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Marple

(54) **DEVICES AND METHODS FOR OVERLAYING BLOOD OR CELLULAR SUSPENSIONS**

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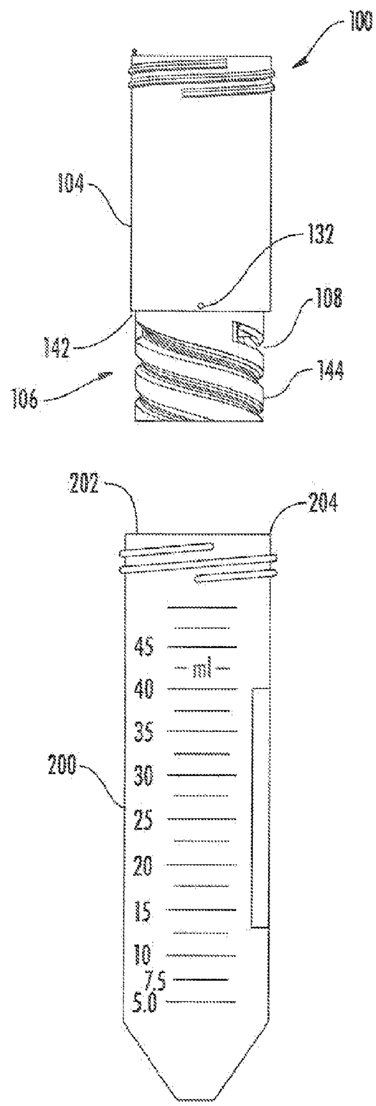
(57) **ABSTRACT**

A device is described that overlays a first fluid, such as blood or a cellular suspension onto a base material, such as a Ficoll liquid density gradient. In some embodiments, the fluid layering device includes a cylindrical reservoir, a fluid barrier, a coupling extension, one or more helical channels in fluid communication with the reservoir, and an exhaust vent. The fluid layering device can be coupled through its coupling extension to an open end of a container, such as a conical centrifuge tube, including the base material. Once attached, the device regulates flow of the first fluid from the reservoir into the conical tube so that a suitable overlay is formed without substantially disturbing a surface of the base material, regardless of the care and skill of the user.



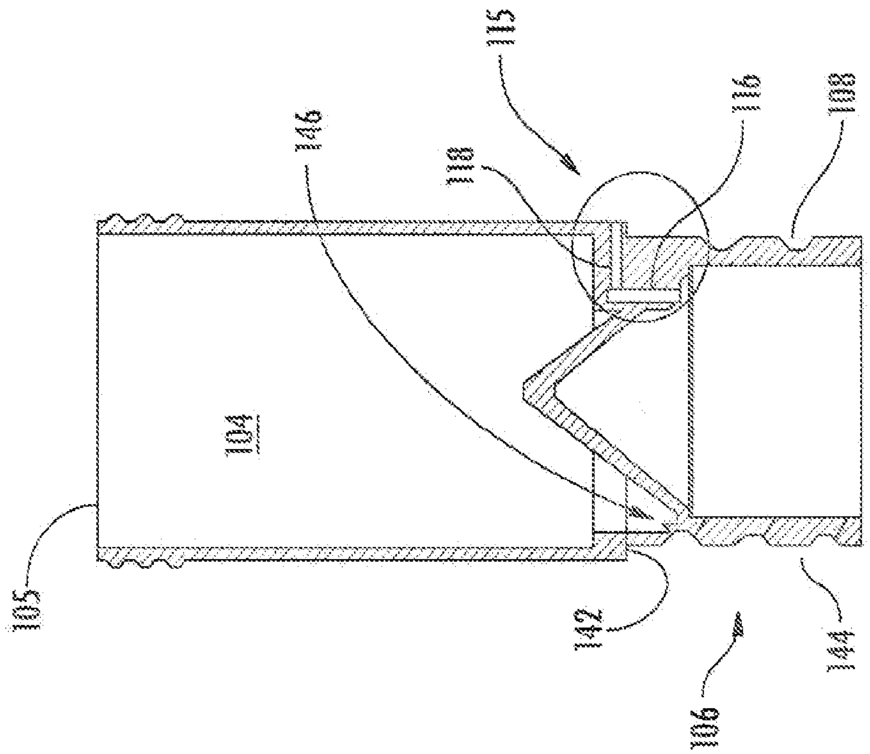

115
116
108
118
146
104
105
142
106
144

FIG. 2

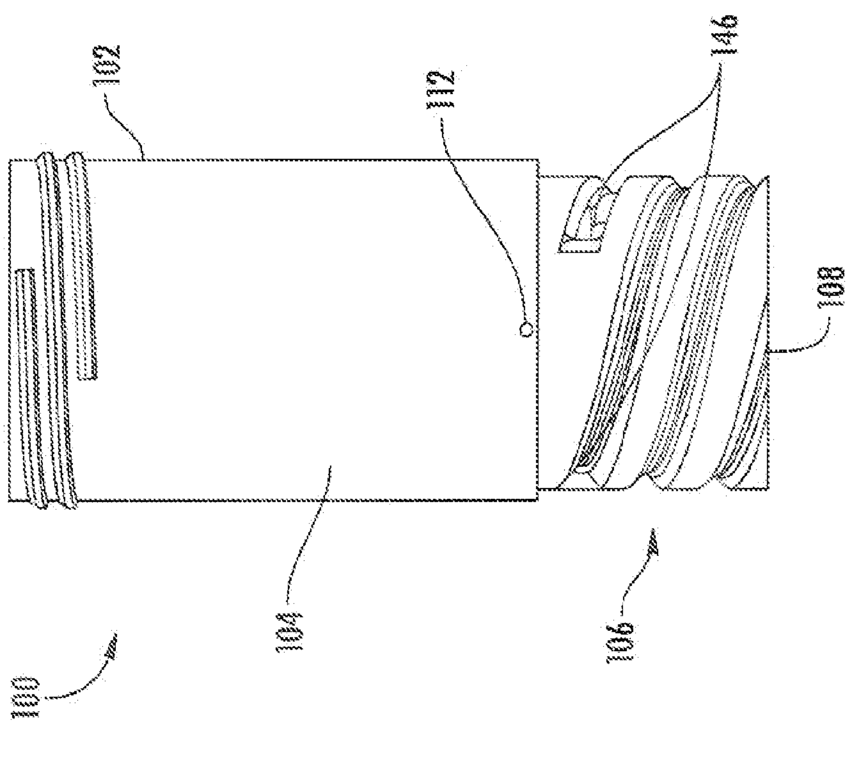

102
112
146
108
104
100
106

FIG. 1

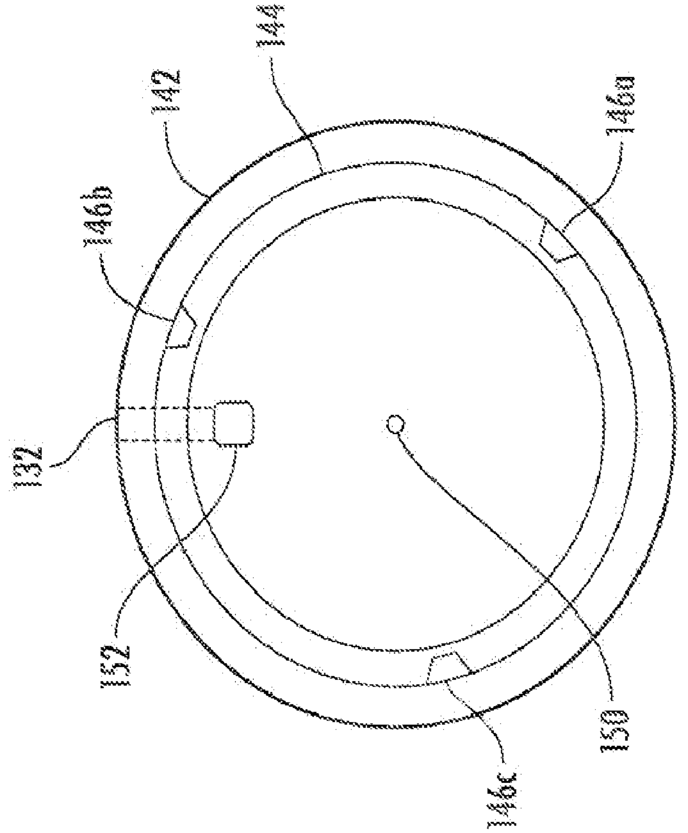

144
142
146a
146b
132
152
150
146c

FIG. 3B

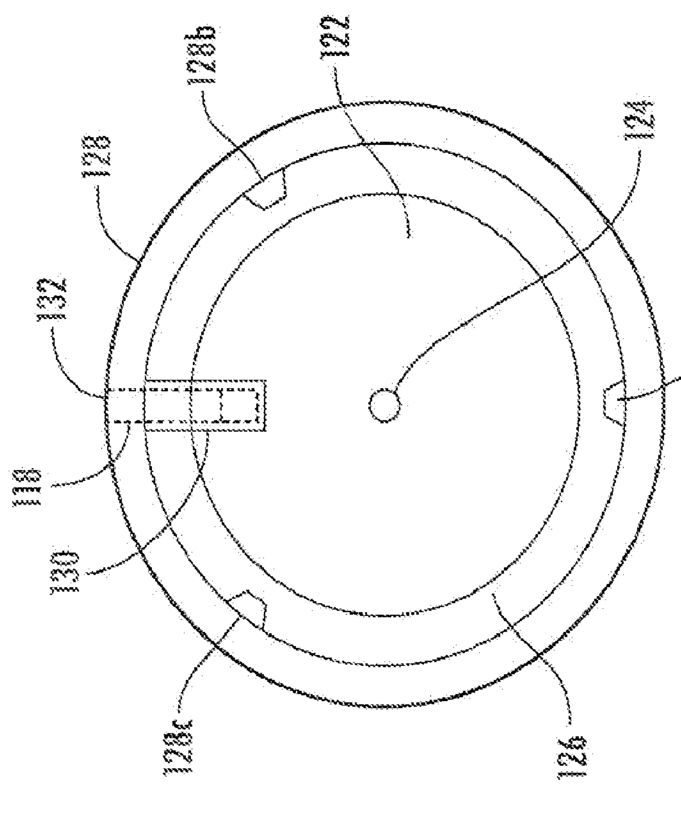

128
128b
122
132
118
130
124
128a
128c
126

FIG. 3A

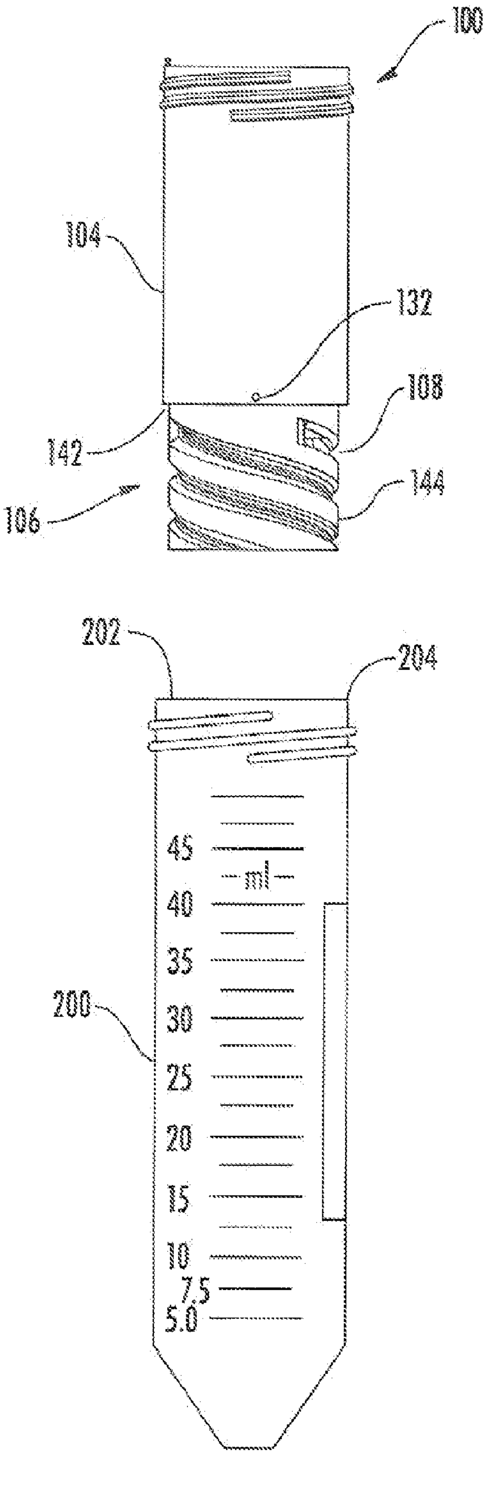

100
104
132
108
142
144
106
202
204
45
ml
40
35
200
30
25
20
15
10
7.5
5.0

FIG. 4A

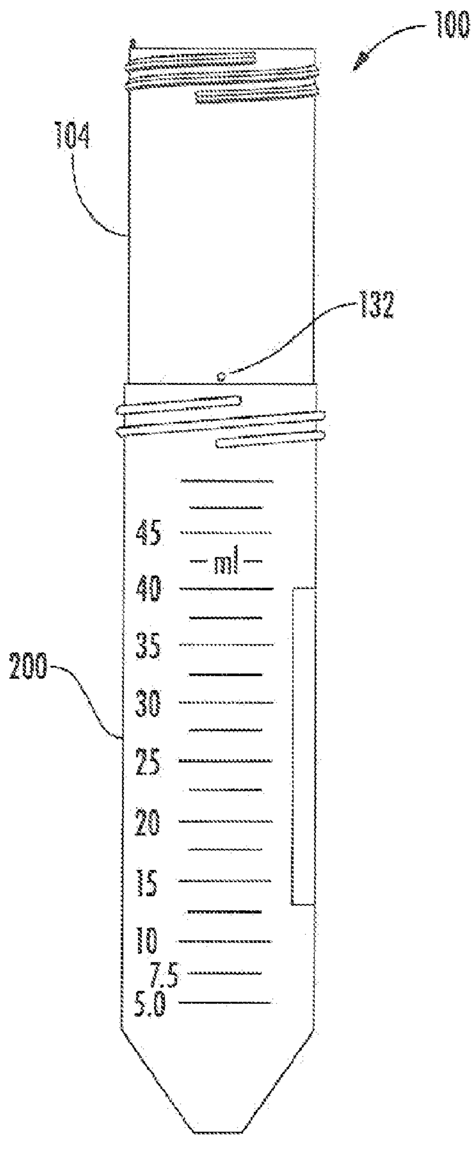

100
104
132
45
ml
40
35
200
30
25
20
15
10
7.5
5.0

FIG. 4B

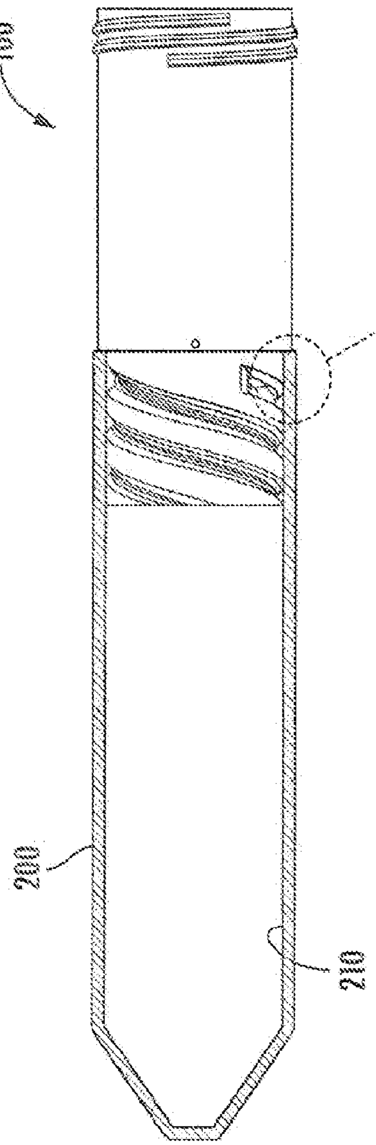


FIG. 5A

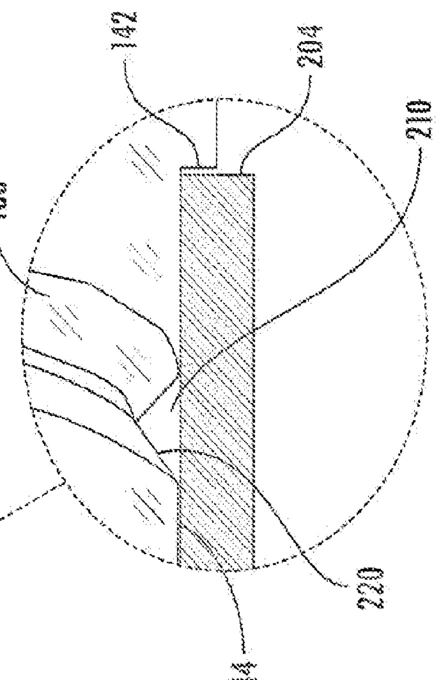


FIG. 5B

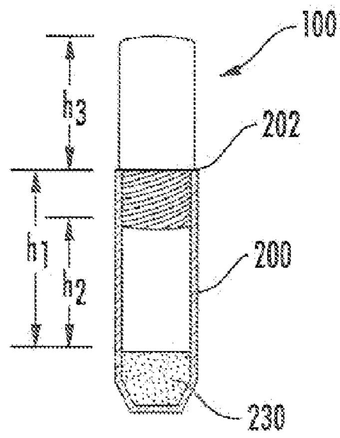


FIG. 6A

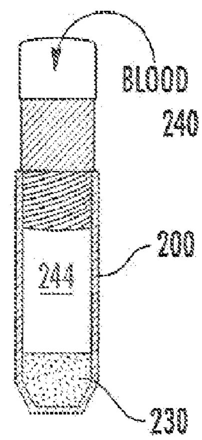


FIG. 6B

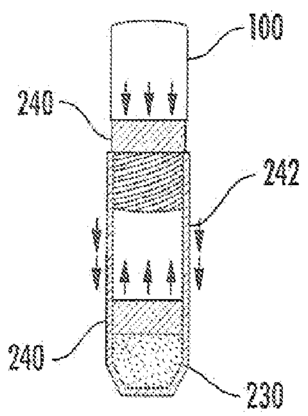


FIG. 6C

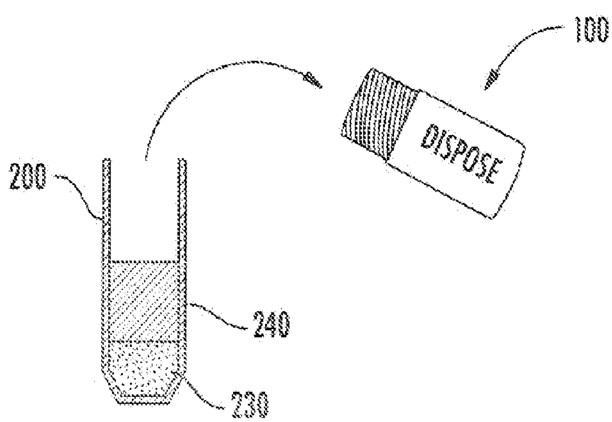


FIG. 6D

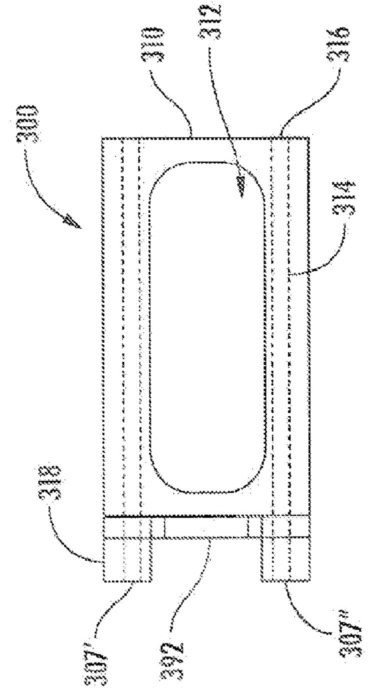


FIG. 7A

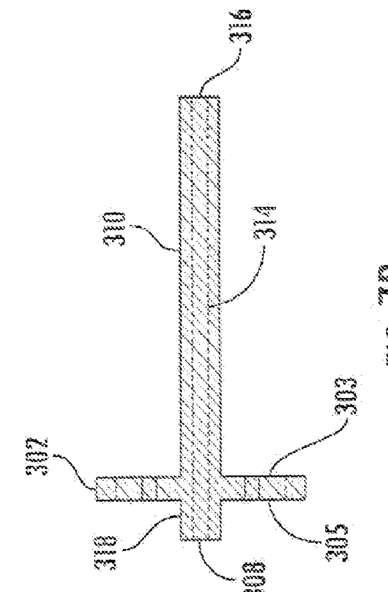


FIG. 7B

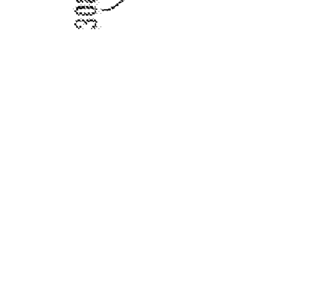


FIG. 7C

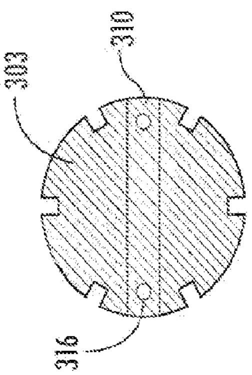


FIG. 7D

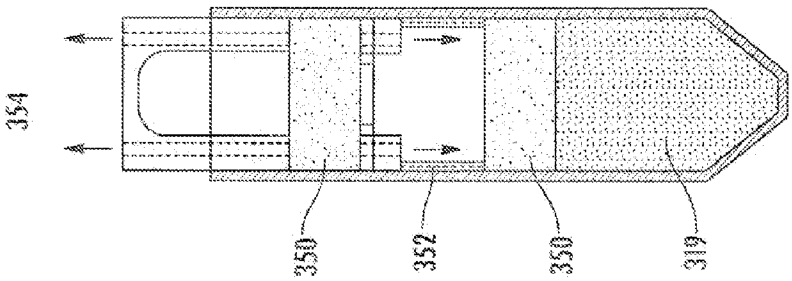


FIG. 8C

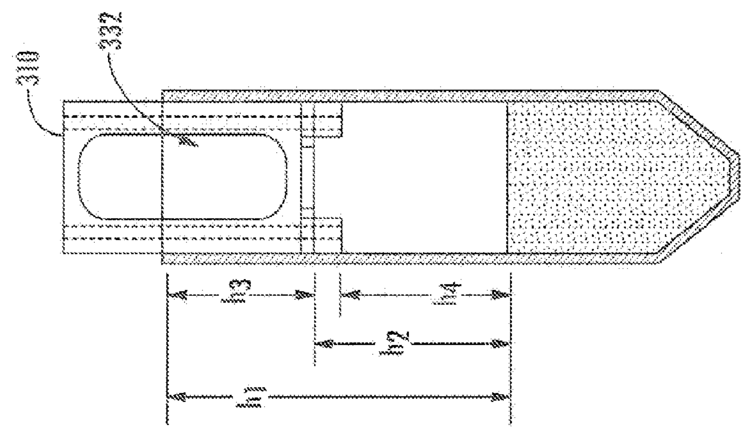


FIG. 8B

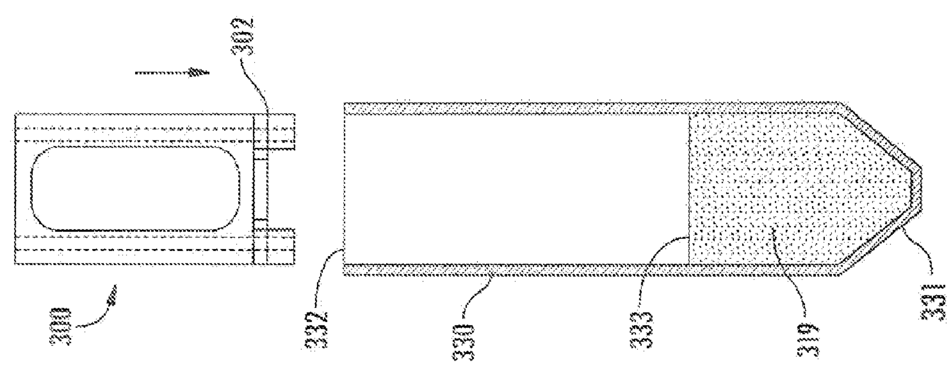


FIG. 8A

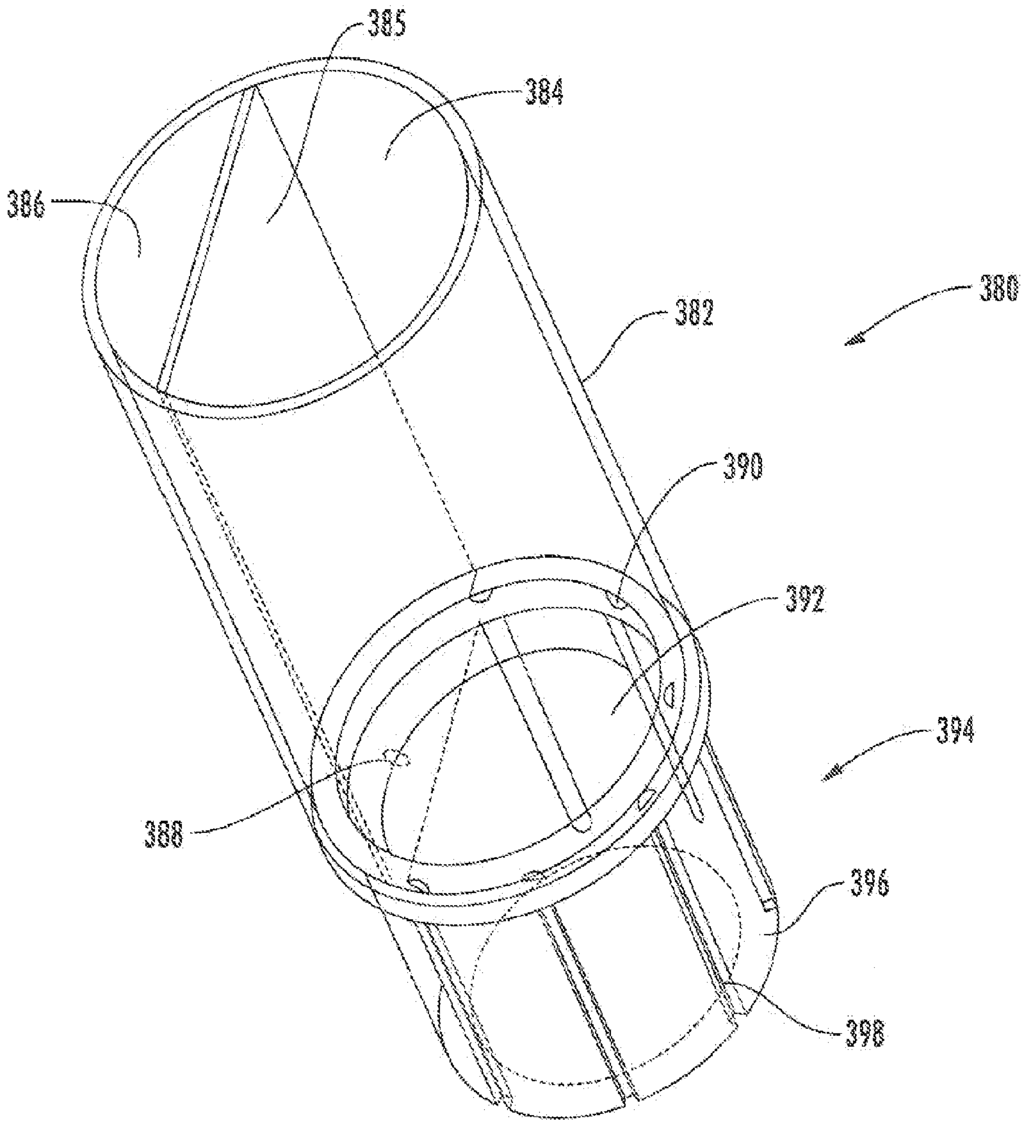


FIG. 9

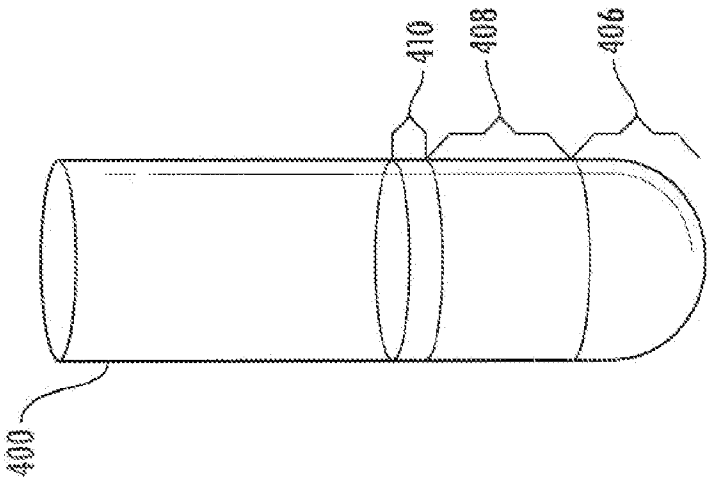


FIG. 10C

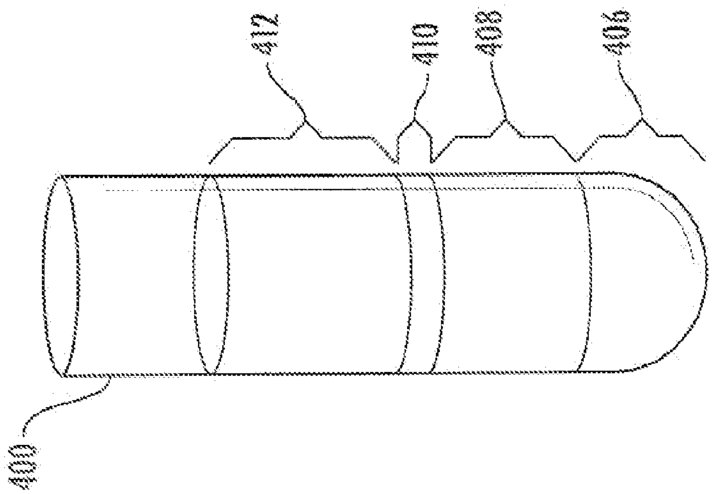


FIG. 10B

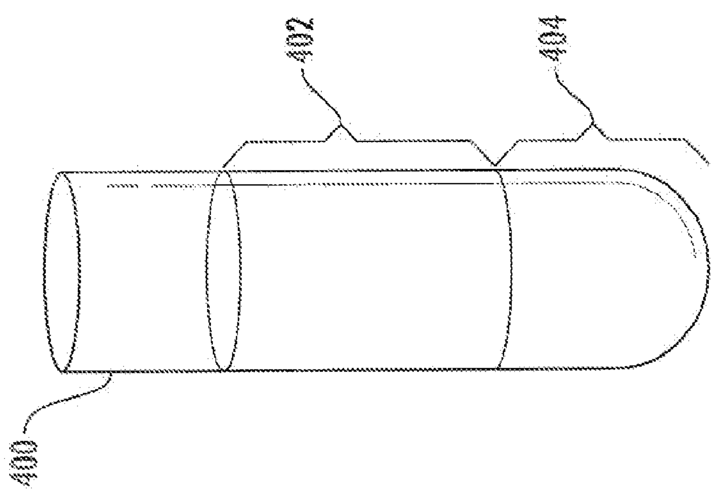


FIG. 10A

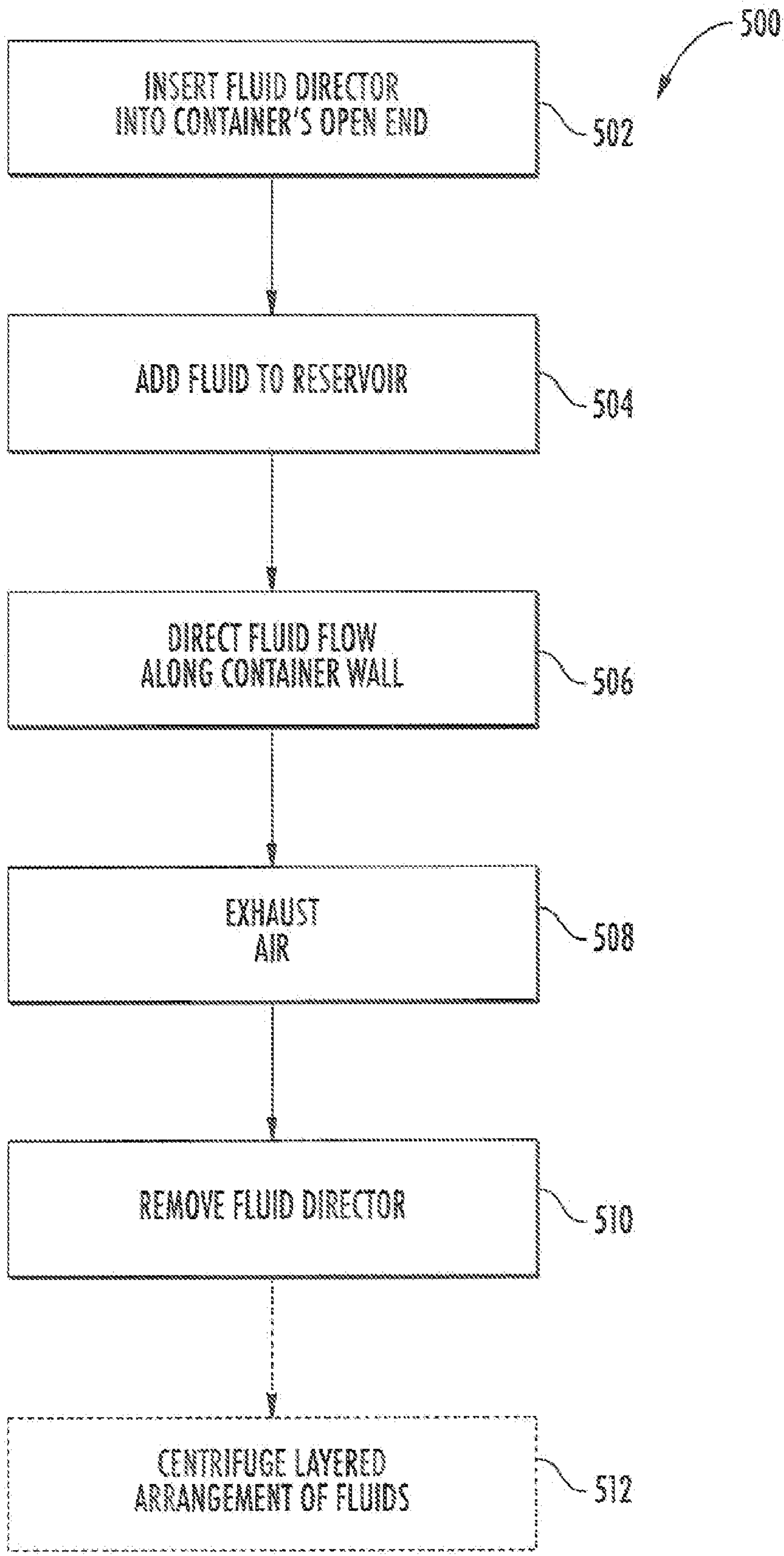

500
INSERT FLUID DIRECTOR INTO CONTAINER'S OPEN END
502
ADD FLUID TO RESERVOIR
504
DIRECT FLUID FLOW ALONG CONTAINER WALL
506
EXHAUST AIR
508
REMOVE FLUID DIRECTOR
510
CENTRIFUGE LAYERED ARRANGEMENT OF FLUIDS
512

FIG. 11

DEVICES AND METHODS FOR OVERLAYING BLOOD OR CELLULAR SUSPENSIONS

BACKGROUND

[0001] This invention relates to a thermoplastic device for overlaying blood or cellular suspension over a volume of Ficoll contained in a 50 ml or 15 ml conical centrifuge tube.

[0002] The isolation and preparation of leukocytes, more generally referred to as "white blood cells" (WBC's), from whole blood or cellular suspension using a Ficoll density gradient is generally the first technique to be carried out in any immunological experiment. WBC's are the main components of our immune system and thus are the main target for experimentation.

[0003] While the Ficoll density gradient protocol is efficient at yielding a high percentage of WBC's from a sample, it is time consuming, messy, and requires hours of hands-on training. The most time consuming part of the Ficoll density protocol is the "overlay", where blood or cellular suspension is carefully poured over the surface of the Ficoll as to prevent any mixing of the two liquids. Two separate layers must be formed with minimal mixing in order to yield a sufficient number of cells. The overlay is completed by holding two conical centrifuge tubes together, one with Ficoll and one with blood or cellular suspension, and slowly pouring the blood or cellular suspension over the Ficoll liquid.

[0004] There are several major drawbacks to the overlay method in the Ficoll density gradient protocol. Primarily, the protocol depends on the lab technician to judge how fast or slow to pour the blood or cellular suspension onto the Ficoll liquid. This dependence on human technique frequently results in spills, mixing the blood/cellular suspension with Ficoll, or total loss of sample. In addition, the overlay method is time consuming and tedious. The majority of the time spent isolating WBC's is spent on the overlay method. Larger experiments that require a lot of WBC's are split into multiple experiments because there is simply not enough time in the day to overlay a large volume of blood or cellular suspension.

[0005] Clearly, then, there is a need for a device used to overlay blood or cellular suspension that can be used by any lab technician without prior training and that can speed up the overlay process. Such a device would virtually eliminate prior training or human error in the overlay process, dramatically reduce the time it takes to overlay, and increase yield by reducing spills.

[0006] Further, such a needed device would be relatively easy to manufacture on a large scale, use, dispose, and would allow much larger experiments to be performed. The present invention fulfills these needs and provides further related advantages.

SUMMARY

[0007] Described and claimed herein are devices and processes for overlaying a fluid, such as blood or a cellular suspension over a volume of a base material, such as Ficoll. In some applications, the base material is initially poured into a conical centrifuge tube (e.g., standard 50 ml or 15 ml tubes). The device at least partially defines a reservoir for temporarily storing the overlay fluid, a fluid barrier separating the reservoir and the container, one or more fluid channels across the fluid barrier, and an exhaust regulator. Certain features of the exhaust regulator and channels allow an adequately regulated, steady flow of blood or cellular composition into the conical centrifuge tube and onto the Ficoll liquid, without substantially disturbing a surface layer of the Ficoll regardless of the level of skill or care of the clinician.

[0008] The devices and processes described and claimed herein offer certain advantages over existing overlay techniques. First, the fluid layering device requires no skill, thus eliminating prior training from others, mixing, spills, or ruined experiments. Next, the device dispenses blood/cellular suspension in an ordered, uniform fashion so that a perfect overlay occurs in the minimal amount of time. Multiple devices can be used at once to dramatically reduce the total time spent overlaying in an experiment. The device is sterile, disposable, and affordable—an economical and feasible alternative to the mainstream overlay technique in PBMC isolation. Other features and advantages of the present invention will become apparent from the following more detailed description, taken in conjunction with the accompanying drawings, by way of example, the principles of the invention.

[0009] In one embodiment, a fluid layering device is configured to control a flow of fluid into an open-ended container. The device includes a fluid barrier configured to prevent passage of fluid from a proximal reservoir toward a distal end of the open-ended container. A peripheral seal extends along an outer perimeter of the fluid barrier and is configured for sealing engagement along an interior surface of the open-ended container. The device further includes at least one groove formed in the peripheral seal. The at least one groove is configured to provide a controlled flow of fluid across the fluid barrier and along the interior surface of the open-ended container. The device also includes an exhaust vent configured to vent from the open-ended container gas displaced by the controlled flow of fluid.

[0010] In some embodiments, the device includes a proximal reservoir defined at least partially by a proximal surface of the fluid barrier.

[0011] In some embodiments, the reservoir is open-ended, defined by an elongated cylindrical wall extending proximally from the fluid barrier.

[0012] In some embodiments, the device includes a coupling arrangement adjacent to the open end of the reservoir.

[0013] In some embodiments, the device includes the coupling arrangement comprises a thread.

[0014] In some embodiments, the device includes the fluid barrier comprises at least one drain port in fluid communication with a proximal end of each of the at least one grooves.

[0015] In some embodiments, the device includes a proximal handle allowing for insertion and removal of the fluid layering device with respect to the open-ended container.

[0016] In some embodiments, the exhaust vent terminates in an exhaust port disposed along an outer surface of the fluid layering device.

[0017] In some embodiments, each of the at least one grooves extends helically along a cylindrical surface defining the peripheral seal.

[0018] In some embodiments, the device includes a shoulder positioned to abut at least a portion of a rim of the open-ended container when the fluid layering device is inserted therein, the shoulder position with respect to a proximal end of the fluid layering device to control height of the fluid barrier along a longitudinal axis of the open-ended container.

[0019] In some embodiments, the device is sterilized.

[0020] In some embodiments, the device includes is formed from material selected from the group consisting of: plastics;

polymers; resins; glass; ceramics; metals; and combinations thereof.

[0021] In some embodiments, fluid stored within the fluid layering device is observable through a sidewall of the device.

[0022] In some embodiments, at least a portion of the fluid layering device is translucent or transparent.

[0023] In another embodiment, a process for controlling a flow of fluid into an open-ended container includes positioning a fluid barrier above a surface of a base material disposed in a distal end of the open-ended container. A first fluid is added into a reservoir positioned proximal to the fluid barrier. A flow of fluid is directed from the reservoir across the fluid barrier and into the distal end of the open-ended container, fluid passing the fluid barrier flowing toward the base material along an interior surface of the open-ended container. Gas displaced by the controlled flow of fluid is exhausted from the distal end of the open-ended container, such that a layer of the first fluid is deposited over the base material without substantially disturbing a surface of the base material.

[0024] In some embodiments, the process includes positioning the fluid barrier comprises inserting at least a proximal portion of a fluid layering device into an open end of the open-ended container.

[0025] In some embodiments, the process includes removing the fluid barrier from the open-ended container.

[0026] In some embodiments, the process includes centrifuging the layered material.

[0027] In some embodiments of the process, the base material is Ficoll.

[0028] In some embodiments of the process, the fluid is a cellular suspension.

[0029] In some embodiments of the process, the cellular suspension is blood.

[0030] In some embodiments a fluid layering device configured to control a flow of fluid into an open-ended container includes means for positioning a fluid barrier above a surface of a base material disposed in a distal end of the open-ended container. The device also includes means for adding a first fluid into a reservoir positioned proximal to the fluid barrier and means for directing a flow of fluid from the reservoir across the fluid barrier and into the distal end of the open-ended container, wherein passing the fluid barrier flowing toward the base material occurs along an interior surface of the open-ended container. The device also includes means for exhausting from the distal end of the open-ended container gas displaced by the controlled flow of fluid.

[0031] In yet another embodiment, a fluid layering device configured to control a flow of fluid into an open-ended container containing a base material includes a longitudinally extending cylindrical side wall open at its proximal end and a fluid barrier disposed across a distal end of the cylindrical side wall, a proximal surface of the fluid barrier and an interior surface of the cylindrical side wall forming an open-ended reservoir. The device also includes an insertable portion extending distally from the fluid barrier. The insertable portion includes a longitudinally extending sealing wall positioned to form a fluid-tight seal along a peripheral interior surface of a proximal portion of the open-ended container and at least one groove extending along the sealing wall and terminating in a peripheral fluid port configured to ensure fluid flowing into the open-ended container flows along an interior surface of the open-ended container. The device also includes at least one drain in fluid communication between the reservoir and a proximal end of each of the at least one grooves and an exhaust vent in fluid communication with the open-ended container. The vent regulates the flow of gas displaced by the controlled flow of fluid, thereby contributing to a rate of fluid flow.

[0032] In some embodiments of the device, the at least one groove extends helically along the sealing wall.

[0033] In some embodiments the device further includes a threaded coupling engagement along a distal portion of the cylindrical wall.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

[0035] FIG. **1** illustrates a side view of one embodiment of a fluid layering device.

[0036] FIG. **2** illustrates a longitudinal cross-section of FIG. **1**.

[0037] FIG. **3**A illustrates a proximal end view of the fluid layering device of FIG. **1** and FIG. **2**

[0038] FIG. **3**B is a distal end view of the fluid layering device of FIG. **1** and FIG. **2**.

[0039] FIG. **4**A is a side view of the fluid layering device of FIG. **1** aligned for insertion into an open ended conical container.

[0040] FIG. **4**B illustrates the fluid layering device after insertion into the conical container.

[0041] FIG. **5**A illustrates a cross-section of the conical open ended container with the fluid layering device of FIG. **1** and FIG. **2** inserted into its open end.

[0042] FIG. **5**B illustrates in more detail, engagement of an insertable portion of the fluid layering device with an interior surface of the open ended conical container.

[0043] FIG. **6**A-**6**D illustrate operation of an embodiment of a fluid layering device in controlling flow of a fluid into an open ended conical container containing a base material.

[0044] FIG. **7**A-**7**D illustrate an alternative embodiment of a fluid layering device.

[0045] FIG. **8**A-**8**C illustrate insertion of the embodiment of the fluid layering device illustrated in FIG. **7**A-**7**D into an open end of the conical container and its direction of a fluid flow into the container.

[0046] FIG. **9** illustrates yet another embodiment of a fluid layering device.

[0047] FIG. **10**A illustrates an open ended container comprising a base material and a blood sample layered according to one embodiment of the invention.

[0048] FIG. **10**B illustrates contents of the open ended container illustrated in FIG. **10**A after applied to a centrifuge process.

[0049] FIG. **10**C illustrates the open ended container contents of FIG. **10**B after further processing.

[0050] FIG. **11** illustrates an embodiment of a process for directing fluid flow into an open ended container.

DETAILED DESCRIPTION

[0051] Described herein are examples of devices and processes configured to overlay a first fluid, such as blood or a cellular suspension onto a base material. The base material can include, but is not limited to, sugar density gradients such as Ficoll, Percoll, Isopercoll, and isopycnic sucrose density gradient. The cellular suspension can include, but is not limited to, monocyte cultures; Tc clones; islets of Langerhans from pancreatic tissue (e.g., Dellê et al., The Use of Iodixanol for the Purification of Rat Pancreatic Islets, TRANSPLANTATION PROCEEDINGS, Vol. 39, No. 2, pages 467-469); neural cells from brain tissue (e.g., Sims & Anderson, Isolation of mitochondria from rat brain using Percoll density gradient centrifugation, NATURE PROTOCOLS, Vol. 3, 2008, pages 1228-1239); ovarian follicles (e.g., Martinez-Madrid et al., Ficoll density gradient method for recovery of isolated human ovarian primordial follicles, FERTILITY AND STERILITY, Vol. 82, No. 6, 2004, pages 1648-1653); spermatozoa from epididymis (e.g., Haldar et al., Ficoll Gradient Isolation of Immature Sperm of High Purity and Intactness From Goat Epididymis, SYSTEMS BIOLOGY IN REPRODUCTIVE MEDICINE, Vol. 24, No. 2, 1990, pages 125-128); and plant cells (e.g., Attree & Sheffield, An evaluation of Ficoll density gradient centrifugation as a method for eliminating microbial contamination and purifying plant protoplasts, JOURNAL PLANT CELL REPORTS, Vol. 5, No. 4, 1986, pages 288-291; Liang et al., Isolation of Spinach Leaf Peroxisomes in 0.25 Molar Sucrose Solution by Percoll Density Gradient Centrifugation, PLANT PHYSIOL., Vol. 70, 1982, pages 1210-**1212**).

[0052] In general, the fluid layering device can be coupled through a coupling extension to an open end of a container, such as a conical centrifuge tube, already including the base material. Once attached, the device regulates flow of the first fluid from the reservoir into the conical tube so that a suitable overlay layer is formed without substantially disturbing a surface of the base material, regardless of the skill and care of the user.

[0053] A side view of an embodiment a fluid layering device **100** is illustrated in FIG. **1**. The device **100** includes an elongated cylindrical housing **102** defining an open ended reservoir **104** at one end and an insertable coupling portion **106** at an opposite end. One or more helical channels **108** are defined along a peripheral side wall **144** of the coupling portion **106**. The helical channels **108** extend from a top or proximal end of the coupling portion **106** to a bottom or distal end of the coupling portion **106**. There are also a number of triangular openings **110** that connect the cylindrical reservoir **104** to helical channels **108** that wrap around the coupling portion or base **106** of the device **100** (the base is fully inserted into the conical centrifuge tube). The number of triangle openings **110** and helical channels **108** depend on the size/model of the invention. There is a circular exhaust vent **118**, which allows gas to be vented from the inside of the conical centrifuge tube to the environment.

[0054] In at least some embodiments, the entire device **100** can be made of a common material. In this embodiment, the device is made from a moldable material, such as a hard, translucent, non-flexible plastic such as polystyrene (PS), acrylonitrile butadiene styrene (ABS), or polycarbonate (PC). The reason for translucency is so the user can view the progress of the blood/cellular suspension in the device in order to determine whether or not the process has completed. The engineering grade plastics (PS, ABS, PC) are required so that the device will retain structurally integrity when inserted to and removed from its conical centrifuge tube.

[0055] FIG. **2** details the cross-section of the device **100** and illustrates the exhaust vent **112** and an interior barrier wall **114**. In the illustrative embodiment, the barrier wall is a conical divider **114**. The exhaust vent is composed of a rectangular tunnel **116** that leads from the conical centrifuge volume to a circular tunnel **118** that exits a proximal side wall of the device. The cross-section of this tunnel **116**, **118** determines the rate of gas exiting the conical tube and thus the rate of the blood/cellular suspension entering the conical tube via the helical channels **108**. The conical divider physically separates the contents of the cylindrical reservoir from the conical centrifuge tube. Its conical shape assists in the complete evacuation of the reservoirs contents into the centrifuge tube.

[0056] FIG. **3**A illustrates a proximal end view of the fluid flow device illustrated in FIG. **1** and FIG. **2**. A proximal end or rim of the reservoir wall **120** defines an open end of the reservoir **104**. A proximal surface **122** of the conical divider **114** defines a bottom of the reservoir **104**. The proximal surface **122** follows the contour of the conical divider **114** terminating in a conical peak **124** centrally located along a base surface of the reservoir **104**. An annular trough **126** extends between a base portion of the proximal surface **122** and the base of the interior surface of the reservoir walls **120**. One or more openings **128***a*, **128***b*, **128***c* (generally **128**) are located along the annular trough **126**. In the illustrative embodiment three triangular openings **128** are disposed about the annular trough. Also visible is a proximal portion of an exhaust vent housing **130**. The exhaust vent exit tunnel **118** is illustrated in phantom, exiting the reservoir wall **120** at the exhaust vent outlet port **132**. A fluid placed in the reservoir **104** will be directed by the conical peak **124** toward the annular trough **126**. The fluid is able to exit the reservoir **104** through the triangular openings **128**.

[0057] Illustrated in FIG. **3**B is a distal end view of the fluid layering device **100**. A proximal extension **106** defines along its outer peripheral surface a peripheral sealing wall **140**. An annular shoulder **142** is defined along a base portion of the reservoir wall **120** extending in radial direction beyond the peripheral sealing wall **144**. One or more fluid ports are defined along a proximal end of the proximal extension **312**. In the illustrative embodiment three fluid ports **146***a*, **146***b*, **146***c* (generally **146**) are disposed evenly about the circumference of the proximal extension **106**. A distal surface **148** of the conical divider **114** extends across an opening of the proximal extension **106**. An inner conical peak **150** is shown centrally to the distal surface **148**. The distal surface **148** includes an exhaust vent inlet port **152**. The exhaust vent inlet port **152** is in fluid communication with the exhaust vent exit tunnel **118** allowing gas to exit through the exhaust vent outlet port **132**.

[0058] Preferring to FIG. **4**A, the fluid layering device **100** is positioned in axial alignment with an elongated open end conical container **200**. The fluid layering device **100** is positioned such that a distal end of the insertable portion **106** faces an open end **202** of the open ended container **200**. Coupling of the two devices is accomplished by advancing the fluid layering device **100** towards the open end **200** advancing the insertable portion **106** within the open end **202** of the container **200**. The fluid layering device **100** is advanced axially until the annular shoulder **142** abuts an annular rim **204** of the open end **202**. An illustration of the fluid layering device **100** fully inserted within the open ended container **200** is shown in FIG. **4**B. Also visible is the exhaust vent outlet port **132** positioned proximally to the annular shoulder **142** such that the exhaust outlet port **132** remains unobstructed after insertion.

[0059] Illustrated in FIG. 5A a is a lateral cross-section of the open ended container **200** with the fluid layering device **100** fully inserted into its open end **202**. FIG. 5B illustrates in more detail arrangement of the peripheral sealing wall **144**, the helical groove **108** and an inner wall surface **210**. In particular a fluid channel **220** is formed along the helical groove **108** being defined by the helical groove **108** and an adjacent portion of the inner wall surface **210**. Also shown in more detail is the abutting arrangement of the shoulder **142** against the annular rim **204**.

[0060] Operation of an embodiment of a fluid layering device in controlling flow of a fluid into an open ended conical container is illustrated in the series of FIG. 6A through FIG. 6D. Initially, a base material, such as Ficoll is inserted into an open end of the upright container **200**. The base material **230** pools along the conical bottom end, such that a surface of the base material is spaced apart from the open end **202** by a distance h_1. Referring to FIG. 6A, the fluid layering device **100** is coupled to the container **200** as described above and illustrated in FIG. 4B and FIG. 5A. When fully inserted, a distal end of the insertable portion **106** resides at a height h_2 above the surface of the base material. The reservoir **104** extends proximally from the open end **202** to a height h_3. As illustrated in FIG. 6B, a first fluid, in this instance a cellular suspension—blood, is poured into an open end **105** of the reservoir **104**. Preferably, the container-fluid layering device arrangement is positioned in upright or vertically, such that gravity will drive the flow of blood into the container **200**. In some embodiments, the arrangement can be positioned at an angle, but preferably not much more than about 30 degrees measured from vertical.

[0061] As shown in FIG. 6C, gravity induces a downward flow, forcing blood **240** from the reservoir **104** into the channels formed between helical grooves **108** and the inner surface of the container **210**. The fluid **240** exits the grooves **108** along the interior walls **210** of the substantially upright container **200**. Gravity continues to drive blood flow **242** downward towards the exposed surface of the base material **230**. Surface tension keeps the blood flow **242** substantially directed along the inner side walls **210**. As the volume of blood increases in a layer above the base material **230**, air pressure within the chamber **244** increases. The exhaust vent **115** allows air to bleed off from the chamber **244**, thereby reducing the pressure and allowing for continued blood flow **242**. Careful selection of the dimensions of the exhaust vent **115** can be used to control flow of blood **242** into the container **200**. For example, a substantially narrow exhaust vent **115** restricts the flow of gas similarly restricting flow of blood **240** into the chamber **244**. When the reservoir **104** contents have been substantially transferred, the fluid layering device **100** can be removed carefully as shown in FIG. 6C. Care must be exercised when separating the device **100** from the container so as not to disturb the layered arrangement of fluid **240** and base material **230** (e.g., blood and Ficoll). In most applications and particularly when working with blood **240**, the fluid layering device **100** is preferably sterilized. Although it may be possible to re-sterilize (e.g., autoclave) the device **100**, it is generally anticipated that it will be disposable based on the relative simplicity of design and minimum costs.

[0062] An alternative embodiment of a fluid layering device **300** is illustrated in FIG. 7A-7D. The device **300** includes a barrier wall **302** defining a perimeter conforming to a lateral cross section of the open ended container **330**. In the illustrative embodiment, the perimeter is substantially circular. The barrier wall **302** defines a proximal surface **303** facing the fluid reservoir and a distal surface **305** facing the base material. A peripheral sealing wall **304** extends between the proximal surface **303** and the distal surface **305** and along the outer perimeter of the barrier wall **302**. When inserted into an open end of the container **330**, the barrier wall prevents fluid flow from the reservoir toward the base material.

[0063] The fluid layering device **300** also includes one or more grooves **306** formed in the peripheral sealing wall **304**, configured to allow a controlled flow of fluid from the reservoir toward the base material. In the illustrative embodiment, six such grooves **306** are evenly distributed around the circular perimeter of the barrier wall **302**. Each groove is formed as rectangular groove **306**. The rectangular shape of the groove **306** is not meant to be limiting in any way. Other shaped grooves are contemplated, such as triangular, elliptical, circular, polygons, random shapes, and combinations of any such shaped. In the illustrative embodiment, the grooves are directed parallel to a longitudinal axis of the device. In some embodiments, the grooves may be angled, and/or curved, for example, in a helix arrangement. It is not necessary that all of the grooves **306** be identical in size, shape, or orientation.

[0064] The fluid layering device **300** also includes one or more exhaust vents. In the illustrative embodiment, the device **300** includes two such vents. Each exhaust vent **307'**, **307"** (generally **307**). Each vent **307** includes an elongated exhaust vent lumen **314** defined by a proximal handle **310** and extending between an exhaust vent inlet port **308** and an exhaust vent exit port **316**. The handle **310** extends proximally away from the proximal surface of the barrier wall **302**. Preferably, the handle **310** extends axially for a length sufficiently longer than any intended insertion depth, such that at least a proximal portion of the handle **310** extends beyond an open end of the container **330**. In some embodiments, the handle includes an open area to facilitate removal of the fluid layering device **300** from the container by providing a surface upon which a finger, fingers, or suitable instrument may apply an axial removing force to remove the device **300** from the container **330**.

[0065] Operation of an embodiment of the alternative embodiment of the fluid layering device illustrated in FIG. 7A-7D and described above is illustrated in the series of FIG. 8A-8C. Once again, a base material, such as Ficoll is inserted into an open end of the upright container **330**. The base material pools along the conical bottom end **331**, such that a surface **333** of the base material **319** is spaced apart from the open end **332** by a distance h_1. Referring to FIG. 8A, a distal portion of the fluid layering device **300** including the barrier wall **302** is inserted into the open end of the container **330**. When fully inserted, a distal surface of the barrier wall **302** resides at a height h_2 above the surface **333** of the base material **319** as illustrated in FIG. 8B. A reservoir **332** extends proximally from a proximal surface of the barrier wall **332** to the open end **332** to a height h_3. A proximal portion of the handle remains exposed, extending to a height h^5 measured from the open end **332**. Preferably, a sufficient portion of the open area **312** remains exposed to allow for insertion of a finger or suitable instrument during removal process.

[0066] A first fluid, in this instance a cellular suspension blood, is poured into the open end **332** of the reservoir **30**. Once again, the container-fluid layering device arrangement is preferably positioned upright or vertically, such that gravity will drive the flow of blood **350** into the container **330**. In some embodiments, the arrangement can be positioned at an angle, but preferably not much more than about 30 degrees measured from vertical.

[0067] As shown in FIG. **8**C, gravity induces a downward flow, forcing blood **350** from the reservoir **332** into the channels formed between grooves **306** (FIG. **7**C and FIG. **7**D) and the inner surface of the container **330**. The blood **350** exits the grooves **306** along the interior walls of the substantially upright container **330**. Gravity continues to drive blood flow **352** downward towards the exposed surface **333** of the base material **319** (e.g., Ficoll). Surface tension keeps the blood flow **352** substantially directed along the inner side walls. As the volume of blood increases in a layer above the base material, air pressure within the chamber increases. The exhaust vent allows exhausted air **354** to bleed off from the chamber, thereby reducing the pressure and allowing for continued blood flow.

[0068] As before, careful selection of the dimensions of the one or more exhaust vents can be used to control flow of blood **352** into the container **330**. For example, a substantially narrow exhaust vent restricts the flow of gas similarly restricting flow of blood into the chamber. When the reservoir contents have been substantially transferred, the fluid layering device **300** can be removed carefully by puling the exposed portion of the handle **310**. Care must be exercised when separating the device from the container so as not to disturb the layered arrangement of blood **352** and Ficoll **319**.

[0069] FIG. **9** illustrates yet another embodiment of a fluid layering device **380**. The device **380** includes a proximal reservoir **382** and an insertable base portion **394**. The reservoir **382** includes an open ended fluid chamber **384** and an elongated side wall **385** extending along a cord dissecting a cylindrical wall of the reservoir **382**. Fluid poured into the open end portion **384** flows toward a barrier wall **392** forming a base portion of the reservoir **382**. One or more drain apertures **390** are disposed along an outer periphery of the barrier wall **392**, allowing fluid stored within the container to enter one or more longitudinally extending channels **398** defined along a peripheral sealing wall **396**. Fluid flows along the longitudinal channels **398** between the channels **398** and an interior wall **210** of the open-ended container **200**. Gas displaced by the inflowing fluid exits the container **200** through an exhaust vent **388**. The exhaust vent directs expelled gas (e.g., air) into a vent portion **386** defined between an opposite side of the elongated separating wall **385** and an opposing portion of the cylindrical side wall, such that exiting exhaust air is not interfered with by fluid stored within the reservoir **382**.

[0070] FIG. **10**A illustrates an open ended container **400** comprising a layered arrangement of blood **402** over Ficoll-paque PLUS **404**. The layered arrangement **402**, **404** can be accomplished using any of the devices and processes described herein. The layered arrangement **402**, **404** can be centrifuged using standard techniques to separate various blood components. FIG. **10**B illustrates contents of the container illustrated in FIG. **10**A after applied to a centrifuge process. A bottom layer **406** of granulocytes and erythrocytes is formed along the very bottom of the container **400**. Disposed above this layer **406**, is a first intermediate layer **408** of Ficoll-paque PLUS. Above the first intermediate layer **408** is a second intermediate layer **410** of lymphocytes, and above that is a top layer **412** of plasma and platelets. In some embodiments, the top layer **412** can be removed, for example by simply pouring it out of the container as illustrated in FIG. **10**C.

[0071] FIG. **11** illustrates an embodiment of a process **500** for directing fluid flow into an open ended container. In an initialization step not shown, an open-ended container is partially filled with a base material. This is generally accomplished as part of protocols known to those skilled in the art, such as a protocol for PBMC isolation. The fluid layering device is then coupled to the container from its open end at **502**. The coupled arrangement defines a fluid reservoir for temporarily storing a first fluid during the layering process. Also defined are one or more fluid channels providing fluid communication between the reservoir and an interior region of the container in a space or chamber formed between a barrier wall and a surface of the base material.

[0072] Next, a fluid is added to the reservoir at **504**. The fluid flows under the influence of gravity through the one or more fluid channels into the chamber above the surface of the base material at **506**. In particular, the fluid flow is directed along an inner surface of the container wall, avoiding any free-falling droplets that would otherwise disturb the surface tension of the base material. The side-wall flow continues under the influence of gravity until it reaches the surface of the base material. The fluid then begins to pool along the surface. As the volume of fluid increases in the chamber, pressure of any gas within the chamber, such as air is raised. Gas (air) is exhausted from the chamber at **508** by pressure induced by the fluid flow. Exhausted air reduces the pressure and allows for continued fluid flow. Ultimately an equilibrium can be reached between the inflow of fluid and outflow of exhaust gas. The rate of flow can be controlled by at least one of the dimension of the fluid channels (e.g., length, shape, direction, diameter) and dimensions of one or more exhaust vents.

[0073] The fluid layering device is removed from the chamber at **510**. Generally, the device is removed after all of the fluid has flowed from the reservoir into the chamber. Removal of the device allows for further processing, such as centrifuging at **510** (optional).

[0074] Other embodiments will be evident to those of skill in the art. It should be understood that the foregoing detailed description is provided for clarity only and is merely exemplary. The spirit and scope of the present invention are not limited to the above examples, but are encompassed by the following claims.

What is claimed is:

1. A fluid layering device configured to control a flow of fluid into an open-ended container, comprising:

a fluid barrier configured to prevent passage of fluid from a proximal reservoir toward a distal end of the open-ended container;

a peripheral seal extending along an outer perimeter of the fluid barrier, the peripheral seal configured for sealing engagement along an interior surface of the open-ended container;

at least one groove formed in the peripheral seal, configured to provide a controlled flow of fluid across the fluid barrier and along the interior surface of the open-ended container; and

an exhaust vent configured to vent from the open-ended container gas displaced by the controlled flow of fluid.

2. The fluid layering device of claim **1**, further comprising a proximal reservoir defined at least partially by a proximal surface of the fluid barrier.

3. The fluid layering device of claim **2**, wherein the reservoir is open-ended, defined by an elongated cylindrical wall extending proximally from the fluid barrier.

4. The fluid layering device of claim **3**, further comprising a coupling arrangement adjacent to the open end of the reservoir.

5. The fluid layering device of claim **4**, wherein the coupling arrangement comprises a thread.

6. The fluid layering device of claim **1**, wherein the fluid barrier comprises at least one drain port in fluid communication with a proximal end of each of the at least one grooves.

7. The fluid layering device of claim **1**, further comprising a proximal handle allowing for insertion and removal of the fluid layering device with respect to the open-ended container.

8. The fluid layering device of claim **1**, wherein the exhaust vent terminates in an exhaust port disposed along an outer surface of the fluid layering device.

9. The fluid layering device of claim **1**, wherein each of the at least one grooves extends helically along a cylindrical surface defining the peripheral seal.

10. The fluid layering device of claim **1**, further comprising a shoulder positioned to abut at least a portion of a rim of the open-ended container when the fluid layering device is inserted therein, the shoulder position with respect to a proximal end of the fluid layering device to control height of the fluid barrier along a longitudinal axis of the open-ended container.

11. The fluid layering device of any of the above claims, wherein the fluid layering device is sterilized.

12. The fluid layering device of any of the above claims, wherein the fluid layering device is formed from material selected from the group consisting of: plastics; polymers; resins; glass; ceramics; metals; and combinations thereof.

13. The fluid layering device of any of the above claims, wherein fluid stored within the fluid layering device is observable through a sidewall of the device.

14. The fluid layering device of claim **13**, wherein at least a portion of the fluid layering device is translucent or transparent.

15. A method for controlling a flow of fluid into an open-ended container, comprising:
- positioning a fluid barrier above a surface of a base material disposed in a distal end of the open-ended container;
- adding a first fluid into a reservoir positioned proximal to the fluid barrier;
- directing a flow of fluid from the reservoir across the fluid barrier and into the distal end of the open-ended container, fluid passing the fluid barrier flowing toward the base material along an interior surface of the open-ended container; and
- exhausting from the distal end of the open-ended container gas displaced by the controlled flow of fluid,
- wherein a layer of the first fluid is deposited over the base material without substantially disturbing a surface of the base material.

16. The method of claim **15**, wherein positioning the fluid barrier comprises inserting at least a proximal portion of a fluid layering device into an open end of the open-ended container.

17. The method of any of claims **15-16**, further comprising removing the fluid barrier from the open-ended container.

18. The method of claim **17**, further comprising centrifuging the layered material.

19. The method of any of claims **15-17**, wherein the base material is Ficoll.

20. The method of any of claims **15-18**, wherein the fluid is a cellular suspension.

21. The method of claim **20**, wherein the cellular suspension is blood.

22. A fluid layering device configured to control a flow of fluid into an open-ended container, comprising:
- means for positioning a fluid barrier above a surface of a base material disposed in a distal end of the open-ended container;
- means for adding a first fluid into a reservoir positioned proximal to the fluid barrier;
- means for directing a flow of fluid from the reservoir across the fluid barrier and into the distal end of the open-ended container, fluid passing the fluid barrier flowing toward the base material along an interior surface of the open-ended container; and
- means for exhausting from the distal end of the open-ended container gas displaced by the controlled flow of fluid.

23. A fluid layering device configured to control a flow of fluid into an open-ended container containing a base material, comprising:
- a longitudinally extending cylindrical side wall open at its proximal end;
- a fluid barrier disposed across a distal end of the cylindrical side wall, a proximal surface of the fluid barrier and an interior surface of the cylindrical side wall forming an open-ended reservoir;
- an insertable portion extending distally from the fluid barrier; the insertable portion comprising:
  - a longitudinally extending sealing wall positioned to form a fluid-tight seal along a peripheral interior surface of a proximal portion of the open-ended container;
  - at least one groove extending along the sealing wall and terminating in a peripheral fluid port configured to ensure fluid flowing into the open-ended container flows along an interior surface of the open-ended container;
- at least one drain in fluid communication between the reservoir and a proximal end of each of the at least one grooves; and
- an exhaust vent in fluid communication with the open-ended container, wherein the vent regulates the flow of gas displaced by the controlled flow of fluid, thereby contributing to a rate of fluid flow.

24. The fluid layering device of claim **23**, wherein at least one groove extends helically along the sealing wall.

25. The fluid layering device of claim **23**, further comprising a threaded coupling engagement along a distal portion of the cylindrical wall.

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