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(54) BUFFY COAT TUBE AND FLOAT SYSTEM AND METHOD

BUFFY COAT RÖHRCHEN MIT SCHWIMMER VORRICHTUNG UND VERFAHREN

TUBE POUR COUCHE LEUCO-PLAQUETTAIRE ET PROCEDE ET SYSTEME A FLOTTEUR

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Description

FIELD OF THE INVENTION

[0001] The present invention relates generally to density-based fluid separation and, in particular, to an improved sample tube and float design for the separation and axial expansion of constituent fluid components layered by centrifugation, and a method employing the same. The present invention finds particular application in blood separation and axial expansion of the buffy coat layers, and will be described with particular reference thereto. However, it will be recognized that the present invention is also amenable to other like applications.

BACKGROUND OF THE INVENTION

[0002] 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 white cells that form between the layer of red cells and the plasma when unclotted blood is centrifuged or allowed to stand.

[0003] 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.

[0004] 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, New Jersey.

[0005] 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.

[0006] 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 employer with the conventional QBC capillary tubes and floats.

[0007] US 4,154,690 and EP-A-1005910 each disclose a device for use in blood centrifuging to separate plasma and red blood cells which uses a rigid axially travelling separator element in a flexibly-walled centrifuge tube.

[0008] The present invention contemplates a new and improved blood separation assembly and method that overcome the above-referenced problems and others.

SUMMARY OF THE INVENTION

[0009] In a first aspect of the invention there is provided, a method in accordance with claim 20 of separating and axially expanding the buffy coat constituents in a blood sample includes introducing the blood sample into a flexible sample tube having an elongate sidewall of a first cross-sectional inner diameter. An elongate rigid volume- occupying float is also inserted into, or is present in, the flexible sample tube.

[0010] The float has a specific gravity intermediate that of red blood cells and plasma. It includes a main body portion and one or more support members protruding from the main body portion of the float to engage and support the sidewall of the sample tube. The main body portion and the support members have a cross-sectional diameter less than the first inner diameter of the tube when the sample tube is subsequently expanded, such as by centrifugation.

[0011] The main body portion of the float, together with an axially aligned portion of the sidewall of the sample tube, defines an annular volume therebetween. The support members protruding from the main body portion of the float traverse the annular volume to engage and support the sidewall of the tube thereby producing one or more analysis areas.

[0012] The sample tube containing the blood sample and float is then centrifuged to effect a density-based separation of the blood sample into discrete layers at a rotational speed that causes a resilient expansion or enlargement of the diameter of the sidewall to a second diameter in response to pressure in the blood caused by the centrifugal force, which diameter expansion is sufficiently large to permit axial movement of the float in the tube. During centrifugation, the float is moved into axial alignment with at least the buffy coat layers of the blood sample due to the density of the float. After centrifugation, the rotational speed is reduced and the tube sidewall returns to essentially its first diameter and engages the float. As a result, the buffy coat constituents are trapped in the analysis areas for review, measurement and/or detection by conventional methods.

[0013] In a further aspect of the invention there is provided, an apparatus in accordance with claim 1 for separation and analysis of a target analyte in a sample of anticoagulated whole blood is produced. The apparatus includes a transparent, or semi-transparent, flexible tube for holding the sample, the tube having an elongate sidewall of a first cross-sectional inner diameter. The apparatus further includes an elongate, rigid, volume-occupying float having a specific gravity which is intermediate that of red blood cells and plasma.

[0014] The float includes a main body portion having one or more support members protruding from the main body portion. The cross-sectional diameter of the main body portion and/or the support members of the float are less than the first cross-sectional inner diameter of the tube when the sample tube is subsequently expanded. In this regard, the sidewall is resiliently radially expandable to a second diameter in response to pressure or force. The second diameter is sufficiently large to permit axial movement of the float in the tube during centrifugation.

[0015] The main body portion of the float, together with an axially aligned portion of the sidewall, defines an annular volume therebetween. The protrusions of the float traverse the annular volume and engage and support the sidewall, forming the analysis area subsequent to centrifugation.

[0016] In a still further aspect there is provided a method for detecting circulating target cells, such as epithelial cancer cells, stem cells, cell fragments, virally-infected cells, trypanosomes, etc., in an anticoagulated whole blood sample is provided. This method includes combining the blood sample with one or more target cell epitope-specific-labeling agents so as to differentiate the target cells from other cells in the blood sample. The blood sample and a volume-occupying separator float are placed into a transparent, or semi-transparent, flexible sample tube. The separator float has a specifically defined specific gravity. It comprises a rigid main body portion and tube support members. The separator float in conjunction with the sidewalls produces one or more areas of analysis. Additionally, the float has a cross-sectional diameter less than an inner diameter of the sample tube when the sample tube is expanded. The blood sample and separator float are centrifuged in the sample tube to effect centrifugally motivated localization of any target cells present in the blood sample to the areas of analysis. The blood sample present in the analysis areas is then examined to identify whether any target cells are present.

[0017] One advantage of the present invention is found in a blood separating apparatus that can separate the entire buffy coat of a relatively large blood sample from the rest of the blood volume.

[0018] Another advantage of the invention resides in the fact that the buffy coat layers can be made available for visualization or imaging in one simple operation, i.e, the application of pressure and/or centrifugation.

[0019] Still another advantage of the invention resides in enhanced puffy coat separation, retention, and, if desired, removal from the sample tube for further processing.

[0020] Yet another advantage of the invention is found in that the tolerance precision between the float and tube is decreased over that necessary for the prior art QBC-type systems, thus reducing the necessary cost of the components.

[0021] Still another advantage is found in that the tube can be supported for improved imaging of the sample, and a more repeatable depth for imaging may be provided.

[0022] Still further advantages of the present invention reside in its relatively simple construction, ease of manufacture, and low cost.

[0023] Still further advantages and benefits of the present invention will become apparent to those of ordinary skill in the art upon reading and understanding the following detailed description of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The invention may take form in various components and arrangements of components, and in various steps and arrangements of steps. The drawings, in which like reference numerals denote like components throughout the several views, are only for purposes of illustrating various embodiments of the invention and are not to be construed as limiting the invention.

FIGURE 1 is a sectional view of a sample tube containing a generally spool-shaped separator float according to an exemplary embodiment of the invention.

FIGURE 2 is an elevational view of a separator float having generally conical ends according to another exemplary embodiment of the invention.

FIGURE 3 is an elevational view of a separator float having generally frustoconical ends according to another exemplary embodiment of the invention.

FIGURE 4 is an elevational view of a separator float according to yet another exemplary embodiment, wherein the ends are generally convex or dome shaped.

FIGURE 5 is an elevational view of a separator float according to still another exemplary embodiment having sealing ridges offset from the ends.

FIGURES 6-8 are elevational views of ribbed separator floats according to further exemplary embodiments of the invention.

FIGURE 9 is an elevational view of a separator float according to another exemplary embodiment of the present invention having generally helical tube support ridges.

FIGURE 10 is an elevational view of a separator float according to a further embodiment of the invention having support ribs, which are tapered in the radial direction.

FIGURE 11 is an elevational view of a separator float according to yet another exemplary embodiment of the present invention having generally tapered, helical tube support ridges.

FIGURE 12 is an elevational view of a separator float according to another embodiment of the invention having support ribs, which are rounded in cross-sectional shape.

FIGURE 13 is an elevational view of a separator float according to another embodiment of the invention having helical support ridges, which are rounded in cross-sectional shape.

FIGURE 14 is an elevational view of a splined separator float according to another exemplary embodiment of the invention.

FIGURE 15 is an enlarged cross-sectional view taken along the lines 15--15 shown in FIGURE 14.

FIGURE 16 is an elevational view of a further splined separator float embodiment of the invention.

FIGURES 17 and 18 are elevational views of additional splined float embodiments in accordance the invention.

FIGURE 19 is a perspective view of yet another splined float embodiment of the present invention.

FIGURE 20 is a perspective view of a float of still another exemplary embodiment wherein the support ridges include intersecting annular ribs and splines.

FIGURES 21-26 are elevational views of knobbed or studded separator floats having generally rounded protrusions in various configurations, in accordance with further exemplary embodiments of the present invention.

FIGURES 27 and 28 are elevational views of spiked or studded separator floats having facet-like protrusions according to additional exemplary embodiments of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0025] Turning now to the drawings, wherein the showings are for purposes of illustrating the preferred embodiments of the invention only and not for limiting the same, FIGURE 1 shows a blood separation tube and float assembly **100,** including a sample tube **130** having a separator float or bobber **110 of** the invention therein.

[0026] The sample tube **130** is generally cylindrical in the depicted embodiment, although tubes having polygonal and other geometrical cross-sectional shapes are also contemplated. The sample tube **130** includes a first, closed end **132** and a second, open end **134** receiving a stopper or cap **140.** Other closure means are also contemplated, such as parafilm or the like. In alternative embodiments, not shown, the sample tube may be open at each end, with each end receiving an appropriate closure device.

[0027] Although the tube is depicted as generally cylindrical, the tube **130** may be minimally tapered, slightly enlarging toward the open end **134,** 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.

[0028] The tube **130** is formed of a transparent or semitransparent material and the sidewall **136** of the tube **130** 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 **136** substantially returns to its original size and shape.

[0029] The tube may be formed of any transparent or semi-transparent, flexible 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 can't 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 invention and trapped near the wall by the float **110** as described in more detail below.

[0030] In a preferred embodiment, the tube **130** is sized to accommodate the float **110** 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 an especially preferred embodiment, the tube **130** has an inner diameter **138** of about 1.5 cm and accommodates at least about ten milliliters of blood in addition to the float **110.**

[0031] The float **110** includes a main body portion **112** and two sealing rings or flanges **114,** disposed at opposite axial ends of the float **110.** The float **110** 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.

[0032] In this regard, one of the objectives of the present invention is 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 **110** must not have interfering or "background" fluorescence at the wavelength of interest.

[0033] The main body portion **112** and the sealing rings or support members **114** of the float **110** are sized to have an outer diameter **118** which is less than the inner diameter **138** of the sample tube **130,** under pressure or centrifugation. The main body portion **112** of the float **110** **is** also less than the sealing or support rings **114,** thereby defining an annular channel **150** between the float **110** and the sidewall **136** of the tube **130.** The main body portion occupies much of the cross-sectional area of the tube, the annular gap **150** being large enough to contain the cellular components of the buffy coat layers and associated target cells when the tube is the non-flexed state. The dimensions **118** and **138** are such that the annular gap **150** has a radial thickness ranging from about 25-250 microns, most preferably about 50 microns.

[0034] While in some instances the outer diameter **118** of the main body portion **112** of the float **110** may be less than the inner diameter **138** of the tube **130,** this relationship is not required. This is because once the tube **130** is centrifuged (or pressurized), the tube **130** expands and the float **110** moves freely. Once the centrifugation (or pressurization) step is completed, the tube **130** constricts back down on the sealing rings or support ridges **114.** The annular gap or channel **150** is then created, and sized by the height of the support ridges or sealing rings **114** (i.e., the depth of the "pool" is equal to the height of the support ridges **114,** independent of what the tube diameter is/was).

[0035] In an especially preferred embodiment, 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.

[0036] The sealing or support flanged ends **114** are sized to be roughly equal to, or slightly greater than, the inner diameter **138** of the tube. The float **110,** being generally rigid, can also provide support to the flexible tube wall **136.** Furthermore, the large diameter portions **114** provide a sealing function to maintain separation of the blood constituent layers. The seal formed between the large diameter regions **114** of the float and the wall **136** 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 **114** and the tube wall **136** providing a substantial seal which is, in most cases, adequate for purposes of the invention.

[0037] The sealing rings **114** 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, the sealing ridges can be discontinuous or segmented bands having one or openings providing a fluid path in and out of the annular gap **150.** The sealing ridges **114** may be separately formed and attached to the main body portion **112.** Preferably, however, the sealing ridges **114** and the main body portion **112** form a unitary or integral structure.

[0038] The overall specific gravity of the separator float **110** should be between that of red blood cells (approximately 1.090) and that of plasma (approximately 1.028). In a preferred embodiment, the specific gravity is in the range of from about 1.089-1.029, more preferably from about 1.070 to about 1.040, and most preferably about 1.05.

[0039] 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 **110** and the volume of the annular gap **150** 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 **110** 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 **150** between the float **110** and the inner wall **136** of the tube **130.** 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.

[0040] In one preferred embodiment, the density of the float **110** 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.

[0041] 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 invention.

[0042] In a preferred exemplary method of using the tube/float system 100 of the invention, 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.

[0043] A fluorescently labeled antibody, which is specific to the target epithelial cells or other target analytes of interest, can be added to the blood sample and incubated. 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.

[0044] The blood is then transferred to the assembly **100** for centrifugation. The float **110** may be fitted into the tube **130** after the blood sample is introduced into the sample tube **130** 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.

[0045] When the centrifugation is started, the resultant hydrostatic pressure deforms or flexes the wall **136** so as to enlarge the diameter of the tube. The blood components and the float **110** are thus free to move under centrifugal force within the tube **130.** The blood sample is separated into six distinct layers according to density, which are, from bottom to top: 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, it occupies the same axial position as the buffy coat layers which thus occupy the narrow annular gap **150,** potentially along with a small amount of the red cell and/or plasma).

[0046] After centrifugal separation is complete and the centrifugal force is removed, the tube **130** returns to its original diameter to capture or retain the buffy coat layers and target analytes within the annular gap **150**. The tube/float system **100** is transferred to a microscope or optical reader to identify any target analytes in the blood sample.

[0047] FIGURES 2-28 illustrate several exemplary modifications of the float according to the invention. FIGURE 2 illustrates a float **210** that is similar to the float **110** shown and described by way of reference to the of FIGURE 1, which includes a main body portion **212** and sealing rings **214,** but which further including a tapered or cone-shaped endcap member **216** disposed at each end. The tapered endcaps **216** are provided to facilitate and direct the flow of cells past the float **210** and sealing ridges **214** during centrifugation.

[0048] FIGURE 3 illustrates a float **310,** which is similar to the float **210** shown and described by way of reference to FIGURE 2, including a main body portion **312** and sealing ridges **314,** but having truncated cone-shaped endcap members **316,** disposed at each end. The frustoconical endcaps **316** are provided to facilitate the movement or flow of cells and the float during centrifugation.

[0049] FIGURE 4 illustrates a float **410,** which is substantially as shown and described by way of reference to the floats **210** and **310** of FIGURES 2 and 3, respectively, but where instead, generally convex or dome-shaped members **416,** which cap the sealing ridges **414.** The endcaps **416** may be hemispherical, hemiellipsoidal, or otherwise similarly sloped, are provided. Again, the sloping ends **416** are provided to facilitate density-motivated cell and float movement during centrifugation.

[0050] The geometrical configurations of the endcap units **216, 316,** and **416** illustrated in FIGURES 2-4, respectively, are intended to be exemplary and illustrative only, and many other geometrical shapes (including concave or convex configurations) providing a curved, sloping, and/or tapered surface around which the blood sample may flow 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.

[0051] FIGURE 5 illustrates a float **510** similar to the embodiment depicted in FIGURE 1, but wherein the sealing ridges are **514** are axially displaced from the ends. Optional endcap members **516** appear as conical in the illustrated embodiment. However, it will be recognized that the endcaps **516,** if present, any other geometrical configuration which provides a sloped or tapered surface may be used, as described above.

[0052] Although the remaining FIGURES 6-28 are illustrated with generally flat ends, i.e., without tapered ends, it will be recognized that each of the illustrated embodiments may optionally be modified to include any of the end cap types shown above in FIGURES 2-5, or other geometrical configuration which provide a sloped or tapered surface.

[0053] FIGURES 6-13 illustrate embodiments of the invention having generally annular tube support members. FIGURE 6 illustrates a ribbed float **610** having a plurality of annular ribs or ridges **620** axially spaced along a central body portion **612.** Optional end sealing ridges **614** are disposed at opposite ends of the float. The ribs **620** and the optional end sealing ridges **614** are sized to provide a sealing engagement with the tube **130** (FIGURE 1) when a centrifugal force is removed. The flexible tube expands during centrifugation to permit flow therearound during the density-based centrifugal separation process. The main body portion **612** has a diameter smaller than the inner diameter of the tube during centrifugation and while supported by rib **614** and, thus, multiple annular channels **650** are defined between the main body portion **612** and the inner tube wall upon completion of the centrifugation process.

[0054] Although the illustrated embodiment in FIGURE 6 depicts continuous ribs, it will be recognized that the support ribs may likewise be broken or segmented to provide an enhanced flow path between adjacent annular channels **650.** Additionally, multiple ribs and/or sealing ridges may be present in order to provide support for the deformable tube and/or to prevent the tube walls from collapsing inwardly.

[0055] FIGURE 7 illustrates a float **710** according to a further embodiment. The float **710** is similar to the float **610** shown in FIGURE 6, and has a plurality of ribs **720** axially spaced along a central body portion **712,** and wherein plural annular channels **750** are defined therebetween as described above, but wherein the tube support ribs **720** are less densely spaced apart than in the FIGURE 6 embodiment. Optional sealing ridges **714** are disposed at opposite ends of the float. Again, the illustrated embodiment depicts continuous ribs, however, it will be recognized that the support ribs may likewise be broken or segmented to provide an enhanced flow path between adjacent annular channels **750.**

[0056] FIGURE 8 illustrates a further float embodiment **810,** similar to the embodiments of FIGURES 6 and 7, the above descriptions of which are equally applicable thereto. However, the float **810** differs in that it lacks sealing ridges at the opposite ends thereof, which may optionally be provided, and the spacing of the ribs **820** is intermediate the rib spacing shown in FIGURES 6 and 7.

[0057] FIGURE 9 illustrates a further float embodiment **910,** wherein a helical support member or ridge **920** is provided. That is, instead of discrete annular bands, multiple turns of the helical ridge 920 provides a series of spaced apart ridges on the main body portion **912,** which defines a corresponding helical channel **950.** The helical ridge 920 is illustrated as continuous, however, the helical band may instead be segmented or broken into two or more segments, e.g., to provide path for fluid flow between adjacent turns of the helical buffy coat retention channel **950.** Optional sealing ridges **914** appear at each axial end of the float **910.**

[0058] FIGURES 10 and 11 illustrate further ribbed and helical float embodiments **1010** and **1110,** respectively. In FIGURE 10, annular support ribs **1020,** on a main body portion **1012,** are tapered in the radial dimension. In FIGURE 11, a tapered helical support **1120** appears, formed on a main body portion **1112.** The floats **1010** and **1110** are otherwise as described above by way of reference to FIGURES 6 and 9, respectively. Although the support members **1020** and **1120** are shown as continuous, they may alternatively be discontinuous or segmented to facilitate axial flow. Option sealing ridges, as described above, at opposite axial ends of the floats **1010** and **1110** are omitted in the illustrated embodiment, and may optionally be provided.

[0059] FIGURES 12 and 13 illustrate still further ribbed and helical float embodiments **1210** and **1310,** respectively. Appearing are support members **1220** and **1320,** formed on respective main body portions **1212** and **1312.** The tube support members **1220** and **1320** each have a generally curved or rounded cross-sectional profile. The floats **1210** and **1310** are otherwise as described above by way of reference to FIGURES 6 and 9, respectively. Again, the support members **1220** and **1320** are shown as continuous but may, in alternative embodiments, be discontinuous or segmented. Optional end sealing ridges **1314** appear in FIGURE 13. Furthermore, end sealing ridges do not appear in FIGURE 12, but may optionally be provided.

[0060] Referring now to FIGURES 14 and 15, there is shown a splined separator float **1410.** The float **1410** includes a plurality of axially-oriented splines or ridges **1424** radially spaced about a central body portion **1412.** Optional end sealing ridges **1414** are disposed at opposite ends of the float. The splines **1424** and the optional end sealing ridges **1414** protrude from the main body **1412** to engage and provide support for the deformable tube. Where provided, the end sealing ridges **1414** provide a sealing function as described above. The axial protrusions **1424** define fluid retention channels **1450,** between the tube inner wall and the main body portion **1412.** The surfaces **1413** of the main body portion disposed between the protrusions **1424** may be curved, e.g., when the main body portion is cylindrical, however, flat surfaces **1413** are also contemplated. Although the illustrated embodiment depicts splines **1424** that are continuous along the entire axial length of the float, segmented or discontinuous splines are also contemplated.

[0061] FIGURE 16 illustrates a further splined float embodiment **1610** similar to the float **1410** as shown and described above by way of reference to FIGURES 14 and 15, but wherein optional end sealing ridges are not provided.

[0062] FIGURES 17 and 18 are elevational views of alternative splined floats **1710** and **1810,** respectively, and are similar to the respective embodiments shown and described above by way of reference to respective FIGURES 14 and 16, but wherein the axial splines **1724** and **1824,** respectively, protruding from respective main body portions **1712** and **1812** are more sparsely radially spaced. The float **1710** includes optional end sealing ridges **1714;** such do not appear on the float **1810** of FIGURE 18. As above, the respective surfaces **1713** and

1813 may be flat or curved.

[0063] Referring now to FIGURE 19, there is shown a perspective view of a splined separator float **1910** in accordance with a further embodiment of the invention. Multiple axially oriented splines **1924** are spaced radially about and protrude from a central body portion **1912** to provide support for the flexible tube. Optional sealing end ridges **1914** are disposed at opposite ends of the float **1910.** Fluid retention channels **1950** formed between adjacent splines **1924** are defined by adjacent splines **1924** and surfaces **1913** on the main body portion **1912.** The surfaces **1913** are depicted as generally flat, although curved surfaces are also contemplated. The axial splines **1924** are depicted as continuous along the length of the tube; however, segmented or discontinuous splines are also contemplated.

[0064] Referring now to FIGURE 20, there is shown yet another embodiment **2010,** including a tube supporting member **2026** protruding with respect to a main body portion **2012.** The support means **2026** can be described as an intersecting network of annular rings or ribs **2020** and axial splines **2024.** Optional end sealing ridges **2014** are disposed at opposite ends of the float. The support member **2026** and the optional sealing ridges **2014** radially protrude from the main body portion **2012** at opposite ends of the float to engage and provide support for the deformable tube. Where provided, the end sealing ridges **2014** provide a sealing function as described above. The raised support member **2026** defines a plurality of fluid retention windows **2050** formed between the tube inner wall and the main body portion **2012.** Surfaces **2013** of the main body portion **2012** corresponding to the windows **2050** may be curved, e.g., when the main body portion is cylindrical, however, flat surfaces **2013** are also contemplated. Although the illustrated embodiment depicts the support member **2026** as a network of annular ribs and axial splines which is continuous, breaks may also be includes in the annular and/or axial portions of the network **2026,** e.g., to provide a fluid path between two or more of the windows **2050.**

[0065] FIGURES 21-26 illustrate several floats having a plurality of protrusions thereon for providing support for the deformable walls of the sample tube. Referring to FIGURES 21 and 22, float **2110** and **2210,** respectively, include multiple rounded bumps or knobs **2128** spaced over the surface of a central body portion **2112.** Optional end sealing ridges **2114** (FIGURE 21) are disposed at opposite ends of the float **2110** and do not appear on the float **2210** of FIGURE 22. The knobs **2128** and the optional end sealing ridges **2114** radially protrude from the main body **2112** and traverse an annular gap **2150** to engage and provide support for the deformable tube wall. Where provided, the end sealing ridges **2114** provide a sealing function as described above. The surface of the main body portion disposed between the protrusions may be curved, e.g., when the main body portion is cylindrical, or, alternatively, may have flat portions or facets.

[0066] In FIGURES 23 and 24, there are illustrated float embodiments **2310** and **2410,** which are as substantially as described above by way of reference to FIGURES 21 and 22, respectfully, but wherein the protrusions **2328** form an aligned rather than staggered pattern over the surface of the main body portion **2312.** Optional end sealing ridges **2314** appear in the FIGURE 23 embodiment.

[0067] Referring now to FIGURES 25 and 26, there are illustrated float embodiments **2510** and **2610,** which are as substantially as described above by way of reference to FIGURES 21 and 22, respectfully, but wherein the protrusions **2528** are less densely spaced over the surface of the main body portion **2512.** Optional end sealing ridges **2514** appear in the FIGURE 25 embodiment.

[0068] FIGURES 27 and 28 illustrate float embodiments **2710** and **2810,** respectively, which include multiple raised facets **2728** spaced over the surface of a central body portion **2712.** Optional end sealing ridges **2714** (FIGURE 27) are disposed at opposite ends of the float **2710,** and do not appear in the FIGURE 28 embodiment. The facets 2728 and the optional end sealing ridges 2714 radially protrude from the main body 2712 and traverse an annular gap to engage and provide support for the deformable tube wall and define a plurality of fluid retention windows 2750. Where provided, the end sealing ridges 2714 provide a sealing function as described above. The surfaces 2713 of the main body portion, disposed between the protrusions 2728 an forming a surface defining the fluid-retention windows 2750, may be curved surfaces, e.g., when the main body portion is cylindrical. Alternatively, the surfaces 2713 may be flat. In alternative embodiments, the size, spacing density, and alignment patterns of the facets 2718 can be modified extensively.

[0069] The exemplary embodiments of FIGURES 21-28 have been described with reference to rounded knobs or square facets as supporting the flexible sample tube, although protrusions of any geometrical configuration may be used. Other geometrical configurations for the protrusions are also contemplated, such as conical or frustoconical spikes, tectiform or truncated tectiform protrusions, cylindrical protrusions, pyramidal or truncated pyramidal protrusions, hemiellipsoidal protrusions, and so forth, as well as any combinations thereof. Likewise, the size, spacing, and pattern of the protrusions can be varied. Where the sample is to be imaged, the size and spacing can be selected in accordance with the imaging field of view and other factors.

[0070] The invention has been described with reference to the preferred embodiments Obviously, modifications and alterations will occur to others upon reading and understanding the preceding detailed description. It is intended that the invention be construed as including all such modifications and alterations insofar as they come within the scope of the appended claims.

Claims

1. An apparatus (100) for separation and analysis of a target analyte in a sample of anticoagulated whole blood, comprising:

   a flexible sample tube (130) for holding the sample, the sample tube having an elongate sidewall (136) of a first cross-sectional inner diameter (TD), which tube is at least semi-transparent, or optionally transparent
   said sidewall (136) being resiliently radially expandable to a second diameter in response to centrifugal force, said second diameter being sufficiently large to permit axial movement of a float in the tube during centrifugation;
   an elongate, rigid, volume-occupying float (110, 210, 310, 410, 510, 610, 710, 810, 910, 1010, 1110, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 2010. 2110, 2210, 2310, 2A 10, 2510, 2610, 2710, 2810) having a specific gravity intermediate that of red blood cells and plasma, said float comprising:

      a main body portion (112) and one or more support members (114) protruding from the main body portion to engage and support the sidewall of the sample tube, said main body portion and said support members having a cross-sectional diameter less than said second inner diameter of the tube when the sample tube is expanded, wherein said main body portion (112) together with an axially aligned portion of said sidewall (136) define an annular volume (150) therebetween; and wherein said support members traverse said annular volume to produce one or more analysis areas;
      and **characterized in that** the annular volume (150) between said main body portion and the axially aligned portion of said tube sidewall has a radial thickness of from 25 μm to 250 μm when the tube is not expanded, wherein the thickness is sized by the height of the support members (114).

2. The apparatus according to claim 1 wherein the blood sample tube comprises a closed first end (132), an open second end (134) adapted to receive a closure device (140), and the float (110).

3. The apparatus according to claim 1 wherein the sample tube (130) is sized to accommodate the float and at least 5 ml of blood sample

4. The apparatus according to claim 1 wherein the float includes opposite axial ends (216, 316, 516) which are tapered in the axial direction,

5. The apparatus according to claim 1 wherein the one or more support members include one or more annular ridges (214, 314, 414, 514, 614, 620, 714, 720, 820, 1020, 1220, 2014, 2026).

6. The apparatus according to claim 1 wherein the one or more support members include two annular ridges (114, 214, 314, 414, 514).

7. The apparatus according to claim 6 wherein the two annular ridges are disposed at opposite axial ends of the float.

8. The apparatus according to claim 1 wherein the one or more support members include three or more axially-spaced annular ridges.

9. The apparatus according to claim 1 wherein the one or more support members comprises a helical ridge (920, 1120, 1320).

10. The apparatus according to claim 1 wherein the one or more support members include a plurality of radially spaced-apart splines (1424, 1724, 1824, 1924,2024).

11. The apparatus according to claim 10 wherein the splines are aligned parallel to an axis of the float.

12. The apparatus according to claim 10 wherein the one or more support members further include annular ridges (1414, 2014) disposed at opposite axial ends of the float.

13. The apparatus according to claim 1 wherein the one or more support members include a plurality of radially spaced-apart splines (2024) intersecting with a plurality of axially spaced-apart splines (2020).

14. The apparatus according to claim 1 wherein the one or more support members include a plurality of raised protrusions (2128, 2718) spaced over the surface of the main body portion.

15. The apparatus according to claim 14 wherein the protrusions are selected from rounded bumps (2128) and faceted bumps (2718).

16. The apparatus according to claim 1 wherein the tube is formed of a flexible transparent polymeric material and the float is formed of a hard polymeric material.

17. The apparatus according to claim 1 wherein the float has a specific gravity in the range of from 1.029 to 1.089.

18. The apparatus according to any of the preceding claims wherein the main body portion and the one

or more support members are integrally formed.

19. The apparatus according to any of the preceding claims wherein the annular volume (150) has a radial thickness of 50 microns.

20. A method of separating and axially expanding the buffy coat constituents of a blood sample using the apparatus (100) of any of claims 1 to 19, which method comprises:

introducing the blood sample into the flexible sample tube (130);
introducing the elongate rigid volume-occupying float (110, 210, 310, 410, 510, 610, 710, 810, 910, 1010, 1110, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 2010, 2110, 2210, 2310, 2410, 2510, 2610, 2710, 2810) into the flexible sample tube;
centrifuging the sample tube to effect a density-based separation of the blood sample into discrete layers at a rotational speed that causes a resilient enlargement of the sidewall (136) to a second diameter in response to centrifugal force, said second diameter being sufficiently large to permit axial movement of the float in the tube;
moving said float into axial alignment with at least the buffy coat constituents of the blood sample in response to centrifugal forces produced in centrifuging the blood; and
thereafter, reducing the rotational speed to cause the tube sidewall to return to said first diameter, thereby capturing the float and trapping the buffy coat constituents in the analysis area.

21. The method according to claim 20 wherein said float is introduced into the blood sample tube before the blood is introduced therein.

22. The method according to claim 20 wherein said blood sample is introduced into the blood sample tube before the float is introduced therein.

23. The method according to claim 20 wherein the blood sample comprises anticoagulated whole blood.

24. The method according to claim 20 wherein the float is further moved into axial alignment with at least one of a portion of a separated red blood cell layer and a portion of a separated plasma layer.

25. A method for detecting circulating target cells in an anticoagulated whole blood sample, which method comprises:

providing the apparatus of any of claims 1 to 19;
combining the blood sample with one or more labeling agents so as to differentiate target cells from other cells in the blood sample;
introducing the blood sample into the at least semi-transparent, flexible sample tube;
inserting the volume-occupying float into the sample tube;
centrifuging the blood sample and float in the sample tube to effect centrifugally-motivated localization of any target cells present in the blood sample within said analysis area; and
after said centrifuging, allowing the sample tube to constrict upon the separator float, and examining the blood sample present in the analysis area to identify any target cells contained therein.

26. The method according to claim 25 wherein the one or more labeling agents includes a fluorescently labeled ligand, and further wherein said examining step includes imaging the blood sample present in the analysis area under illumination and magnification.

27. The method according to claim 25 and further comprising combining the blood sample with a stain.

Patentansprüche

1. Vorrichtung (100) für ein Separieren und eine Analyse eines Zielanalyten in einer Probe aus antikoaguliertem Vollblut, aufweisend:

ein flexibles Probenröhrchen (130) zum Halten der Probe, wobei das Probenröhrchen eine längliche Seitenwand (136) von einem ersten Querschnittsinnendurchmesser (TD) aufweist, wobei das Röhrchen zumindest halbtransparent, oder optional transparent ist,
wobei die Seitenwand (136) auf einen zweiten Durchmesser elastisch radial aufgeweitet werden kann, und zwar ansprechend auf eine Zentrifugalkraft, wobei der zweite Durchmesser ausreichend groß ist, um eine axiale Bewegung eines Schwimmers in dem Röhrchen während einer Zentrifugierung zu ermöglichen, und
einen länglichen, starren, raumnehmenden Schwimmer (110, 210, 310, 410, 510, 610, 710, 810, 910, 1010, 1110, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 2010, 2110, 2210, 2310, 2410, 2510, 2610, 2710, 2810), dessen spezifisches Gewicht zwischen dem von roten Blutzellen und Plasma liegt, wobei der Schwimmer aufweist:

einen Hauptkörperabschnitt (112) und eines oder mehrere Tragelemente (114), die von dem Hauptkörperabschnitt hervorstehen, um ein Eingreifen und Abstützen der Seitenwand des Proberöhrchens zu bewirken, wobei der Hauptkörperabschnitt und die Tragelemente einen Querschnittdurchmesser haben, der geringer als der zweite Innendurchmesser des Röhrchens ist, wenn das Proberöhrchen aufgeweitet ist, wobei der Hauptkörperabschnitt (112) gemeinsam mit einem axial fluchtenden Abschnitt der Seitenwand (136) einen Ringraum (150) zwischen diesen definiert; und wobei die Tragelemente sich durch den Ringraum hindurch erstrecken, um eine oder mehrere Analysezonen zu erzeugen;

dadurch gekennzeichnet, dass der Ringraum (150) zwischen dem Hauptkörperabschnitt und dem axial fluchtenden Abschnitt der Seitenwand des Röhrchens eine radiale Dicke von 25 μm bis 250 μm aufweist, wenn das Röhrchen nicht aufgeweitet ist, wobei die Dicke in ihrem Ausmaß durch die Höhe der Tragelemente (114) bestimmt ist.

2. Vorrichtung nach Anspruch 1, wobei das Blutproberöhrchen ein geschlossenes erstes Ende (132), ein offenes zweites Ende (134), das ausgebildet ist, um eine Verschlussvorrichtung (140) aufzunehmen, und den Schwimmer (110) aufweist.

3. Vorrichtung nach Anspruch 1, wobei das Probenröhrchen (130) solche Abmessungen hat, dass es den Schwimmer und mindestens 5 ml einer Blutprobe aufnehmen kann.

4. Vorrichtung nach Anspruch 1, wobei der Schwimmer gegenüberliegende axiale Enden (216, 316, 516) beinhaltet, die sich in axialer Richtung verjüngen.

5. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente eine oder mehrere ringförmige Rippen (214, 314, 414, 514, 614, 620, 714, 720, 820, 1020, 1220, 2014, 2026) beinhalten.

6. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente zwei ringförmige Rippen (114, 214, 314, 414, 514) beinhalten.

7. Vorrichtung nach Anspruch 6, wobei die zwei ringförmigen Rippen an gegenüberliegenden axialen Enden des Schwimmers angeordnet sind.

8. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente drei oder mehr axial beabstandete ringförmige Rippen beinhalten.

9. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente eine helixförmige Rippe (920, 1120, 1320) beinhalten.

10. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente eine Mehrzahl von radial beabstandeten Keilverzahnungen (1424, 1724, 1824, 1924, 2024) beinhalten.

11. Vorrichtung nach Anspruch 10, wobei die Keilverzahnungen parallel zu einer Achse des Schwimmers ausgerichtet sind.

12. Vorrichtung nach Anspruch 10, wobei das eine oder die mehreren Tragelemente weiter zwei ringförmige Rippen (1414, 2014) beinhalten, die an entgegengesetzten axialen Enden des Schwimmers angeordnet sind.

13. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente eine Mehrzahl von radial beabstandeten Keilverzahnungen (2024) beinhalten, die sich mit einer Mehrzahl von axial beabstandeten Keilverzahnungen (2020) schneiden.

14. Vorrichtung nach Anspruch 1, wobei das eine oder die mehreren Tragelemente eine Mehrzahl von erhöhten Vorsprüngen (2128, 2718) beinhalten, die über die Fläche des Hauptkörperabschnitts beabstandet angeordnet sind.

15. Vorrichtung nach Anspruch 14, wobei die Vorsprünge aus abgerundeten Höckern (2128) und facettierten Höckern (2718) gewählt sind.

16. Vorrichtung nach Anspruch 1, wobei das Röhrchen aus einem flexiblen transparenten Polymermaterial ausgebildet ist und der Schwimmer aus einem harten Polymermaterial ausgebildet ist.

17. Vorrichtung nach Anspruch 1, wobei der Schwimmer ein spezifisches Gewicht zwischen 1,029 und 1,089 hat.

18. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei der Hauptkörperabschnitt und das eine oder die mehreren Tragelemente integral ausgebildet sind.

19. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei der Ringraum (150) eine radiale Dicke von 50 μm hat.

20. Verfahren zum Separieren und axialem Aufweiten der Buffy-Coat-Bestandteile einer Blutprobe unter Verwendung der Vorrichtung (100) nach einem der Ansprüche 1 bis 19, wobei das Verfahren umfasst:

Einbringen der Blutprobe in das flexible Proberöhrchen (130);
Einbringen des länglichen, starren, raumnehmenden Schwimmers (110, 210, 310, 410, 510,

610, 710, 810, 910, 1010, 1110, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 2010, 2110, 2210, 2310, 2410, 2510, 2610, 2710, 2810) in das flexible Proberöhrchen;
Zentrifugieren des Proberöhrchens, um eine Dichte-basierte Separierung der Blutprobe in separate Schichten zu bewirken, und zwar mit einer Drehzahl, die eine elastische Aufweitung der Seitenwand (136) auf einen zweiten Durchmesser ansprechend auf eine Zentrifugalkraft bewirkt, wobei der zweite Durchmesser ausreichend groß ist, um eine axiale Bewegung des Schwimmers in dem Röhrchen zu erlauben;
Bewegen des Schwimmers zu einer axialen Fluchtung mit zumindest den Buffy-Coat-Bestandteilen der Blutprobe ansprechend auf Zentrifugalkräfte, die beim Zentrifugieren des Blutes erzeugt werden; und
danach, Verringern der Drehzahl, um zu bewirken, dass die Seitenwand des Röhrchens auf den ersten Durchmesser zurückgeht, wodurch der Schwimmer gefasst wird und die Buffy-Coat-Bestandteile in der Analysezone gefangen werden.

21. Verfahren nach Anspruch 20, wobei der Schwimmer in das Blutproberöhrchen eingebracht wird, bevor das Blut in dieses eingebracht wird.

22. Verfahren nach Anspruch 20, wobei die Blutprobe in das Blutproberöhrchen eingebracht wird, bevor der Schwimmer in dieses eingebracht wird.

23. Verfahren nach Anspruch 20, wobei die Blutprobe antikoaguliertes Vollblut beinhaltet.

24. Verfahren nach Anspruch 20, wobei der Schwimmer weiter in axiale Fluchtung mit einem Abschnitt einer separierten Schicht von roten Blutzellen und/oder einem Abschnitt einer separierten Plasma-Schicht gebracht wird.

25. Verfahren zum Erkennen von zirkulierenden Zielzellen in einer antikoagulierten Vollblutprobe, wobei das Verfahren beinhaltet:

Bereitstellen der Vorrichtung nach einem der Ansprüche 1 bis 19;
Kombinieren der Blutprobe mit einem oder mehreren Markierungsagentien, um so Zielzellen von anderen Zellen in der Blutprobe zu unterscheiden;
Einbringen der Blutprobe in das zumindest halbtransparente, flexible Proberöhrchen;
Einsetzen des raumnehmenden Schwimmers in das Proberöhrchen;
Zentrifugieren der Blutprobe und des Schwimmers in dem Proberöhrchen, um eine durch Zentrifugieren bewirkte Lokalisierung jeglicher in der Blutprobe vorhandener Zielzellen in der Analysezone zu bewirken;
nach dem Zentrifugieren lässt man das Proberöhrchen sich über dem Separatorschwimmer zusammenziehen, und untersucht die in der Analysezone vorhandene Blutprobe, um jegliche dort enthaltene Zielzellen zu identifizieren.

26. Verfahren nach Anspruch 25, wobei das eine oder die mehreren Markierungsagentien einen fluoreszierend markierten Liganden beinhalten, und wobei weiter der Untersuchungsschritt beinhaltet, dass die in der Analysezone vorhandene Blutprobe unter Beleuchtung und Vergrößerung bildlich dargestellt wird.

27. Verfahren nach Anspruch 25, das weiter beinhaltet, dass die Blutprobe mit einem Anfärbereagens kombiniert wird.

Revendications

1. Appareil (100) de séparation et d'analyse d'un analyte cible dans un échantillon de sang entier anticoagulé, qui comprend :

un tube à échantillon flexible (130) destiné à contenir l'échantillon, le tube à échantillon ayant une paroi latérale allongée (136) d'un premier diamètre intérieur transversal (TD), ledit tube étant au moins semi-transparent, ou optionnellement transparent,
ladite paroi latérale (136) étant radialement extensible de manière élastique jusqu'à un second diamètre en réaction à la force centrifuge, ledit second diamètre étant suffisamment grand pour permettre le mouvement axial d'un flotteur dans le tube durant la centrifugation ;
un flotteur allongé, rigide et qui occupe un volume (110, 210, 310, 410, 510, 610, 710, 810, 910, 1010, 1110, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 2010, 2110, 2210, 2310, 2410, 2510, 2610, 2710, 2810), qui présente une gravité spécifique située entre celle des globules rouges et du plasma, ledit flotteur comprenant :

une partie de corps principal (112) et un ou plusieurs élément(s) de soutien (114) qui sortent de la partie de corps principal afin d'engager et de soutenir la paroi latérale du tube à échantillon, ladite partie de corps principal et lesdits éléments de soutien ayant un diamètre transversal inférieur audit second diamètre intérieur du tube lorsque le tube à échantillon s'allonge, dans lequel ladite partie de corps principal (112),

avec une partie axialement alignée de ladite paroi latérale (136), définit un volume annulaire (150) entre celles-ci ; et dans lequel lesdits éléments de soutien traversent ledit volume annulaire afin de produire une ou plusieurs zone(s) d'analyse ;
et **caractérisé en ce que** le volume annulaire (150) situé entre ladite partie de corps principal et la partie axialement alignée de ladite paroi latérale du tube possède une épaisseur radiale comprise entre 25 μm et 250 μm lorsque le tube n'est pas allongé, l'épaisseur étant mesurée selon la hauteur des éléments de soutien (114).

2. Appareil selon la revendication 1, dans lequel le tube à échantillon sanguin comprend une première extrémité fermée (132), une seconde extrémité ouverte (134) adaptée pour recevoir un dispositif de fermeture (140), et le flotteur (110).

3. Appareil selon la revendication 1, dans lequel le tube à échantillon (130) est dimensionné pour recevoir le flotteur et au moins 5 ml d'échantillon sanguin.

4. Appareil selon la revendication 1, dans lequel le flotteur comprend des extrémités axiales opposées (216, 316, 516) qui sont effilées dans la direction axiale.

5. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent une ou plusieurs arête(s) annulaire(s) (214, 314, 414, 514, 614, 620, 714, 720, 820, 1020, 1220, 2014, 2026).

6. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent deux arêtes annulaires (114, 214, 314, 414, 514).

7. Appareil selon la revendication 6, dans lequel les deux arêtes annulaires sont disposées aux extrémités axiales opposées du flotteur.

8. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent trois arêtes annulaires axialement espacées ou plus.

9. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent une arête hélicoïdale (920, 1120, 1320).

10. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent une pluralité de cannelures radialement espacées (1424, 1724, 1824, 1924, 2024).

11. Appareil selon la revendication 10, dans lequel les cannelures sont alignées parallèlement à un axe du flotteur.

12. Appareil selon la revendication 10, dans lequel le ou les élément(s) de soutien comprend/comprennent en outre des arêtes annulaires (1414, 2014) disposées aux extrémités opposées axiales du flotteur.

13. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent une pluralité de cannelures radialement espacées (2024) qui s'entrecroisent avec une pluralité de cannelures axialement espacées (2020).

14. Appareil selon la revendication 1, dans lequel le ou les élément(s) de soutien comprend/comprennent une pluralité de saillies surélevées (2128, 2718) réparties sur la surface de la partie de corps principal.

15. Appareil selon la revendication 14, dans lequel les saillies sont choisies parmi des bosses arrondies (2128) et des bosses à facettes (2718).

16. Appareil selon la revendication 1, dans lequel le tube est formé d'un matériau polymère transparent souple et le flotteur est formé d'un matériau polymère dur.

17. Appareil selon la revendication 1, dans lequel le flotteur possède une gravité spécifique de l'ordre de 1,029 à 1,089.

18. Appareil selon l'une quelconque des revendications précédentes, dans lequel la partie de corps principal et le ou les élément(s) de soutien sont formés intégralement.

19. Appareil selon l'une quelconque des revendications précédentes, dans lequel le volume annulaire (150) possède une épaisseur radiale de 50 microns.

20. Procédé de séparation et d'extension axiale des constituants de couche leuco-plaquettaire d'un échantillon sanguin à l'aide de l'appareil (100) selon l'une quelconque des revendications 1 à 19, le procédé comprenant :

l'introduction de l'échantillon sanguin à l'intérieur du tube à échantillon flexible (130) ;
l'introduction du flotteur allongé, rigide et qui occupe un volume (110, 210, 310, 410, 510, 610, 710, 810, 910, 1010, 1210, 1310, 1410, 1610, 1710, 1810, 1910, 200, 2110, 2210, 2310, 2410, 2510, 2610, 2710, 2810) à l'intérieur du tube à échantillon flexible ;
la centrifugation du tube à échantillon afin d'effectuer une séparation de l'échantillon sanguin, sur la base de la densité, en des couches dis-

crètes à une vitesse de rotation qui provoque un élargissement élastique de la paroi latérale (136) jusqu'à un second diamètre en réaction à la force centrifuge, ledit second diamètre étant suffisamment grand pour permettre un mouvement axial du flotteur dans le tube ;
le déplacement dudit flotteur en alignement axial avec au moins les constituants de la couche leuco-plaquettaire de l'échantillon sanguin en réaction aux forces centrifuges produites lors de la centrifugation du sang ; et
par la suite, la réduction de la vitesse de rotation afin que la paroi latérale du tube revienne audit premier diamètre, de façon à capturer le flotteur et à piéger les constituants de la couche leuco-plaquettaire dans la zone d'analyse.

21. Procédé selon la revendication 20, dans lequel ledit flotteur est introduit dans le tube à échantillon sanguin avant que le sang y soit introduit.

22. Procédé selon la revendication 20, dans lequel ledit échantillon sanguin est introduit dans le tube à échantillon sanguin avant que le flotteur y soit introduit.

23. Procédé selon la revendication 20, dans lequel l'échantillon sanguin comprend du sang entier anticoagulé.

24. Procédé selon la revendication 20, dans lequel le flotteur est en outre déplacé en alignement axial avec au moins l'une d'une partie d'une couche de globule rouge séparée et d'une partie de couche de plasma séparée.

25. Procédé de détection de cellules cibles en circulation dans un échantillon de sang entier anticoagulé, le procédé comprenant :

le fait de prévoir l'appareil selon l'une quelconque des revendications 1 à 19,
la combinaison de l'échantillon sanguin avec un ou plusieurs agent(s) de marquage afin de différencier les cellules cibles des autres cellules dans l'échantillon sanguin ;
l'introduction de l'échantillon sanguin dans au moins un tube à échantillon semi-transparent souple ;
l'insertion du flotteur qui occupe un volume à l'intérieur du tube à échantillon ;
la centrifugation de l'échantillon sanguin et du flotteur dans le tube à échantillon afin d'effectuer une localisation centrifuge de n'importe quelle cellule cible présente dans l'échantillon sanguin à l'intérieur de ladite zone d'analyse ; et
après ladite centrifugation, le fait de laisser le tube à échantillon se resserrer sur le flotteur séparateur, l'examen de l'échantillon sanguin présent dans la zone d'analyse afin d'identifier toute cellule cible contenue à l'intérieur.

26. Procédé selon la revendication 25, dans lequel le ou les agent(s) de marquage comprend/comprennent un ligand marqué de façon fluorescente, et, en outre, dans lequel ladite étape d'examen comprend l'imagerie de l'échantillon sanguin présent dans la zone d'analyse sous éclairage et grossissement.

27. Procédé selon la revendication 25, et qui comprend en outre la combinaison de l'échantillon sanguin avec une tâche.

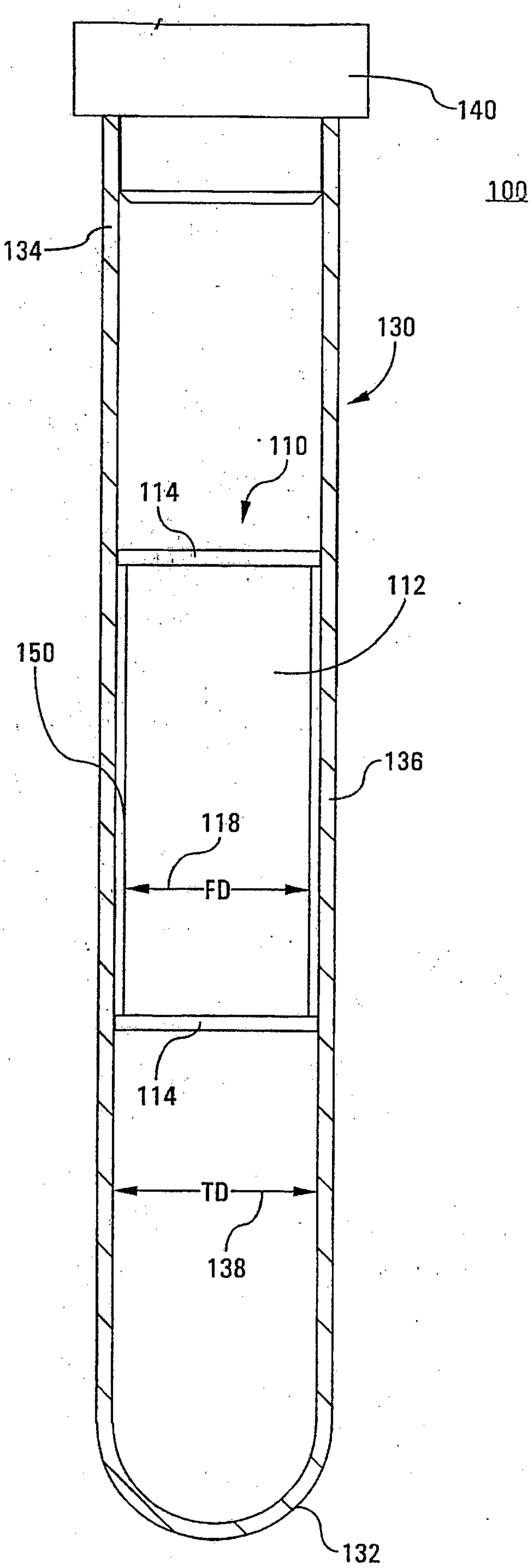

FIG. 1

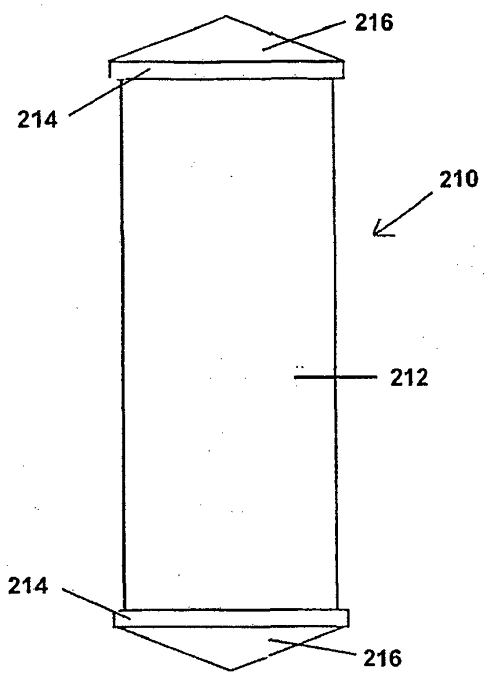

FIG. 2

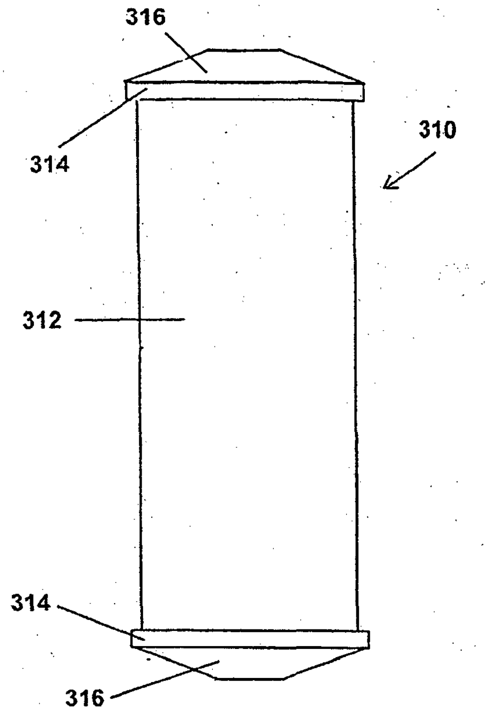

FIG. 3

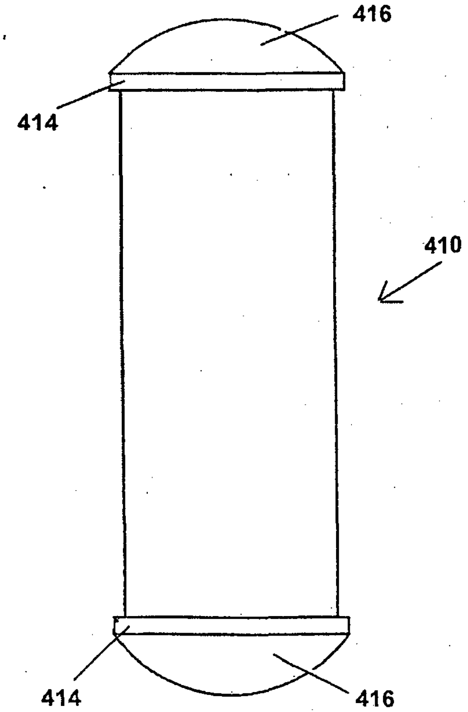

FIG. 4

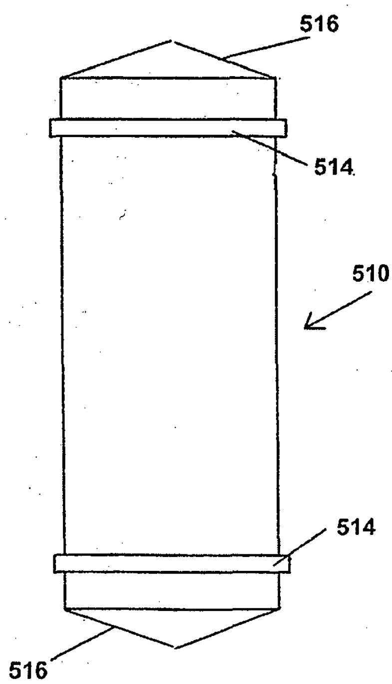

FIG. 5

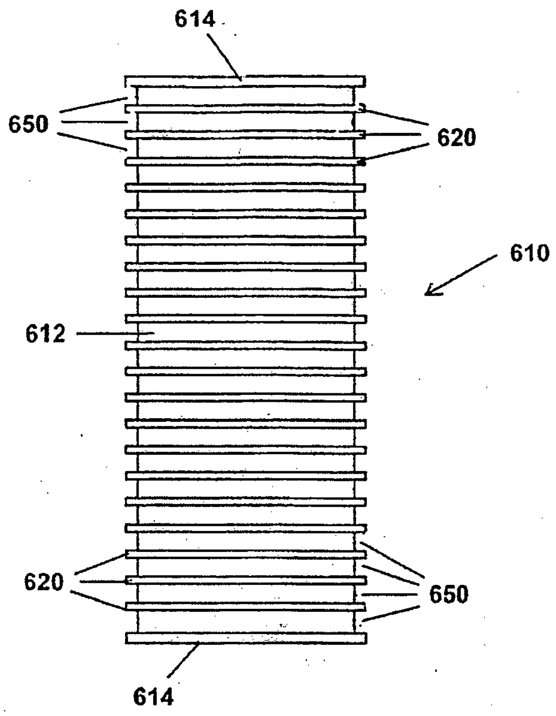

FIG. 6

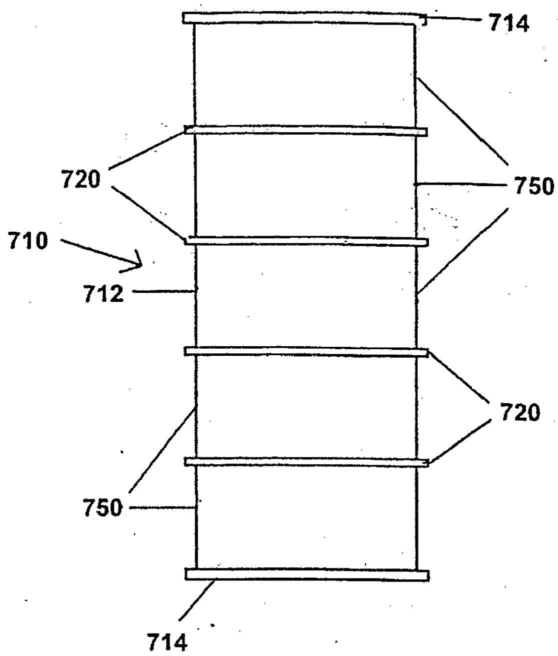

FIG. 7

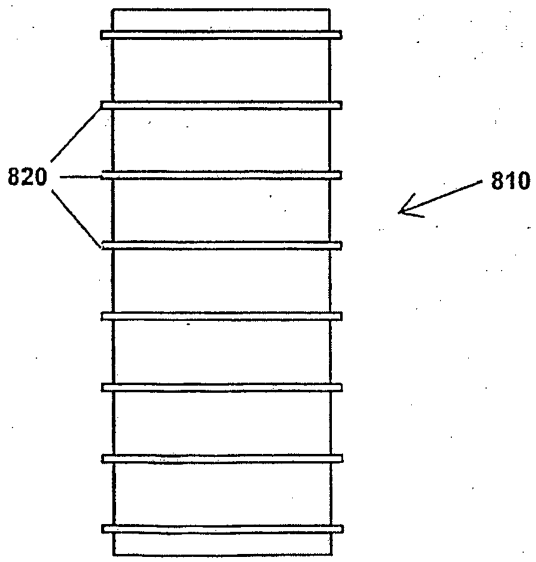

FIG. 8

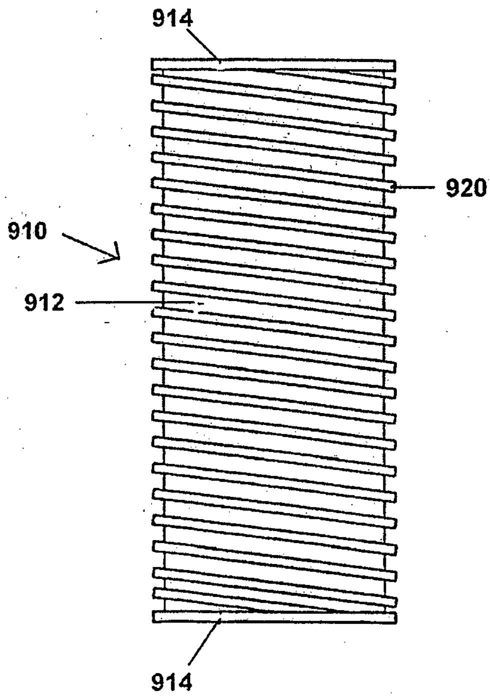

FIG. 9

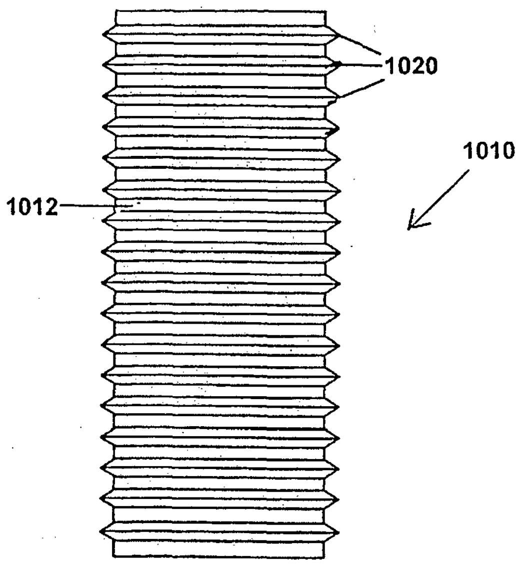

FIG. 10

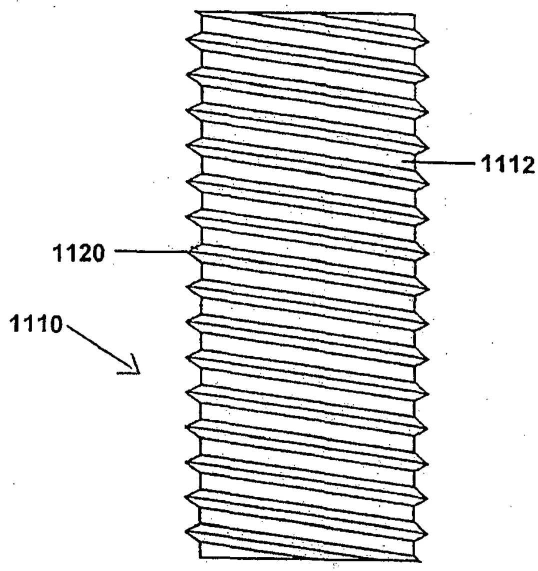

FIG. 11

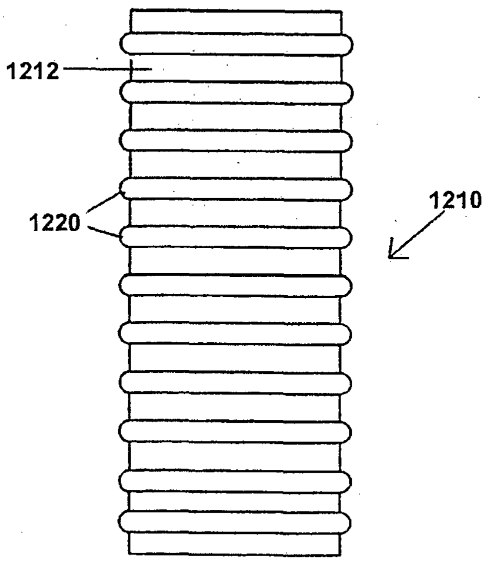

FIG. 12

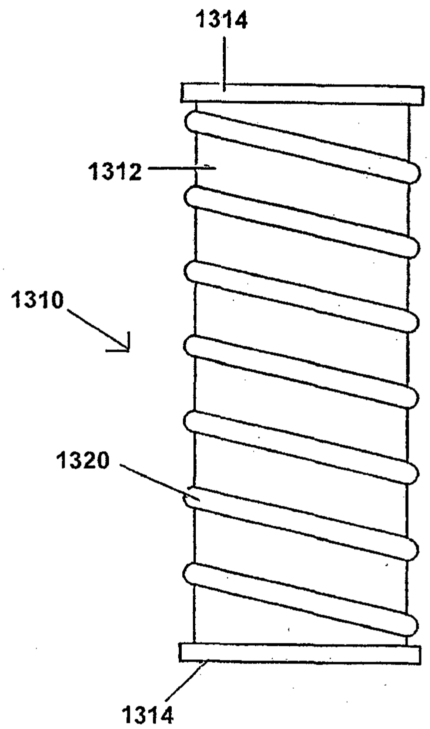

FIG. 13

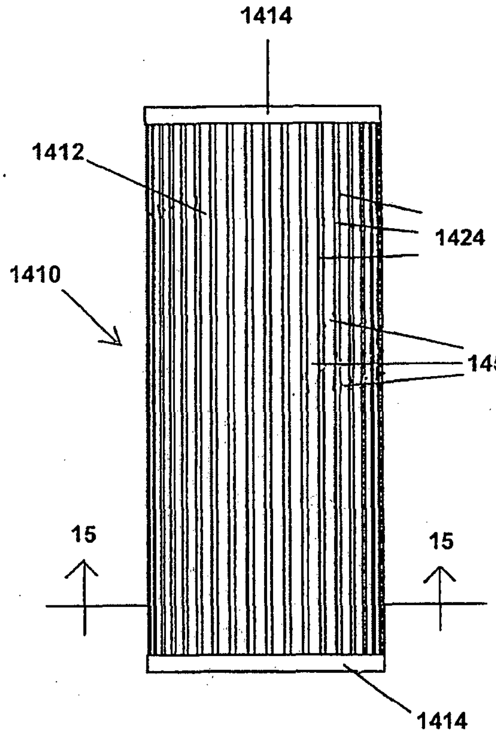

FIG. 14

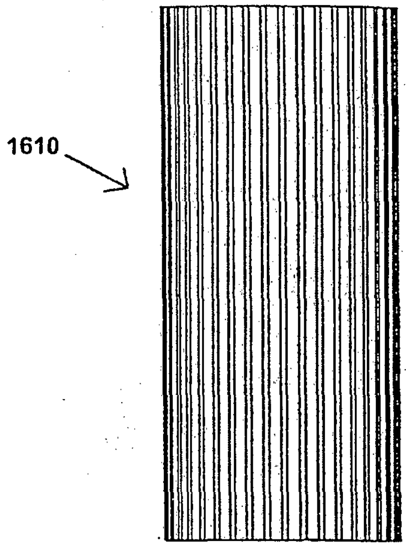

FIG. 16

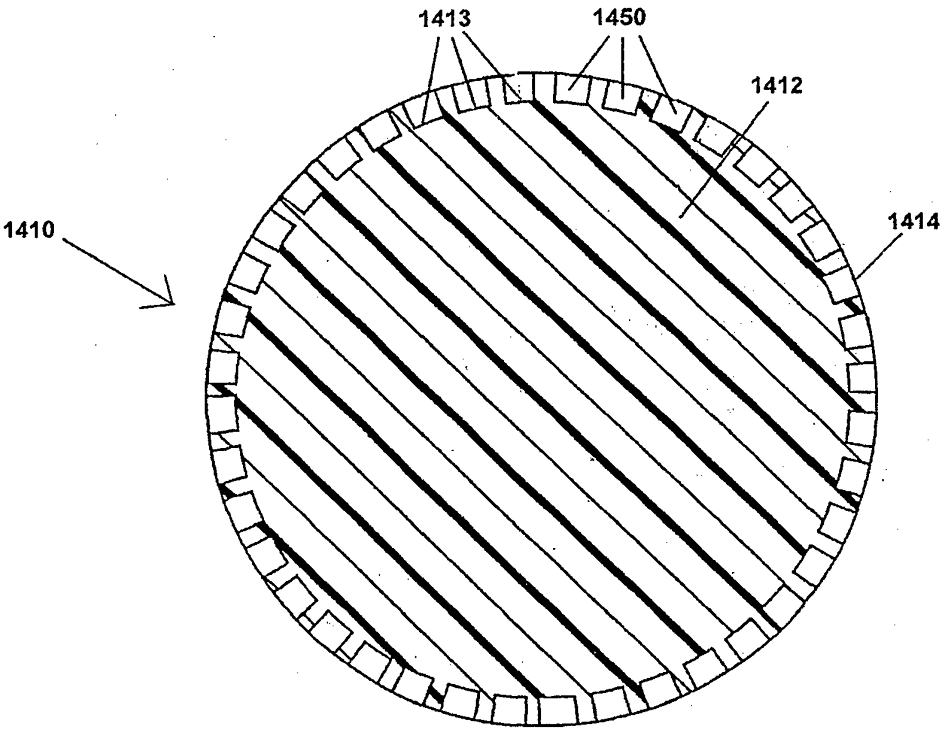

FIG. 15

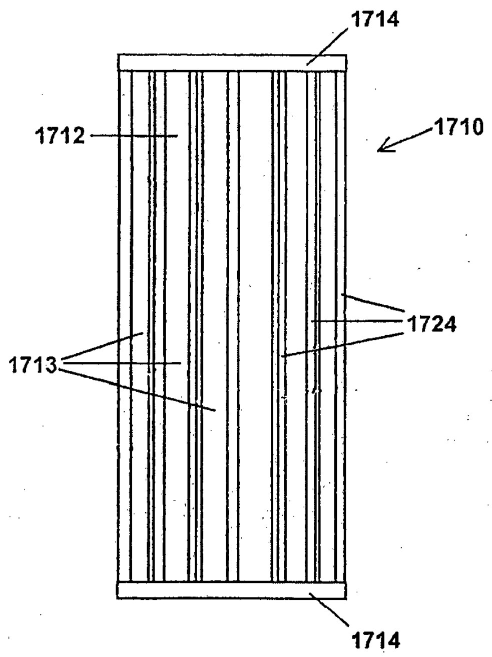

FIG. 17

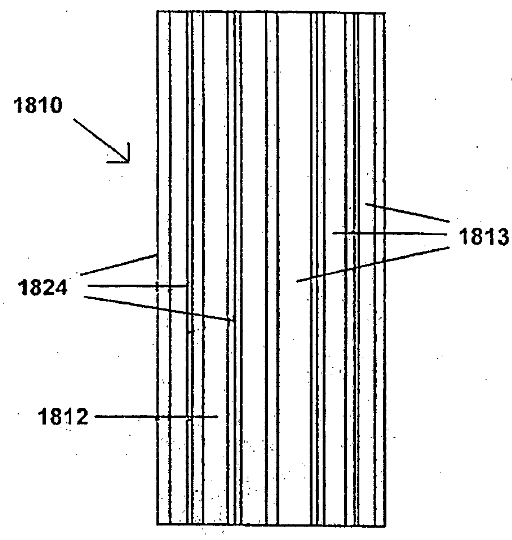

FIG. 18

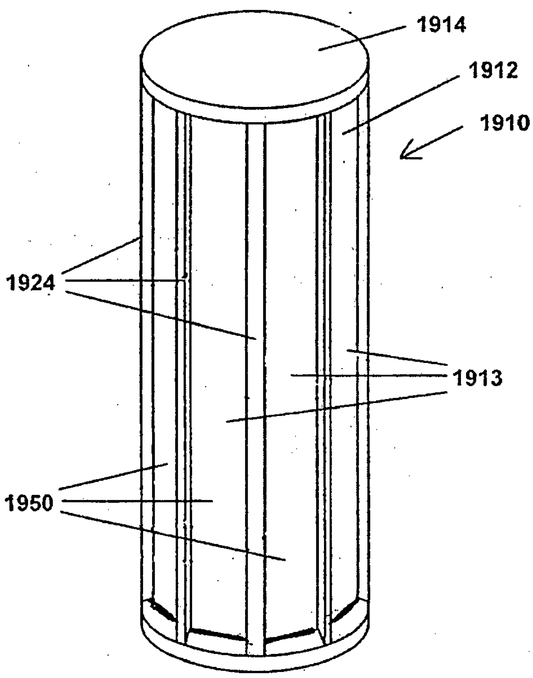

FIG. 19

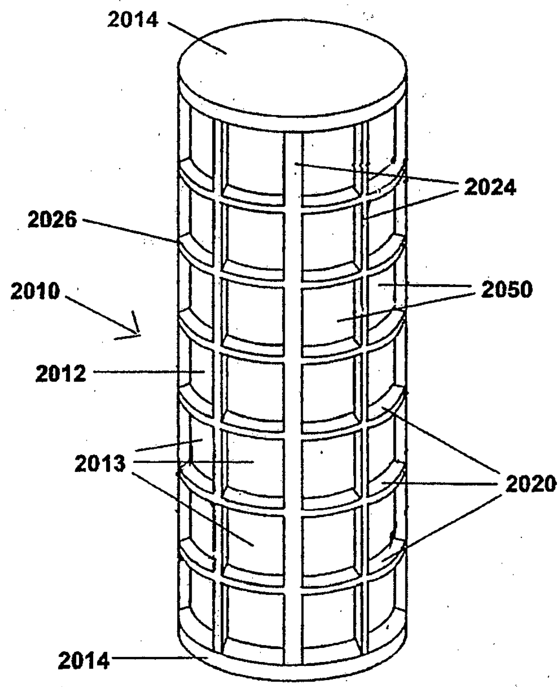

FIG. 20

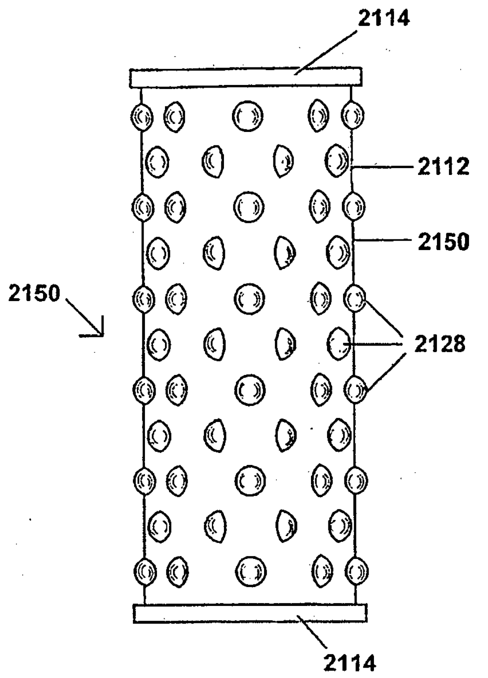

FIG. 21

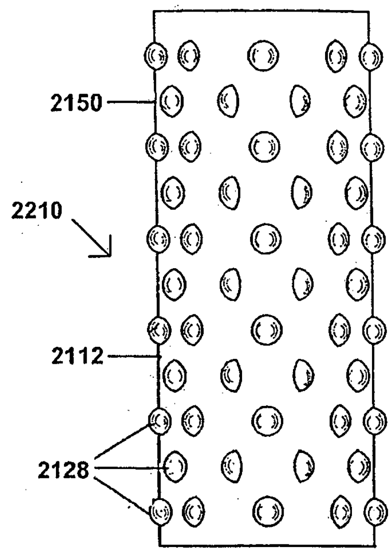

FIG. 22

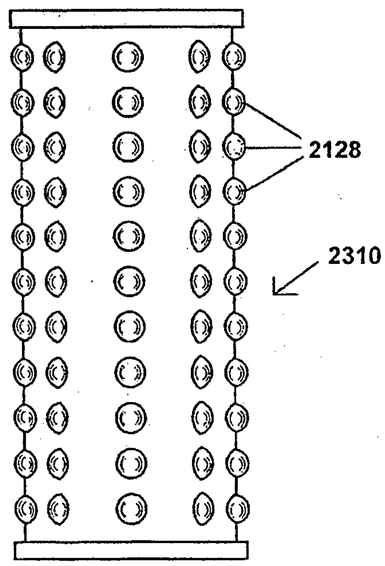

FIG. 23

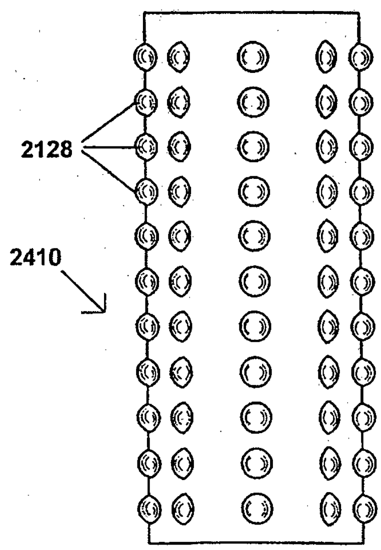

FIG. 24

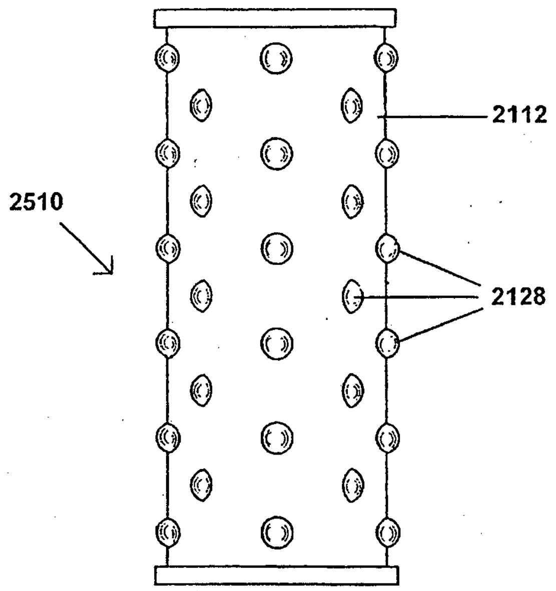

FIG. 25

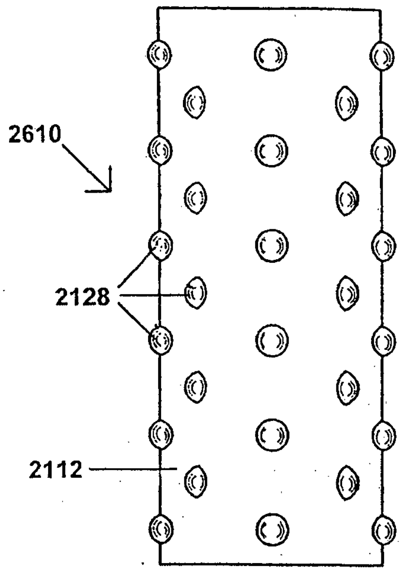

FIG. 26

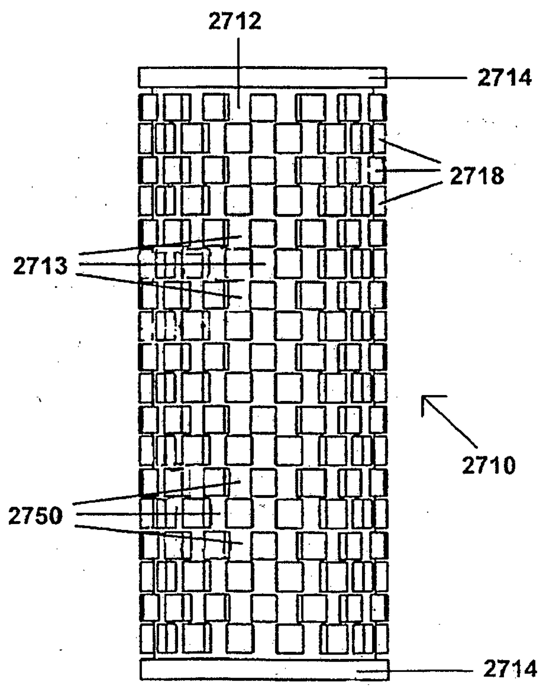

FIG. 27

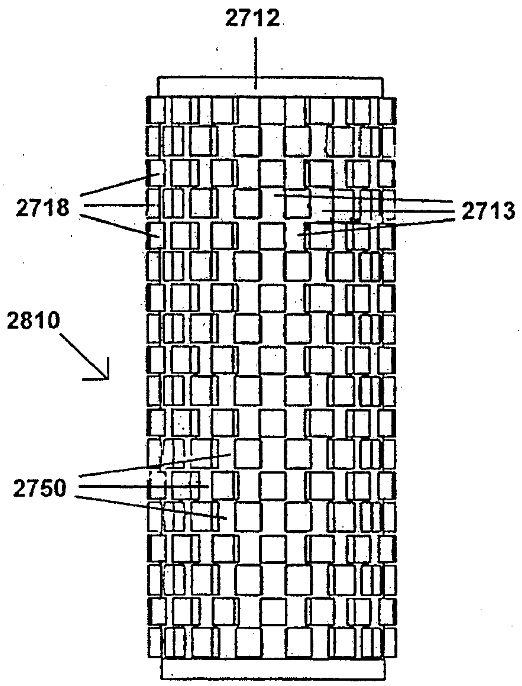

FIG. 28

REFERENCES CITED IN THE DESCRIPTION

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- US 4154690 A **[0007]**
- EP 1005910 A **[0007]**
- US 6197523 B **[0041]**

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专利名称(译)	Buffy涂层管和浮子系统和方法		
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申请号	EP2003799393	申请日	2003-10-02
[标]申请(专利权)人(译)	巴特勒记忆研究所		
申请(专利权)人(译)	巴特尔纪念研究所		
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IPC分类号	B01L3/00 B01L3/14 G01N33/49 G01N33/53		
CPC分类号	B01L3/50215 B01L3/505 B01L2400/0409 G01N33/49 Y10S435/81 Y10S436/81		
优先权	10/263975 2002-10-03 US		
其他公开文献	EP1546720A4 EP1546720B1		
外部链接	Espacenet		

摘要(译)

本发明提供了一种在管浮系统中使用的血沉棕黄浮体，其用于血沉棕黄层的分离和轴向膨胀。该系统包括透明或半透明的柔性样品管（130）和刚性分离器浮子（110），其比重在红血球和血浆的比重之间。样品管具有细长的侧壁（136），该侧壁具有第一横截面内径。浮子包括主体部分和从主体部分突出以接合并支撑样品管的侧壁的一个或多个支撑构件。当例如通过离心使样本管膨胀时，浮子的主体部分和支撑构件的横截面直径小于管（138）的第一横截面内径的横截面直径。浮子的主体部分与侧壁的轴向对准部分一起在它们之间限定了环形容积。从浮子的主体部分突出的支撑构件横越所述环形体积以产生一个或多个分析区域。在离心过程中，离心力使管的直径增大，以允许浮子在管中基于密度的轴向运动。此后，减小离心力以使管侧壁返回其第一直径，从而捕获浮子并将血沉棕黄层成分捕获在分析区域中。