (needle pointing vertically) on the meter scale constitutes a detection criterion. Once the "redox-monitoring" potential, after being initially zeroed, exceeds this criterion, contact is made between the meter needle 61 and the pin 62 which completes a circuit that causes lamp 66 to light. The lighting of the lamp is a physical indication that the detection criterion has been exceeded and that there is bacteria present in the "redox-monitoring" cell 30.

Figure 4 is a flow chart indicating how samples of biological fluid should be processed by a bacteriological laboratory using the invention. As shown, first a test vial is prepared by mixing the biological fluid with a culture media and incubating the vial in order to initiate bacterial growth. Next, the test vial is screened using the "redox-monitoring" technique. If bacteria are found not to be present, which would occur for

the majority of samples, a report to the physician is made. On the other hand, if bacteria are found to be present, a plate is innoculated with the sample and it is incubated for 24 hrs. The plate is then read to determine the type of bacteria present after which a report is made to the physician.

Although several embodiments of this invention have been illustrated and described, it will be appreciated that modification is possible without departing from its spirit and scope.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