(19) DANMARK

(10) **DK/EP 1816461 T3**

Patent- og Varemærkestyrelsen

(12) Oversættelse af europæisk patentskrift

---

(51) Int.Cl.: ***G 01 N 1/31 (2006.01)*** ***A 01 N 1/00 (2006.01)*** ***B 01 L 3/14 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2020-04-14**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-01-08**

(86) Europæisk ansøgning nr.: **07008752.3**

(86) Europæisk indleveringsdag: **2003-10-16**

(87) Den europæiske ansøgnings publiceringsdag: **2007-08-08**

(30) Prioritet: **2002-10-16 US 418978 P**

(62) Stamansøgningsnr: **03256535.0**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT RO SE SI SK TR**

(73) Patenthaver: **Streck Laboratories, Inc., 7002 South 109th Street, La Vista, NE 68128, USA**

(72) Opfinder: **Ryan, Wayne L., 1606 S. 187 Circle, Omaha, Nebraska 68130, USA**

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **Fremgangsmåde og indretning til indsamling og sikring af celler til brug for analyse**

(56) Fremdragne publikationer:
US-A- 4 675 159
US-A- 5 260 048
US-A- 5 618 664
US-A- 5 849 517
US-A- 5 977 153

DESCRIPTION

[0001] The present invention relates to a method of preparing and the use for stabilising a sample of whole blood, epithelial or spinal fluid cells.

[0002] In biological and biochemical analysis, and related arts, it is often necessary to collect and preserve biological tissues (i.e., cells and cellular components), for useful periods of time. The collected and preserved cells are often utilized-in a wide variety of applications, including but not limited to instructional aids and the diagnosis-and treatment of diseases. For example, such cells are often utilized in histological, cytological, immunological, and proteinaceous studies and the like.

[0003] Various methods are known in the art for analyzing histological, cytological, immunological, and proteinaceous materials. For example, surface marker analysis has developed as a laboratory tool, which is particularly useful for clinical diagnosis through the investigation of immunodeficiency states, differentiation of cell types and development stages, and other cell processes. The expansion of uses for surface marker analysis has resulted in the use of flow cytometry and antibody probes to evaluate cellular properties. While other means of assaying for surface marker analysis exist, flow cytometry provides rapid, objective and quantitative assessment of surface markers. Furthermore, even though the microscope is still the conventional means for examining preserved and stained biological materials, biological materials may also be examined with a flow cytometer. The flow cytometer is an important method for examining a plurality of cells in a brief time.

[0004] Flow cytometry and flow cytometers are generally described in Keran's text, Flow cytometry in Clinical Diagnosis (1989). Flow cytometers operate in principle by multiparameter analysis of heterogeneous cell populations (or cellular components) on a cell-by-cell basis. Flow cytometry allows biological and physical properties of cells and cellular components to be determined. In flow cytometry, cells in suspension are forced single file, via hydrodynamic focusing, through the path of a laser beam. Interaction of the cells with the laser beam scatters some of the light and causes excitation and emission from fluorescent molecules present on the surface or interior of the cell. A series of lenses collect the scattered or emitted light and pass it to a photomultiplier. Each photomultiplier measures a separate parameter. Parameters measured include: forward light scatter, which measures relative particle size; side light scatter, which measures relative granularity or other internal structure; and fluorescence emission. The optical signals are converted by a computer to a data display for analysis and interpretation. Cells collected and preserved using conventional methods and instruments generally require further dilution and/or treatment before they can be analyzed by flow cytometry. Thus, it is desirable in the art to obtain a method and a collection device that allow the cells to be directly analyzed by flow cytometry without further dilution and/or treatment. (There is need for a method to collect and transport human blood specimens for flow cytometric analysis. Current methods are inadequate in that the samples have to be analyzed soon after collection.)

[0005] The primary objective of tissue preservation is to provide as much structural detail of cells and components thereof as possible. To do this, it is necessary to maintain the cells in their original unaltered morphology so that maximum cellular detail may be observed. With the clinical application of immunostaining, there is also the requirement that antigens are not altered by the method of preservation. Thus, it is desirable in the art to obtain a method and a collection device that maintain the cells in their original unaltered morphology and preserve their antigenic sites.

[0006] The usual formulations for preservation of cells contain one or more agents, which react vigorously with the proteins of the cells to denature and insolubilize the components of the cell. Typical of this type of agent is picric acid, mercuric ions, formaldehyde and glutaraldehyde. In addition, some less toxic compounds can also be utilized which denature and stabilize the proteins such as acetic and formic acid. Unfortunately, the toxicity associated with such compounds renders their use less than satisfactory. For example, a 37% solution of formaldehyde, the most common of these fixatives, is a noxious gas which is also toxic, flammable, and carcinogenic. Although efforts are made when this chemical is used to protect workers and avoid contamination of the drainage system when disposed, these efforts are usually both expensive and inconvenient, and fixatives such as formaldehyde still present a danger to laboratory workers and health care professionals. Thus, it is highly desirable to develop a method and a collection device, which can preserve the cells in a low toxicity and non-flammable environment so that it can be used safely, effectively and conveniently in histological and other studies.

[0007] From US5849517 A a method and composition for fixing and stabilizing tissues, cells, and cell components such that the antigenic sites and nucleic acids are preserved is provided. The fixative is selected from the group consisting of imidazolidinyl urea, diazolidinyl urea, and mixtures thereof.

[0008] For even easier handling, it is also desirable to develop a method and a collection device that allow transportation (e.g., from the collection site to the analysis site) of the cells in ambient temperature. This problem is solved by the subject-matter of claims 1-5.

[0009] The present application addresses many of the challenges encountered when using conventional methods and instruments to collect and preserve cells by providing a method and a collection device that are capable of maintaining the cells in their original unaltered morphology; preserving the cell antigenic sites; and allowing the cells to be transported at ambient temperature, to be handled in a low toxicity and non-flammable environment, and to be directly analyzed by flow cytometry without further dilution and/or treatment. The claimed subject matter more specifically relates to a method and a device that allow cells (e.g., whole blood, epithelial cells, spinal fluid, and the like.) to be collected and preserved for analysis and addresses many of the challenges encountered when using conventional methods and instruments. Specifically, the claimed subject matter describes a method and a collection device that (1) use a less toxic and non-flammable reagent for fixing and stabilizing cells; (2) allow the cells to stay in their original unaltered morphology; (3) allow the cell antigenic sites to

be preserved for a useful period of time; (4) allow the cells to be transported at ambient temperature; and/or (5) allow the cells to be directly analyzed by flow cytometry without further dilution and/or treatment.

[0010] The present application includes a device to collect and preserve cells comprising of: (1) a collection container comprised of a tube having an open end and a closed end, a closure in the open end of the tube, a vacuum drawn to a predetermined level inside the container; and (2) compounds including an anticoagulant agent and a fixative agent selected from the group consisting of: diazolidinyl urea, imidazolidinyl urea, dimethoylol-5,5-dimethylhydantoin, dimethylol urea, 2-bromo-2-nitropropane-1,3-diol, oxazolidines, sodium hydroxymethyl glycinate, 5-hydroxymethoxymethyl-1-laza-3, 7-dioxabicyclo[3.3.0]octane, 5-hydroxymethyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, 5-hydroxypoly[methyleneoxy]methyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, quaternary adamantine and combinations thereof. The claimed subject matter may optionally include polyacrylic acid or a suitable acid having a pH ranging from about one to about seven inside the tube. The compounds of the device must be in a sufficient amount to preserve the collected cells' original morphology and antigenic sites without significant dilution of the cells (i.e., in a volume that is not clinically significant), and thereby allowing the cells, stored with the compounds, to be directly analyzed by a flow cytometer.

[0011] The present application also includes a method comprised of (1) providing a tube with an open end and a closed end, (2) preloading the tube with compounds including: an anticoagulant agent, a fixative agent selected from the group consisting of: diazolidinyl urea, imidazolidinyl urea, dimethoylol-5,5-dimethylhydantoin, dimethylol urea, 2-bromo-2-nitropropane-1,3-diol, oxazolidines, sodium hydroxymethyl glycinate, 5-hydroxymethoxymethyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, 5-hydroxymethyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, 5-hydroxypoly[methyleneoxy]methyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, and quaternary adamantine, and optionally a polyacrylic acid or a suitable acid having a pH ranging from about one to about seven, wherein the compounds are in a sufficient amount to preserve the collected cells' original morphology and antigenic sites without significant dilution of the cells, and thereby allowing the cells, stored with the compounds; to be directly analyzed by a flow cytometer; inserting a closure into the open end of the tube; and drawing a vacuum to a predetermined level inside the tube.

[0012] The method and device of the present application may also optionally include other art-disclosed components conventionally used in cell collection and analysis such as gauze, glove, tourniquet, lancet, needle, test strip (e.g., immunoassay), alcohol swab, tube holder, additional cell collection tubes (with or without conventional cell analysis additives inside these tubes), adhesive strip, syringe, glass or plastic strip, packaging means to store the desired components and the device, and packaging means to transport at least the collected and preserved cells stored in the device. The method of the claimed subject matter may also optionally include additional art disclosed methods and instruments used for cell analysis such as a flow cytometer, a hematology analyzer, and other hematology instruments, etc.

[0013] Embodiments of the present invention will now be described, by way of examples only have reference to the accompanying drawings, in which:

FIG. 1. A cross-sectional illustration of an exemplary embodiment of the collection device of the claimed subject matter; and

FIG. 2 A flow diagram illustrating a method for making the collection device illustrated in the FIG. 1.

[0014] Turning now to the drawings, FIG. 1 shows a cross-sectional illustration of a device 100 that incorporates a preferred embodiment of this claimed subject matter and can be used to collect and preserve biological tissues such as cells and cellular components for analysis. The device 100 is particularly useful in the collection of whole blood, but can be use to collect other types of bodily fluids and/or biological tissues (e.g., epithelial cells, bone marrow, spinal fluid and the like) including, without limitation, abnormal tissue samples such as leukemias, cancer tissue cancer, and the like as long as the tissue samples-can be transformed into a cellular suspension.

[0015] The device 100 includes an evacuated collection container 10 comprised of (1) a tube 12 having an open end 14 and a closed end 16; a closure (e.g., stopper) 18 in the open end of the tube 12, and a predetermined level of vacuum (not shown) inside the container 10. It is preferred that the tube 12 is made of a transparent material such as glass or plastic for better visibility. It is also preferred that the tube 12 has an interior surface that is sterile and resists adherence to the cells 20 (not shown) during collection, storage, and analysis. The closure 18 is preferably puncturable by a needle and resealable allowing easy transfer of the cells 20 (e.g., the cells 20 from its host to the container 10 and from the container 10 to another substrate if desired). It is also preferred that the closure 18 and the tube 12 together form a seal capable of maintaining a pressure differential between atmospheric pressure and a pressure less than atmospheric pressure within the tube 12.

[0016] The size of the container 10 is not narrowly critical and is dependent upon the cell sample volume that is desired to be collected and preserved. For example, a typical size for the container 10 may have an internal volume of between 100µl to 10 ml. The container 10 can be constructed using art-disclosed methods and is commercially available (e.g., Vacutainer Plus Plastic Tubes with Hemogard Closure available from Becton Dickinson and Company located in Franklin Lakes, New Jersey; the evacuated sample collection tube described in U.S. Patent No. 5,860,937, which is incorporated by reference). Of course, it should be understood that a wide range of changes and modifications can be made to the preferred embodiment described above for the container 10.

[0017] For preservation (e.g., fixation, stabilization and the like) of the cells 20, the device 100 further includes compounds 22 including an anticoagulant agent 24, a fixative agent 26 selected from the group consisting of: diazolidinyl urea, imidazolidinyl urea, dimethoylol-5,5-

dimethylhydantoin, dimethylol urea, 2-bromo-2-nitropropane-1,3-diol, oxaizolidines, sodium hydroxymethyl glycinate, 5-hydroxymethoxymethyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, 5-hydroxymethyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, 5-hydroxypoly[methyleneoxy]methyl-1-laza-3,7-dioxabicyclo[3.3.0]octane, and quaternary adamantine, and optionally a polyacrylic acid 28 or any suitable acid having a pH ranging from about one to about seven, wherein the compounds are in a sufficient amount to preserve the collected cells' original morphology and antigenic sites without significant dilution of the cells 20, and thereby allowing the cells 20, stored with the compounds 22, to be directly analyzed by a flow cytometer. It is preferred that the compounds 22 have been sterilized (e.g., by sterilizing filtration).

[0018] A suitable amount of any art-disclosed anticoagulant agent such as ethylene diamine tetra acetic acid (EDT A) and its salts, ethylene glycol tetra acetic acid (EGTA) and its salts, hirudin, heparin, citric acid, salts of citric acid, oxalic acid, salts of oxalic acid, or mixtures thereof may be used. A preferred anticoagulant agent 24 is K_3EDTA. The suitable amount of the anticoagulant agent 24 for the claimed subject matter is that effective to prevent coagulation of the cells 20 (e.g., whole blood) without causing significant dilution of the cells 20 (i.e., not clinically significant), and thereby allowing the cells 20, stored with the compounds 22, to be directly analyzed by a flow cytometer). For example, in a preferred embodiment, K_3EDTA is the anticoagulant agent 24 and its concentration weight/volume is preferably less than about 0.3 g/ml, more preferably less than about 0.2 g/ml, and most preferably about less than about 0.15 g/ml.

[0019] The preferred fixative agent 26 is a heterocyclic urea (e.g., diazolidinyl urea (known as DU), imidazolidinyl urea (known as IDU) or a mixture thereof). The most preferred fixative agent is diazolidinyl urea. The suitable amount of the fixative agent 26 for-the claimed subject matter is that effective to fix or stabilize the cells 20 without causing significant dilution of the cells 20 (i.e., not clinically significant), and thereby allowing the cells 20, stored with the compounds 22, to be directly analyzed by a flow cytometer. For example, in a preferred embodiment, diazolidinyl urea is the fixative agent 26 and its concentration weight/volume is preferably about less than about 1 g/ml, more preferably less than about 0.75 g/ml, and most preferably less than about 0.5 g/ml. (concentration of solution of DU before blood sample is added.)

[0020] It is known that the acid 28 may rise signal to noise ratio during flow cytometry; and therefore, the acid 28 may be optionally added as one of the compounds 22 in the device 100. The preferred acid 28 is a polycarboxylic acid, and more preferably a polyacrylic acid with a molecular weight of 5,000. The suitable amount of the acid 28 for the claimed subject matter is that effective to rise signal to noise ratio during flow cytometry but without causing significant dilution to the cells 20 (i.e., not clinically significant) so that the cells 20, stored with the compounds 22, can be directly analyzed by a flow cytometry. For example, in a preferred embodiment, polyacrylic acid with a molecular weight of 5,000 is included in the container 10.

[0021] Additional compounds may optionally be added as one of the compounds 22 in the device 100. Such additional and optional compounds may include: cell permeabilizing agents

for substantially gaining access to intracellular analytes/epitopes and7or for lysing red blood cells; proteins that substantially protect the cells during processing and/or substantially reduce non-specific binding of probes; serum/lipoproteins that substantially protect cells during processing and/or substantially reduce non-specific binding of probes; RNAse inhibitors which substantially inhibit digestion of RNA and/or substantially maintain RNA integrity; nucleic acid stabilizers which substantially inhibit the degradation of nucleic acids and nucleic acid containing compounds; amino acids / polypeptides which substantially enhance binding of probes/antibodies to epitopes and/or substantially increases the observable signal; fixatives which substantially preserve cell integrity especially for permeabilization agents, and may preserve some epitopes; anticoagulants which substantially decreases clotting of red blood cells, chelates calcium and / or may help maintain WBC integrity/viability; protease inhibitors which substantially decreases degradation of protein epitopes; antioxidants/ reducing agents which substantially prevent hemolysis of red blood cells and/ or substantially prevent oxidation of peptides, and/ or substantially maintain epitopes; nucleic acid dyes that generally serve to label/identify nucleic acid; carbohydrates which substantially maintain cellular integrity and/or osmolarity; and, polyacrylic acids which substantially enhance the binding of probes and/or antibodies to epitopes; and /or substantially increases signal. One of skill in the art should be able to determine the usefulness and quantities of such optional compounds by routine testing and knowledge of the art. Within multiple specific embodiments the above additional and optional compounds may be more specifically include: Cell permeabilizing agents such as: DMSO (Dimethyl Sulfoxide), Ethylene glycol, Polyethylene glycol, Glycerin, Cellosolves (ethylene glycol dimethyl ether) (phenoxyethanol), Triton X 100, Triton X 705 (non-ionic detergents), 1-methyl-2-pyrrolidinone, Tween 20, Tween 40 (non-ionic), Brij 35 (detergent), Polyoxyethylene ether (Polyox), Sodium cholate, Ethylene oxide polymers, Monensin, Monactin, Pentachlorophenol, 2,4 dinitrophenol, saponin, SDS (sodium dodecyl sulfate); Proteins such as: Biotin, Albumins (egg, bovine), Gelatin, and similar such compounds as should be known to one of skill in the art; RNAse inhibitors such as: human placenta derived RNAse inhibitor, and similar such compounds should be known to one of skill in the art; Nucleic acid stabilizers such as: Guanidinium hydrochloride, Polycations such as Polyethylenimine), and similar such compounds as should be known to one of skill in the art; Amino acids/polypeptides such as: Glutamic acid, Glycine, Aspartic acid, and similar such compounds as should be known to one of skill in the art; Fixatives such as: Aldehydes (formaldehyde and glutaraldehyde), Alcohols (ethanol, methanol), and similar such compounds as should be known to one of skill in the art; Anticoagulants such as: EDTA (Ethylene Diamine Tetra acetic acid.), and similar such compounds as should be known to one of skill in the art; ACD (Acid Citrate Dextrose), Heparin; and similar such compounds as should be known to one of skill in the art; Protease -Inhibitors such as: EDTA, PMSF (phenyl methyl sulfonyl fluoride), AEBSF (2-Aminoethyl benzene sulfonyl fluoride), and similar such compounds as should be known to one of skill in the art; Antioxidants/ Reducing agents such as: Trolox, a-tocopherol, B-mercaptoethanol, and similar such compounds as should be known to one of skill in the art; Nucleic Acid Dyes such as: DAPI (Diamidino 2-phenylindole), Propidium Iodide, Fluorescein diacetate, and similar such compounds as should be known to one of skill in the art; Carbohydrates such as: Sugars (sucrose), cellulose, and similar such compounds as should be known to one of skill in the art. It should be appreciated that the above specific listings of

compounds may contain a measure of overlap, which recognizes the sometimes-overlapping function of certain specific compounds. One of skill in the art should understand and appreciate this aspect of the disclosure.

[0022] The claimed subject matter allows the final composition 30 to be transported in ambient temperature. Thereafter, it is preferred that the final composition 30 be stored at temperature less than about 40°C. The cells 20 stored in the final composition 30 have more than about 3 days, preferably more than about 5 days, more preferably more than about 7 days stability. The claimed subject matter allows the cells 20 stored in the final composition 30 to be directly analyzed by a flow cytometer without further dilution and/or treatment because the compounds 22 can preserve the cells 20 without significantly diluting the cells 20. Any significant dilution of the cells 20 is likely to cause error in flow cytometry measurements (e.g., lowering the lymphocytes' count). To avoid significant dilution, the compounds 22 (comprising of the anticoagulant agent 24, the fixative agent 26, and optionally, the acid 28) are in concentrated forms, preferably in a ratio with the final composition 30 that is less than about 2:100, more preferably less than about 1.5:100, and most preferably less than about 1:100.

[0023] The device 100 may be included in a kit of the claimed subject matter containing components 32 (not shown) conventionally used to collect and analyze the cells 20 such as alcohol swab, gauze, tube holder, tourniquet, glove, other cell collection tube (with or without conventional cell analysis additives inside such tube), needle (with hub, part of a syringe assembly including barrel and plunger, or with wings connected via a hub and tubing to another needle for delivery to the device 100 or other collection tubes), lancet, adhesive strip, syringe, test strip (allowing the cells 20 to flow directly onto a glass or plastic strip containing reagents for cell analysis), glass or plastic strip containing reagents for cell analysis (e.g., immunoassay), packaging means (e.g., plastic bag, compartmentalized plastic enclosure, and the like) to store the desired components 32 and the device 100, and packaging means to transport the cells 20 stored in the device 100 after collection. It is preferred that the packaging means and any other components 32 that may become in physical contact with the cells 20 be sterilized and the packaging means is constructed to maintain this sterile environment.

[0024] Unlike the typical histological fixing agents, the fixative agent 26 of the claimed subject matter has extremely low toxicity. For example, toxicity studies comparing diazolidinyl urea with formaldehyde of the prior art show the following:

	INHALATION TOXICITY	DERMAL TOXICITY	LD50
Formaldehyde	500 mg/kg	270 mg/kg	800 mg/kg
Diazolidinyl Urea	None	2000 mg/kg	2570 mg/kg

[0025] This reduced toxicity makes disposal and handling less of a problem. In addition, since there is no inhalation toxicity, there are no badge detection devices required as there are for formaldehyde.

[0026] Another advantage offered by the fixative agent 26 is the fact that it is not flammable and therefore does not present a fire hazard as do many of the prior art fixative agents.

[0027] The mechanism by which the fixative agent 26 provides the desired tissue and cell membrane stabilization is not known for certain. It is believed that the fixative agent binds in some fashion to the cell membrane or tissue. This hypothesis is drawn because many members of the active agent are known disinfectants, which kill bacteria by binding to cell structure. This is not a full explanation of the mechanism responsible for the results of the claimed subject matter since many other disinfectants such as KATHON and OMADINE fail to provide tissue and cell stabilizing effects.

[0028] The ability of the fixative agent 26 to preserve antigenic sites is also not understood but it is probably due to a difference in the reaction between proteins and the fixative agent 26 compared to prior art preservatives such as formaldehyde-Formaldehyde cross-links with itself and proteins to obscure the antigen. To determine if this is true, diazolidinyl urea was added to the protein, albumin. After incubation of the diazolidinyl urea and protein mixture for 24 hours, disc-gel electrophoresis indicated no change in the rate of migration of the protein. When this experiment is conducted with formaldehyde, a large number of insoluble proteins result and the electrophoretic migration is altered.

[0029] Referring to FIG. 2, a method of making the device 100 of the claimed subject matter is comprised of providing the tube 12 having the open end 14 and the closed end 16 (202). It is preferred that the tube 12 is sterile. The method is further comprised of preloading (i.e., introducing) the compounds 22 comprising of the anticoagulant agent 24, the fixative agent 26, and optionally the acid 28 into the tube 12 using art-disclosed methods (204). The types and amounts of the anticoagulant agent 24, the fixative agent 26, and optionally, the acid 28 including the ratio between the compounds 22 and the final composition 30 are the same as described above for the device 100 of the claimed subject matter. It is preferred the compounds have been sterilized (e.g., by sterile filtration). The method of the preloading step 204 may optionally include freeze drying the compounds in the tube 12. The method 200 further includes inserting the closure 18 into the open end 14 of the tube 12 (206). The method 200 further includes drawing a vacuum inside the tube 12 to a predetermined level (208) using art-disclosed methods. The amount of vacuum to be drawn is dependent upon the nature and volume of the cells desired to be collected and preserved. For example, for whole blood collection, the vacuum should be drawn to a level that allows the pressure of the whole blood to cause it to flow into and fill the tube 12 to the desired level. The method 200 may optionally include providing the components 32 conventionally used to collect and analyze the cells 20. The components 32 are the same as for the device 100 of the claimed subject matter as described above.

[0030] The method may also optionally include collecting the cells 20 using art-disclosed methods (e.g., venipuncture, use of a lancet, etc.). It may optionally include screening the cells 20 using art-disclosed instruments such as flow cytometers (eg., FACScan, FACSCalibur by BD and EPICS by Beckman Coulter), other hematology instruments (e.g., H3 by Bayer

Corporation, the Beckman Coulter STKS or Gen-S Systems, the Abbott Cell-Dyn 4000 Hematology System, Bayer ADVIA 120 System, the Sysmex XE2100 System, and the like. The screening of the cells may be for any purpose including, without limitation, for HIV, HPV, hepatitis, leukemia, cancer, and the like; other art-disclosed screening such as immunoassay, AIDS panel, and the like; and screening by methods disclosed in commonly owned United States Patent No. 4,788,139 (Ryan) titled "Platelet aggregation reagent, reagent container and method of determining platelet aggregation in EDTA-anticoagulated blood", which is hereby incorporated by reference. Cells 20 collected and preserved using the claimed subject matter may undergo histological study in any known conventional manner, such as through the use of paraffin sectioning equipment, staining, mounting on slides, or other common steps utilized prior to microscopic or other examination. The claimed subject matter thus provides a safe, convenient, and effective solution to collect and preserve cells for analysis.

[0031] It should be noted that the claimed subject matter may be used by those skilled in the art to preserve antigenic sites on or within cells (or components thereof) derived from any source including normal blood, bone marrow, lymph, or solid tissues, or may be derived from abnormal tissues such as leukemias or solid tissue cancers. The claimed subject matter may also be utilized with any cellular component or biological material that has at least one antigenic site.

[0032] It should be noted that in preferred embodiments of the claimed subject matter cell clumping is prevented, light scattering properties are preserved, antigenic sites are preserved, and nucleic acids may be analyzed.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US5849517A [0007]
- US5860937A [0016]
- US4788139A [0030]

Non-patent literature cited in the description

- Keran's text, Flow cytometry in Clinical Diagnosis, 1989, [0004]

Patentkrav

1. Anvendelse til stabilisering i et rør af en prøve af fuldblod, epithel- eller spinalvæskeceller, der suges ind i røret med henblik på en efterfølgende analyse af højst 2 dele af en blanding af et antikoaguleringsmiddel og et fikseringsmiddel pr. 100 dele af prøven, antikoaguleringsmiddel og fikseringsmiddel, hvor fikseringsmidlet (26) er udvalgt fra gruppen bestående af diazolidinyl-urinstof og imidazolidinyl-urinstof, og antikoaguleringsmidlet omfatter ethylendiamintetra-eddikesyre eller salte deraf.

2. Anvendelse ifølge krav 1, hvor mængden af antikoaguleringsmidlet er en sådan, at den tilvejebringer mindre end ca. 0,3 g/ml.

3. Anvendelse ifølge krav 1 eller krav 2, hvor mængden af fikseringsmidlet er en sådan, at den tilvejebringer mindre end ca. 1 g/ml.

4. Anvendelse ifølge et af kravene 1 til 3, hvor sammensætningen indeholder en polyacrylsyre.

5. Fremgangsmåde til fremstilling af fuldblod, epithel- eller spinalvæskeceller til analyser omfattende at suge en celleprøve under vakuum ind i et rør, hvor røret indeholder en stabiliseringssammensætning omfattende en blanding af et fikseringsmiddel og et antikoaguleringsmiddel, hvor fikseringsmidlet er udvalgt blandt diazolidinyl-urinstof og imidazolindyl-urinstof, og antikoaguleringsmidlet er udvalgt blandt ethylendiamintetra-eddikesyre og salte deraf, hvor mængden af stabiliseringssammensætningen inden i røret tilvejebringer mindre end 2 dele pr. 100 dele af prøven, antikoaguleringsmiddel og fikseringsmiddel, når prøven suges ind i røret.

6. Fremgangsmåde ifølge krav 5, hvor stabiliseringssammensætningen omfatter glycin.

7. Fremgangsmåde ifølge krav 5 eller krav 6, hvor cellerne transporteres til analyse ved omgivelsestemperatur.

8. Fremgangsmåde ifølge et af kravene 5 til 7, omfattende at transportere celleprøven ved omgivelsestemperatur med henblik på at analysere den direkte ved hjælp af flowcytometri uden yderligere fortynding eller behandling.

9. Fremgangsmåde ifølge et af kravene 5 til 8, hvor koncentrationen af diazolidinyl-urinstof er mindre end 1 g/ml.

10. Fremgangsmåde ifølge et af kravene 5 til 9, hvor antikoaguleringsmidlet er K_3EDTA, og koncentrationen af antikoaguleringsmidlet er mindre end 0,3 g/ml.

11. Fremgangsmåde ifølge et af kravene 5 til 10, hvor fremgangsmåden omfatter screening af celleprøven med henblik på analyse for kræft i fast væv.

12. Fremgangsmåde ifølge et af kravene 5 til 11, hvor stabiliseringssammensætningen inden i røret tilvejebringer mindre end 1 del pr. 100 dele af prøven, antikoaguleringsmiddel og fikseringsmiddel.

13. Fremgangsmåde ifølge et af kravene 5 til 12, omfattende at suge celleprøven ind ved hjælp af venepunktur.

14. Fremgangsmåde ifølge et af kravene 5 til 13, hvor røret er af transparent plast.

DRAWINGS

FIG. 1

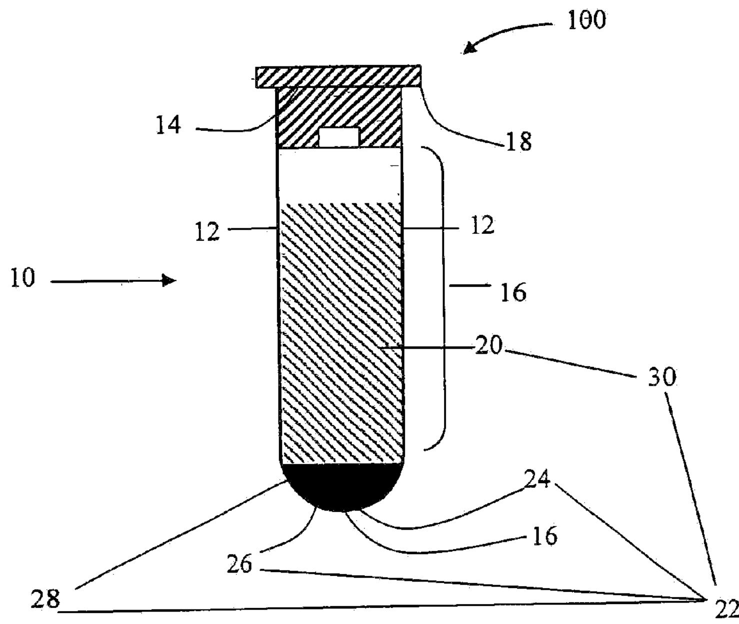

FIG. 2