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Kirkham et al.

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[54] BLOOD AND FLUID CULTURING SYSTEM

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[52] U.S. Cl. 195/103.5, 99/104, 99/139

[51] Int. Cl. C12k 1/04

[58] Field of Search 195/103.5, 127, 139, 104

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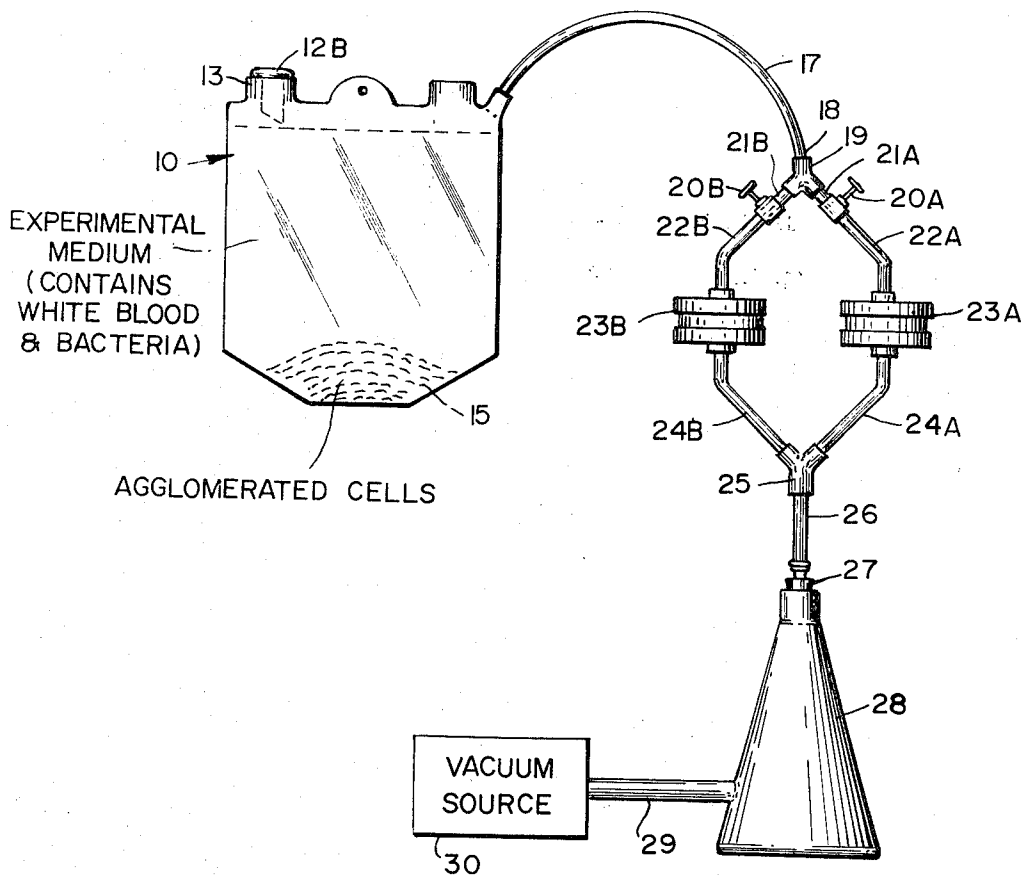
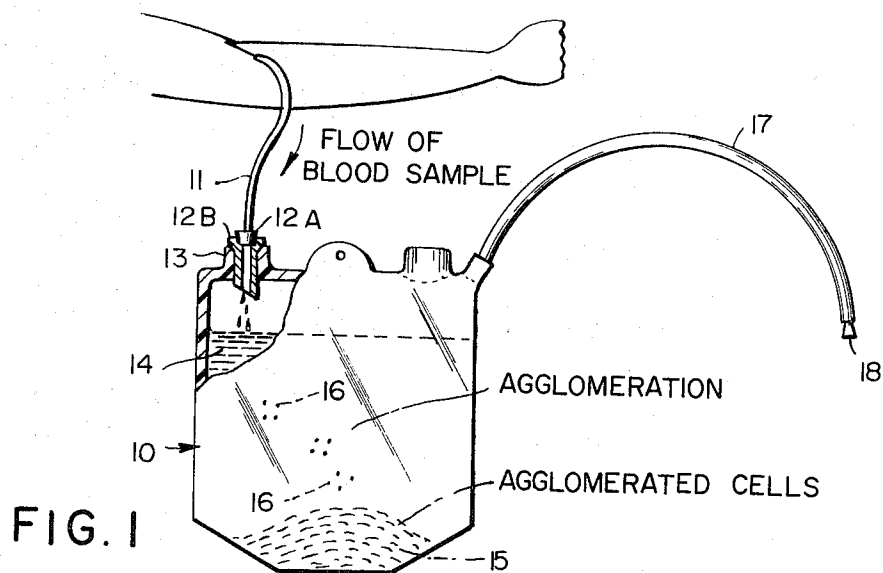
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[57]

ABSTRACT

Method of culturing blood by agglomerating red blood cells in a container containing a solution of "agglomerating" agents and a nutrient medium for any bacteria which may be present in the blood, filtering the solution and unagglomerated blood elements whereby bacteria are trapped upon the filter, adding a culture medium to the filter and incubating the bacteria for growth and identification. The filtering unit may be composed of two separate filters so that aerobic and anaerobic bacterial growth may be carried out. The apparatus for the method is also disclosed.

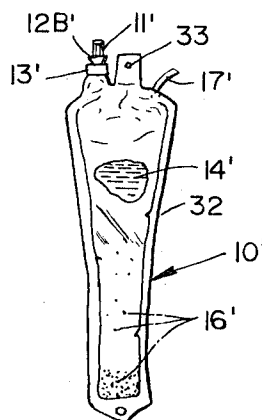
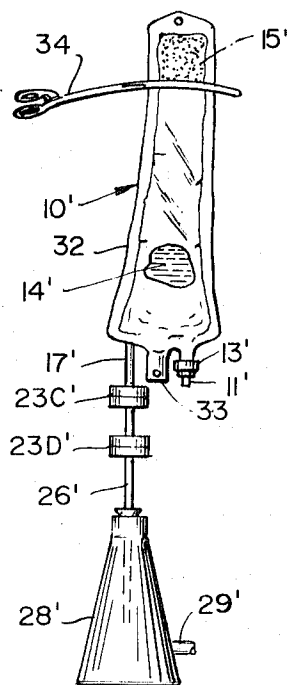
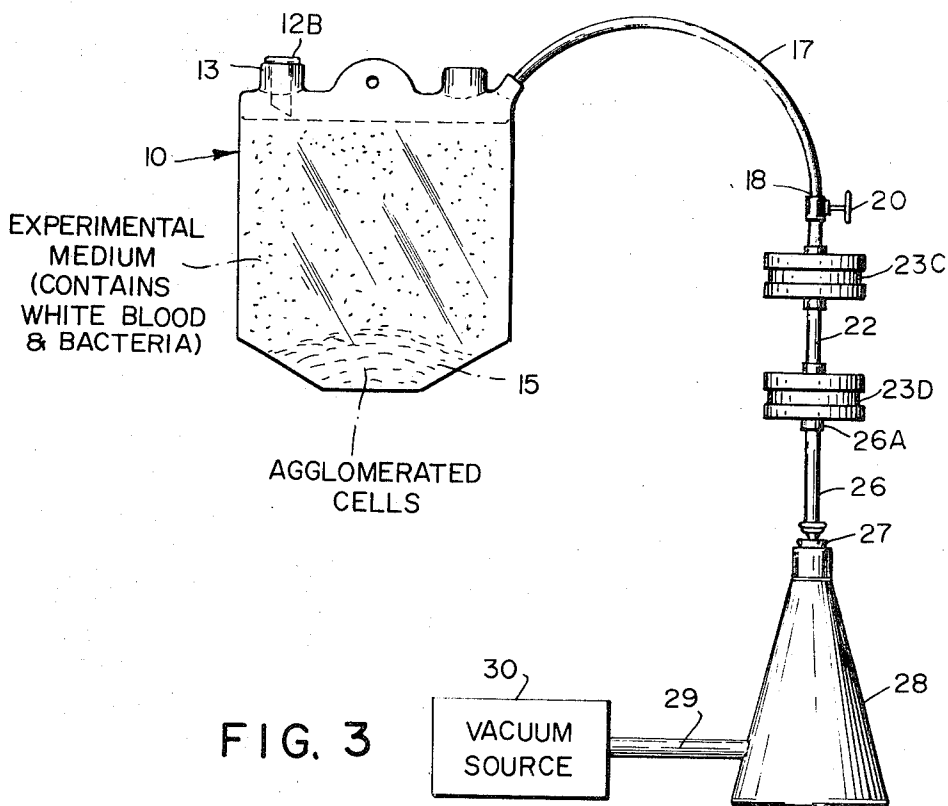
6 Claims, 10 Drawing Figures



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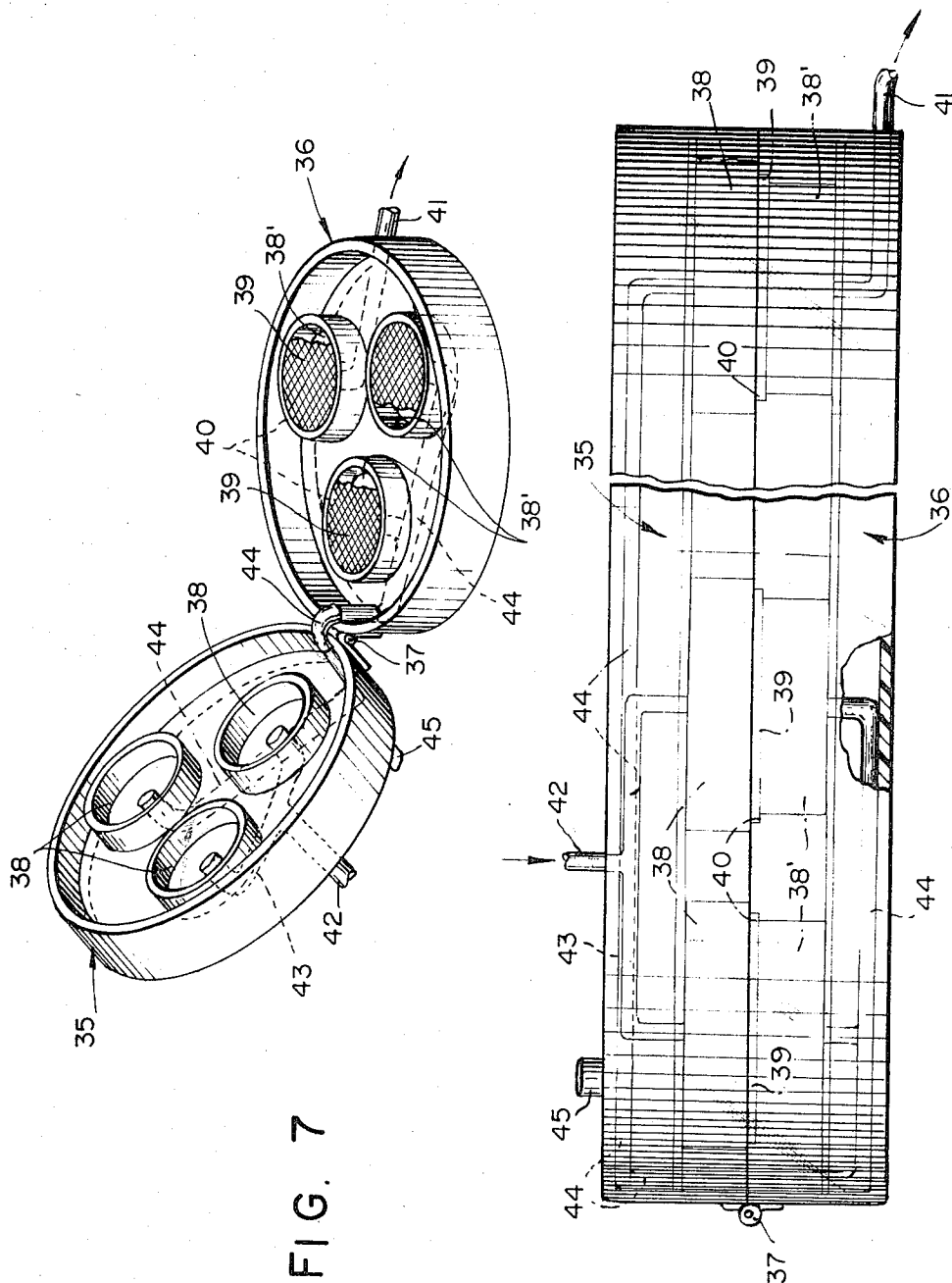


FIG. 7

FIG. 6

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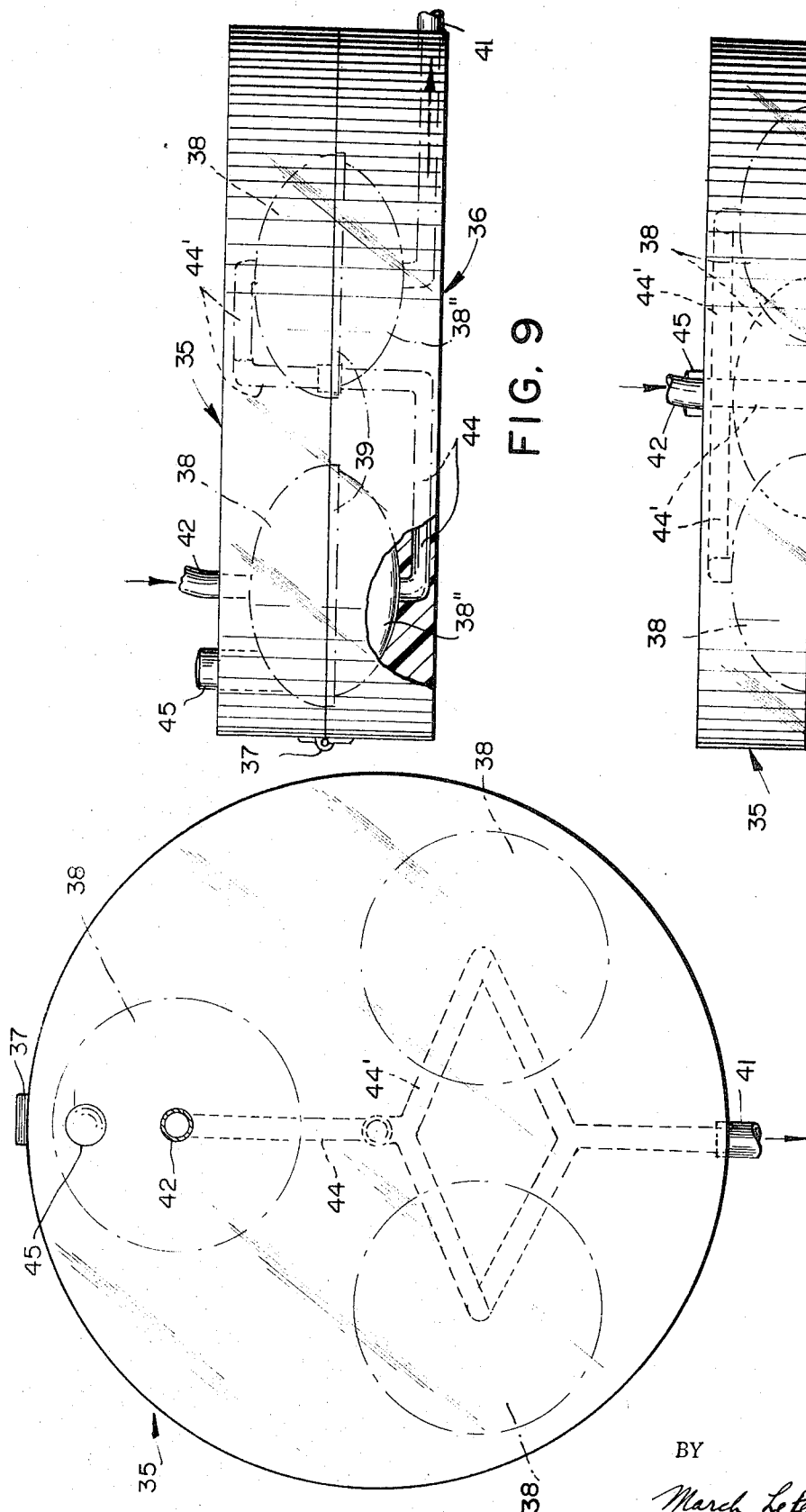


FIG. 8

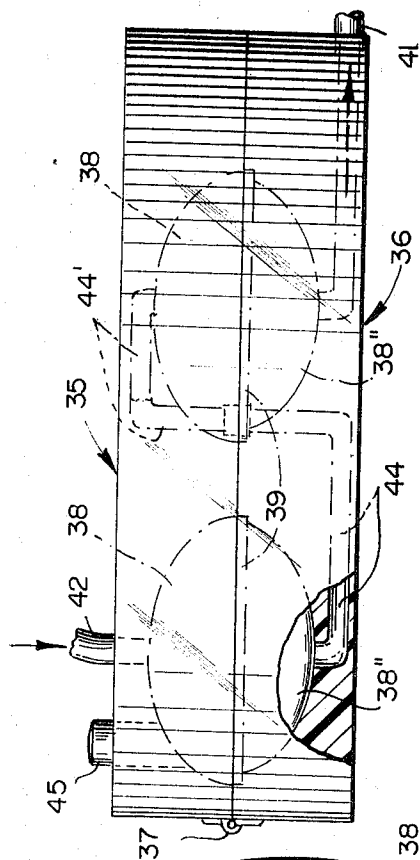


FIG. 9

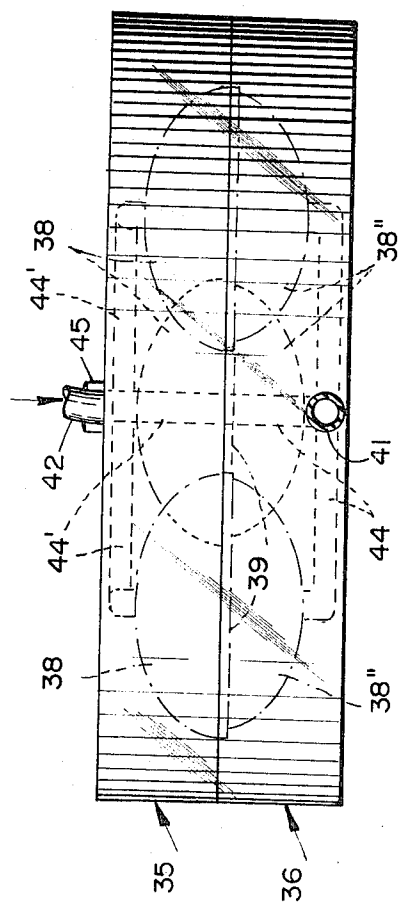


FIG. 10

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BLOOD AND FLUID CULTURING SYSTEM

This application is a continuation-in-part of Ser. No. 717,708 filed Apr. 1, 1968, now abandoned.

BACKGROUND OF INVENTION

When culturing blood or other fluids for bacteria and micro-organisms, it is generally desirable to dilute the sample by at least a factor of one to 10 by adding an appropriate quantity of solution, such as a nutrient broth medium. The one to 10 dilution of the blood is generally recognized as the minimum dilution that will reduce the concentration of antibiotics and inhibiting factors in the blood sufficiently to permit the organisms present to grow. The nutrient medium, of course, provides the vehicle upon which the organisms can grow and multiply.

In a common and current blood-culturing practice, 10 ml. of blood is removed from a patient's arm, 5 ml. of this blood is then diluted to 50 ml. with a nutrient broth in a closed sterile bottle, and the remaining 5 ml. of blood is added to a second bottle containing the same amount of broth. One bottle provides an aerobic culture because air is not introduced or the bottle is placed in an incubator devoid of oxygen. The bottles are then incubated and examined periodically for bacterial growth.

With the foregoing described technique, it is not feasible to analyze a large quantity of blood in a single sample or to dilute a 5 ml. sample greater than by a factor of one to 10. For example, to sample 10 ml. of blood on a 1 to 10 basis or to dilute a 5 ml. sample by a factor of 1 to 20 would require 100 ml. on a routine operating basis.

Both of these limitations are undesirable. First of all, for certain suspected causative organisms such as *Brucella*, it is necessary that larger samples of blood be taken and analyzed since this particular organism is found in the blood stream only in extremely small numbers. The larger the sample of blood taken, the greater is the possibility of recovering and identifying this organism and any other organism when few are present. Secondly, it is often desirable to dilute a 5 ml. sample by more than a factor of one to 10. This is often the case where there has been extensive and massive antibiotic therapy. With only a one to 10 dilution the antibiotic concentration may still be high enough to kill or inhibit the causative organism.

With present practices, samples are incubated, examined periodically, and when evidence of bacteria growth is discovered a portion of diluted sample is placed on a culture growth medium such as a sterile nutrient gel. The nutrient gel is then further incubated and subsequently examined to ascertain the nature of the bacteria. Detection of growth in the broth culture medium must be made in the presence of red blood cells, precipitated fibrous strands and other opaque debris so that early detection tends to be delayed.

It is known to introduce blood samples directly from a patient's arm into a closed bottle containing a nutrient solution. The mixture is then incubated and examined for growth and identification of the bacteria.

A major disadvantage of present filter methods is that red blood cells or erythrocytes, cannot be separated quickly and easily from the sample so that only the fluid portion of the sample containing the suspected bacteria may be used for analysis. The presence of the red blood cells quickly clogs filters and thus impedes efforts to quickly isolate the bacteria. If the red blood cells could be quickly separated from the fluid portion of blood, the remainder of the sample could be easily filtered, regardless of its size, in order to isolate and identify the bacteria present. Methods involving centrifuges are known for separating the red blood cells from the rest of the sample. However, the centrifuging process is inherently limited to small samples, requires considerable sample manipulation with the possibility of contamination, is time consuming, expensive, and is thus not satisfactory for everyday use.

BRIEF SUMMARY OF INVENTION

It is, therefore, an object of the invention to provide improved blood and other body fluid-culturing facilities.

It is a further object to provide improved culturing facilities which permit samples to be diluted as much as may be desired and which also permit larger samples of blood to be taken when attempting to identify infrequently occurring bacteria. Further objectives are to permit micro-organisms present in a large volume to be collected in a small area for ease of detection and to free micro-organisms from growth inhibiting influences by dilution.

In accordance with our invention we provide improved blood-culturing facilities in accordance with which a blood sample is taken from a patient's arm directly into a flexible plastic bag containing a solution that has both nutrient and agglomerating qualities. As soon as the blood enters and becomes mixed with the solution, the red blood cells begin to agglomerate and fall to the bottom of the bag. This leaves only the white blood cells, or leukocytes, platelets and bacteria in suspension in the solution. The quantity of blood taken and the amount of solution in the bag can be varied as desired so as to (1) obtain the desired dilution ratio (2) obtain the amount of blood desired.

As the agglomeration of the red blood cells takes place, the bacteria may multiply due to the nutrient qualities of the solution. Subsequently, the bag is gently compressed and the solution is forced through an exit tube connected to filters which pass the solution but trap the bacteria to be identified. The filters may be removed and placed on any of a number of solid media or permeated with differential culture media.

It may be seen from the foregoing that the culturing system of our invention overcomes the restrictions imposed by prior art culturing methods, i.e., dilution ratios, quantity of a blood sample that can conveniently be analyzed and lack of concentration and delayed detection of micro-organisms.

These and other objects and features of the invention will become more apparent upon a reading of the following description taken in conjunction with the drawing in which:

FIG. 1 shows an arrangement of the elements of our systems as a blood sample is being withdrawn from a patient's arm.

FIG. 2 shows an arrangement of the elements of our systems as the solution and bacteria are transferred from the plastic bag to the filters.

FIG. 3 shows an alternative arrangement of elements in accordance with our invention for transferring the solution and bacteria to the filters.

FIG. 4 shows another embodiment of the blood culture bag of the invention in position to receive the blood sample from the patient;

FIG. 5 shows this embodiment of the blood culture bag in position for filtration.

FIGS. 6-10 illustrate the novel filtration unit of the invention, showing respectively, a side view of the unit in closed position, a perspective view in its opened position, a top plan view in closed position, a view from the left side in closed position, and a view from the front in closed position.

DETAILED DESCRIPTION OF INVENTION

Turning now to the drawings, and with particular reference to FIGS. 1-3, blood to be cultured flows from a patient's arm punctured by a needle, through bleeding tube 11, through plug 12a at the end of tube 11, through the neck of 13 and bag 10, and into solution 14 which acts as a diluent for the blood, as an agglomerating medium for the red blood cells and as a nutrient broth for micro-organisms.

The solution for performing the above enumerated functions is preferably of the following composition:

Tryptose ¹	0.75 g.
Proteose Peptone ₁	0.5 g.
Yeast Extract	0.50 g.
Na ₂ HPO ₄	25 mg.
NaH ₂ PO ₄	10 mg.
NaCl	15 mg.

Dextrose
H₂O

45 g.
1,000 ml.

1 Difco Laboratories, Detroit

The solution is sterilized by autoclaving and has a final pH of about 6.4 to 6.8. It is cooled after autoclaving and then aseptically dispensed into bag 10 which is incubated overnight to check for sterility of the medium. An alternative method of preparing sterile fluid in the container is to first fill the container and autoclave the container and the fluid together. The fluid can also be sterilized by filtration.

Although the composition of the fluid set out above is satisfactory for the purpose of this system it is to be understood that other solutions which combine the functions of agglomeration of red blood cells, nutrition of any organisms present and dilution of the blood sample may be used. Other nutrient solutions with ingredients which promote agglomeration of the erythrocytes may be used. Agglomerating agents such as phytohemagglutinins, lectins, antibodies, dextran, enzymes and other substances which reduce the repulsive forces (Zeta potential) between erythrocytes may be used instead of the sugar and nutrient medium described above.

It must be kept in mind that the purpose of the medium is to dilute the sample, to nourish bacteria, and to cause agglomeration of red blood cells, and any solution which accomplishes these objectives may be used.

As soon as the blood becomes mixed with solution 14, the red blood cells 16, agglomerate and settle as a mass 15 on the bottom of the bag. This leaves only the white blood cells and bacteria in suspension. The bacteria feed upon the solution 14 because of its nutrient qualities. The tube 17 may remain closed at its end 18 during the blood drawing process. With reference to FIG. 1, tube 11 may be clamped or removed, and the bag sealed by the self-sealing plug 12a in neck 13 (FIG. 1) after the desired quantity of blood has been transferred to bag 10.

Subsequently, when the red blood cells have agglomerated and settled to the bottom of the self-sealing container, a plug in the end 18 of hose 17 is connected to a Y (wye) (FIG. 2) member 19 which is connected by legs 21A and 21B to clamp valves 20A and 20B which in turn, are connected by legs 22A and 22B to filters 23A and 23B. The output (lower end) of the filters are connected by tubes 24A and 24B to Y member 25 which is connected by tube 26 and plug 27 to the top opening of jar 28. An outlet tube 29 in the side of the jar is connected to a vacuum source 30.

With the foregoing arrangement, when it is desired to extract the solution and suspended bacteria from the bag 10 and pass it through the filters, the vacuum source 30 is energized and the valves 20A and 20B are opened either simultaneously or sequentially to permit the desired quantity of suspended bacteria and solution flow from the bag, hose 17, through each filter and into jar 28 under influence of the vacuum.

Each of filters 23A and 23B are placed on or permeated with a suitable culture growth medium upon which the bacteria collected by the filter may incubate and grow. If desired, one filter may be exposed to sterile air and the other flushed with oxygen-free gas in order to provide growth conditions for both aerobic and anaerobic bacteria, respectively. After a suitable incubation and growth period, the filtered bacteria may be macroscopically and microscopically examined and identified.

The arrangement of FIG. 3 is similar to that of FIG. 2 except that the end 18 of hose 17 is connected to a clamp valve 20 and two filters 23C and 23D which are series connected by a hose 22. The output 26A (lower end) of the filter 23D is connected by hose 26 and plug 27 to the top opening of jar 28. An outlet tube 29 in the side of the jar is connected to a vacuum source 30. Filter 23C may be selected of such pore size that it will retain any suspended red blood cells, white blood cells, platelets and fibrous strands and some bacteria. Filter 23D may be selected of smaller pore size to retain bacteria. This combination promotes fluid flow through the filter system, at the same time retains the bacteria on the filters.

With the foregoing arrangement, when it is desired to extract the solution and suspended bacteria from the bag 10 and pass it through the filters, the vacuum source 30 is energized and valve 20 is opened to permit the desired quantity of suspended bacteria and solution to flow from the bag 10 through hose 17, through each filter and into jar 28 under influence of the vacuum. Each of filters 23C and 23D are placed on or permeated with a suitable culture growth medium; for example: blood agar plate, nutrient media, upon which the bacteria collected by the filter may incubate and grow. If desired, one filter, or part of the filter, may be exposed to sterile air and the other filter, or part of the filter, flushed with oxygen-free gas in order to provide growth condition for both aerobic and anaerobic bacteria. After a suitable incubation and growth period, the filtered bacteria may be macroscopically and microscopically examined and identified.

It may be seen that our invention overcomes the limitations of existing blood-culturing techniques. Specifically, as little or as much blood as may reasonably be desired may be withdrawn for analysis; the dilution ratio may also be made as large as desired. Further, since the red cells are agglomerated, almost all of the solution may be withdrawn from the bag and filtered without the filter being clogged by the red blood cells. This permits all bacteria present to be filtered and collected in a small area. This means that the vast majority of micro-organisms present in the initial blood sample are collected on one or more small filters. It also means that a much larger number of micro-organisms may be collected on the filter surfaces, due to the growth stimulating properties of the solution. The increased concentration of micro-organisms on the filters provides for a greatly increased rate of bacterial growth after suitable culture gel is added. This, in turn, provides for quicker identification of the bacteria in the analyzed blood samples. Detection and identification of micro-organisms is facilitated.

An important feature of the inventive concept is the receiving container which holds the dilution—agglomerating—nutrient solution for the fluid sample. A separate embodiment of this device is illustrated in FIGS. 4 and 5.

In this embodiment, container 10', preferably of a plastic material with heat-sealed edges, 32, is elongated in shape and is equipped with a neck, 13', a self-sealing plug, 12b', and a bleeding tube, 11', through which a blood sample is introduced into bag 10' containing the nutrient-agglomerating dilution solution, 14'.

FIG. 4 illustrates the container in its upright position—the position in which the sample is introduced therein. The container may be supported in this position for introduction of the blood sample by means of eyelet, 33, and supporting means, not shown. Red blood cells, which are beginning to agglomerate and settle to the bottom of container 10', are shown at 16'.

When agglomeration of red blood cells is complete, and when they have all settled to the bottom of container 10', a clamping means, such as clamp 34, illustrated as a large hemostat, may be placed across the narrow portion of container 10' to confine the agglomerated red blood cells, as at 15', in a small area of container 10', leaving the bulk of solution 14', containing the diluted sample of blood less the red blood cells, in the larger portion of the container. Usually the agglomeration and sedimentation of the red blood cells takes place in from ½ to 2 hours at 37° C., leaving the free organisms and leukocytes, which may contain viable phagocytized organisms, in the supernatant fluid.

Container 10' may then be inverted, as shown in FIG. 5, and the supernatant filtered through exit tube 17', filters 23C' and 23D', tube 26', and into flask 28', using a vacuum source, not shown, attached to tube 29'.

Container 10' is shown as having a narrow elongated shape in FIGS. 4 and 5, and this is the preferred shape. However, it is to be noted that any shape is contemplated in this embodiment which has neck or restricted portion which permits segregation of the agglomerated erythrocytes, or red blood cells, after separation by simple clamping means. Other designs or shapes

will suggest themselves to those familiar with the art, and are intended to be included in the inventive embodiment.

Another aspect of the instant invention in the novel blood-culturing techniques, is the new and unique unitary filtration device which is illustrated in FIGS. 6-10.

As was described above, the blood sample, diluted with the novel nutrient-diluting agglomerating solution of this invention contains red blood cells or erythrocytes, white blood cells, or leukocytes, microbial organisms, such as bacteria, etc. The erythrocytes agglomerate and settle to the bottom of the container leaving in the supernatant liquid the leukocytes, any viable phagocytized organisms, and the free organisms which are to be identified. These organisms, or bacteria, are filtered from the solution and are usually subjected to further procedures to identify and type them.

One step in the process of the identification may be visual examination either naturally or with magnification prior to removal of the filters to grow the media for further development of the bacteria. The novel filtration unit of this invention is adapted to enable the observer to easily and quickly examine the filter plates for morphological purposes and to examine and compare the various filtered bacteria or bacterial colonies developed in the medium.

The novel filtration unit illustrated in FIGS. 6-10 is preferably a set of matched discs, connected by hinges, each having two or more recesses therein to serve as filtration chambers. The bottom of at least one filtration chamber is connected to the top of at least one other chamber so that the fluid flows serially from the bottom of one chamber to the top of another. Although the fluid flow is preferably by means of channelled passageways to make the desired serial connection, it may also be by means of flexible tubes.

In the embodiment illustrated in FIGS. 6 and 7, for example the pair of matching discs, 35, 36, are hanged by hinge means, 37, and contain matching recesses, 38 and 38' which, when the unit is closed (FIG. 6) form filtration chambers. Recesses 38' are adapted to receive and support filter plates 39, usually by means of shoulders as at 40. Other means of supporting filter plates 39, such as projections, platform supports, "frogs," etc., may of course be used.

When the unit is closed, filter plates in position, and in condition for operation a vacuum is applied by vacuum-producing means, not shown, to outlet 41, fluid enters through inlet 42 passes through flexible tubes 43, through filter plates 39, through flexible tube 44 and into the top of a serial filter chamber, out the bottom and into a collection vessel, not shown.

Preferably the first filtration is through a coarser filter, say a Millipore filter plate of from 8.0 to 10.0 μ in pore size and then through a finer filter, say one of a pore size of from 0.40 to 0.50 μ . The unit can be adapted and the tubing arranged, of course, so that the fluid may be filtered first through one coarse filter and then through two finer filters, if desired, in the embodiment shown. Any number of filtration chambers and filtration plates may be formed in matching discs 35, 36, without departing from the concept of the invention.

The matching discs may be fitted with sealing rings, not shown, or other means of assuring an airtight seal for efficiency of operation. When the filtration step is concluded, an air vent 45 may be opened to break the vacuum holding the unit together, (although an air vent is not necessary), disc 35 is moved upwardly about hinge means 37 and the filter discs containing the organisms are exposed all in the same plane. This feature enables the observer to scrutinize all the filter discs with their retained material at one time on a side-by-side relation without the necessity of handling and the risk of contamination. After the preliminary observation step, either with or without magnification, and recordal of the data obtained therefrom, the filter discs may be removed aseptically and placed on the usual culture media for further identification by known procedures.

FIGS. 8-10 illustrates another embodiment of the invention in which the chambers are made from hemispheres instead of

the cylinders of FIG. 6-7. In this embodiment the conduct of the fluid through the two opposing discs and through the filters is by means of channels molded or otherwise introduced into the rigid material of the discs. In this instance channel 44 is connected with 44' by a sealing ring, a male-female receptacle arrangement, or other means not shown, to provide the continuity of the channel. FIGS. 8-10 illustrate the preferred arrangement with inflow of fluid in 42. Fluid passes through filter 39, into the lower portion of the filter chamber 38'', through 44 and 44' where it enters one or more additional filter chambers 38' and flows through the filters 39. The lower portion of these filter chambers 38'' may be connected by a single channel 41 which is connected to a vacuum source. Of course, any numbers of filters can be arranged serially with channels leading from the bottom of one chamber to the top of the next. Furthermore, groups of chambers may be arranged in parallel by dividing the channel to the top of recipient filter chambers as depicted in FIGS. 8-10. Such serial and parallel or mixed serial and parallel arrangements of filters may be made without departing from the concept of the invention.

Although specific embodiments of the invention have been shown herein for purposes of description, it may be appreciated by those skilled in the art that numerous variations may be made without departing from the spirit or scope of the invention. For example, although the invention has been described with reference to blood culturing, it may be also used in the culturing and identification of bacteria in other body fluids; in water, such as in wells and community water systems; in biological and medicinal fluids requiring sterility testing and other fluids; gases and solids in which it is desirable to first dilute the specimen and then collect micro-organisms in a small area for concentrating the micro-organisms and for ease of handling and detection. The apparatus in which this dilution, agglomeration and subsequent filtration is carried out need not be necessarily a flexible container, although such is preferred.

To summarize briefly, this invention relates to a novel body fluid-culturing technique, and apparatus for performing the same, which comprises the steps of transferring a quantity of body fluid, preferably blood, to a container containing a sterile diluting solution having nutrient and erythrocyte agglomerating agents therein, retaining the mixture of blood and solution in said container for a period of time sufficient for the erythrocytes to agglomerate and sediment, and filtering the supernatant fluid to retain any bacteria in said solution. The concept of the invention includes a container which is of a flexible material and of a shape such that the sedimented agglomerated erythrocytes may be separated from the supernatant solution by clamping means to and in the filtration step.

The invention also relates to a unitary filtration unit which is designed to serially filter the supernatant liquid through two or more filters whose filter plates are in the same horizontal plane.

What is claimed is:

1. A process of culturing blood comprising the steps of (1) transferring a quantity of blood from the body of a patient to a container containing a diluting solution having nutrient and agglomerating agents, (2) retaining the mixture of blood and solution in said container for a time sufficient for the red blood cells to agglomerate and any bacteria in said blood to feed upon the nutrient agents in said solutions, (3) causing the solution and the unagglomerated blood elements to flow out of said container to a filter where bacteria are trapped upon said filter.

2. The process of claim 1, which further includes the step of subsequently adding a culture medium to said filter, and storing said filter and said bacteria at a suitable temperature to facilitate bacterial incubation, growth and identification.

3. The process of claim 2 which further includes the steps of causing a portion of the solution and unagglomerated blood elements to flow through a second filter which is similar to said first mentioned filter, exposing one filter to sterile air for

aerobic bacterial growth, exposing the second filter to an environment lacking free oxygen and facilitating anaerobic growth, and storing said filters at a suitable temperature to facilitate bacterial incubation, growth and identification.

4. A blood culture apparatus which comprises:
 - a container having inlet means for allowing blood to be inserted therein for culture and outlet means for permitting controlled outflow of the contents of said container;
 - filter means connected to the outlet of said container; and means for recovering the contents of said container passing through said filter means
 - said filter means comprising a first and second filter, each having an inlet and an outlet;
 - a Y-shaped tube member having an upper leg connected to said container outlet means;
 - a first lower leg of said Y tube connected to an inlet of said first filter;
 - a second lower leg of said Y tube connected to an inlet of said second filter;
 - means for individually connecting the outlet of said filters with the separate legs of a second Y-shaped member;
 - a third leg of said second Y-shaped member connected to said recovering means;
 - individual valve means intermediate said first Y-shaped member and the inlets of said filters for permitting the

contents of said container to flow through said outlet means and to each of said filters under control of each of said valve means so that flow may occur in any selected one of said filters or in both of said filters concurrently.

5. A blood culture apparatus which comprises:
 - a container having inlet means for allowing blood to be inserted therein for culture and outlet means for permitting controlled outflow of the contents of said container;
 - filter means connected to the outlet of said container; and means for recovering the contents of said container passing through said filter means
 - said filter means comprising a first and second series connected filter;
 - means for connecting the outlet of said container with the input of said first filter means;
 - means for connecting the outlet of said first filter means with the inlet of said second filter means; and
 - means for connecting the outlet of said second filter means with said means for recovering the contents of said container.
6. An apparatus according to claim 5 wherein said means for connecting the outlet of said container to said first filter means includes valve means for permitting the selective flow of said container contents through said filters.

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