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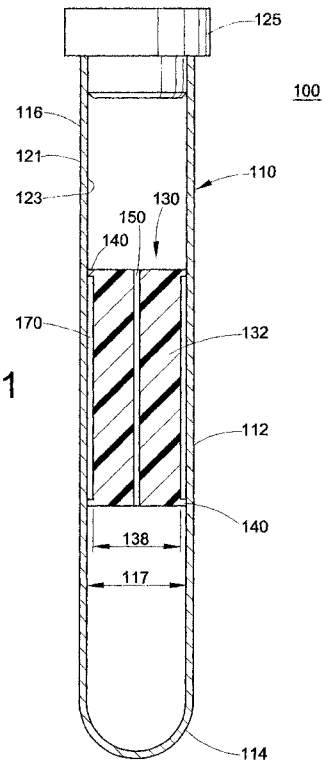

FIG. 1

(57) Abstract: Tube and float systems for separation and axial expansion of the buffy coat are provided. Generally, the systems include a flexible sample tube and a rigid separator float having a specific gravity intermediate that of red blood cells and plasma. The sample tube has an elongated sidewall having a first cross-sectional inner diameter. The float has a main body portion and one or more support members protruding from the main body portion to engage and support the sidewall of the sample tube. During centrifugation, the centrifugal force enlarges the diameter of the tube to permit density-based axial movement of the float in the tube. After centrifugation is ended, the tube sidewall returns to its first diameter, thereby capturing the float and trapping the buffy coat constituents in an annular volume. Several different systems for capturing and retrieving the buffy coat constituents are described.

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BUFFY COAT SEPARATOR FLOAT SYSTEMS AND METHODS

BACKGROUND OF THE DISCLOSURE

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/318,912, filed on March 30, 2010, and to U.S. Provisional Patent Application Serial No. 61/372,900, filed on August 12, 2010. The disclosure of these applications is hereby fully incorporated by reference.

[0002] The present disclosure relates generally to density-based fluid separation, and in particular to improved sample tubes and float designs for the separation and axial expansion of constituent fluid components layered by centrifugation, and methods employing the same. The present disclosure finds particular application in separation and axial expansion of the buffy coat layers of the blood, and will be described with particular reference thereto.

[0003] Quantitative Buffy Coat (QBC) analysis is routinely performed in clinical laboratories for the evaluation of whole blood. The buffy coat is a series of thin, light-colored layers of mostly white blood cells that form between the layer of red blood cells and the plasma when unclotted blood is centrifuged or allowed to stand.

[0004] QBC analysis techniques generally employ centrifugation of small capillary tubes containing anticoagulated whole blood, to separate the blood into essentially six layers: (1) packed red cells, (2) reticulocytes, (3) granulocytes, (4) lymphocytes/monocytes, (5) platelets, and (6) plasma. The buffy coat consists of the layers, from top to bottom, of platelets, lymphocytes and granulocytes and reticulocytes.

[0005] Based on examination of the capillary tube, the length or height of each layer is determined during the QBC analysis and converted into a cell count, thus allowing quantitative measurement of each layer. The length or height of each layer can be measured with a manual reading device, i.e., a magnification eyepiece and a manual pointing device, or photometrically by an automated optical scanning device that finds the layers by measuring light transmittance and fluorescence along the length of the tube. A series of commonly used QBC instruments are manufactured by Becton-Dickinson and Company of Franklin, Lakes, N.J.

[0006] Since the buffy coat layers are very thin, the buffy coat is often expanded in the capillary tube for more accurate visual or optical measurement by placing a plastic cylinder, or float, into the tube. The float has a density less than that of red

blood cells (approximately 1.090 g/ml) and greater than that of plasma (approximately 1.028 g/ml) and occupies nearly all of the cross-sectional area of the tube. The volume-occupying float, therefore, generally rests on the packed red blood cell layer and expands the axial length of the buffy coat layers in the tube for easier and more accurate measurement.

[0007] There exists a need in the art for an improved sample tube and float system and method for separating blood and/or identifying circulating cancer and/or other rare cells, organisms or particulates or objects (i.e., stem cells, cell fragments, virally-infected cells, trypanosomes, etc.) in the buffy coat or other layers in a blood sample. However, the number of cells expected to be typically present in the buffy coat is very low relative to the volume of blood, for example, in the range of about 1-100 cells per millimeter of blood, thus making the measurement difficult, particularly with the very small sample sizes employed with the conventional QBC capillary tubes and floats.

[0008] The present disclosure contemplates new and improved blood separation assemblies and methods that overcome the above-referenced problems and others.

BRIEF DESCRIPTION

[0009] The present application discloses, in various embodiments, apparatuses and methods for separating and axially expanding the buffy coat constituents in a blood sample. The apparatuses include separator floats and sample tubes.

[0010] Disclosed herein are methods of separating and axially expanding buffy coat constitutents in a blood sample; detecting target cells in a blood sample; and capturing or extracting buffy coat constitutents / target cells in a blood sample. Those methods require introducing the blood sample and a rigid volume-occupying float into a flexible sample tube. The rigid float has a specific gravity intermediate that of red blood cells and plasma, and comprises a main body portion spacedly surrounded radially by the sidewall of the sample tube to form an annular volume therebetween; and one or more support members protruding from the main body portion and engaging the sidewall. The sample tube is centrifuged at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and movement of the float into alignment with at least the buffy coat constituents of the blood sample. The rotational speed is reduced to cause the sidewall to capture the

float and trap buffy coat constituents in the annular volume, which might be divided into one or more analysis areas.

[0011] Disclosed in some embodiments is a volume-occupying separator float comprising at least a first piece and a second piece. Each piece has a first end, a second end, an exterior surface, and an interior surface. The interior surfaces of the first and second pieces cooperate to form an open passage extending between the first end and the second end. The first and second pieces can be connected together.

[0012] The first and second pieces may have substantially the same three-dimensional shape. In some embodiments, the interior surface of the first piece comprises a semi-cylindrical surface. Put another way, a lateral cross-sectional view of the first piece may have a semi-annular shape. Sometimes, the interior surface of the first piece is substantially planar, i.e. a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line. In some floats, a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line, and a lateral cross-sectional view of the interior surface of the second piece is substantially a straight line with a central indent. In specific embodiments, a lateral cross-sectional view of the open passage may have a rectangular shape or a circular shape.

[0013] The exterior surface of the first and second pieces may each substantially conform to an inner surface of the sidewall of the sample tube in which the two-piece float is used. The exterior surface of the first and second pieces may also each comprise at least one support member for engaging an inner surface of the sidewall.

[0014] The first and second pieces can be joined together using clips, clamps, and other joining mechanisms or devices.

[0015] Sometimes, the first and second piece each further comprise at least one side surface. The first and second pieces can be connected together on the at least one side surface.

[0016] The two-piece or multiple-piece float can be used in conjunction with a flexible bag to capture buffy coat constituents in a blood sample. A blood sample is introduced into a flexible bag, and a float is placed around the flexible bag, then the bag and float are placed in a flexible sample tube. During centrifugation, the float moves into alignment with at least the buffy coat constituents. Upon reducing the rotational speed, the sidewall captures the float. The flexible bag can then be sealed

at the first end and the second end of the float to capture the buffy coat constituents. The flexible bag can be sealed by welding the first end and the second end of the float. The welding may be performed ultrasonically. Other sealing and/or enclosure devices or mechanisms are also contemplated.

[0017] Disclosed in other embodiments is a volume-occupying separator float comprising a main body portion. The main body portion has a first end and a second end and at least one pressure seal. A buffy coat passage extends from the second end to the first end and has a centrifugation valve oriented to open during centrifugation, the valve being located at the second end.In some specific embodiments, there is a first pressure seal around the first end and a second pressure seal around the second end of the main body portion. In additional embodiments, a pressure relief passage extends from the second end to the first end and has a pressure relief valve oriented to open when pressure at the second end is greater than pressure at the first end by a specified value. In other embodiments, there is a pressure relief valve but not a buffy coat passage in the separator float.

[0018] During centrifugation, the pressure seal(s) substantially prevent the blood sample from traveling between the float and the inner surface of the sample tube. The buffy coat constituents are trapped in the buffy coat passage, and the float can then be removed from the sample tube to obtain the buffy coat constituents.

[0019] Alternatively, a volume-occupying separator float comprises a main body portion. The main body portion has a first end and a second end. One or more centrifugation valves are circumferentially disposed about the main body portion.

[0020] Disclosed in still other embodiments is a volume-occupying separator float comprising a first piece and a second piece. The first and second pieces cooperate to form a rectangular passage between a first end and a second end of the float, and wherein the first and second pieces are joined at the first end of the float. A slide can be placed or located in the rectangular passage.

[0021] During use, this two-piece float containing a slide is placed in a sample tube and centrifuged. This causes the slide to be coated with the buffy coat constituents of the blood sample. The float can then be removed from the sample tube; and the slide can be extracted from the float. This float can also be used to surround a flexible bag, as described above.

[0022] In other embodiments, a two-piece float comprises a top float and a bottom float. The top float has a density intermediate that of plasma and the buffy

coat constituents. The top float comprises (i) a lower support member having an upper surface and a lower surface, and (ii) a pitot tube extending axially from the lower surface of the top float through the upper surface and having a top end located distally from the upper surface. The bottom float has a density intermediate that of the buffy coat constituents and red blood cells. The top float lower support member lower surface and the bottom float are complementarily shaped.

[0023] The two-piece float may be designed to relieve pressure below the bottom float. The top float further comprises a second passage extending from the lower surface to the upper surface of the lower support member. The bottom float comprises a support member having an upper surface and a lower surface. A pressure relief tube extends axially from the lower surface of the bottom float support member through the upper surface and terminates at an upper end. The upper end of the bottom float pressure relief tube extends through the top float second passage.

[0024] The top float may further comprise a manipulator extending axially from the upper surface of the lower support member. The manipulator is used to push the top float towards the bottom float. The manipulator can be considered a handle for handling the top float.

[0025] During centrifugation, the buffy coat constituents become aligned between the top float and the bottom float. The top float is then pushed toward the bottom float to remove the buffy coat constituents through the pitot tube.

[0026] The pitot tube may be integral, or made as a separate piece. When the pitot tube is separate, the top float includes (ii) a first passage from the lower surface of the lower support member to the upper surface of the lower support member. After centrifugation, the pitot tube engages the first passage of the top float, and the top float can then be pushed toward the bottom float to remove the buffy coat constituents through the pitot tube.

[0027] Still other embodiments of a two-piece float comprise a top float and a bottom float. The top float has a density intermediate that of plasma and the buffy coat constituents. The top float comprises (i) a lower lateral support member having an upper surface and a lower surface, and (ii) a manipulator extending axially from the upper surface of the lower lateral support member. The bottom float has a density intermediate that of the buffy coat constituents and red blood cells. The bottom float comprises an upper lateral support member having an upper surface

and a lower surface. The top float lower lateral support member and the bottom float upper lateral support member are complementarily shaped to form a recess. The recess can be used to trap the buffy coat constituents, and/or can enclose a slide.

[0028] The bottom float may further comprise a support member extending axially from the lower surface of the upper lateral support member.

[0029] The recess can be substantially formed in the bottom float upper lateral support member, with the top float lower lateral support member covering the recess. Alternatively, the recess can be formed in the top float lower lateral support member and the bottom float upper lateral support member covering the recess.

[0030] During use, the buffy coat constituents become aligned between the top float and the bottom float. The top float is then pushed toward the bottom float to push the buffy coat constituents into the recess, and coat the slide. The two-piece float and the sample tube can be separated, and the buffy coat constituents or the slide can then be removed from the two-piece float.

[0031] In other embodiments, the top float comprises (i) a lower lateral support member having an upper surface and a lower surface, and (ii) a hollow member open on the lower surface of the lower lateral support member and extending axially from the upper surface of the lower lateral support member. The hollow member can be adapted to receive a slide. The bottom float comprises an upper lateral support member for sealing the hollow member.

[0032] Also disclosed in embodiments are additional designs for a volume-occupying separator float. The float comprises a main body portion having a top end and a bottom end. One or more support members protrude laterally from the main body portion. The main body portion and the one or more support members define an annular volume. A pitot tube extends axially from the top end of the main body portion. An internal passage passes through the main body portion and connects the pitot tube to an opening in the annular volume. An upper piece comprises (i) a passageway through which the pitot tube extends, and (ii) a manipulator extending axially away from the main body portion. The upper piece has a lower density than the main body portion. The manipulator acts as a handle for moving the upper piece.

[0033] The upper piece passageway can be located inside the manipulator.

[0034] The float may further comprise a one-way valve in the opening oriented to permit flow from the annular volume into the internal passage. The main body

portion internal passage can also connect the pitot tube to a plurality of openings in the annular volume.

[0035] The main body portion may further comprise a pressure relief passage extending from the first end to the second end, the pressure relief passage not intersecting the internal passage.

[0036] In particular embodiments, one support member is located on the bottom end of the main body portion, and the opening connecting to the annular volume is located proximally to the one support member.

[0037] In other embodiments, the float comprises one support member extending laterally from a bottom end of the main body portion and at least one helical support member located proximal to the top end of the main body portion.

[0038] In use, the upper piece is pushed down to force fluid towards the annular volume and push the buffy coat constituents through the pitot tube. These embodiments contemplate the pitot tube being integral with the main body portion.

[0039] Sometimes, the pitot tube is separate from the main body portion. Then, only the main body portion is placed in the tube with the blood sample. Centrifugation causes alignment of the annular volume with at least the buffy coat constituents. The pitot tube is then engaged with the internal passage at the top end of the main body portion. The upper piece, comprising a hollow member that extends axially away from the main body portion and surrounds the pitot tube, is threaded around the pitot tube. The upper piece is then pushed down to force fluid towards the annular volume and push the buffy coat constituents through the pitot tube.

[0040] These and other non-limiting characteristics of the disclosure are more particularly disclosed below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0042] **FIG. 1** is a side view of a sample tube containing a volume-occupying separator float.

[0043] **FIG. 2** is a diagram illustrating the methods of the present disclosure.

[0044] **FIG. 3A** is a side cross-sectional view of a sample tube containing a separator float surrounding a flexible bag.

[0045] **FIG. 3B** is a top cross-sectional view of a first exemplary embodiment of a two-piece float for surrounding a flexible bag.

[0046] **FIG. 3C** is a top cross-sectional view of a second exemplary embodiment of a two-piece float for surrounding a flexible bag.

[0047] **FIG. 3D** is a top cross-sectional view of a third exemplary embodiment of a two-piece float for surrounding a flexible bag.

[0048] **FIG. 4A** is a side cross-sectional view of a first exemplary embodiment of a separator float containing a buffy coat passage for trapping / catching the buffy coat constituents and using a centrifugation valve to control flow through the buffy coat passage.

[0049] **FIG. 4B** is a side cross-sectional view of a second exemplary embodiment of a separator float containing a buffy coat passage for trapping / catching the buffy coat constituents and using a centrifugation valve to control flow through the buffy coat passage.

[0050] **FIG. 4C** is a side cross-sectional view of a third exemplary embodiment of a separator float using a centrifugation valve to control flow through an annular volume.

[0051] **FIG. 4D** is a side cross-sectional view of another exemplary embodiment of a separator float.

[0052] **FIG. 5A** is a side cross-sectional view of a two-piece separator float containing a slide.

[0053] **FIG. 5B** is a perspective view of the float of **FIG. 5A** in a closed position.

[0054] **FIG. 5C** is a perspective view of the float of **FIG. 5A** in an open position.

[0055] **FIG. 6** is a side cross-sectional view of a two-piece float that traps buffy coat constituents and removes them through a pitot tube.

[0056] **FIG. 7A** is a side cross-sectional view of a first exemplary embodiment of a two-piece float that traps buffy coat constituents in a recess.

[0057] **FIG. 7B** is a side cross-sectional view of a second exemplary embodiment of a two-piece float that traps buffy coat constituents in a recess.

[0058] **FIG. 7C** is a side cross-sectional view of a third exemplary embodiment of a two-piece float that traps buffy coat constituents.

[0059] **FIG. 8** is a side cross-sectional view of a first exemplary embodiment of a two-piece float for extracting buffy coat constituents.

[0060] **FIG. 9** is a side cross-sectional view of a second exemplary embodiment of a two-piece float for extracting buffy coat constituents.

DETAILED DESCRIPTION

[0061] A more complete understanding of the components, processes, and apparatuses disclosed herein can be obtained by reference to the accompanying drawings. These figures are merely schematic representations based on convenience and the ease of demonstrating the present disclosure, and are, therefore, not intended to indicate relative size and dimensions of the devices or components thereof and/or to define or limit the scope of the exemplary embodiments.

[0062] Although specific terms are used in the following description for the sake of clarity, these terms are intended to refer only to the particular structure of the embodiments selected for illustration in the drawings, and are not intended to define or limit the scope of the disclosure. In the drawings and the following description below, it is to be understood that like numeric designations refer to components of like function.

[0063] The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). When used in the context of a range, the modifier "about" should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the range of "from about 2 to about 10" also discloses the range "from 2 to 10."

[0064] The present disclosure relates generally to apparatuses and assemblies which are useful for separating the various components of a blood sample, based on the density of the various components. Those apparatuses include volume-occupying separator floats, sample tubes, and combinations thereof.

[0065] **FIG. 1** is an axial cross-section of a blood separation tube and float assembly **100**. The assembly includes a sample tube **110** and a separator float or bobber **130** placed therein.

[0066] The sample tube **110** is generally cylindrical. However, sample tubes having polygonal and other geometrical cross-sectional shapes are also contemplated. In other words, the sample tube may have a cross-section that is a polygon having *n* sides. For example, when n=3, the sample tube has a triangular cross-section. In particular, the sample tube may have a regular polygonal cross-section (i.e. the lengths of each side are substantially equal).

[0067] The sample tube **110** includes a first, closed end **114** and a second, open end **116** receiving a stopper or cap **125**. Other closure means are also contemplated, such as parafilm or the like. In alternative embodiments, discussed further herein, the sample tube may be open at each end, with each end receiving an appropriate closure device.

[0068] Although the tube is depicted as generally cylindrical, the tube **110** may be minimally tapered, slightly enlarging toward the open end **116**, particularly when manufactured by an injection molding process. This taper or draft angle is generally necessary for ease of removal of the tube from the injection molding tool.

[0069] The tube **110** is formed of a transparent or semi-transparent material and the sidewall **112** of the tube **110** is sufficiently flexible or deformable such that it expands in the radial direction during centrifugation, e.g., due to the resultant hydrostatic pressure of the sample under centrifugal load. As the centrifugal force is removed, the tube sidewall **112** substantially returns to its original size and shape.

[0070] The tube may be formed of any transparent or semi-transparent, flexible polymeric material (organic and inorganic), such as polystyrene, polycarbonate, styrene-butadiene-styrene ("SBS"), styrene/butadiene copolymer (such as "K-Resin®" available from Phillips 66 Co., Bartlesville, Oklahoma), etc. Preferably, the tube material is transparent. However, the tube does not necessarily have to be clear, as long as the receiving instrument that is looking for the cells or items of interest in the sample specimen can "see" or detect those items in the tube. For example, items of very low level of radioactivity that cannot be detected in a bulk sample can be detected through a non-clear or semi-transparent wall after it is separated by the process of the present disclosure and trapped near the wall by the float **130** as described in more detail below. Desirably, the sample tube is seamless, at least along those portions of the tube along which the float will travel.

[0071] In some embodiments, the tube **110** is sized to accommodate the float **130** plus at least about five milliliters of blood or sample fluid, more preferably at least about eight milliliters of blood or fluid, and most preferably at least about ten milliliters of blood or fluid. In particular embodiments, the tube **110** has an inner diameter **117** of about 1.5 cm and accommodates at least about ten milliliters of blood in addition to the float **130**.

[0072] The float **130** depicted here includes a main body portion **132** and two sealing rings or flanges **140**, disposed at opposite axial ends of the float **130**. The main body portion **132** and the sealing rings or support members **140** of the float **130** are sized to have an outer diameter which is less than the inner diameter **117** of the sample tube **110**, under pressure or centrifugation. Put another way, the outer diameter of the support members is substantially equal to the inner diameter **117** of the sample tube **110** in a non-flexed state, so that the float can be held in a particular location by the sample tube. The main body portion **132** of the float **130** also has a smaller outer diameter **138** which is less than the diameter of the sealing or support rings **140**, thereby defining an annular volume **170** between the float **130** and the sidewall **112** of the tube **110**. The main body portion occupies much of the cross-sectional area of the tube, with the annular volume **170** being large enough to contain the cellular components of the buffy coat layers (i.e. buffy coat constituents) and associated target cells when the tube is in the non-flexed state. Preferably, the dimensions **138** and **117** are such that the annular volume **170** has a radial thickness ranging from about 25 microns to about 250 microns, most preferably about 50 microns. It should be noted that the term "annular" is used to refer to the ring-like shape formed by the float within the tube, and should not be construed as requiring the shape to be defined by two concentric circles. Rather, the tube and the float may each have different shapes and "annular" refers to the shape formed between them. The number of support members **140** may also vary, as will be seen further herein.

[0073] A bore or channel **150** extends axially through the float **130**. When the tube/float system is centrifuged, the tube expands, freeing the float in the blood sample. As centrifugation is slowed, the float is captured by the sidewall **112** of the tube as the sube returns to its original diameter. As the tube continues to contract, pressure may build up in the blood fraction trapped below the float, primarily red blood cells. This pressure may cause red cells to be forced into the annular volume **170** containing the captured buffy coat constituents, thus diluting the contents or

making imaging of the contents of the buffy coat more difficult. Alternatively, the collapse of the side wall of the sample tube during deceleration may produce excessive or disruptive fluid flow through the separated buffy coat layers. The bore **150** allows for any excessive fluid flow or any resultant pressure in the dense fractions trapped below the float **130** to be relieved. The excessive fluid flows into the bore **150**, thus preventing degradation of the buffy coat sample. This bore can be considered a pressure relief means for inhibiting excessive fluid flow through the buffy coat constituents. The bore is depicted here as being central and axially aligned within the float **130**, but other configurations are contemplated so long as the bore extends completely through the float from one end to the other. In some embodiments, the bore **150** is centrally located and axially extending.

[0074] While in some instances the outer diameter **138** of the main body portion **132** of the float **130** may be less than the inner diameter **117** of the tube **110**, this relationship is not required. This is because once the tube **110** is centrifuged (or pressurized), the tube **110** expands and the float **130** moves freely. Once the centrifugation (or pressurization) step is completed, the tube **130** constricts back down on the sealing rings or support ridges **140** to capture the float. The annular volume **170** is then created, and sized by the length of the support ridges or sealing rings **140** (i.e., the depth of the "pool" is equal to the length of the support ridges **140**, independent of what the tube diameter is/was).

[0075] In desired embodiments, the float dimensions are 3.5 cm tall x 1.5 cm in diameter, with a main body portion sized to provide a 50-micron gap for capturing the buffy coat layers of the blood. Thus, the volume available for the capture of the buffy coat layer is approximately 0.08 milliliter. Since the entire buffy coat layer is generally less than about 0.5% of the total blood sample, the preferred float accommodates the entire quantity of buffy layer separated in an eight to ten milliliter sample of blood.

[0076] The sealing or support flanged ends **140** are sized to be substantially equal to, or slightly greater than, the inner diameter **117** of the tube. The float **130**, being generally rigid, can also provide support to the flexible tube wall **112**. Furthermore, the support members **140** provide a sealing function to maintain separation of the blood constituent layers. The seal formed between the support members **140** of the float and the wall **112** of the tube may form a fluid-tight seal. As used herein, the term "seal" is also intended to encompass near-zero clearance or

slight interference between the flanges **140** and the tube wall **112** providing a substantial seal which is, in most cases, adequate for purposes of the disclosure.

[0077] The support members **140** are most preferably continuous ridges, in which case the sample may be centrifuged at lower speeds and slumping of the separated layers is inhibited. However, in alternative embodiments which are discussed further herein, the support members can be discontinuous or segmented bands having one or openings providing a fluid path in and out of the annular gap **170**. The support members **140** may be separately formed and attached to the main body portion **132**. Preferably, however, the support members **140** and the main body portion **132** form a unitary or integral structure.

[0078] The geometrical configuration of the support members are exemplary only, and different configurations are contemplated. For example, the support member **140** in **FIG. 1** is flat but support members that are tapered away from the main body portion **132** or concave curved are also contemplated. These shapes can provide a surface that encourages flow of the blood around the float during centrifugation. Additional exemplary shapes contemplated include, but are not limited to, tectiform and truncated tectiform; three, four, or more sided pyramidal and truncated pyramidal, ogival or truncated ogival; geodesic shapes, and the like.

[0079] The overall specific gravity of the separator float **130** should be between that of red blood cells (approximately 1.090) and that of plasma (approximately 1.028). In more specific embodiments, the specific gravity is in the range of from about 1.089 to about 1.029, more preferably from about 1.070 to about 1.040, and most preferably about 1.05.

[0080] The float may be formed of multiple materials having different specific gravities, so long as the overall specific gravity of the float is within the desired range. The overall specific gravity of the float **130** and the volume of the annular gap **170** may be selected so that some red cells and/or plasma may be retained within the annular gap, as well as the buffy coat layers. Upon centrifuging, the float **130** occupies the same axial position as the buffy coat layers and target cells and floats on the packed red cell layer. The buffy coat is retained in the narrow annular gap **170** between the float **130** and the inner wall **112** of the tube **110**. The expanded buffy coat region can then be examined, under illumination and magnification, to identify circulating epithelial cancer or tumor cells or other target analytes.

[0081] In embodiments, the density of the float **130** is selected to settle in the granulocyte layer of the blood sample. The granulocytes settle on, or just above, the packed red-cell layer and have a specific gravity of about 1.08-1.09. In this preferred embodiment, the specific gravity of the float is in this range of from about 1.08 to about 1.09 such that, upon centrifugation, the float settles in the granulocyte layer. The amount of granulocytes can vary from patient to patient by as much as a factor of about twenty. Therefore, selecting the float density such that the float settles in the granulocyte layer is especially advantageous since loss of any of the lymphocyte/monocyte layer, which settles just above the granulocyte layer, is avoided. During centrifugation, as the granulocyte layer increases in size, the float settles higher in the granulocytes and keeps the lymphocytes and monocytes at essentially the same position with respect to the float. In other embodiments described further herein, the float may be made from two pieces, and the specific gravity of each piece may differ.

[0082] The float **130** is formed of one or more generally rigid organic or inorganic materials, preferably a rigid plastic material, such as polystyrene, acrylonitrile butadiene styrene (ABS) copolymers, aromatic polycarbonates, aromatic polyesters, carboxymethylcellulose, ethyl cellulose, ethylene vinyl acetate copolymers, nylon, polyacetals, polyacetates, polyacrylonitrile and other nitrile resins, polyacrylonitrile-vinyl chloride copolymer, polyamides, aromatic polyamides (aramids), polyamide-imide, polyarylates, polyarylene oxides, polyarylene sulfides, polyarylsulfones, polybenzimidazole, polybutylene terephthalate, polycarbonates, polyester, polyester imides, polyether sulfones, polyetherimides, polyetherketones, polyetheretherketones, polyethylene terephthalate, polyimides, polymethacrylate, polyolefins (e.g., polyethylene, polypropylene), polyallomers, polyoxadiazole, polyparaxylene, polyphenylene oxides (PPO), modified PPOs, polystyrene, polysulfone, fluorine containing polymer such as polytetrafluoroethylene, polyurethane, polyvinyl acetate, polyvinyl alcohol, polyvinyl halides such as polyvinyl chloride, polyvinyl chloride-vinyl acetate copolymer, polyvinyl pyrrolidone, polyvinylidene chloride, specialty polymers, and so forth., and most preferably polystyrene, polycarbonate, polypropylene, acrylonitrite butadiene-styrene copolymer ("ABS") and others.

[0083] In this regard, it is desirable to avoid the use of materials and/or additives that interfere with the detection or scanning method. For example, if fluorescence is

utilized for detection purposes, the material utilized to construct the float **130** should not have interfering or "background" fluorescence at the wavelength of interest.

[0084] In some aspects, the compressibility and/or rigidity of the flexible tube and rigid float can be reversed. The float is flexible and designed to shrink in diameter at the higher pressures and moves freely within a rigid tube. The use of a compressible float allows for usage of transparent glass tubes which, in some instances, exhibit enhanced optical properties over polymeric tubes. Furthermore, this aspect generally reduces the tolerance requirements for the glass tubes (since the float would expand up against the tube wall after the pressure decreases), and a full range of float designs is possible.

[0085] The method for detecting circulating epithelial cancer cells in a blood of a subject is disclosed in U.S. Patent No. 6,197,523 may advantageously be modified to employ the sample tube and float system of the subject disclosure. The aforementioned U.S. Patent No. 6,197,523 is incorporated herein by reference in its entirety.

[0086] In an exemplary method of using the tube/float system **100** of the disclosure, a sample of anticoagulated blood is provided. For example, the blood to be analyzed may be drawn using a standard Vacutainer® or other like blood collection device of a type having an anticoagulant predisposed therein.

[0087] A tag, such as a fluorescently labeled antibody or ligand, which is specific to the target epithelial cells or other target analytes of interest, can be added to the blood sample and incubated prior to centrifugation. In an exemplary embodiment, the epithelial cells are labeled with anti-epcam having a fluorescent tag attached to it. Anti-epcam binds to an epithelial cell-specific site that is not expected to be present in any other cell normally found in the blood stream. A stain or colorant, such as acridine orange, may also be added to the sample to cause the various cell types to assume differential coloration for ease of discerning the buffy coat layers under illumination and to highlight or clarify the morphology of epithelial cells during examination of the sample.

[0088] The blood is then transferred to the assembly **100** for centrifugation. The float **130** may be introduced into the tube **110** after the blood sample is introduced into the sample tube **110** or otherwise may be placed therein beforehand. The tube and float assembly **100** containing the sample is then centrifuged. Operations required for centrifuging the blood by means of the subject tube/float system **100** are

not expressly different from the conventional case, although, as stated above, reduced centrifuge speeds may be possible and problems of slumping may be reduced. An adaptor may optionally be utilized in the rotor to prevent failure of the flexible tube due to stress.

[0089] During centrifugation, the sample tube is spun at a rotational speed sufficient to cause several effects. In particular, the resultant hydrostatic pressure deforms or flexes the wall **112** so as to enlarge the diameter of the tube from a first cross-sectional inner diameter to a second diameter, the second diameter being greater than the first diameter. The second diameter is sufficiently large to permit the blood components and the float **130** to move axially under centrifugal force within the tube **110**. The blood sample is separated into six discrete and distinct layers according to density, which are, from bottom to top (most dense to least dense): packed red blood cells, reticulocytes, granulocytes, lymphocytes/monocytes, platelets, and plasma. The epithelial cells sought to be imaged tend to collect by density in the buffy coat layers, i.e., in the granulocyte, lymphocyte/monocyte, and platelet layers. Due to the density of the float, the float occupies the same axial position within the sample tube as the buffy coat layers/constituents which thus occupy the narrow annular volume **188**, potentially along with a small amount of the red cell and/or plasma). Put another way, the float moves into alignment with at least the buffy coat constituents of the blood sample.

[0090] After centrifugal separation is complete and the centrifugal force is removed, the tube **110** returns to its original diameter to capture or retain the float and the buffy coat layers and target analytes within the annular volume **188**. The tube/float system can be transferred to a microscope or optical reader to identify any target analytes in the blood sample. Depending on the subsequent use of the float, the annular volume may be considered to make up one or more analysis areas.

[0091] Centrifugation may not be required. Sometimes the application of pressure alone to the inside of the tube, or simply the expansion of the tube (or the compression of the float) is required. For example, such pressure can be produced through the use of a vacuum source on the outside of the tube. Such an application also allows for the top of the sample tube to be kept open and easily accessible. Additionally, the use of a vacuum source may be easier to implement in some situations than the application of a centrifugal force. Additionally, any method of tubular expansion/contraction (or float compression) such as mechanical, electrical,

magnetic, etc., can be implemented. Once the tube is expanded (or the float is compressed), the float will move to the proper location due to buoyancy forces created by the density variations within the sample.

[0092] In additional embodiments described herein, a removal device, such as a syringe, is then used to extract the buffy coat layers / constituents from the annular volume. The intent here is to extract the target cells of interest, so it is acceptable to remove some of the red blood cells and/or plasma during this process as well. If tags have not yet been added, they may be added now to tag or label the "target" cells of interest. Again, the tags are any kind that an analytical instrument or detector could detect, e.g. fluorescent, radioactive, etc. The tags may be in the removal device itself, or they can be added separately.

[0093] The sample is then "squirted" through the instrument / detector and the tagged cells are analyzed. It may be sufficient to count the number of tagged cells. However, in further embodiments, the 'positive' sample cells are diverted into a holder for further analysis. Means of separating such cells are known in the art and can be similar to those used in flow cytometry, for example by coordinating the timing of the instrument / detector with the holder. The positive sample can then be further analyzed, for example by preparing a slide for further examination. This 'squirt-n-divert' method results in a smaller sample volume that is easier to analyze compared to the original blood sample, which was many times larger.

[0094] The float can comprise a part of a collection tube system or assembly. Thus, it is not necessary to transfer the buffy coat sample from a collection container to an analysis tube. The blood or sample fluid can be collected immediately and then tested. Such a system is somewhat faster, and also safer from a biohazard standpoint. For example, this system is desirable in very contagious situations (i.e. Ebola virus, HIV, etc.) where any type of exposure of the blood must be minimized.

[0095] **FIG. 2** is a diagram illustrating the general methods described above. In step **2**, the target cells in the buffy coat layers of the blood sample can be tagged prior to centrifugation. In step **4**, the buffy coat is isolated, e.g. by centrifugation. In step **6**, the sample containing the buffy coat, and reduced in volume compared to the original blood sample, is extracted from the sample tube. In step **8**, if the target cells were not already tagged, they can be tagged now. Alternatively, they can be tagged using different tags suitable for use with the given instrument / detector. In step **10**, the reduced volume is run through the detector. As illustrated here, the reduced

volume with the tagged target cells begin in syringe **20** and are injected into detector **25** which separates the 'positive' sample (i.e. target cells) and diverts them into holder **30**. The 'negative' sample goes to waste, i.e. is disposed of. Finally, in step **12**, the positive sample is further analyzed.

[0096] The sample tubes, separator floats, and methods described above provide a general idea of the present disclosure. Several further concepts are described herein.

[0097] **FIGs. 3A-3D** illustrate one concept of a blood separation apparatus **300** where rather than placing the blood sample and float into a sample tube, the blood sample is placed into a flexible bag **302**. **FIG. 3A** is an axial cross-sectional view. Instead of being placed in the bag and contacting the blood sample, the float **320** is placed around the bag **302**, i.e. on the exterior of the flexible bag. The flexible bag **302** and the float **320** are then placed into a sample tube **310** and centrifuged. After centrifugation, the buffy coat layers / constituents are in the portion of the bag **302** located between a first end **324** of the float and a second end **326** of the float. The flexible bag **302** is then sealed at the first end **324** and the second end **326** to capture the buffy coat constituents. The sealing may be done, for example, by welding. In particular embodiments, the welding is performed ultrasonically. Ultrasonic welding is an industrial technique commonly used for plastics, whereby high-frequency ultrasonic acoustic vibrations are locally applied to two items being held together to create a solid-state weld between the two items. The term "welding" is used here to indicate the action of closing the bag off in a specific location, and is synonymous with melting.

[0098] Separator floats made from two or more pieces are especially suitable for use with this concept. In embodiments, the separator float **320** comprises a first piece **340** and a second piece **350**. The first piece **340** has a first end **341**, a second end **342**, an interior surface **344**, and an exterior surface **346**. In some embodiments, the first piece also includes at least one side surface **348**. The second piece **350** has a first end **351**, a second end **352**, an interior surface **354**, and an exterior surface **356**. In some embodiments, the second piece also includes at least one side surface **358**. The first piece interior surface **344** and the second piece interior surface **354** cooperate to form an open passage **380** extending between the first end **324** of the float and the second end **326** of the float, or in other words between the first end **341**, **351** and second end **342**, **352** of each piece **340**, **350**.

Each piece **340**, **350** may also include optional support members **327** on their exterior surface **346**, **356** for engaging the sidewall **312**. In some embodiments, the exterior surface of each piece **340**, **350** comprises at least one support member, and in particular embodiments, the exterior surface of each piece has two support members, i.e. a first support member at the first end and a second support member at the second end.

[0099] In particular embodiments, the first and second pieces have substantially the same three-dimensional shape. **FIGs. 3B-3D** show lateral cross-sectional views of different first and second pieces. In **FIG. 3B**, the first piece **340** and second piece **350** are substantially of the same shape. The interior surface **344** of the first piece **340** is substantially planar, i.e. is substantially a straight line in this lateral cross-sectional view. The interior surface **354** of the second piece **350** is also substantially planar. The two interior surfaces are substantially parallel to each other, i.e. the open passage **380** has a rectangular shape. It should be noted that the exterior surface **346**, **356** is shown contacting the interior surface **344**, **354** at both ends, and that the exterior surface **346**, **356** is substantially conforming to an inner surface of the sample tube, for example having a semi-cylindrical surface. In some embodiments, the first piece **340** and/or second piece **350** may have at least one side surface **348**, **358** (shown here as a dotted line). In such embodiments having a side surface, the exterior surface may be described as an arcuate surface, or in a lateral cross-sectional view the exterior surface is an arc. The side surface **348**, **358** is generally perpendicular to the interior surface **344**, **354**.

[0100] The first piece **340** and second piece **350** can be connected together using any means. For example, in **FIG. 3B**, the first piece and second piece are joined on one side by a hinge mechanism **374**, and on the other side by clips **375**. Other connecting mechanisms, such as tongue-and-groove, detent-and-catch, hook-and-loop, etc., can also be used. The connecting mechanism can be located on the interior surface or a side surface.

[0101] In **FIG. 3C**, the interior surface **344** of the first piece **340** is substantially planar. The second piece **350** has a semi-annular cross-sectional shape. Put another way, the interior surface **354** is substantially a straight line with a central indent. Put yet another way, the interior surface **354** of the second piece comprises a semi-cylindrical surface **355**.

[0102] In **FIG. 3D**, the first piece **340** and the second piece **350** each have a semi-annular cross-sectional shape. The open passage **380** has a circular shape.

[0103] **FIG. 4A** shows another concept of a blood separation apparatus **400** including a sample tube **410** and a separator float **420**. The sample tube **410** is formed from a sidewall **412** and has a first, closed end **414** and a second, open end **416**. The separator float **420** includes a main body portion **422** having a first end **424** and a second end **426.** A pressure relief passage **487** extends from the second end **426** to the first end **424,** and has a first one-way pressure relief valve **434** oriented to open when pressure at the second end **426** is greater than pressure at the first end **424** by a specified value. Put another way, the first pressure relief valve **434** does not open until there is a specified difference between the pressure at the second end **426** and the pressure at the first end **424**. A buffy coat passage **478** extends from the second end **426** to the first end **424** and has a centrifugation valve **435** oriented to open during centrifugation. At least one pressure seal wraps around the main body portion. Two pressure seals **404** are shown here, one extending radially from the first end **424** and the other from the second end. The pressure seals effectively prevent fluid flow between the main body portion **422** and the sidewall **412** of the sample tube.

[0104] When the apparatus of **FIG. 4A** is used, during centrifugation, the centrifugation valve **435** opens as necessary to allow the blood sample to separate into discrete layers. The centrifugation valve **435** may be thought of as a weight on a spring. When exposed to higher forces during centrifugation, the valve opens and allows red blood cells, plasma, and buffy coat constituents to flow through the buffy coat passage **478**. As the centrifuge begins to slow down, the valve closes to seal the buffy coat passage. After centrifugation, buffy coat constituents reside in the buffy coat passage **478**. Pressure built up underneath the float **420** can be relieved through the pressure relief passage **487** with minimal disturbance to the buffy coat constituents in the buffy coat passage **478**. The float **420** can be removed from the sample tube **410** with the buffy coat constituents being present in the buffy coat passage **478**.

[0105] **FIG. 4B** depicts an apparatus similar to the apparatus of **FIG. 4A**, except that there is no pressure relief passage **487**. It is contemplated here that in the event of a pressure difference between the first end **424** and the second end **426**, the pressure might cause the float to slide upward along the tube. However, this

movement would be acceptable because the centrifugation valve **435** does not permit the movement of fluid through the buffy coat passage **487**.

[0106] **FIG. 4C** is a cross-sectional view of another exemplary embodiment of a separator float similar to the apparatus of **FIG. 4A**. Here, the separator float **420** includes a main body portion **422** having a first end **424** and a second end **426**. The main body portion has an outer diameter **438**, while the sample tube **410** has an inner diameter **417**. Again, there is no pressure relief passage **487**. Rather than pressure seals **404**, at least one support member extends radially outwards from the main body portion towards the sample tube **410**, and has a diameter substantially equivalent to the inner diameter **417**. Here, two support members **440** are located at the first end **424** and the second end **426**. The support member(s) can also be described as being circumferentially disposed about the main body portion. The support members are centrifugation valves, as described above. The support member(s) and the main body portion **422** define an annular volume **470**. The centrifugation valve(s) itself can be considered to have an annular shape when considered in isolation. The centrifugation valves are oriented to allow fluid to flow through the annular volume **470** from second end **426** to first end **424** during centrifugation. When centrifugation ends, the centrifugation valves close, no longer permitting fluid flow through the annular volume. It is again contemplated that any pressure difference between the first end **424** and the second end **426** would result only the float sliding upward along the tube.

[0107] **FIG. 4D** is a cross-sectional view of yet another exemplary embodiment of a separator float of the present disclosure. The separator float **420** is similar to the float of **FIG. 4A,** except the float **420** does not include a buffy coat passage **478** or a centrifugation valve **435**. It is contemplated that the separator float initially begins near the open end **416** of the sample tube. During centrifugation, the float is pushed downwards into the blood. The resulting pressure on the first one-way pressure relief valve **434** is sufficient to open the valve, allowing blood components to flow from one end to the other. The various components then settle into layers based on density, with the target cells residing within the pressure relief passage **487**. After centrifugation, the relief valve remains closed, keeping the target cells in the pressure relief passage **487**.

[0108] Another concept is illustrated in **FIGs. 5A-C**. **FIG. 5A** is a side view showing a blood separation apparatus **500** including a sample tube **510** and a

separator float **520**. The sample tube **510** is formed from a sidewall **512** and has a first, closed end **514** and a second, open end **516**.

[0109] **FIG. 5B** is a perspective view of the float in a closed position, and **FIG. 5C** is a view of the float in an open position. The separator float **520** is formed from a first piece **540** and a second piece **550**. The float **520** has a first end **526** and a second end **524**. The first piece **540** has a first end **546** and a second end **544**. Similarly, the second piece **550** has a first end **556** and a second end **554**. The pieces are joined together at their first end **546**, **556**, for example by a hinge **574**. The first piece **540** and second piece **550** cooperate to form a rectangular passage **584** between the first end **526** and second end **524** of the float **520**. The float **520** contains a slide **508** within the rectangular passage **584**. **FIG. 5B** shows the float **520** after removal from the sample tube. **FIG. 5C** shows the float **520** after the float **520** has been opened and the slide has been removed.

[0110] In use, the slide **508** is placed in the float **520**, and the float is centrifugated with the blood sample. Buffy coat constituents present in the rectangular passage **584** adhere to the slide **508** within the rectangular passage **584**. After centrifugation, the float **520** may be removed from the sample tube **510** and opened at the hinge **574** to extract the slide **508**. The slide, with the buffy coat constituents already adhered to it, can then be examined.

[0111] In other specific embodiments, it is contemplated that the float **520** can be used with the flexible bag **302**, shown in **FIG. 3**. In these embodiments, the flexible bag **302** is placed in the rectangular passage **584** instead of the slide **508**.

[0112] **FIG. 6** illustrates still another concept. A side view of a blood separation apparatus **600** includes a sample tube **610** and a two-piece float **620**. The sample tube **610** is formed from a sidewall **612** and has a first, closed end **614** and a second, open end **616**.

[0113] The two-piece float **620** includes a top float **660** and a bottom float **668**. The top float **660** includes a lower support member **662** having an upper surface **663** and a lower surface **664**.

[0114] The top float **660** is formed from a lower support member **662** and a pitot tube **690**. The lower support member **662** has an upper surface **663** and a lower surface **664**. The pitot tube **690** extends from the lower surface **664** through the upper surface **663** and has a top end **694** located distally from the upper surface **663**. The pitot tube forms a passage from the lower surface **663** to the top end **694**.

In this respect, a pitot tube acts like a straw; fluid flows through the pitot tube when the pressure at the top of the pitot tube is lower than the pressure at the bottom of the pitot tube.

[0115] The bottom float **668** may comprise a support member **670** having an upper surface **671** and a lower surface **672**. The top float lower support member lower surface **664** and the bottom float support member upper surface **671** are complementarily shaped.

[0116] Generally, the density of the top float **660** and the bottom float **668** are independently from about 1.029 to about 1.09. The top float **660** has a density intermediate that of plasma and the buffy coat constituents, or in other words a specific gravity of from about 1.029 to about 1.08. The bottom float **668** has a density intermediate that of the buffy coat constituents and red blood cells, or in other words a specific gravity of from about 1.08 to about 1.09. Regardless of the value of the density, it is generally contemplated in specific embodiments that the top float has a density which is less than the bottom float.

[0117] In use, the apparatus of **FIG. 6** traps buffy coat constituents in the volume **685** between the lower surface **664** of the lower support member **662** of the top float **660** and the upper surface **671** of the support member **670** of the bottom float **668**. The top float **660** is then pushed downwards towards the bottom float **668** to extract buffy coat constituents through the pitot tube **690**.

[0118] In these embodiments, the pitot tube **690** is integral with the lower support member **662**.

[0119] However, it is also contemplated that the pitot tube is made separately from the lower support member. In such embodiments, only the lower support member **662** of the top float **660** is centrifuged with the blood sample. The lower support member in this case is made with a first passage from the lower surface **664** to the upper surface **663**. After centrifugation ends, the pitot tube **690** is inserted to engage the first passage. The top float **660** is then pushed down towards the bottom float 668 to push buffy coat constituents through the pitot tube **690**.

[0120] Some additional variations on this two-piece float **620** are contemplated. The pitot tube **690** itself is contemplated as being rigid, so as to be suitable for use in pushing the top float **660** downwards. In some embodiments, however, and as depicted here, the top float **660** may further comprise a manipulator **666**, such as a handle, extending axially from the upper surface **663** of the top float **660**, and this

manipulator can be used to push the top float downwards. The manipulator is generally made or situated on the upper surface **663** so that its presence will not affect the final alignment of the lower support member **662** with the buffy coat constituents.

[0121] After centrifugation, excess pressure may form below the bottom float **668**. This pressure may be relieved through a pressure relief tube **676** which extends axially from the lower surface **672** of the bottom float support member **670** through the upper surface **671** and terminates at an upper end **677**. The top float **660** includes a second passage **675** from the lower surface **664** to the upper surface **663**, and the pressure relief tube **676** extends through the second passage.

[0122] In addition, if desired, an axial support member **673** may extend axially from the lower surface **672** of the support member **670** of the bottom float **668**. It is contemplated that this axial support member **673** would contact the closed end **614** of the sample tube **610** and provide additional resistance when the top float **660** is pushed towards the bottom float **668**. It should be noted, however, that the length of the axial support member may be uncertain, as the level at which the bottom float support member **670** rests after centrifugation would depend partially on the size of the blood sample, and the size of the blood sample with which the float is used is not a factor that can be controlled during manufacture of the float.

[0123] **FIGS. 7A-7C** show three exemplary embodiments of another concept. Here, a blood separation apparatus **700** includes a sample tube **710** and a two piece float **720**. The sample tube **710** is formed from a sidewall **712** and has a first, closed end **714** and a second, open end **716**. Generally speaking, the buffy coat is captured between the two pieces of the float.

[0124] The two-piece float can include a recess, in which the buffy coat layer is trapped or contained. The recess is placed in different locations in **FIG. 7A** and **FIG. 7B**.

[0125] A first exemplary embodiment is shown in **FIG. 7A**. The two-piece float **720** comprises a top float **760** and a bottom float **768**. The top float **760** includes a lower lateral support member **762** which has an upper surface **763** and a lower surface **764**. A member or manipulator **766** extends axially from the upper surface **763** of the lower lateral support member **762**. The manipulator **766** is used to push the top float **760** towards the bottom float **768**.

[0126] The bottom float **768** comprises an upper lateral support member **770** having an upper surface **771** and a lower surface **772**. The top float lower lateral support member **762** and the bottom float upper lateral support member **770** are complimentarily shaped to form a recess **796**. For example, **FIG. 7A** shows five sides of the recess **796** being formed in the bottom float upper lateral support member **770**, while the top float lower lateral support member **762** covers the recess. It is contemplated that this arrangement could be reversed, with the top float lower lateral support member **762** containing the recess and the bottom float upper lateral support member **770** covering the recess. The recess resides in a lateral plane, in other words perpendicularly to the long axis of the sample tube **710**.

[0127] A second exemplary embodiment is shown in **FIG. 7B**. Here, the recess **796** is located in the member **766**. The hollow member **766** is open on the lower surface **764** of the lower lateral support member **762**, and is adapted to receive the buffy coat layer. The upper lateral support member **770** of the bottom float **768** is used to seal the hollow member.

[0128] In **FIG. 7C**, there is no recess. Rather, the buffy coat layers are simply trapped between the top float **760** and the bottom float **768**.

[0129] Generally, the density of the top float **760** and the bottom float **768** are independently from about 1.029 to about 1.09. The top float **760** has a density intermediate that of plasma and the buffy coat constituents, or in other words a specific gravity of from about 1.029 to about 1.08. The bottom float **768** has a density intermediate that of the buffy coat constituents and red blood cells, or in other words a specific gravity of from about 1.08 to about 1.09. Regardless of the value of the density, it is generally contemplated in specific embodiments that the top float has a density which is less than the bottom float. Preferably, the densities are selected in **FIG. 7A** and **FIG. 7B** so that there is little to no additional space between the two floats, so that the buffy coat is located in the recess.

[0130] In use, the apparatus of **FIGS. 7A-7C** traps buffy coat constituents in the volume between the lower surface **764** of the lower support member **762** of the top float **760** and the upper surface **771** of the support member **770** of the bottom float **768**. The buffy coat constituents are then removed from the float. In the case of **FIG. 7A** and **FIG. 7B**, this may be done by breaking the sample tube to retrieve the float. In the case of **FIG. 7C**, one end of the sample tube is broken off, and one

piece of the two-piece float is then removed so that the buffy coat constituents can be drained out of the remainder of the tube.

[0131] When the apparatuses of **FIGS. 7A** and **B** is used to separate buffy coat constituents, after centrifugation, the buffy coat constituents reside in the volume between the lower surface **764** of the lower lateral support member **762** of the top float **760** and the upper surface **771** of the upper lateral support member **770** of the lower float **768**. The member or manipulator **766** is pushed down to push buffy coat constituents in the recess **796**. The float can then be separated from the sample tube, and the buffy coat can be extracted onto a slide for examination.

[0132] In particular embodiments, it is contemplated that a suitable slide **708** could be placed within the recess **796**, and the buffy coat constituents could adhere to the slide **708** within the recess **796**. The two-piece float **720** could then be separated from the sample tube **710** and the slide **708** would be removed from the recess **796** in the two-piece float **720**. The slide, with the buffy coat constituents already adhered to it, could then be examined.

[0133] Some additional variations on this two-piece float **720** are contemplated. After centrifugation, excess pressure may form below the bottom float **768**. This pressure may be relieved through a pressure relief tube **776** which extends axially from the lower surface **772** of the bottom float upper lateral support member **770** through the upper surface **771** and terminates at an upper end **777**. The top float **760** includes a second passage **775** from the lower surface **764** to the upper surface **763**, and the pressure relief tube **776** extends through the second passage.

[0134] In addition, if desired, an axial support member **773** may extend axially from the lower surface **772** of the upper lateral support member **770** of the bottom float **768**. It is contemplated that this axial support member **773** would contact the closed end **714** of the sample tube **710** and provide additional resistance when the top float **760** is pushed towards the bottom float **768**. It should be noted, however, that the length of the axial support member may be uncertain, as the level at which the bottom float support member **770** rests after centrifugation would depend partially on the size of the blood sample, and the size of the blood sample with which the float is used is not a factor that can be controlled during manufacture of the float

[0135] **FIG. 8** and **FIG. 9** illustrate another concept with two exemplary embodiments. Briefly, the separator is a two-piece float. The upper piece is used to

push fluid down towards the lower piece. The lower piece contains a pitot tube through which the buffy coat constituents are extracted from the tube.

[0136] **FIG. 8** shows a side view of a sample tube **810** and a separator float **820**. The sample tube **810** is formed from a sidewall **812** and has a first, closed end **814** and a second, open end **816**.

[0137] The separator float **820** includes a lower piece **862** and an upper piece **860**. The lower piece **862** is formed from a main body portion **822**. The main body portion **822** has a top end **824** and a bottom end **826**. One or more support members **827** protrude from the main body portion. The main body portion and the support members define an annular volume **880**. A pitot tube **890** extends axially from the top end **824** of the main body portion **822**. The main body portion contains an internal passage **882** that connects a distal end **892** of the pitot tube **890** to an opening **833** in the annular volume **880**. In particular embodiments, the main body portion has a specific gravity of from about 1.029 to about 1.09, including from about 1.08 to about 1.09.

[0138] The upper piece **860** includes a passageway **865** through which the pitot tube **890** extends. The upper piece also includes a member **866** or handle extending axially away from the main body portion **822**. The upper piece **860** has a lower density than the main body portion **822,** i.e. is less dense. The upper piece and the lower piece both have a diameter sufficient to engage the sidewall **812** of the sample tube **810**. Put another way, the upper piece **860** and the lower piece **862** both have substantially the same diameter.

[0139] In some embodiments, the passageway **865** is located inside the member **866**, i.e. the member is hollow or the member **866** surrounds the pitot tube **890**. The float **820** may further include one or more one-way valves **834** in the opening **833** of the main body portion which are oriented to permit flow from the annular volume **880** into the internal passage **882**. Put another way, the one-way valves are oriented to open when the pressure in the annular volume is greater than the pressure in the internal passage. In other embodiments, the internal passage **882** connects the pitot tube **890** to a plurality of openings in the annular volume **880**.

[0140] When the apparatus of **FIG. 8** is used, both the upper piece **860** and the lower piece **862** are aligned with each other so that the pitot tube **890** extends through the passageway **865**, and both pieces are placed into the sample tube with the blood sample prior to centrifugation. During centrifugation, the main body portion

822 aligns with the buffy coat constituents and traps them in the annular volume **880** after centrifugation ends. The upper piece **860**, which has a lower density than the main body portion, remains floating and not in contact with the main body portion. The upper piece **860** is then pushed downwards towards the main body portion to force fluid into the annular volume, which in turn pushes the buffy coat constituents upwards through the pitot tube **890**. The one-way valves **834** can be used to keep fluid from entering the internal passage **882**, particularly during centrifugation. In these embodiments, the pitot tube **890** is integral with the main body portion **822**.

[0141] However, it is also contemplated that the pitot tube is made separately from the main body portion. In such embodiments, only the main body portion **822** is centrifuged with the blood sample. The internal passage **882** connects the opening **833** in the annular volume **880** to the top end **824** of the main body portion. After centrifugation ends, the pitot tube **890** is inserted to engage the internal passage **882** at the top end **824**. The upper piece **860** is then threaded onto the pitot tube, so that the pitot tube extends through the member **866**, and the upper piece **860** is pushed down towards the main body portion **822** to force fluid into the annular passage and push buffy coat constituents through the pitot tube **890**. It should be noted that in addition, the upper piece **860** does not need to have a lower density than the lower piece **862** because the upper piece **860** is not centrifuged.

[0142] An optional pressure relief passage **887** may also be present for relieving excessive pressure. The pressure relief passage **887** is an independent passage between the top end **824** and the bottom end **826**, and does not intersect the internal passage **882**, any openings **833**, or any one-way valves **834**.

[0143] In specific embodiments, the one or more support members **827** includes a bottom support member **830** which protrudes from the bottom end **826** of the main body portion. The opening **833** may be located proximally to the bottom support member **830**. The one or more support members **827** may also include a top support member **828** which protrudes from the top end **824** of the main body portion, i.e. which is proximal to the top end **824** of the main body portion.

[0144] **FIG. 9** is a side view of a second exemplary embodiment similar to **FIG. 8**. Here, the one or more support members **827** include a bottom support member **830** which protrudes from the bottom end **826** of the main body portion. Here, the top support member **828** is a helical support member which is proximal to the top end **824** of the main body portion. One advantage to using a helical support member

instead of a flat support member is that when the upper piece **860** is pushed towards a flat support member as in **FIG. 8**, fluid is forced into the annular volume **880** around the entire perimeter of the support member. This can cause mixing of the desired buffy coat constituents with the fluid, increasing the volume which must be extracted through the pitot tube and analyzed later for target cells. However, when the upper piece is pushed towards a helical support member, the fluid enters the annular volume along a smaller periphery and acts like a wave front that "pushes" the buffy coat into the pitot tube. The smaller periphery reduces the volume in which mixing occurs.

[0145] While particular embodiments have been described, alternatives, modifications, variations, improvements, and substantial equivalents that are or may be presently unforeseen may arise to applicants or other skilled in the art. Accordingly, the appended claims as filed and as they are amended are intended to embrace all such alternatives, modifications, variations, improvements, and substantial equivalents.

CLAIMS:

1. A volume-occupying separator float, the float comprising:

a first piece and a second piece, each piece having a first end, a second end, an exterior surface, and an interior surface;

wherein the interior surfaces of the first and second pieces cooperate to form an open passage extending between the first end and the second end; and

wherein the first and second pieces can be connected together.

2. The float of claim 1, wherein the first and second pieces have substantially the same three-dimensional shape.

3. The float of claim 1, wherein the interior surface of the first piece comprises a semi-cylindrical surface.

4. The float of claim 1, wherein a lateral cross-sectional view of the first piece has a semi-annular shape.

5. The float of claim 1, wherein the interior surface of the first piece is substantially planar.

6. The float of claim 1, wherein a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line.

7. The float of claim 1, wherein a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line, and wherein a lateral cross-sectional view of the interior surface of the second piece is substantially a straight line with a central indent.

8. The float of claim 1, wherein a lateral cross-sectional view of the open passage has a rectangular shape.

9. The float of claim 1, wherein a lateral cross-sectional view of the open passage has a circular shape.

10. The float of claim 1, wherein the exterior surface of the first and second pieces each substantially conform to an inner surface of the sidewall.

11. The float of claim 1, wherein the exterior surface of the first and second pieces each comprise at least one support member for engaging an inner surface of the sidewall.

12. The float of claim 11, wherein the exterior surface of the first and second pieces each have two support members.

13. The float of claim 1, wherein the first piece and the second piece each have a specific gravity of from about 1.029 to about 1.09.

14. The float of claim 1, wherein the first piece and the second piece each have a specific gravity of from about 1.040 to about 1.070.

15. The float of claim 1, wherein the first piece and the second piece each have a specific gravity of from about 1.08 to about 1.09.

16. The float of claim 1, wherein the first piece and the second piece are each formed from a rigid plastic material.

17. The float of claim 1, wherein the first and second pieces can be joined together using clips.

18. The float of claim 1, wherein the first and second piece each further comprise at least one side surface.

19. The float of claim 18, wherein the first and second pieces can be connected together on the at least one side surface.

20. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing a blood sample into a flexible bag;

placing a float around the flexible bag, the float having a first end and a second end and the float having a specific gravity intermediate that of plasma and red blood cells;

placing the flexible bag and float in a flexible sample tube, the sample tube having a sidewall, wherein the float engages the sidewall;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and movement of the float into alignment with at least the buffy coat constituents;

reducing the rotational speed to cause the sidewall to capture the float; and

sealing the flexible bag at the first end and the second end of the float to capture the buffy coat constituents.

21. The method of claim 20, wherein the flexible bag is sealed by welding the first end and the second end of the float.

22. The method of claim 21, wherein the welding is performed ultrasonically.

23. The method of claim 20, wherein the float comprises a first piece and a second piece, each piece having an exterior surface and an interior surface, the interior surfaces of the first and second pieces cooperating to form an open passage extending between the first end and the second end for the flexible bag.

24. The method of claim 23, wherein the first and second pieces have substantially the same three-dimensional shape.

25. The method of claim 23, wherein the interior surface of the first piece comprises a semi-cylindrical surface.

26. The method of claim 23, wherein a lateral cross-sectional view of the first piece has a semi-annular shape.

27. The method of claim 23, wherein the interior surface of the first piece is substantially planar.

28. The method of claim 23, wherein a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line.

29. The method of claim 23, wherein the first and second pieces are separate.

30. The method of claim 23, wherein the first and second pieces are joined together on at least one side.

31. The method of claim 30, wherein the first and second pieces are joined together on two sides.

32. The method of claim 30, wherein the first and second pieces are joined together using clips.

33. The method of claim 30, wherein the first and second pieces are joined at the first end of the float.

34. The method of claim 23, wherein a lateral cross-sectional view of the interior surface of the first piece is substantially a straight line, and wherein a lateral cross-sectional view of the interior surface of the second piece is substantially a straight line with a central indent.

35. The method of claim 23, wherein a lateral cross-sectional view of the open passage has a rectangular shape.

36. The method of claim 23, wherein a lateral cross-sectional view of the open passage has a circular shape.

37. The method of claim 23, wherein the exterior surface of the first and second pieces each substantially conform to an inner surface of the sidewall.

38. The method of claim 23, wherein the exterior surface of the first and second pieces each comprise at least one support member for engaging an inner surface of the sidewall.

39. The method of claim 38, wherein the exterior surface of the first and second pieces each have two support members.

40. The method of claim 23, wherein the first and second pieces of the float each have a specific gravity of from about 1.029 to about 1.089.

41. The method of claim 23, wherein the first and second pieces of the float are each formed from a rigid plastic material.

42. The method of claim 20, further comprising combining the blood sample with a stain.

43. The method of claim 20, wherein the sample tube is seamless at least along the path of axial movement of the float.

44. The method of claim 20, wherein the flexible bag is formed of a transparent polymeric material.

45. A two-piece float for capturing buffy coat constituents of a blood sample, comprising:

a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower support member having an upper surface and a lower surface, and (ii) a pitot tube extending axially from the lower surface of the top float through the upper surface and having a top end located distally from the upper surface; and

a bottom float having a density intermediate that of the buffy coat constituents and red blood cells;

wherein the top float lower support member lower surface and the bottom float are complementarily shaped.

46. The two-piece float of claim 45, wherein the top float further comprises a second passage extending from the lower surface to the upper surface of the lower support member;

wherein the bottom float comprises (i) a support member having an upper surface and a lower surface, and (ii) a pressure relief tube extending axially from the lower surface of the bottom float support member through the upper surface and terminating at an upper end; and

wherein the upper end of the bottom float pressure relief tube extends through the top float second passage.

47. The two-piece float of claim 45, wherein the top float has a specific gravity of from about 1.029 to about 1.09.

48. The two-piece float of claim 45, wherein the bottom float has a specific gravity of from about 1.029 to about 1.09.

49. The two-piece float of claim 45, wherein the top float further comprises a manipulator extending axially from the upper surface of the lower support member.

50. The two-piece float of claim 45, wherein the top float and the bottom float are each formed from a rigid plastic material.

51. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a sample tube, the sample tube having a sidewall;

introducing a two-piece float into the sample tube, the two-piece float comprising:

a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower support member having an upper surface and a lower surface, and (ii) a pitot tube extending axially from the lower surface of the top float through the upper surface and having a top end located distally from the upper surface; and

a bottom float having a density intermediate that of the buffy coat constituents and red blood cells;

wherein the top float lower support member lower surface and the bottom float are complementarily shaped;

wherein the top float lower support member and the bottom float engage the sidewall;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and alignment of the buffy coat constituents between the top float and the bottom float; and

pushing the top float toward the bottom float to remove the buffy coat constituents through the pitot tube.

52. The method of claim 51, wherein the top float further comprises a second passage extending from the lower surface to the upper surface of the lower support member;

wherein the bottom float comprises (i) a support member having an upper surface and a lower surface, and (ii) a pressure relief tube extending axially from the lower surface of the bottom float support member through the upper surface and terminating at an upper end; and

wherein the upper end of the bottom float pressure relief tube extends through the top float second passage.

53. The method of claim 51, wherein the top float has a specific gravity of from about 1.029 to about 1.09.

54. The method of claim 51, wherein the bottom float has a specific gravity of from about 1.029 to about 1.09.

55. The method of claim 51, wherein the top float further comprises a manipulator extending axially from the upper surface of the lower support member, the manipulator being used to push the top float toward the bottom float.

56. The method of claim 51, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

57. The method of claim 51, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

58. The method of claim 51, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

59. The method of claim 51, wherein the blood sample comprises anticoagulated whole blood.

60. The method of claim 51, wherein the sample tube is self-supporting.

61. The method of claim 51, further comprising combining the blood sample with a stain.

62. The method of claim 51, wherein the sample tube is seamless at least along the path of axial movement of the float.

63. The method of claim 51, wherein the flexible sample tube is formed of a flexible polymeric material.

64. The method of claim 63, wherein the flexible sample tube is semi-transparent.

65. The method of claim 63, wherein the flexible sample tube is transparent.

66. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a sample tube, the sample tube having a sidewall;

introducing a two-piece float into the sample tube, the two-piece float comprising:

a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower support member having an upper surface and a lower surface, and (ii) a first passage from the lower surface of the lower support member to the upper surface of the lower support member; and

a bottom float having a density intermediate that of the buffy coat constituents and red blood cells;

wherein the top float lower support member lower surface and the bottom float are complementarily shaped;

wherein the top float lower support member and the bottom float engage the sidewall;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and alignment of the buffy coat constituents between the top float and the bottom float; and

engaging a pitot tube with the first passage of the top float;

pushing the top float toward the bottom float to remove the buffy coat constituents through the pitot tube.

67. A two-piece float for capturing buffy coat constituents of a blood sample, comprising:

a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower lateral support member having an upper surface and a lower surface, and (ii) a manipulator extending axially from the upper surface of the lower lateral support member; and

a bottom float having a density intermediate that of the buffy coat constituents and red blood cells, wherein the bottom float comprises an upper lateral support member having an upper surface and a lower surface.

68. The float of claim 67, wherein the top float lower lateral support member and the bottom float upper lateral support member are complementarily shaped to form a recess.

69. The float of claim 68, further comprising a slide in the recess.

70. The float of claim 68, wherein the recess is substantially formed in the bottom float upper lateral support member, and the top float lower lateral support member covers the recess.

71. The float of claim 68, wherein the recess is substantially formed in the top float lower lateral support member, and the bottom float upper lateral support member covers the recess.

72. The float of claim 68, wherein the recess includes an opening on the lower surface of the lower lateral support member and extends axially from the upper surface of the lower lateral support member.

73. The float of claim 67, wherein the bottom float further comprises a support member extending axially from the lower surface of the upper lateral support member.

74. The float of claim 67, wherein the top float has a specific gravity of from about 1.029 to about 1.09.

75. The float of claim 67, wherein the bottom float has a specific gravity of from about 1.029 to about 1.09.

76. The float of claim 67, wherein the top float and the bottom float are each formed from a rigid plastic material.

77. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a sample tube, the sample tube having a sidewall;

introducing a two-piece float into the sample tube, the two-piece float comprising:

a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower lateral support member having an upper surface and a lower surface, and (ii) a manipulator extending axially from the upper surface of the lower lateral support member; and

a bottom float having a density intermediate that of the buffy coat constituents and red blood cells, wherein the bottom float comprises an upper lateral support member having an upper surface and a lower surface;

wherein the top float lower support member and the bottom float upper lateral support member engage the sidewall;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and alignment of the buffy coat constituents between the top float and the bottom float; and

retrieving the buffy coat constituents.

78. The method of claim 77, wherein the bottom float further comprises a support member extending axially from the lower surface of the upper lateral support member.

79. The method of claim 77, wherein a recess is formed by the top float lower lateral support member and the bottom float upper lateral support member; and wherein the buffy coat constituents are retrieved by pushing the top float toward the bottom float to push the buffy coat constituents into the recess and separating the two piece float from the sample tube.

80. The method of claim 79, wherein the recess is substantially formed in the bottom float upper lateral support member and the top float lower lateral support member covers the recess.

81. The method of claim 79, wherein the recess is substantially formed in that the top float lower lateral support member and the bottom float upper lateral support member covers the recess.

82. The method of claim 79, wherein the recess includes an opening on the lower surface of the lower lateral support member and extends axially from the upper surface of the lower lateral support member.

83. The method of claim 79, wherein a slide is located in the recess.

84. The method of claim 77, wherein the top float has a specific gravity of from about 1.029 to about 1.09.

85. The method of claim 77, wherein the bottom float has a specific gravity of from about 1.029 to about 1.09.

86. The method of claim 77, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

87. The method of claim 77, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

88. The method of claim 77, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

89. The method of claim 77, wherein the blood sample comprises anticoagulated whole blood.

90. The method of claim 77, wherein the sample tube is self-supporting.

91. The method of claim 77, further comprising combining the blood sample with a stain.

92. The method of claim 77, wherein the sample tube is seamless at least along the path of axial movement of the float.

93. The method of claim 77, wherein the flexible sample tube is formed of a flexible polymeric material.

94. The method of claim 93, wherein the flexible sample tube is semi-transparent.

95. The method of claim 93, wherein the flexible sample tube is transparent.

96. A volume-occupying separator float, comprising:

a main body portion having a top end and a bottom end;

one or more support members protruding laterally from the main body portion;

wherein the main body portion and the one or more support members define an annular volume;

a pitot tube extending axially from the top end of the main body portion;

an internal passage that passes through the main body portion and connects the pitot tube to an opening in the annular volume; and

an upper piece comprising (i) a passageway through which the pitot tube extends, and (ii) a member extending axially away from the main body portion;

wherein the upper piece has a lower density than the main body portion.

97. The float of claim 96, wherein the upper piece passageway is located inside the member.

98. The float of claim 96, further comprising a one-way valve in the opening oriented to permit flow from the annular volume into the internal passage.

99. The float of claim 96, wherein the main body portion internal passage connects the pitot tube to a plurality of openings in the annular volume.

100. The float of claim 96, wherein the main body portion further comprises a pressure relief passage extending from the first end to the second end, the pressure relief passage not intersecting the internal passage.

101. The float of claim 96, wherein one support member is located on the bottom end of the main body portion, and the opening connecting to the annular volume is located proximally to the one support member.

102. The float of claim 96, wherein the float comprises one support member extending laterally from a bottom end of the main body portion and at least one helical support member located proximal to the top end of the main body portion.

103. The float of claim 96, wherein the main body portion has a specific gravity of from about 1.029 to about 1.09.

104. The float of claim 96, wherein the main body portion has a specific gravity of from about 1.08 to about 1.09.

105. The float of claim 96, wherein the float is formed from a rigid plastic material.

106. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a sample tube, the sample tube having an inner surface;

introducing a two-piece float into the sample tube, the two-piece float comprising:

a lower piece comprising (i) a main body portion having a top end and a bottom end, (ii) one or more support members protruding laterally from the main body portion and engaging the inner surface of the sample tube, wherein the main body portion and the one or more support members define an annular volume; (iii) a pitot tube extending axially from the top end of the main body portion; and (iv) an internal passage that passes through the main body portion and connects the pitot tube to an opening in the annular volume; and

an upper piece comprising (i) a passageway through which the pitot tube extends, and (ii) a member extending axially away from the main body portion;

wherein the upper piece has a lower density than the main body portion; and

wherein the main body portion has a density intermediate that of red blood cells and plasma;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and alignment of the annular volume with at least the buffy coat constituents; and

pushing the upper piece down to force fluid towards the annular volume and push the buffy coat constituents through the pitot tube.

107. The method of claim 106, wherein the upper piece passageway is located inside the member.

108. The method of claim 106, further comprising a one-way valve in the opening oriented to permit flow from the annular volume into the internal passage.

109. The method of claim 106, wherein the main body portion internal passage connects the pitot tube to a plurality of openings in the annular volume.

110. The method of claim 106, wherein the main body portion further comprises a pressure relief passage extending from the first end to the second end, the pressure relief passage not intersecting the internal passage.

111. The method of claim 106, wherein one support member is located on the bottom end of the main body portion, and the opening connecting to the annular volume is located proximally to the one support member.

112. The method of claim 106, wherein the float comprises one support member extending laterally from a bottom end of the main body portion and at least one helical support member located proximal to the top end of the main body portion.

113. The method of claim 106, wherein the main body portion has a specific gravity of from about 1.029 to about 1.09.

114. The method of claim 106, wherein the main body portion has a specific gravity of from about 1.08 to about 1.09.

115. The method of claim 106, wherein the float is formed from a rigid plastic material.

116. The method of claim 106, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

117. The method of claim 106, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

118. The method of claim 106, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

119. The method of claim 106, wherein the blood sample comprises anticoagulated whole blood.

120. The method of claim 106, wherein the sample tube is self-supporting.

121. The method of claim 106, further comprising combining the blood sample with a stain.

122. The method of claim 106, wherein the sample tube is seamless at least along the path of axial movement of the float.

123. The method of claim 106, wherein the flexible sample tube is formed of a flexible polymeric material.

124. The method of claim 123, wherein the flexible sample tube is semi-transparent.

125. The method of claim 123, wherein the flexible sample tube is transparent.

126. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a sample tube, the sample tube having an inner surface;

introducing a volume-occupying float into the sample tube, the float comprising:

a main body portion having a top end and a bottom end;

one or more support members protruding laterally from the main body portion and engaging the inner surface of the sample tube;

wherein the main body portion and the one or more support members define an annular volume; and

an internal passage that passes through the main body portion and connects the top end of the main body portion to an opening in the annular volume;

wherein the main body portion has a density intermediate that of red blood cells and plasma;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and alignment of the annular volume with at least the buffy coat constituents;

engaging a pitot tube with the internal passage at the top end of the main body portion;

threading an upper piece around the pitot tube, the upper piece engaging the inner surface of the sample tube, the upper piece comprising a hollow member that extends axially away from the main body portion and surrounds the pitot tube; and

pushing the upper piece down to force fluid towards the annular volume and push the buffy coat constituents through the pitot tube.

127. The method of claim 126, further comprising a one-way valve in the opening oriented to permit flow from the annular volume into the internal passage.

128. The method of claim 126, wherein the main body portion internal passage connects the pitot tube to a plurality of openings in the annular volume.

129. The method of claim 126, wherein the main body portion further comprises a pressure relief passage extending from the first end to the second end, the pressure relief passage not intersecting the internal passage.

130. The method of claim 126, wherein one support member is located on the bottom end of the main body portion, and the opening connecting to the annular volume is located proximally to the one support member.

131. The method of claim 126, wherein the float comprises one support member extending laterally from a bottom end of the main body portion and at least one helical support member located proximal to the top end of the main body portion.

132. A volume-occupying separator float, the float comprising:

a main body portion having a first end and a second end;

at least one pressure seal around the main body portion; and

a buffy coat passage extending from the second end to the first end; and having a centrifugation valve at the second end, the centrifugation valve being oriented to open during centrifugation.

133. The float of claim 132, having a first pressure seal around the first end of the main body portion and a second pressure seal around the second end of the main body portion.

134. The float of claim 132, further comprising a pressure relief passage extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value.

135. The float of claim 132, wherein the float has a specific gravity of from about 1.029 to about 1.09.

136. The float of claim 132, wherein the float has a specific gravity of from about 1.040 to about 1.070.

137. The float of claim 132, wherein the float has a specific gravity of from about 1.08 to about 1.09.

138. The float of claim 132, wherein the float is formed from a rigid plastic material.

139. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a flexible sample tube, the sample tube having an inner surface;

introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma;

said float comprising:

a main body portion having a first end and a second end;

at least one pressure seal around the main body portion and engaging the inner surface; and

a buffy coat passage extending from the second end to the first end and having a centrifugation valve at the second end, the centrifugation valve being oriented to open during centrifugation;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and capture of the buffy coat constituents in the buffy coat passage of the float, wherein the first pressure seal and the second pressure seal substantially prevent the blood sample from traveling between the float and the inner surface of the sample tube;

reducing the rotational speed to cause the inner surface to capture the float; and

removing the float from the sample tube.

140. The method of claim 139, wherein the float has a first pressure seal around the first end of the main body portion and a second pressure seal around the second end of the main body portion.

141. The method of claim 139, wherein the float further comprises a pressure relief passage extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value.

142. The method of claim 139, wherein the float has a specific gravity of from about 1.029 to about 1.09.

143. The method of claim 139, wherein the float has a specific gravity of from about 1.040 to about 1.070.

144. The method of claim 139, wherein the float has a specific gravity of from about 1.08 to about 1.09.

145. The method of claim 139, wherein the float is formed from a rigid plastic material.

146. The method of claim 139, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

147. The method of claim 139, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

148. The method of claim 139, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

149. The method of claim 139, wherein the blood sample comprises anticoagulated whole blood.

150. The method of claim 139, wherein the sample tube is self-supporting.

151. The method of claim 139, further comprising combining the blood sample with a stain.

152. The method of claim 139, wherein the sample tube is seamless at least along the path of axial movement of the float.

153. The method of claim 139, wherein the flexible sample tube is formed of a flexible polymeric material.

154. The method of claim 153, wherein the flexible sample tube is semi-transparent.

155. The method of claim 153, wherein the flexible sample tube is transparent.

156. A volume-occupying separator float, the float comprising:

a main body portion having a top end and a bottom end; and

at least one centrifugation valve circumferentially disposed about the main body portion;

wherein the centrifugation valve is configured to open during centrifugation.

157. The float of claim 156, wherein the at least one centrifugation valve is located at the top end of the main body portion.

158. The float of claim 156, wherein the at least one centrifugation valve is located at the bottom end of the main body portion.

159. The float of claim 156, having two centrifugation valves, wherein one centrifugation valve is located at the top end of the main body portion, and the other centrifugation valve is located at the bottom end of the main body portion.

160. The float of claim 156, wherein the float has a specific gravity of from about 1.029 to about 1.09.

161. The float of claim 156, wherein the main body portion is formed from a rigid plastic material.

162. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a flexible sample tube, the sample tube having an inner surface;

introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma;

said float comprising:

a main body portion having a top end and a bottom end; and

a centrifugation valve circumferentially disposed about the main body portion, wherein the centrifugation valve is configured to open during centrifugation;

centrifuging the sample tube at a rotational speed that causes

enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers and to open the centrifugation valve, and capture of the buffy coat constituents in the buffy coat passage of the float;

reducing the rotational speed to cause the inner surface to capture the float, wherein the centrifugation valve closes when the rotational speed is reduced; and

removing the float from the sample tube.

163. The method of claim 162, wherein the at least one centrifugation valve is located at the top end of the main body portion.

164. The method of claim 162, wherein the at least one centrifugation valve is located at the bottom end of the main body portion.

165. The method of claim 162, having two centrifugation valves, wherein one centrifugation valve is located at the top end of the main body portion, and the other centrifugation valve is located at the bottom end of the main body portion.

166. The method of claim 162, wherein the float has a specific gravity of from about 1.029 to about 1.09.

167. The method of claim 162, wherein the float is formed from a rigid plastic material.

168. The method of claim 162, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

169. The method of claim 162, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

170. The method of claim 162, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

171. The method of claim 162, wherein the blood sample comprises anticoagulated whole blood.

172. The method of claim 162, wherein the sample tube is self-supporting.

173. The method of claim 162, further comprising combining the blood sample with a stain.

174. The method of claim 162, wherein the sample tube is seamless at least along the path of axial movement of the float.

175. The method of claim 162, wherein the flexible sample tube is formed of a flexible polymeric material.

176. The method of claim 175, wherein the flexible sample tube is semi-transparent.

177. The method of claim 175, wherein the flexible sample tube is transparent.

178. A volume-occupying separator float, the float comprising:

a main body portion having a first end and a second end;

at least one pressure seal around the main body portion; and

a pressure relief passage extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value.

179. The float of claim 178, having a first pressure seal around the first end of the main body portion and a second pressure seal around the second end of the main body portion.

180. The float of claim 178, wherein the float has a specific gravity of from about 1.029 to about 1.09.

181. The float of claim 178, wherein the float has a specific gravity of from about 1.040 to about 1.070.

182. The float of claim 178, wherein the float has a specific gravity of from about 1.08 to about 1.09.

183. The float of claim 178, wherein the float is formed from a rigid plastic material.

184. A method of capturing buffy coat constituents in a blood sample, comprising:

introducing the blood sample into a flexible sample tube, the sample tube having an inner surface;

introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma;

said float comprising:

a main body portion having a first end and a second end;

at least one pressure seal around the main body portion and engaging the inner surface; and

a pressure relief passage extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value;

centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and capture of the buffy coat constituents in the buffy coat passage of the float, wherein the first pressure seal and the second pressure seal substantially prevent the blood sample from traveling between the float and the inner surface of the sample tube;

reducing the rotational speed to cause the inner surface to capture the float; and

removing the float from the sample tube.

185. The method of claim 184, wherein the float has a first pressure seal around the first end of the main body portion and a second pressure seal around the second end of the main body portion.

186. The method of claim 184, wherein the float has a specific gravity of from about 1.029 to about 1.09.

187. The method of claim 184, wherein the float has a specific gravity of from about 1.040 to about 1.070.

188. The method of claim 184, wherein the float has a specific gravity of from about 1.08 to about 1.09.

189. The method of claim 184, wherein the float is formed from a rigid plastic material.

190. The method of claim 184, wherein the float is introduced into the sample tube before the blood sample is introduced therein.

191. The method of claim 184, wherein the blood sample is introduced into the sample tube before the float is introduced therein.

192. The method of claim 184, wherein the sample tube is sized to receive a blood sample of approximately ten milliliters in volume.

193. The method of claim 184, wherein the blood sample comprises anticoagulated whole blood.

194. The method of claim 184, wherein the sample tube is self-supporting.

195. The method of claim 184, further comprising combining the blood sample with a stain.

196. The method of claim 184, wherein the sample tube is seamless at least along the path of axial movement of the float.

197. The method of claim 184, wherein the flexible sample tube is formed of a flexible polymeric material.

198. The method of claim 197, wherein the flexible sample tube is semi-transparent.

199. The method of claim 197, wherein the flexible sample tube is transparent.

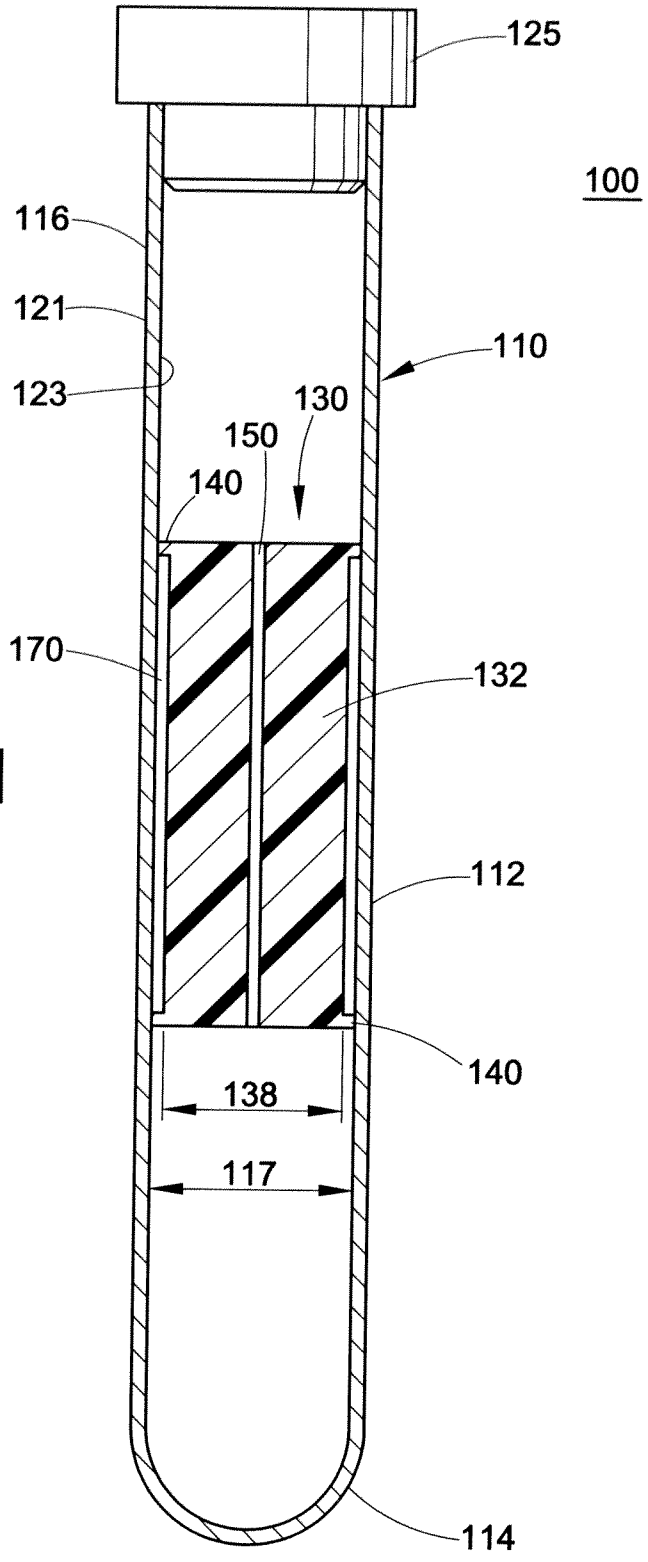

FIG. 1

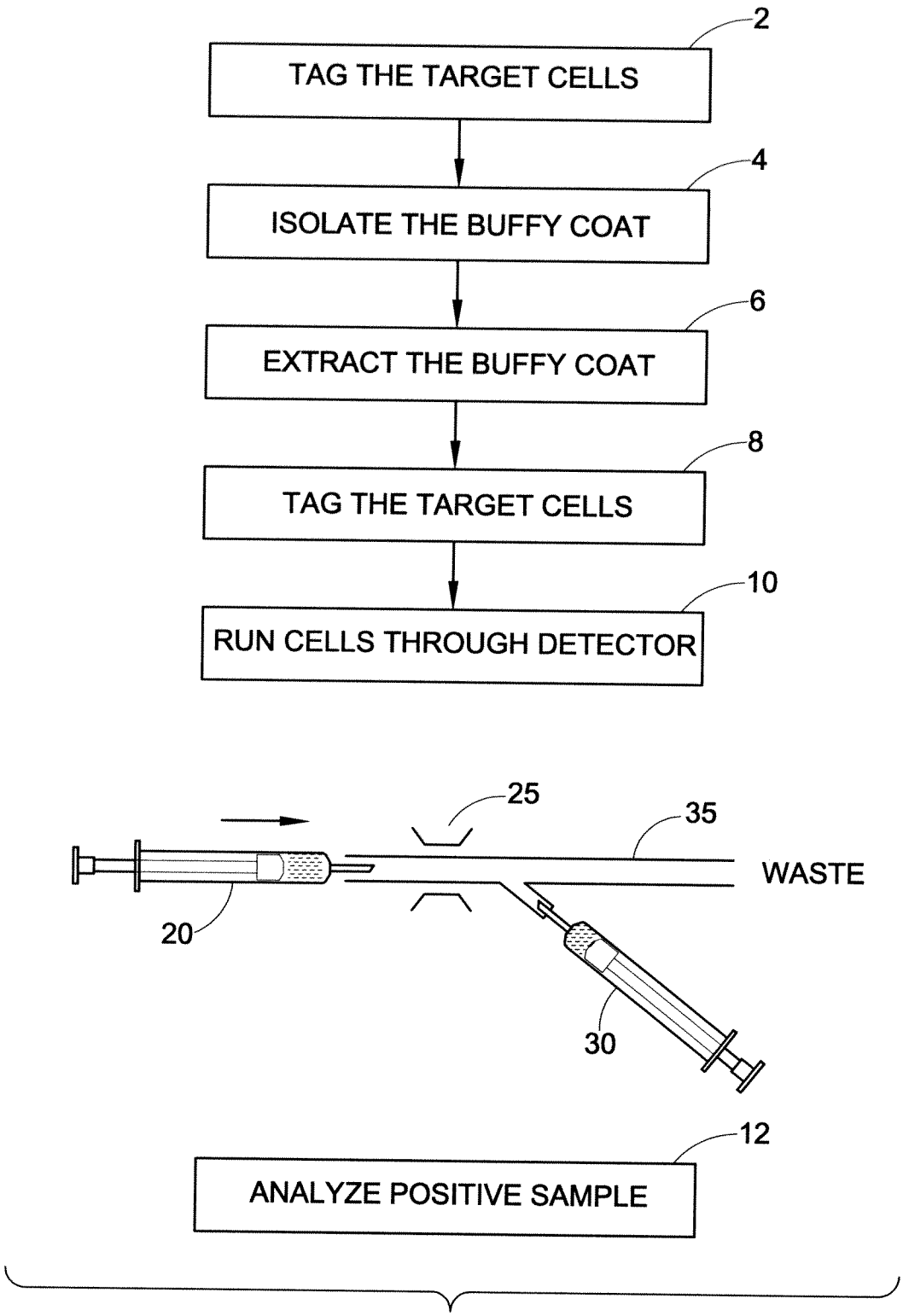
2
TAG THE TARGET CELLS
4
ISOLATE THE BUFFY COAT
6
EXTRACT THE BUFFY COAT
8
TAG THE TARGET CELLS
10
RUN CELLS THROUGH DETECTOR
25
35
WASTE
20
30
12
ANALYZE POSITIVE SAMPLE

FIG. 2

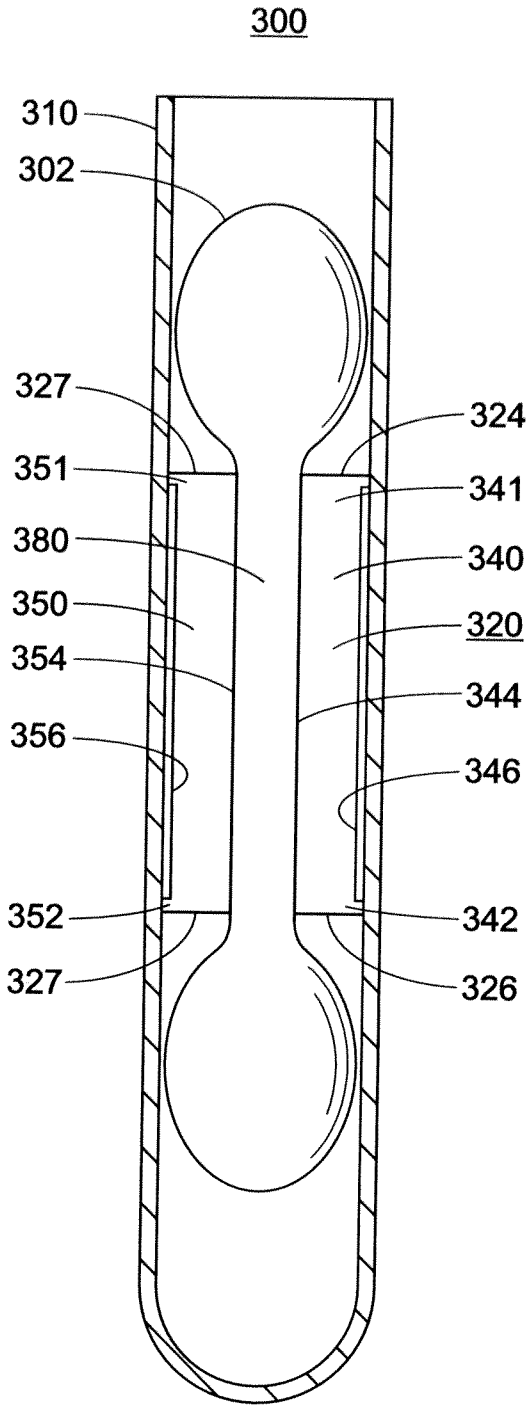
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FIG. 3A

FIG. 3B

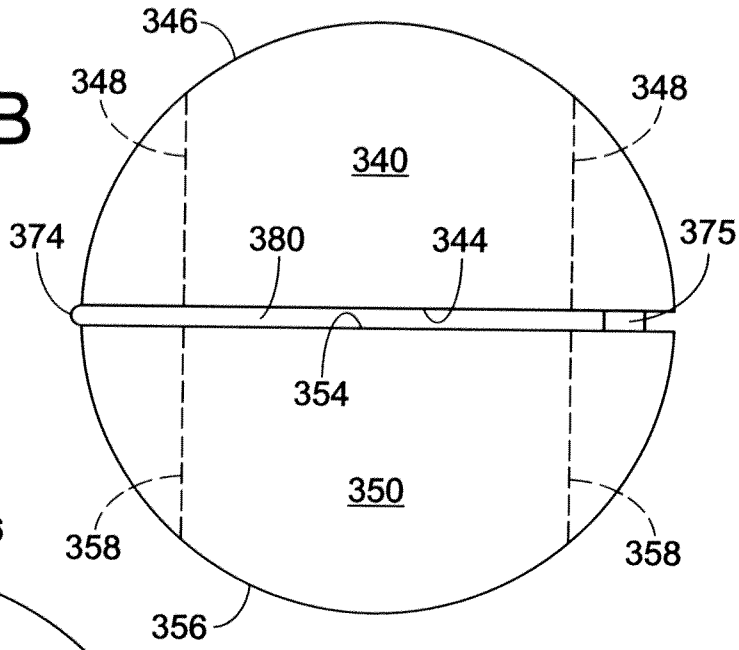
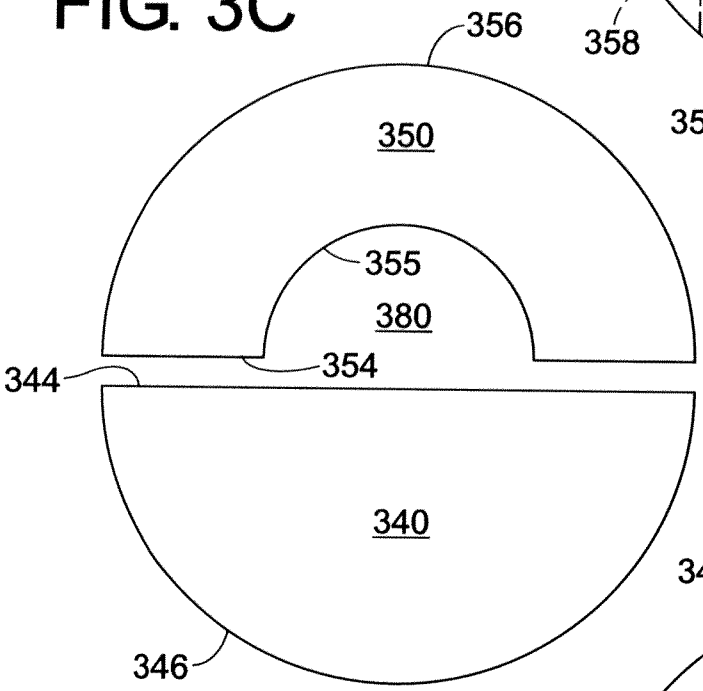


FIG. 3C



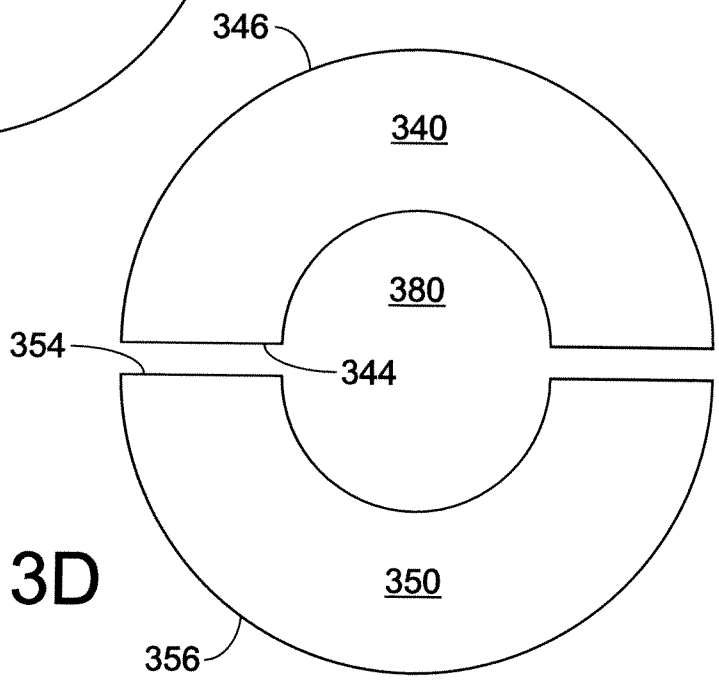


FIG. 3D

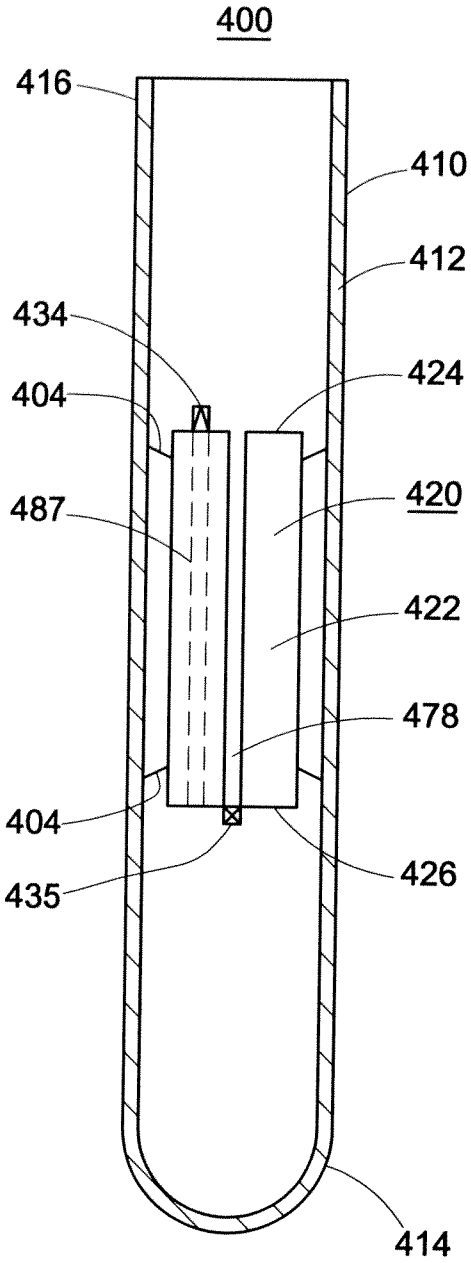

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FIG. 4A

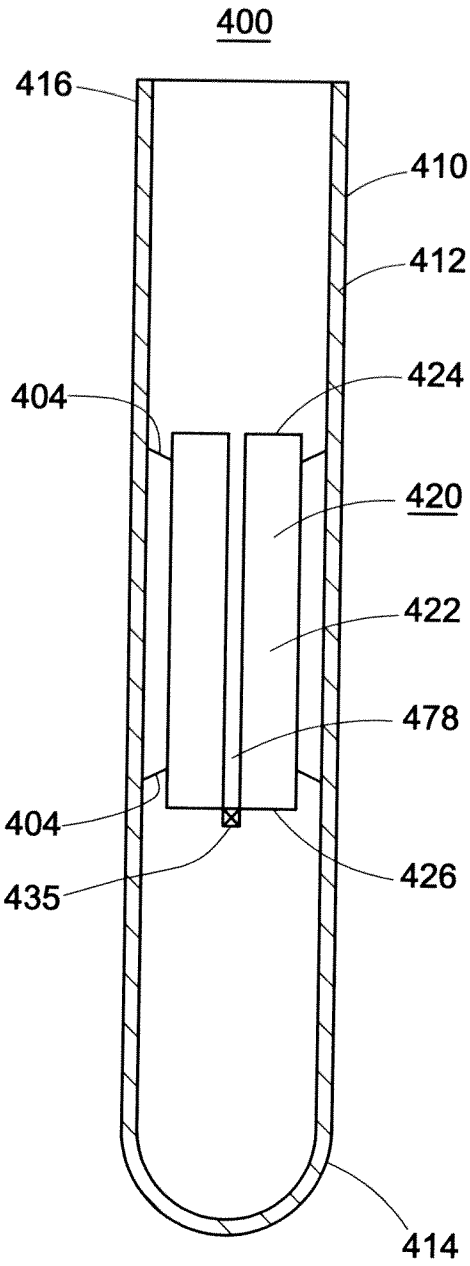
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FIG. 4B

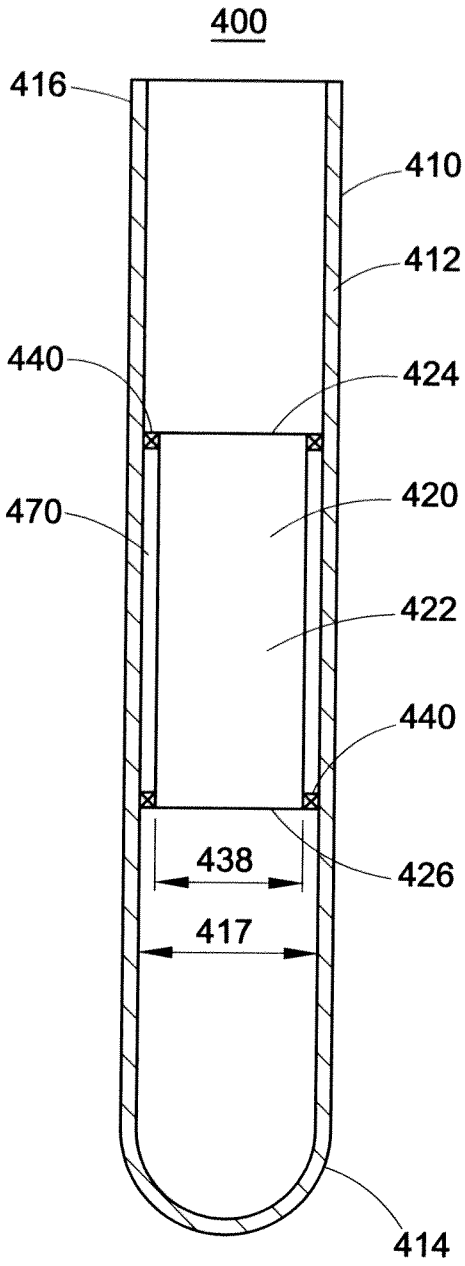

FIG. 4C

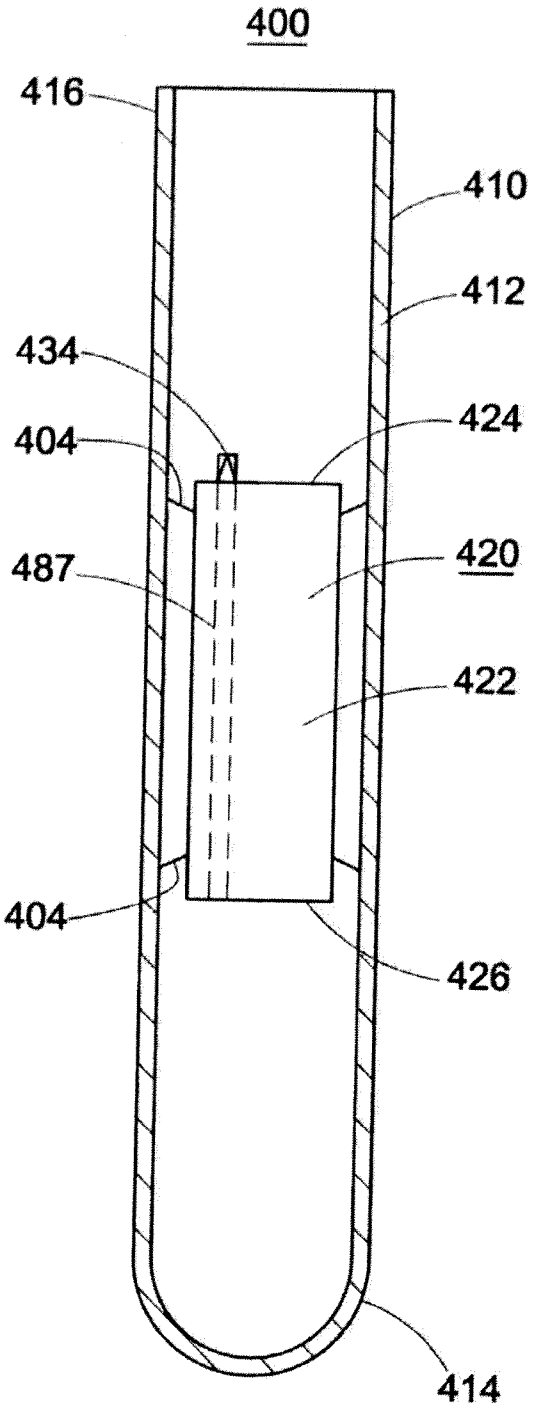

FIG. 4D

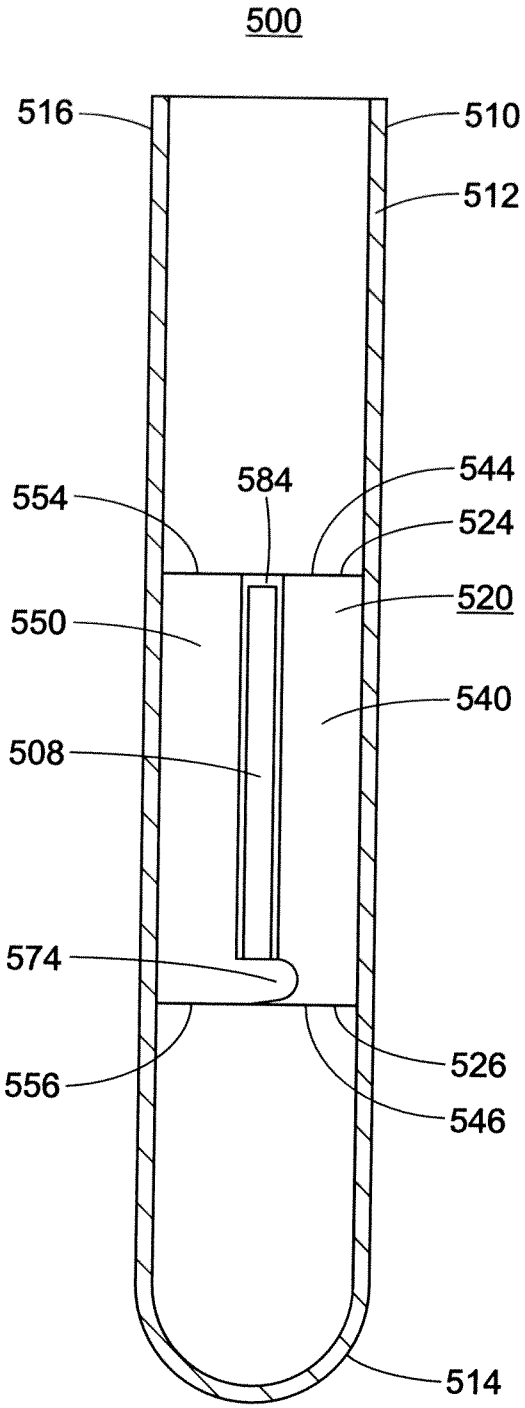
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FIG. 5A

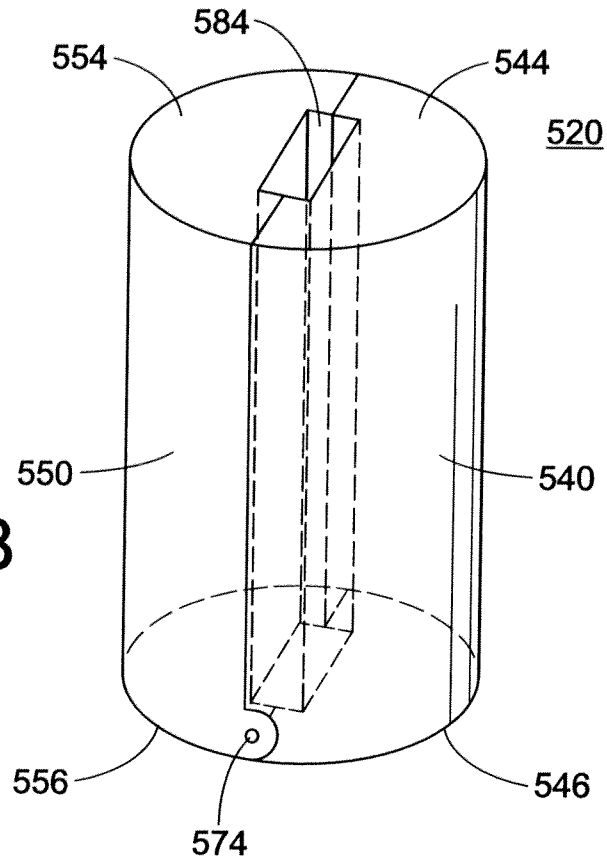

FIG. 5B

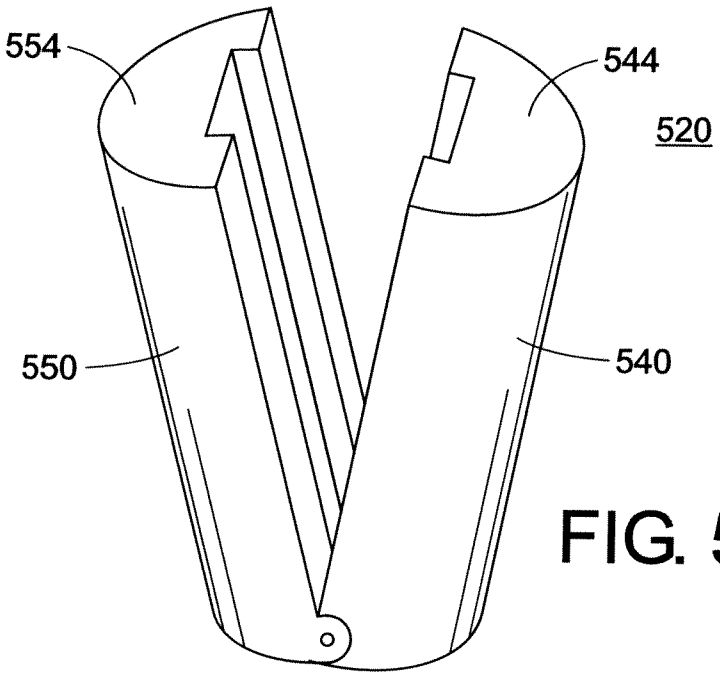

FIG. 5C

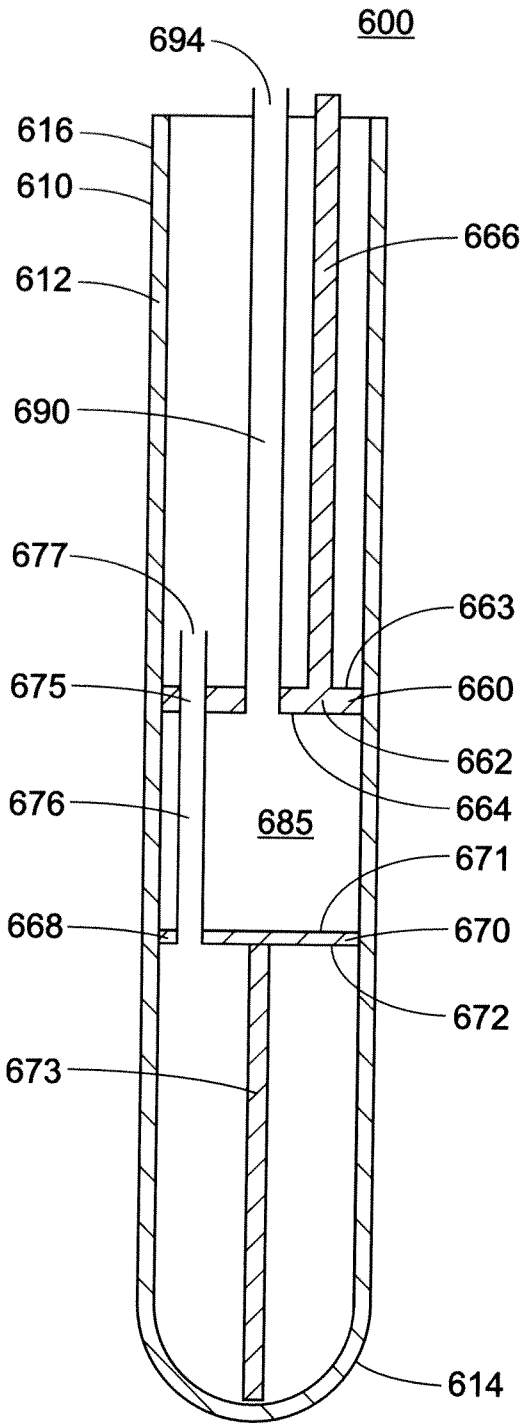
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FIG. 6

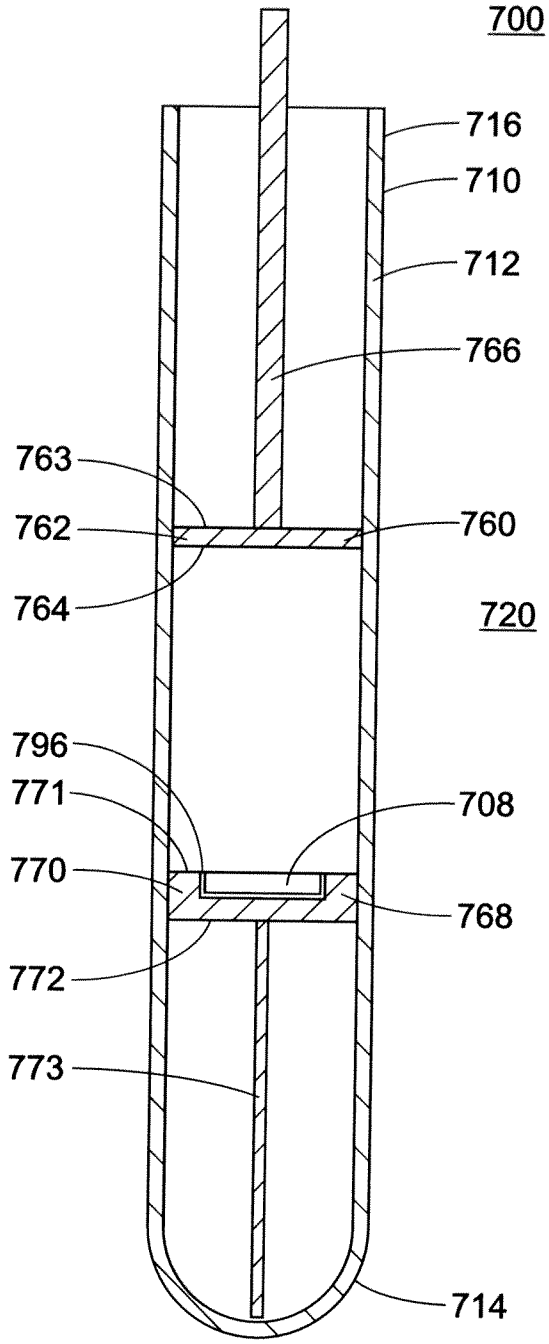
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FIG. 7A

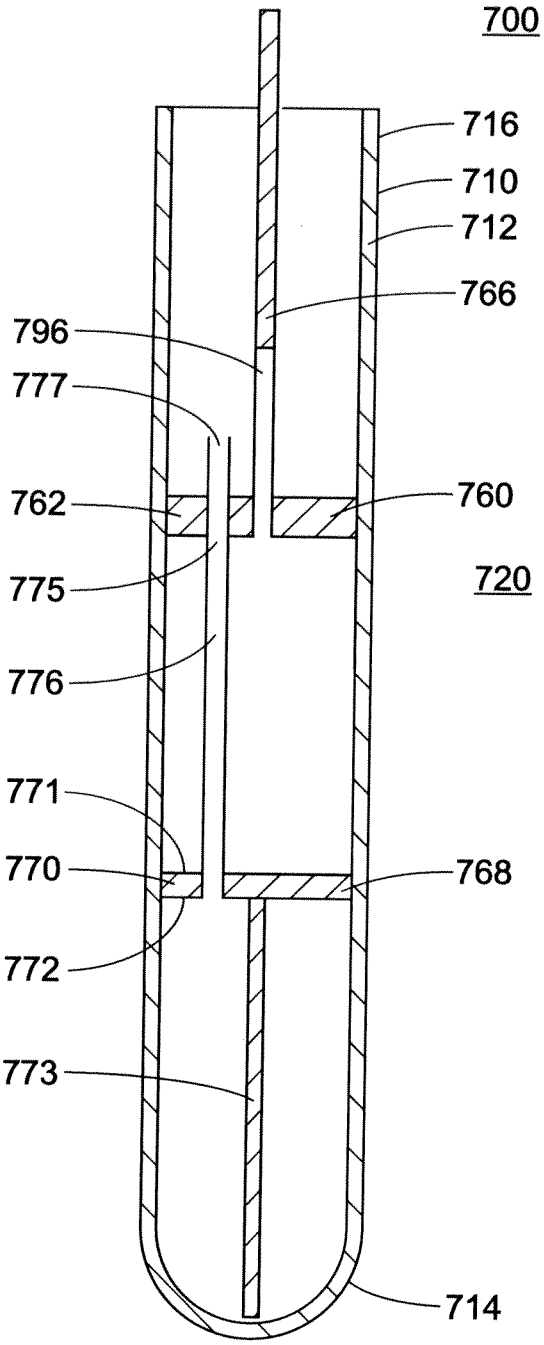
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FIG. 7B

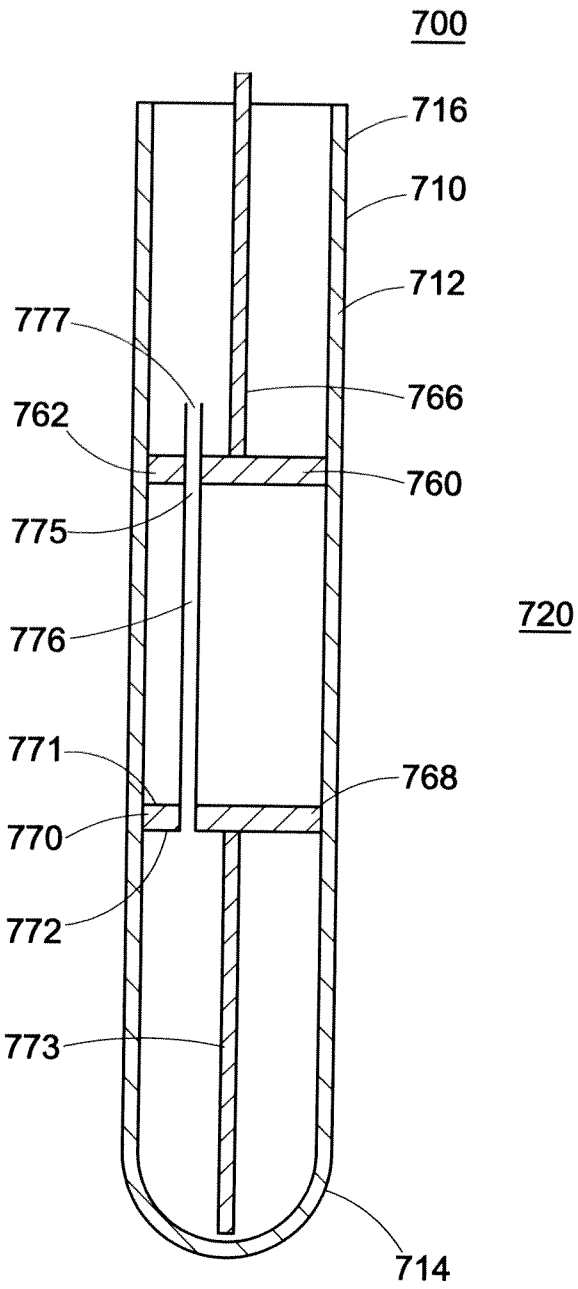
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FIG. 7C

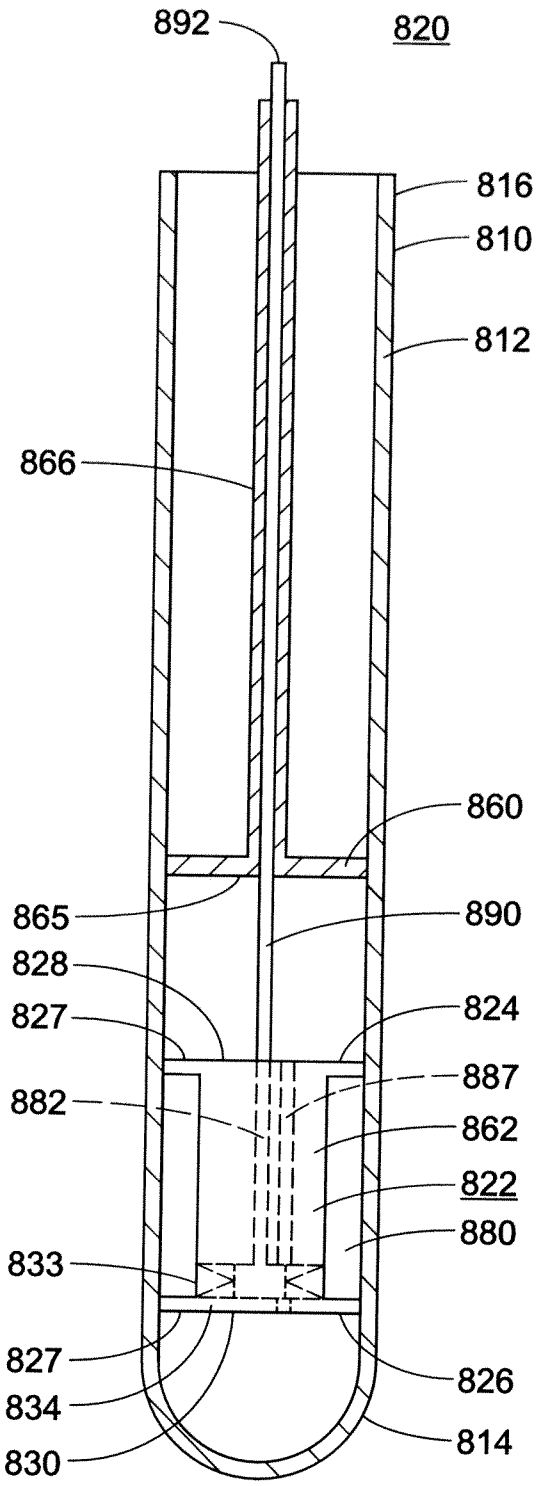
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FIG. 8

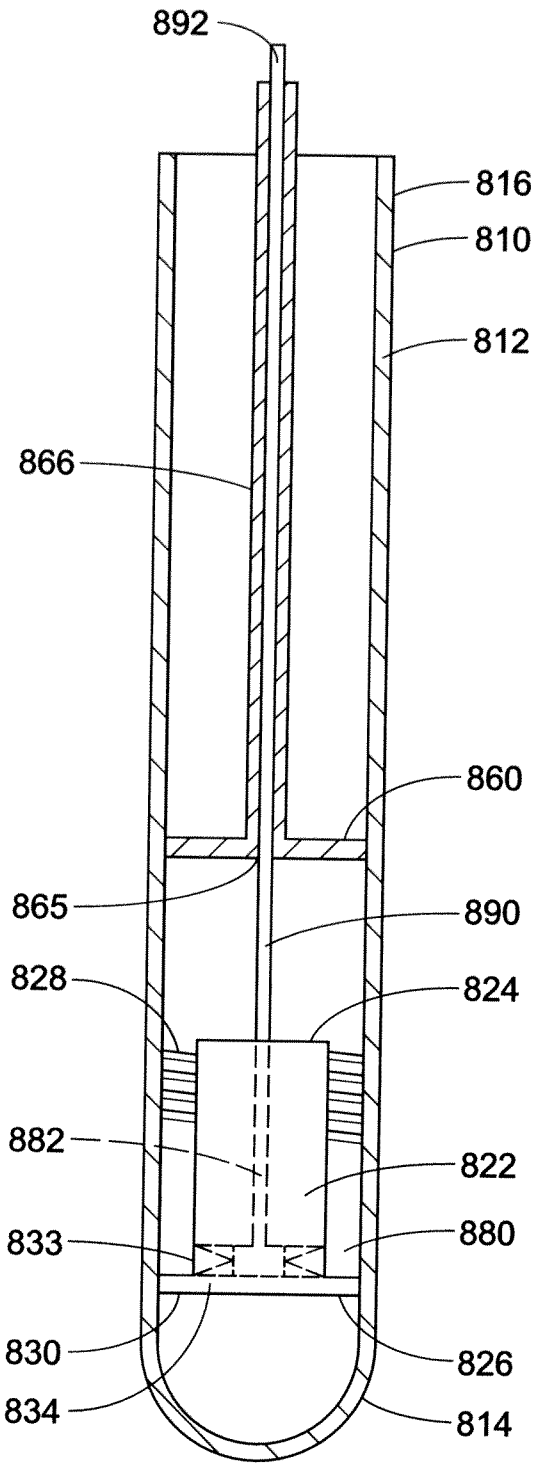
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FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/30417

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 1/18; G01N 33/48; C12M 1/34 (2011.01)
USPC - 436/177 ; 210/782; 422/548; 422/72; 422/73; 435/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8)- G01N 1/18; G01N 33/48; C12M 1/34 (2011.01);
USPC- 436/177 ; 210/782; 422/548; 422/72; 422/73; 435/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Pat, PgPub, EPO, JPO), GoogleScholar (PL, NPL), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL);
Search terms used: volume, separator, float, cylinder, first, second, end, portion, piece, part, connect, join, fit, snap, screw, affix, mate, profile, shape, rectangular, circular, annular, clip

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0131868 A1 (HAUBERT et al.) 05 June 2008 (05.06.2008), Figs. 5-12; para [0007], [0031], [0036], [0038], [0043]-[0046], [0056], [0062], [0068], [0069]	1-19
Y	US 2004/0040599 A1 (PAYNE) 04 March 2004 (04.03.2004), para [0009]-[0013]	3-6
Y	US 2006/0207323 A1 (RAUCHHAUS) 21 September 2006 (21.09.2006), Fig. 3; para [0016], [0025], [0027], [0030]	17-19
Y	US 2008/0087209 A1 (YOSHIDA et al.) 17 April 2008 (17.04.2008), para [0086]	1-19
Y	US 5,333,499 A (GASTON) 02 August 1994 (02.08.1994), Figs. 2-5; col 2-3	1-19

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 August 2011 (02.08.2011)	12 AUG 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/30417

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-19

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/30417

Continuation of Box No. III – Observations where unity of invention is lacking:

The following claim groups were found:
Group I: Claims 1-19
Group II: Claims 20-44
Group III: Claims 45-66, 96-131
Group IV: Claims 67-95
Group V: Claims 132-138
Group VI: Claims 139-155
Group VII: Claims 156-161
Group VIII: Claims162-177
Group IX: Claims 178-183
Group X: Claims 184-199

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: is drawn to a volume-occupying separator float, the float comprising: a first piece and a second piece, each piece having a first end, a second end, an exterior surface, and an interior surface; wherein the interior surfaces of the first and second pieces cooperate to form an open passage extending between the first end and the second end; and wherein the first and second pieces can be connected together (Fig 5; para [0056]; claim 2).

Group II: is drawn to a method of capturing buffy coat constituents in a blood sample, comprising: introducing a blood sample into a flexible bag; placing a float around the flexible bag, the float having a first end and a second end and the float having a specific gravity intermediate that of plasma and red blood cells; placing the flexible bag and float in a flexible sample tube, the sample tube having a sidewall, wherein the float engages the sidewall; centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and movement of the float into alignment with at least the buffy coat constituents; reducing the rotational speed to cause the sidewall to capture the float; and sealing the flexible bag at the first end and the second end of the float to capture the buffy coat constituents. (para [0033]; claim 17 - enlarging sidewall)

Group III: is drawn to a two-piece float for capturing buffy coat constituents of a blood sample, comprising: a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower support member having an upper surface and a lower surface, and (ii) a pitot tube extending axially from the lower surface of the top float through the upper surface and having a top end located distally from the upper surface; and a bottom float having a density intermediate that of the buffy coat constituents and red blood cells; wherein the top float lower support member lower surface and the bottom float are complementarily shaped.

Group IV: is drawn to a two-piece float for capturing buffy coat constituents of a blood sample, comprising: a top float having a density intermediate that of plasma and the buffy coat constituents, wherein the top float comprises (i) a lower lateral support member having an upper surface and a lower surface, and (ii) a manipulator extending axially from the upper surface of the lower lateral support member; and a bottom float having a density intermediate that of the buffy coat constituents and red blood cells, wherein the bottom float comprises an upper lateral support member having an upper surface and a lower surface.

Group V: is drawn to a volume-occupying separator float, the float comprising: a main body portion having a first end and a second end; at least one pressure seal around the main body portion; and a buffy coat passage extending from the second end to the first end; and having a centrifugation valve at the second end, the centrifugation valve being oriented to open during centrifugation (Ayres col 3, ln 29-48)

Group VI: is drawn to a method of capturing buffy coat constituents in a blood sample, comprising: introducing the blood sample into a flexible sample tube, the sample tube having an inner surface; introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma; said float comprising: a main body portion having a first end and a second end; at least one pressure seal around the main body portion and engaging the inner surface; and a buffy coat passage extending from the second end to the first end and having a centrifugation valve at the second end, the centrifugation valve being oriented to open during centrifugation; centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and capture of the buffy coat constituents in the buffy coat passage of the float, wherein the first pressure seal and the second pressure seal substantially prevent the blood sample from traveling between the float and the inner surface of the sample tube; reducing the rotational speed to cause the inner surface to capture the float; and removing the float from the sample tube.

Group VII , is drawn to a volume-occupying separator float, the float comprising: a main body portion having a top end and a bottom end; and at least one centrifugation valve circumferentially disposed about the main body portion; wherein the centrifugation valve is configured to open during centrifugation.

Group VIII , is drawn to a method of capturing buffy coat constituents in a blood sample, comprising: introducing the blood sample into a flexible sample tube, the sample tube having an inner surface; introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma; said float comprising: a main body portion having a top end and a bottom end; and a centrifugation valve circumferentially disposed about the main body portion, wherein the centrifugation valve is configured to open during centrifugation; centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers and to open the centrifugation valve, and capture of the buffy coat constituents in the buffy coat passage of the float; reducing the rotational speed to cause the inner surface to capture the float, wherein the centrifugation valve closes when the rotational speed is reduced; and removing the float from the sample tube.

continued on following Extra Sheet

continued from previous Extra Sheet:

Group IX: is drawn to a volume-occupying separator float, the float comprising: a main body portion having a first end and a second end; at least one pressure seal around the main body portion; and a pressure relief passage (pressure relief bore extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value.

Group X: is drawn to a method of capturing buffy coat constituents in a blood sample, comprising:
introducing the blood sample into a flexible sample tube, the sample tube having an inner surface;
introducing a volume-occupying float into the sample tube, said float having a specific gravity intermediate that of red blood cells and plasma; said float comprising: a main body portion having a first end and a second end; at least one pressure seal around the main body portion and engaging the inner surface; and a pressure relief passage extending from the second end to the first end and having a one-way pressure relief valve oriented to open when pressure at the second end exceeds pressure at the first end by a first value;
centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, separation of the blood into discrete layers, and capture of the buffy coat constituents in the buffy coat passage of the float, wherein the first pressure seal and the second pressure seal substantially prevent the blood sample from traveling between the float and the inner surface of the sample tube;
reducing the rotational speed to cause the inner surface to capture the float; and removing the float from the sample tube.

The inventions listed as Groups I-X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I and II lack unity of invention, because even though the inventions of these groups require the technical feature of a float comprising: a first piece and a second piece, each piece having a first end, a second end, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of US 2008/0131868 A1 to Haubert et al. (hereinafter Haubert), which discloses a float comprising: a first piece and a second piece, each piece having a first end, a second end (Fig 5; para [0056]; claim 2).

Groups II, VI, VIII, and X lack unity of invention, because even though the inventions of these groups require the technical features of a flexible sample tube, the float having a specific gravity intermediate that of plasma and red blood cells, centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float, and reducing the rotational speed to cause the sidewall to capture the float, these technical features are not special technical features as they do not make a contribution over the prior art in view of Haubert, which discloses treating a flexible sample tube (para [0007], the float having a specific gravity intermediate that of plasma and red blood cells (para [0007], [0046], [0057], [0061]), centrifuging the sample tube at a rotational speed that causes enlargement of the sidewall to a diameter sufficiently large to permit axial movement of the float (para [0052]; claim 17), and reducing the rotational speed to cause the sidewall to capture the float (para [0008]; claim 17).

Groups III and IV lack unity of invention, because even though the inventions of these groups require the technical feature of a lower support member having an upper surface and a lower surface 3, 4 note group 4 is drawn to a lateral support member, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of Haubert, which discloses a lower support member having an upper surface and a lower surface (para [0009]).

Groups V, VI, IX, and X lack unity of invention, because even though the inventions of these groups require the technical feature of at least one pressure seal around the main body portion, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of Haubert, which discloses at least one pressure seal around the main body portion (para [0036], Fig 3, item 320; Fig 1 - sealing rings 114).

Groups V-VIII lack unity of invention, because even though the inventions of these groups require the technical feature of a buffy coat passage extending from the second end to the first end; and having a centrifugation valve at the second end, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of US 3,931,010 A to Ayres et al., which discloses a buffy coat passage extending from the second end to the first end; and having a centrifugation valve at the second end (col 3, ln 38-48; col 2, ln 43-45; Fig 4). It would have been obvious to one of skill in the art to use the valve, as disclosed by Ayres, in the blood separation system, as disclosed by Haubert, to maintain the separate phases after rotation of the centrifuge is terminated.).

Groups IX and X lack unity of invention, because even though the inventions of these groups require the technical feature of a pressure relief passage, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of Haubert, which discloses a pressure relief passage (abstract; para [0055], [0010] - pressure relief bore).

Groups VI, VIII, and IX lack unity of invention, because even though the inventions of these groups require the technical feature of removing the float, this technical feature is not a special technical feature as it would have been obvious to one of skill in the art to remove the float after use for cleaning and preventative maintenance.

Groups I-X therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.