

[54] **METHOD FOR WASHING BLOOD CELLS**

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Related U.S. Application Data

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[51] Int. Cl. **B04b 5/02**

[58] Field of Search 233/1 D, 14 R, 14 A, 15, 233/26, 27; 210/83, 84, 516, DIG. 23

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[57] **ABSTRACT**

Method for washing blood cells by forcing blood cells to the bottom of a stationary liquid in a container, where the liquid has a greater density at the bottom of the container than at the top.

9 Claims, 9 Drawing Figures

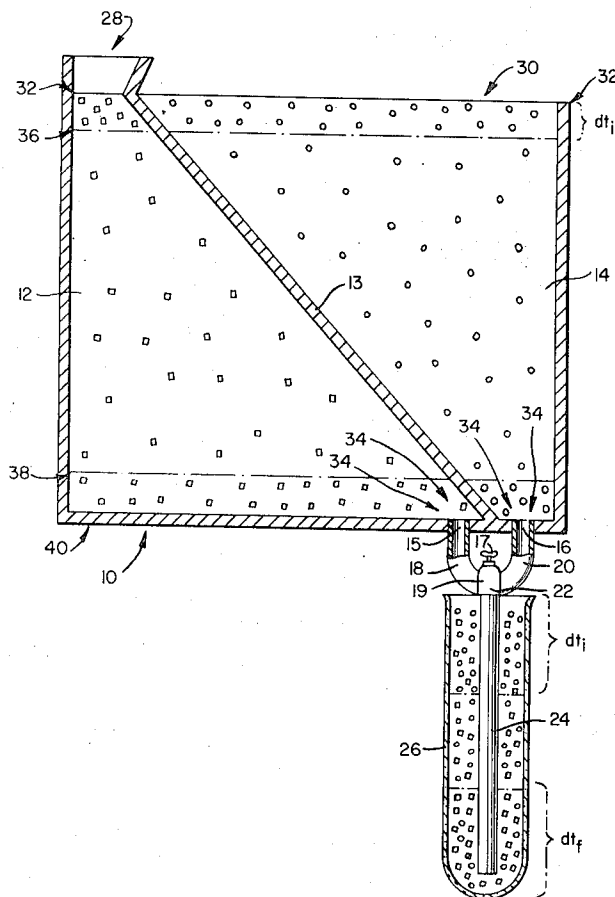


FIG. 2.

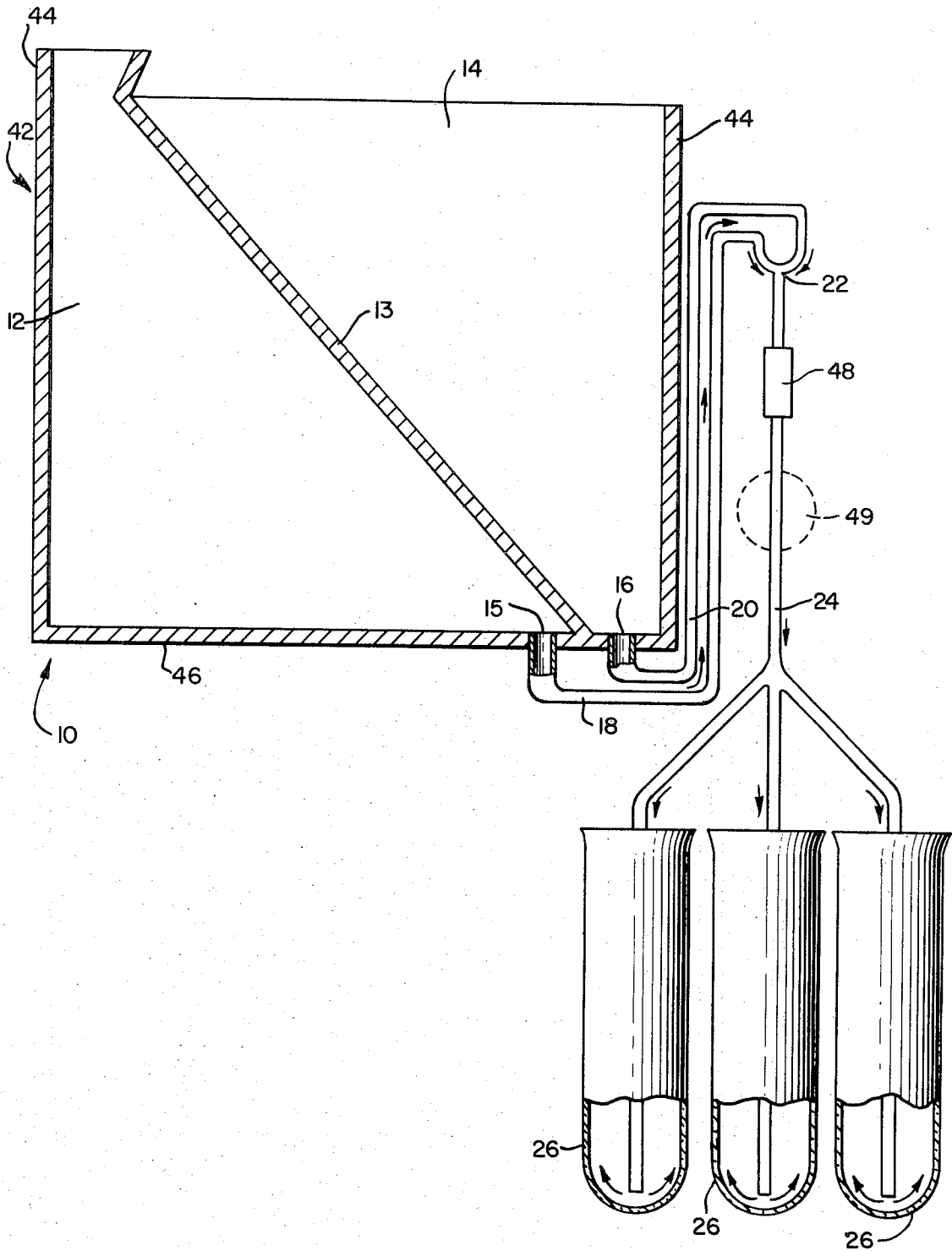


FIG. 4.

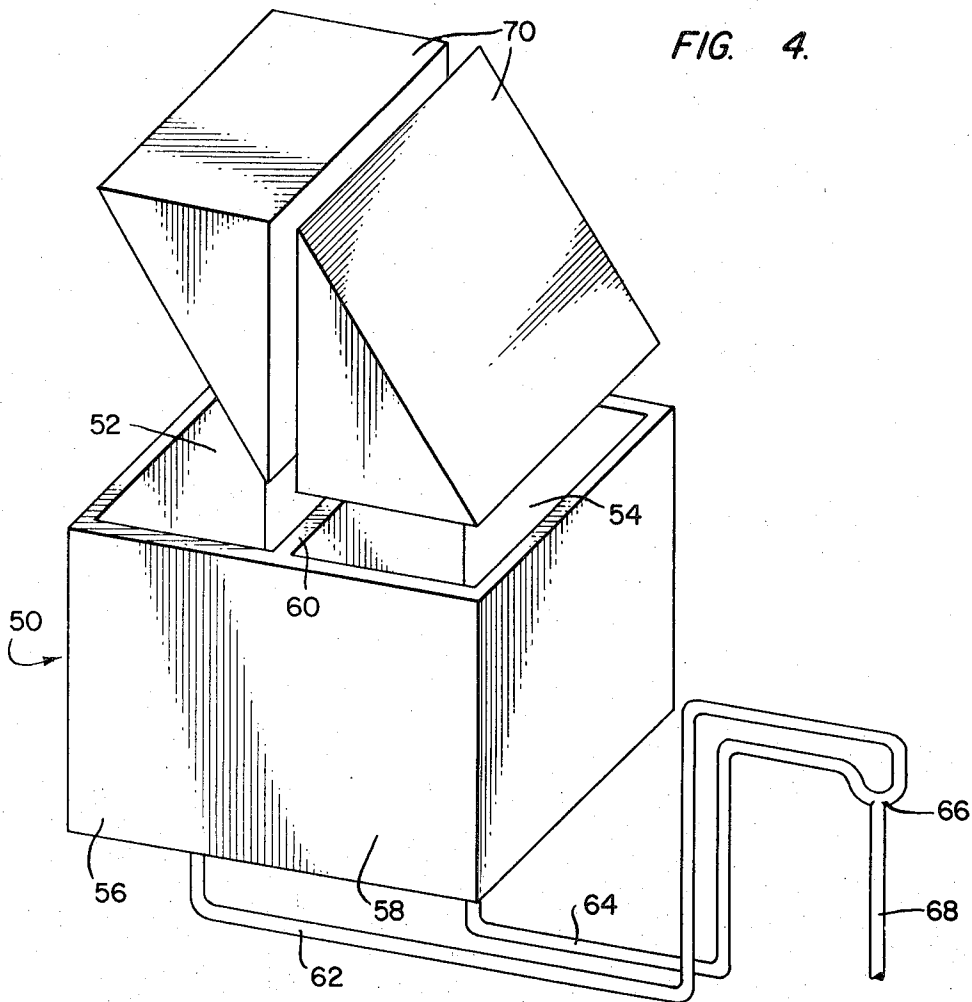


FIG. 3.

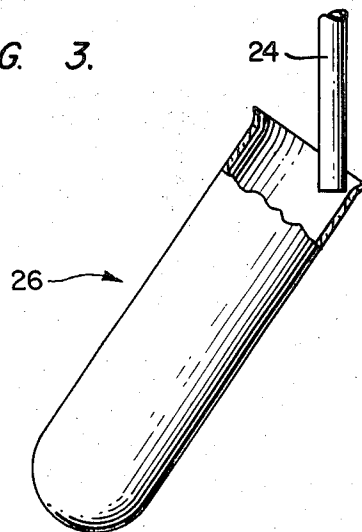


FIG. 5.

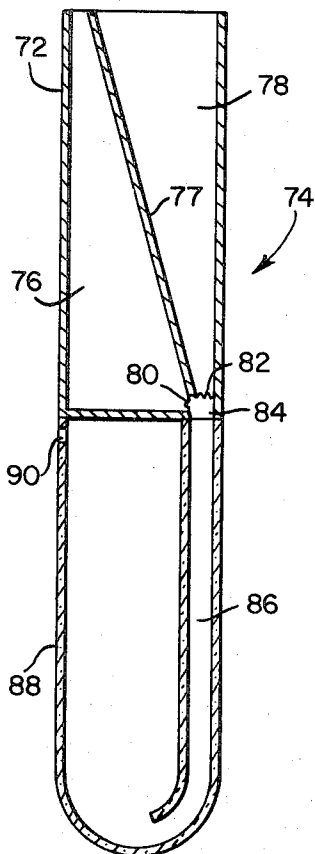


FIG. 6.

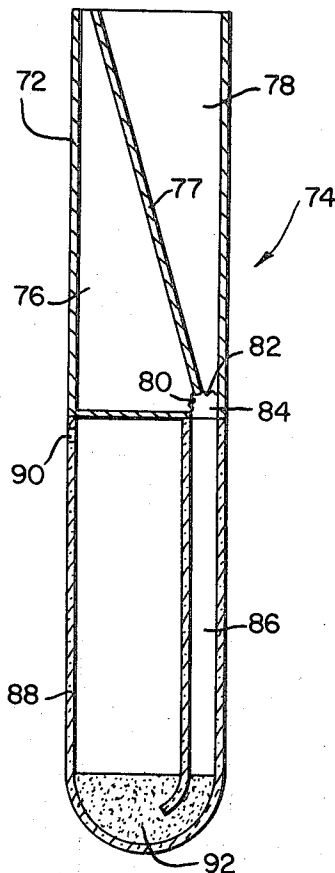


FIG. 7.

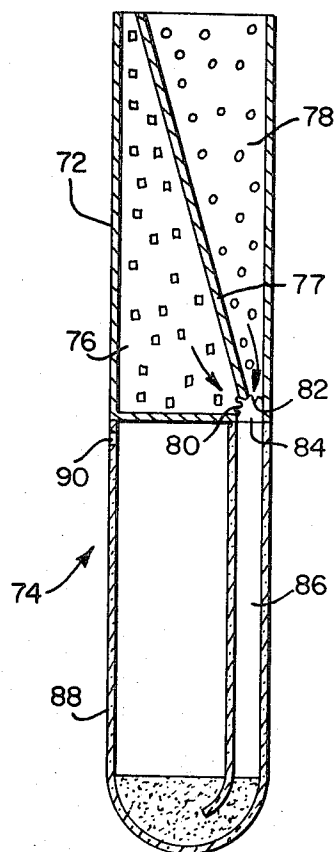


FIG. 8.

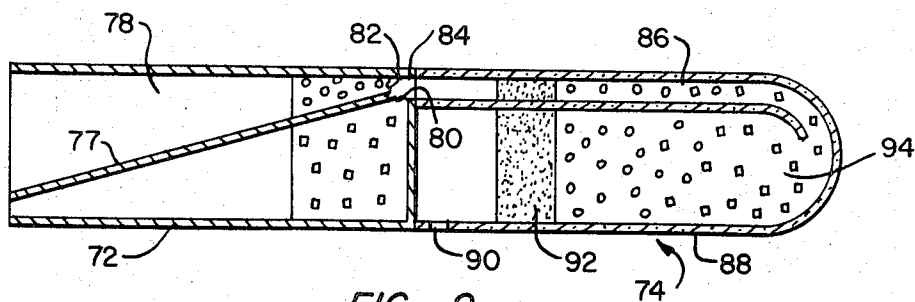
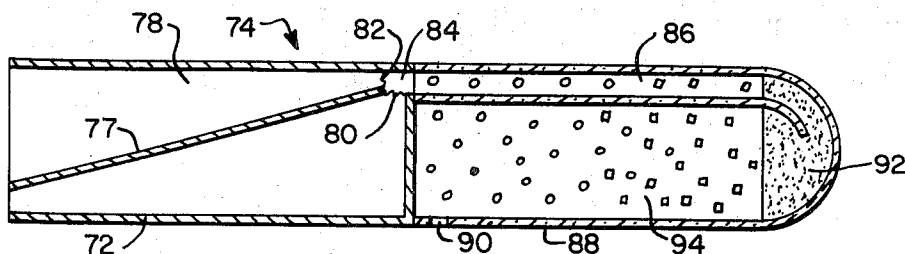


FIG. 9.



METHOD FOR WASHING BLOOD CELLS

This is a continuation, division, of application Serial No. 330,880 filed February 8, 1973 now U.S. Pat. No. 3,813,618.

BACKGROUND OF THE INVENTION

The method and apparatus disclosed in the following specification is especially useful for washing red blood cells with two different liquids so that the red blood cells can be analyzed.

Before a patient requiring a blood transfusion can safely receive blood from a donor, the donor's blood must be tested in order to ascertain that it is compatible with the blood of the patient. In order to test blood for such compatibility, the blood cells must be washed to strip the blood cells of the plasma or serum that surrounds them. Such washing is normally accomplished by repeatedly diluting the blood cells with a saline solution. Such dilution is repeated until a sample of the blood cells is sufficiently free of plasma or serum such that an effective test with Coomb's serum or other reagent may be performed. Such dilutions often require as many as four washing and decanting steps prior to the addition of Coomb's serum or cross-matching reagents.

SUMMARY OF THE INVENTION

The present invention provides a means for stripping plasma or serum from red blood cells by passing the red blood cells through a liquid density gradient. Using the generator of the present invention, a test tube may be efficiently filled with two liquids having different densities in a configuration such that the concentration of a first, or lower density, liquid is greatest adjacent the top of the test tube, while the concentration of a second, or higher density, liquid is at a maximum at the bottom of the test tube.

When red blood cells are floated on a liquid in a test tube containing such a density gradient and then centrifuged, red blood cells are forced to pass downwardly into the test tube through a density gradient which strips all the plasma from the red blood cells before the red blood cells reach the bottom of the test tube. When the test tube is removed from the centrifuge and decanted, the blood cells are retained in the bottom of the test tube and are ready for testing.

Accordingly, it is an object of the present invention to provide a novel apparatus for producing a known and controllable liquid concentration gradient.

Another object of the present invention is to provide an apparatus which will enable the attainment of a predictable volume gradient in a container from two different liquids.

A further object of the invention is to provide a new method for producing a liquid concentration gradient from two different liquids.

Still another object of the present invention is to provide an apparatus for producing a liquid concentration gradient which will enable the washing of red blood cells in a single centrifuging step.

Yet another object of the present invention is to provide a new method for washing red blood cells whereby the plasma surrounding the red blood cells is stripped therefrom as the red blood cells are forced through a liquid having a density gradient.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a diagram showing the operation of the liquid concentration gradient generator of the present invention;

FIG. 2 is a cross-sectional view of a generator of the type shown diagrammatically in FIG. 1;

FIG. 3 is a diagram showing a suitable orientation for a test tube in relationship to a delivery conduit when the gradient generator of FIG. 2 delivers the most dense liquid initially;

FIG. 4 is a perspective view of an alternate embodiment of a gradient generator in which changeable inserts are used to vary the proportion of the gradient which results;

FIG. 5 is a cross-sectional view showing an alternate embodiment of the generator of the present invention; and

FIGS. 6-9 are schematic views showing the utilization of the generator set forth in FIG. 5.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The operation of concentration gradient generator 10 of the present invention is shown diagrammatically in FIG. 1 and includes compartment 12 and compartment 14 which are formed by a barrier 13. At the bottom of generator 10 are liquid outlets 15 and 16 which allow passage or flow of liquid from compartments 12 and 14 into conduits 18 and 20 respectively. Outlets 15 and 16 comprise equal size apertures in generator 10. Conduits 18 and 20 are joined at a junction 22 to form single delivery conduit 24. A suitable valve 17 is provided in order to stop the flow of liquid through delivery conduit 24 during the filling cycle.

To produce a gradient with generator 10, both compartments 12 and 14 are filled with liquids. Since the purpose of generator 10 is to produce a known and controllable concentration gradient in a vessel such as test tube 26, the liquid in compartment 12 differs in some way from the liquid in compartment 14.

For purposes of washing blood cells, in accordance with the present invention, the liquids in the two compartments differ from each other by being solutions of different concentrations or two sugar solutions of different concentrations. For the reasons set forth below, when generating liquid density gradients, it is desirable to fill compartment 12 with the higher density liquid and compartment 14 with the lower density liquid.

Although the invention is primarily directed to forming liquid density gradients, it is to be understood, however, that the gradient generator of the present invention has a utility for producing many kinds of liquid gradients, for example, a gradient generator in accordance with the present invention can be advantageously utilized to produce refractive index gradients, ionic strength gradients, viscosity gradients, pH gradients, and optical density gradients.

In order to facilitate understanding the operation of generator 10, in FIG. 1, the liquid in compartment 12 is represented diagrammatically by a plurality of squares, whereas the liquid in compartment 14 is represented diagrammatically by a plurality of circles. It is to be understood, however, that such symbols have absolutely no relationship to the properties of the liquids which are intended to be placed in compartments 12 and 14. The use of circles and squares is simply a de-

vice to aid in understanding how concentration or volume gradients are produced by the apparatus of the present invention.

Prior to the allowance of any flow through outlets 15 and 16 of the gradient generator 10, compartment 12 is filled with an appropriate liquid by introduction of that liquid through the top of compartment 12 as is indicated by arrow 28. Compartment 14 is similarly filled by introducing a different liquid in compartment 14 through the top as is indicated by arrow 30.

For best operation of generator 10, compartments 12 and 14 are initially filled to an equal level. Compartments 12 and 14 will remain essentially equal. However, for liquids with widely different densities, the levels will have heights approximately inversely proportional to the densities. Thus, prior to operation of generator 10, the level of liquid in both compartments 12 and 14 is equal as is indicated at level 32. With delivery conduit 24 in an open or flow condition, for any given interval of time, dt_i , the level of liquid in both compartments 12 or 14 will remain equal. Thus, for a first unit interval of time, dt_i , during which liquid flows through outlets 15 and 16, as shown by arrows 34, the level of the upper surface of the liquids in both compartments 12 and 14 will drop to a lower but equal level as is shown by level 36.

The time that it takes for the level to drop from level 32 to level 36, due to flow of liquid through outlets 15 and 16, is shown by the bracket dt_i in FIG. 1. During the initial time interval, dt_i , the amount and relative proportion of liquid which flows through outlets 15 and 16 is shown diagrammatically in test tube 26 by the bracket dt_i on test tube 26. As is shown in FIG. 1, as the liquid level in compartments 12 and 14 drops from level 32 to level 36, the amount of liquid which will be delivered into test tube 26 from compartment 14 is that amount represented schematically by the circles between levels 32 and 36, whereas the amount of liquid from compartment 12 that will be delivered into test tube 26 is represented by the squares between levels 32 and 36.

Thus, as can be seen from FIG. 1, initially a large volume of low density liquid from compartment 14 is delivered into test tube 26 along with a relatively smaller volume of high density liquid from compartment 12.

The volume of high density liquid from compartment 12 that is delivered through delivery conduit 24, increases as the level of liquid in the compartments 12 and 14 drops, while on the other hand, the volume of liquid which is delivered from compartment 14 decreases as the level in compartment 14 drops. This relationship continues until both compartments 12 and 14 are empty.

As is shown in FIG. 1, the volume relationship during the final time interval (dt_f), is reversed from the volume relationship during the initial time interval (dt_i). That is, as the level of liquid in compartments 12 and 14 drop from level 38 to bottom 40, the volume proportion of the liquids delivered into test tube 26 is equal but opposite to that proportion delivered during the initial time interval. The foregoing is based on the fact that the actual intervals of time represented by dt_i and dt_f are equal and that barrier 13 is set to provide such a linear gradient. Of course, the invention is not intended to be limited to embodiments in which barrier 13 provides such a gradient. As is also apparent, the volumes of the compartments may be unequal, but the

invention is more easily understood when the volumes of compartments 12 and 14 are equal.

It should also be noted that because a large amount of low density fluid is delivered during the initial interval, that liquid will be displaced by the heavier liquids which follow. For this reason, the liquids delivered during the initial period, dt_i , are shown on the top of test tube 26.

Although it is possible to reverse the relationship of the liquids within compartments 12 and 14 so that high density liquid is in compartment 14 and low density liquid is in compartment 12 and thereby provide for the delivery of more dense fluid followed by less dense fluid, such an arrangement is not as desirable for blood washing applications as the arrangement shown in FIG. 1. The reason for this fact is that if the more dense fluid is delivered first, the less dense fluid which follows percolates through the more dense fluid. Such percolation results in a certain amount of mixing within test tube 26. Of course, as is apparent, any mixing of a liquid gradient is undesirable when the purpose of the concentration gradient is to provide stabilized sedimentation for the transport of suspended matter from one liquid to another by gravitational or centrifugal forces.

An acceptable arrangement for delivering high density fluid first, results with the delivery conduit 24 terminated at the upper end of the test tube, as is shown in FIG. 3. One undesirable aspect of the embodiment shown in FIG. 3 is that some splattering occurs when the end of the delivery conduit 24 is spaced a great distance from the bottom of the test tube. Thus, the lower density liquid, which follows the higher density liquid, can splatter and result in some mixing within test tube 26. At this point, it should be noted, however, that a small degree of mixing is not always a significant problem. Furthermore, as set forth below, in connection with the discussion of the embodiment of the invention shown in FIG. 3, with wettable liquids and with the test tube orientation shown in FIG. 3, it is possible to deliver the less dense liquid first without any appreciable mixing. For blood test applications, however, the preferred embodiment is to deliver the lower density liquid first as shown in FIG. 1.

A more detailed view of the generator shown diagrammatically in FIG. 1 appears in FIG. 2, where generator 10 comprises a vessel 42 which is adapted to contain a quantity of a liquid. Generator 10 is inclusive of four walls 44 and a bottom 46. Since FIG. 2 has a front portion broken away, the front wall does not appear in the view. Vessel 42 is divided into two equal compartments 12 and 14 by a barrier 13 running diagonally within vessel 42. When conduits 18 and 20 are open, fluids contained in compartments 12 and 14 will merge at junction 22 and flow into mixer 48. Mixer 48 may be any conventional in-line laboratory mixer. In fact, acceptable gradients result without a mixer in the delivery system.

Because outlets 15 and 16 comprise equal size apertures in communication with conduits of equal diameters, as the liquid level in compartments 12 and 14 drops, larger amounts of liquid from compartment 14 is mixed with decreasing amounts of liquid from compartment 12.

Should the level of liquid in vessel 42 reach a point that is lower than the highest point of the conduits, a siphon that is established causes the liquids to flow under the influence of gravity, provided no significant

pressure drop is created in the mixer 48. If such a pressure drop is created, however, a suitable pump 49 may be employed after the mixer 48 to draw the liquids through the conduits. Thus, either a pump or a gravity feed may be used to deliver the liquids in conduit 24 to a container. Also, as is shown in FIG. 2, conduit 24 may be branched to enable liquid gradients to be formed simultaneously in test tubes 26.

Since one important application of gradient generator 10 is to make density gradients, as for example, solutions of different concentrations, design precautions are employed to prevent transfer of the less dense fluid in one compartment into the other compartment and domination of flow by viscosity factors rather than by gravitational or body force factors. At this point, it should be noted that the size of outlets 15 and 16, as well as the inside diameter of conduits 18 and 20 are selected so that the resistance to flow of liquids through the outlets and conduits are controlled by the relative pressure in the chambers, with the viscosity of the two liquids are appreciably affecting the flow. At this point, it should be noted that aperture sizes from each compartment may differ since the viscosities of the two liquids may dictate certain desirable balanced flow resistances between each chamber and the mixing zone. For example, a 20 percent sucrose solution is about twice as viscous as water. If the more viscous fluid (20 percent sucrose) were to flow through a conduit whose diameter is larger than the diameter of the conduit of the water by a factor of $\sqrt{2}$, then the resistance to flow per unit length per unit flow rate would be equal. Of course, in some embodiments of the invention such a feature might be desirable. Furthermore, since the flow rates are always varying, it is desirable to locate the junctions of the conduits near the entry to the compartments (as is shown in FIG. 1) so that the pressure drop or flow resistance is governed by a single conduit.

As is set forth above, the system shown in FIG. 2 produces the least dense end of the gradient initially. Thus, liquid emerging from conduit 24 at the bottom of test tubes 26 floats on top of the more dense phases which follow. A suitable variation for the delivery of liquids through conduit 24 is possible when the solutions in compartments 12 and 14 are reversed so that the denser end of the gradient is delivered into test tube 26 initially. When the denser end of the gradient is delivered initially, it is advantageous to position test tube 26 at an angle relative to delivery conduit 24 and orient test tube 26 and delivery conduit 24 relative to each other so that the end of delivery conduit 24 is in the vicinity of the upper end of test tube 26. The arrangement shown in FIG. 3 allows wettable liquids flowing through conduit 24 to slide down the side of the test tube 26 without disturbing the gradient formed therein and without splattering.

Another embodiment of the liquid concentration-volume gradient generator, in accordance with the present invention, is shown in FIG. 4. In FIG. 4, generator 50 is comprised of two symmetrical rectangular compartments 52 and 54. Like generator 10 of FIG. 2, generator 50 is inclusive of liquid containing vessel 56 having four walls 58 and a bottom. A barrier wall 60 divides vessel 56 into compartments 52 and 54. Equal size apertures at the bottom of compartments 52 and 54 allow the passage of liquid into conduits 62 and 64. Conduits 62 and 64 have equal diameters and are also joined at a junction 66 to a delivery conduit 68. Both

compartments 52 and 54 are open at the top. As is also apparent, for some gradient configurations, compartments 52 and 54 may have unequal volumes. Furthermore, as stated above, the apertures may be unequal.

With the device, as shown in FIG. 4, variations of the gradient that can be produced is accomplished with various inserts 70, which are shaped to yield the desired gradient.

In all embodiments of the invention, the concentration of the liquids emerging from the delivery conduit will depend upon the volumetric portion of each liquid leaving their respective compartment. These volumetric portions depend on the liquid surface area exposed on each liquid in its respective compartment which is determined by the combined effect of the barrier separating the compartments and the depth of each liquid in each compartment. The depth of the liquid is a function of the density of the liquid in the compartment and the gravitational force acting on the liquid. For practical consideration, with most liquids, both of these factors are constant. Therefore, for compartments with equal size apertures, the volumetric portioning of the liquids flowing from the compartments is solely a function of the specific positioning of the barrier separating the compartments. For example, in FIG. 2, barrier 13 is shown as a straight angled partition which generates an approximately linear gradient whose shape is approximately equivalent to the rate of change of volume with depth in the compartments.

Another embodiment of a generator, in accordance with the present invention, is shown in FIG. 5. In FIG. 5, the gradient generator 72 is formed in the upper part of a vessel 74 which has a test tube-like configuration enabling vessel 74 to be placed in a centrifuge. Vessel 74 is both a gradient generator and a test tube in which the blood cells to be washed are centrifuged. The upper portion of vessel 74 which forms gradient generator 72 contains two chambers 76 and 78 which are formed by a barrier 77. With the embodiment of the present invention represented by vessel 74 of FIG. 5, it is advantageous to pre-load chambers 76 and 78 with the liquids from which a gradient is to be produced. It should be immediately apparent that chambers 76 and 78 can advantageously be constructed to conform to chambers 12 and 14 of the embodiment described in connection with FIG. 2. Thus, like the embodiment as represented by FIG. 2, the preferred manner of pre-loading generator 72 is to place the less dense liquid in chamber 78, with the more dense liquid being placed in chamber 76. Chambers 76 and 78 each contain a capillary opening 80 and 82 communicating with a capillary passageway 86. The diameters of opening 80 and 82 and the inside diameter of passageway 86 are preferably of a value between the range of 0.5 mm to 2.0 mm. Capillary passageway 86 terminates at the bottom portion of vessel 74 which forms a test tube 88 for blood to be tested. Capillary opening 80 and 82 are designed so that under a normal gravitational field, no liquid flows through these openings. However, under an increased gravitational field, such as the field produced by a centrifuge, liquid in chambers 76 and 78 would flow through openings 80 and 82. Alternatively, openings 80 and 82 can be plugged with a non-wetting material. When vessel 74, having such a plug, is centrifuged, the additional gravitational field created by the centrifuge breaks the plug causing liquid to flow through the openings.

Test tube portion 88 and generator portion 72 of vessel 74 are joined together so that generator 72 can be broken away from test tube portion 88.

The test tube portion 88 has an opening 90 formed therein for loading a sample of blood cells to be washed. Once vessel 74 is loaded, placed in a centrifuge and centrifuged, the liquids in chambers 76 and 78 flow into test tube portion 88. The sample of blood to be tested, which is injected into test tube 88, initially floats on the concentration gradient delivered into test tube portion 88.

Upon further centrifugation, the blood sample will be forced down through the liquid in test tube portion 88 and thereby washed. After completion of such washing and the removal of vessel 74 from the centrifuge, vessel 74 is broken to yield a test tube containing washed blood cells which are readily accessible for further testing. In one embodiment of the invention, vessel 74 is formed of a compliant moldable thermoplastic material such as polyvinyl chloride. The advantage of such a material is that it is easily cut by a pair of scissors. Thus, a vessel formed of this material can be cut, after the cells are washed, above the liquid in the vessel to yield a detached test tube portion 88. Thereafter, the liquid above the washed cells can be poured off. To store washed blood cells, the polyvinyl chloride walls of test tube portion 88 are pinched above the washed blood cells. Pinching seals the test tube portion 88 making storage of the blood cells in test tube portion 88 convenient.

FIGS. 6-9 are schematic diagrams showing the utilization of the embodiment of the invention set forth in FIG. 5.

As is shown in FIG. 6, a sample of blood cells to be washed 92 is injected into test tube portion 88 of vessel 74.

As is shown in FIG. 7, the gradient liquid materials are loaded into chambers 76 and 78 with high density fluid being loaded into chamber 76 and low density fluid being loaded into chamber 78. It is also advantageous to include the total required amount of Coomb's serum as two equal aliquots in chambers 76 and 78, although this step is optional.

As is apparent, the actual order of steps set forth in FIGS. 6 and 7 may be reversed, for example, it is advantageous to pre-package vessel 74 with fluids in compartments 76 and 78 and add the blood sample in the laboratory. With such pre-loading, the only liquid that is added to vessel 74 in the laboratory is the blood sample itself.

With generator 72 and test tube portion 88 loaded, vessel 74 is placed in a centrifuge and centrifuged as is shown in FIG. 8. During the initial phases of centrifuging, a density gradient 94 is generated in test tube portion 88 with the sample of blood cells to be washed 92 floating on density gradient 94. The centrifugally induced forces are strong enough to allow liquids to flow from compartments 76 and 78 to form the gradient in test tube portion 88. After continued centrifugation, red blood cells are forced through gradient 94 to the bottom of test tube portion 88 as is shown in FIG. 9.

Thereafter, vessel 74 is broken in two to yield test tube portion 88 which contains washed red blood cells.

If desired, excess supernatant is poured off and the washed red blood cells are examined.

By utilization of the liquid gradient generator of the present invention, more efficient washing of blood cells

is possible. Examples illustrating the technique for washing blood cells with the generator of the present invention appear below.

EXAMPLE 1

When utilizing the embodiment of the generator represented by FIG. 2 of the drawing, the generator is loaded with liquids of varying density. Into compartment 12 is placed a 12 percent by weight solution of ficoll in normal saline, with compartment 14 being filled to an equal level with normal saline. The specific gravity of the ficoll solution is 1.07, and the specific gravity of normal saline is 1.03. The holding capacity of generator 10 is calculated and designed so that when both compartments 12 and 14 are filled to the top, test tubes 26 will be able to receive and contain all of the liquid held in the generator compartments. Thus, each test tube receives an aliquot portion of the total gradient produced by the generator. After test tubes 26 are filled, a sample of blood to be tested is placed on top of the liquid in the test tube. For a 10 ml test tube containing about 8 mls of the liquid gradient, 1 ml of blood to be tested is placed in the test tube.

The test tube containing the sample of blood to be washed is then placed in a centrifuge and centrifuged at a speed of 3,000 rpms for 1 minute, or 1,000 rpms for 10 minutes. The test tube is removed from the centrifuge and the liquid above the washed blood cells is decanted. The washed blood cells on the bottom of the test tube are then tested with Coomb's serum or another antibody preparation. Of course, the result of the Coomb's serum test is controlled by the type of blood tested.

EXAMPLE 2

When utilizing the embodiment of the invention represented by FIG. 5 of the drawing, blood cells are washed in the following manner. Chambers 76 and 78 are pre-loaded with liquids from which the gradient is to be formed. Chamber 76 is loaded with 2.5 mls of a 12 percent by weight solution of ficoll in normal saline, and chamber 78 is loaded with 2.5 mls of normal saline. A 0.2 mls sample of blood is then injected through opening 90 of vessel 74. Vessel 74 is then placed in a centrifuge and centrifuged at a speed of 2,000 rpms for one half a minute which forces the liquids in chambers 76 and 78 into test tube portion 88. To accomplish the foregoing flow, capillary openings 80 and 82 are 0.5 mm in diameter. Thereafter, the vessel 74 is centrifuged at a speed of 3,000 rpms for 1 minute which forces the blood cells through the liquids in test tube portion 88. The vessel 74 is then removed from the centrifuge and broken in half to yield test tube portion 88. The liquid in test tube portion 88 is then decanted to yield the blood cells which are ready for further examination.

In the foregoing examples, the dense phase of the gradient was formed from a 12 percent solution of ficoll in normal saline. In connection with the term "ficoll," ficoll is a synthetic high polymer made by the copolymerization of sucrose and epichlorohydrin. The molecules have a branched structure and have a high content of hydroxyl groups leading to a very good solubility in aqueous media. No ionized groups have been found in ficoll. The average molecular weight of ficoll is $400,000 \pm 100,000$. It has an intrinsic viscosity of around 0.17 dl/g. Ficoll can be purchased from Phar-

macia Fine Chemicals, Inc., 800 Centennial Avenue, Piscataway, New Jersey 08854.

It should be noted that a gradient for washing blood cells can be formed from a number of materials. The important consideration is that the most dense portion of the gradient be less dense than the blood cells. In this regard, the specific gravity of blood cells is about 1.20. In accordance with the present invention, the gradient through which the blood is to be washed should have a specific density varying between about 1.03 to 1.15, although excellent results are possible with a gradient having a specific gravity variation of 1.03 to 1.09. When a ficoll solution is utilized for the dense phase of the gradient, it is advantageous to use normal saline which has a specific gravity of 1.03 as the less dense phase of the gradient.

Blood cells can also be treated in accordance with the present invention preparatory to freezing. In such freezing preparation, washed cells are "glycerized" after being removed from their serum or plasma. This step can be integral with the wash procedure in accordance with the present invention either by:

1. including the glycerine or desired reagent (Coomb's serum) at the end of the gradient as a separate zone denser than the gradient's heavy end, but lighter than the blood cells; or

2. as a constant concentration in the gradient itself similar to saline. The gradient generated will be of uniform reagent concentration but of varying concentration gradient solute.

It is also desirable to have a dense cushion on the bottom of the test tube to prevent the blood cells from reaching the bottom of the test tube. In this regard, a liquid on the bottom of the test tube having a specific gravity of 1.25 can be effectively utilized for such a cushion. A dense cushion is particularly desirable when the washed blood cells are to be frozen. When ficoll in normal saline is employed, the solution can include ficoll in a range of about 12 - 20 percent by weight.

In addition to ficoll, dextran may also be used to form gradients. In accordance with the present invention, a 12 - 20 percent by weight solution of dextran in normal saline can be employed to form density gradients. Dextran is an anhydroglucose polymer, produced by numerous strains of *Leuconostoc* and closely related bacteria in sucrose-containing solutions. Most of the glucosidic linkages are α -D-1 $\rightarrow$ 6, but to a lesser extent, 1 $\rightarrow$ 3- and 1 $\rightarrow$ 4-linkages also appear. The presence of these non-1 $\rightarrow$ 6-linkages is evidence of the branching of the chains. The ratio of 1 $\rightarrow$ 6 to non-1 $\rightarrow$ 6-linkages can vary within wide limits (3-14) for dextran from different sources, with consequent differences in chemical and physical properties. Pharmacia dextran, produced by *Leuconostoc mesenteroides*, strain B 512 has 5 - 10 percent of non-1 $\rightarrow$ 6-linkages.

Native dextran is soluble in water, resulting in very viscous solutions. Its molecular weight distribution is extremely wide, covering a range of molecular weights from hundreds of millions down to oligosaccharides. Dextran can also be purchased from Pharmacia Fine Chemicals, Inc., 800 Centennial Avenue, Piscataway, New Jersey 08854.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being in-

dicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

I claim:

1. A method for washing blood cells comprising the steps of loading a vessel with a liquid which is less dense than blood cells in such a manner that the liquid increases in density from the top of the vessel to the bottom thereof, thereafter forcing blood cells to be washed from the top of the vessel toward the bottom of the vessel through the stationary liquid to strip the blood cells and thereby produce washed blood cells.

2. The method as set forth in claim 1 wherein said blood cells are forced through said liquid by a centrifugation.

3. A method for washing blood cells comprising the step of producing a density gradient within a liquid contained by a vessel by introducing two liquids of different densities both of which are less dense than blood cells into separate compartments of a generator, initially allowing the liquids to flow from said compartments to a mixing zone, varying continuously the amount of liquid flowing from one compartment relative to the amount of liquid flowing from a second compartment and delivering said liquids from said mixing zone into a vessel and thereafter forcing blood cells to be washed from the top of the vessel containing a liquid to the bottom thereof.

4. The method as set forth in claim 3 wherein said blood cells are forced through said liquid by a centrifugation.

5. A method for washing blood cells comprising the steps of:

introducing two liquids of different densities both of which are less dense than blood cells into separate compartments of a generator, said generator being located in the upper portion of a vessel having a configuration which enables it to be placed in a centrifuge, each of said compartments having an opening for the flow of liquid into the lower portion of said vessel, said compartments being separated by a partition which causes the amount of liquid flowing from one compartment to continuously vary relative to the amount of liquid flowing from the other compartment, said liquids being introduced into said compartments so that a liquid gradient which increases in density is produced in the lower portion of the vessel;

loading a sample of blood to be washed into the lower portion of said vessel;

centrifuging the vessel to cause the liquid in the compartments to flow into the lower portion of the vessel and produce a liquid density gradient which increases in density from the top of the vessel to the bottom thereof with the sample of the blood floating on the liquid in the lower portion; and, continuing centrifuging to force the blood cell through the stationary liquid gradient to strip the blood cells and thereby produce washed blood cells.

6. The method as set forth in claim 5 including the step of detaching the lower portion of the vessel away from the upper portion to yield readily accessible washed blood cells in the lower portion of the vessel.

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- 7. The method as set forth in claim 6 wherein the lower portion is detached from the upper portion by cutting the vessel with a pair of scissors.
- 8. The method as set forth in claim 6 wherein the lower portion is sealed to provide a container for storing the washed blood cells.
- 9. The method as set forth in claim 5 wherein the

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lower portion of the vessel is loaded into liquid which is denser than the blood cells to provide a dense cushion which prevents the blood cells from reaching the bottom of the lower portion of the vessel when centrifuged.

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,858,795 Dated January 7, 1975

Inventor(s) John E. Joyce

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 5, line 21 change "are" to --not--.

Signed and sealed this 1st day of April 1975.

(SEAL)

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents
and Trademarks