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EP 1221342 A2 **EP 0588964 A1**
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US 5236604 A **US 4722790 A**
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(54) Abstract Title: **Cell separation device**

(57) The present invention relates to a sample handling device and in particular to a flexible cell processing device, such as a plastic bag with attached cell specific ligands (antibodies, proteins, aptamers etc.) for the purpose of processing specific cells, such as stem cells. Also shown is a method for separation, comprising the following steps: a) suspending a sample in suspending media, b) sampling the sample in a device according to the invention, c) allowing specific substances in said sample to interact with the affinity ligands and bind thereto; and d) separating the remainder of the sample from the bound substances. Optionally the bound substances are detached from the sample handling device. The sample handling device may be centrifuged and the sample suspending media may be density gradient media.

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SAMPLE HANDLING DEVICE

Field of the invention

The present invention relates to a sample handling device. In a preferred embodiment, the invention relates to a cell processing device, such as a plastic bag with attached cell specific ligands for the purpose of processing specific cells, such as stem cells.

Background of the invention

Stem cells are immature subpopulations of cells that have the potential to differentiate into a wide variety of specialized cell types such as bone, muscle, pancreas, liver, or blood cells. These undifferentiated cells have the ability of self-renewal which preserves their continuous supply. Embryonic stem cells (ESCs) are commonly derived from 4- to 5-day-old embryos. At this stage, the embryos are spherical and are known as blastocysts. Each blastocyst consists of 50 to 150 cells and includes three structures: an outer layer of cells, a fluid-filled cavity, and a group of about 30 pluripotent cells at one end of the cavity. This latter group of cells called the inner cell mass, form all the cells of the body. Adult stem cells on the other hand are undifferentiated cells that are found in small numbers in most adult tissues. They are also found in humans of other ages and can be extracted from umbilical cord blood. The primary roles of adult stem cells in the body appear to be to maintain and repair the tissues in which they are found. They are usually thought of as multipotent cells, giving rise to a closely related family of cells within the tissue. An example is haematopoietic stem cells, which form all the various cells in the blood. Pluripotent haematopoietic stem cells are currently of interest in research, as they can differentiate into neurons, glia, skeletal muscle cells, heart muscle cells, and liver cells. A variety of other stem or progenitor cells are now known and most would appear to offer promise for use in various cellular based therapies. In this they match several other cell types (e. g. lymphocytes, dendritic cells) which in a native or genetically modified form also hold promise as regards cell therapeutic agents and products.

Blood from the placenta and umbilical cord, which are left over after birth, is a rich source of haematopoietic stem cells. As noted above so-called umbilical cord stem cells have been shown to be able to differentiate into bone cells and neurons, as well as the cells lining the inside of blood vessels.

In cell therapy, it is often necessary to both purify and concentrate subpopulations of cells in a sample. In some stem cell therapies there is a need to use adult stem cells from the patient to be

treated, and to expand said cells in culture, treat them to differentiate into the desired cells, and then to reintroduce them into the patient. The use of the patient's own homologous cells for transplantation will reduce the possibility that they might be rejected by the immune system. However such samples might contain cancerous or other cells that should be removed before the sample can be of further use. Of course heterologous transplantation is also of great value if there is correct matching of transplanted cells with the host. This is analogous to the everyday transplantation of blood (cells) into a person during a blood transplant. In the latter case it can be particularly important to remove certain (lymphocyte subclass) or other cell types that can lead to complications such as graft versus host disease.

Methods for separating cells may be divided into three basic types. The first involves methods where separation is dependent on cell physical properties such as density and size. This includes methods based on differential migration in a fluid flow (field flow fractionation) or in sedimentation field (centrifugation) or in combination (elutriation). Differential sedimentation is often performed in combination with solution or hydrogels of varying inherent densities, either in discontinuous or continuous (gradient) form.

The second type involves methods where separation is dependent on cell surface properties such as unit surface charge (electrophoresis), interfacial free energy (phase partition) or presence of specific.surface molecular groups (which interact with various affinity substances). Examples of the latter include affinity substances such as monoclonal antibodies to cell surface receptors or other determinants. Such surface groups are often related to cells' physiological state and function. Thus affinity methods can be used to both separate a subpopulation of cells and provide information about their function. In many applications specific cell types are isolated on the basis of density based methods (e. g. "white" blood cells) followed by affinity methods (cell subtypes such as B and T lymphocytes). Affinity methods can involve either negative selection (ligand interaction to remove an unwanted cell subpopulation) or positive selection (ligand interaction with "target" cells). Negative selection might also be used to remove other unwanted substances, some of which may occur in relatively small but still practically significant concentrations (e. g. bacterial cells, toxins).

The third type of separation includes methods utilising a combination of properties – typically both physical and surface properties. For example density gradient media used to isolate white blood cells (WBCs) can be combined with addition of dense microparticles coated with antibodies which recognize a desired or undesired subpopulation of white blood cells. Thus one might get a density based separation of WBC sample depleted of some specific WBCs.

Cell samples, such as blood samples, may be obtained in or put into bags such as standard blood bags. For sterility and other reasons it is desirable to undertake as little sample exposure as possible. Therefore it is advantageous that sample handling, such as centrifugation based separation as described above, would be conducted in the same bags. Two examples of cell separation devices which function on the basis of centrifugation, and are specialised with regard to isolation of stem cell enriched subpopulations, are the AXP™ device from Thermogenesis and the Sepax system from Biosafe SA. These two systems are based on density based cell separation (and washing). In these approaches the bags used to process the cell samples are in one sense just sample holders of appropriate geometry which provide a closed sterile environment. However cell to bag (and bag related tube) surface to surface interactions (such as affinity interactions) are not a significant or operator controlled factor in the above separations. In fact the opposite is true. Bag materials are typically chosen to offer little nonspecific adsorption of cells.

US 5 236 604 describes a medical apparatus for separating and partitioning a blood sample into lighter and heavier phases, wherein affinity means are operatively associated with a container of the device. The affinity means has affinity for material interfering with the sampling of the separated sample and has no affinity for the cells.

A combination of density gradient media and affinity media for cell separation has been described, for example in US 5 474 867. However in this case the affinity interacting substances are in solution or in suspension in solution.

There presently exists no simple device using a combination of multiple separation approaches, or handling and isolation functions. Especially there is a need of an approach which involves safe, disposable, proven medical products such as sterile (blood) fluid handling bags.

Future clinical use of human stem cells faces several roadblocks which require technical solutions and products. These include the need to selectively isolate, culture, bank, plus stimulate or modify various cells including immune cells plus stem and related progenitor cells. User friendly approaches which combine functions are especially desirable in research and clinical settings.

Summary of the invention

The present invention relates to attaching ligands to the surfaces of cell handling bags (and related tubes) so as to achieve desirable effects related to isolation of targeted cells by affinity interactions. Such interactions may be related to target cell enrichment or removal of unwanted cells or other substances, for example bacteria. The ligands may be based on antibody or other ligands, and could be related to positive or negative selection. According to the invention, bags and related tubes are not only simple sample holders and conduits but converted to participants in cell sample selection and processing.

Separation can be either positive (interacting with target cells) or negative. Negative selection is used in cases when it is desired to remove specific cells (e. g. cancer cells, or activated lymphocytes, or PMN cells) from a sample prior to storage or use. For this reason it may often be advantageous to use methods which combine affinity and density based isolations as (a) sequential operations or (b) in the same operation.

For the purposes of the invention, the surface area afforded by the bags and related tubes has to provide the needed ligand density per bag (i. e. total number of ligands) necessary to handle a significant fraction of the total number of ligand targets. In many cases the desired target cells in a sample (e. g. stem cells in peripheral blood sample) or unwanted cells (e. g. bacteria or perhaps transplant rejection inducing WBCs in a cell transplant sample) may be only 1% of the total number of cells (or volume of cells) in the sample. Thus, there are several situations where the surface area of a bag and related tubes is sufficient. Note that in many applications (e. g. cancer cell removal) use of ligand coated bags and related tubes may be useful even if they only remove some of the unwanted cells, and further affinity or other cell separations are needed. That is because any advantage gained by the ligand coated bags will improve the efficiency of the overall process.

Thus, the invention provides a novel way of cell processing relying in part on the involved surface areas being suitable to handle ligand densities commensurate with the levels of ligand targets needed when processing cell and related particle samples.

In one embodiment density gradient media (DGM) is placed in the affinity-ligand provided device of the invention, such as user-friendly sterile bags similar to those used universally in various clinical settings. Such bags (and tubes), including those commonly used for blood storage and transfusion, may be treated with a coating which reduces blood cell and protein adsorption and alteration (e. g.

aggregation and formation of microclots). Such bags and coatings are for example made from polyvinylchloride (PVC) and similar polymer plastics and may be coated with a variety of silicone or other coatings to reduce nonspecific protein adsorption.

Thus in a first aspect, the invention relates to a sample handling device for separating one or more substance(s) from a sample, comprising a flexible container with affinity ligands attached on the inside of the container, wherein said affinity ligands are specific for said substance(s).

Preferably, the sample handling device is made of transparent polymer plastic such as PVC or other material commonly used in containers. For direct sampling and processing, the sample handling device is preferably a blood sampling bag. The sample may be suspended in any fluid which does not negatively affect the processing of sample.

The affinity ligands may be antibodies, fractions of antibodies, proteins, nucleic acids, aptamers, lectins, lipids or any other affinity substance directed against specific substance(s) in the sample.

In a preferred embodiment, the substances are human cells, including various native or altered (including recombinantly altered) immune system cells, or blood cells including platelets.

The specific cells may be stem cells, or cancer cells or other cells of specific medical interest in regard to disease pathology, treatment or diagnosis. If the cells are stem cells the affinity ligands might be directed against CD34, CD133 or other stem cell related surface antigens or molecules.

If the cells are platelets the affinity substances might be directed against platelet surface proteins or glycoproteins; or be substances which platelets have natural affinity for (e. g. platelets have affinity for fibrinogen/fibrin proteins) or various ligands (e. g. oligopeptides or glycopeptides) related to such substances.

The inner wall of the container is preferably provided with a coating comprising affinity ligands, such as affinity ligands attached to an underlying poly(ethylene glycol), polymer, responsive polymer, organosilane, plasma RF treatment, bi-reactive reagent, or otherwise generated underlying reactive coating.

In one embodiment, the container comprises inlet(s) provided with affinity ligands on the inside.

Optionally, the inner surface of the container and/or inlet(s) comprises baffles to enhance the surface area for ligand binding. The ligands on the baffles may be the same or different from those in the container and/or inlet(s).

Preferably, the container is adopted for centrifugation. For example, the container could be a centrifugation bag or any flexible container which may be centrifuged.

The affinity ligands on the inner surfaces of the bag and related conduits are coated either randomly or non-randomly, i.e. at specific locations, with one or more affinity substances.

The device could have any shape, such as a pouch, bag or tube, and separation efficiency, and could be improved by optimization of both container geometry, as well as the topographical location of ligands relative to chamber geometry. Such optimization would be expected to vary depending of the particular situation and associated apparatus such containers might be employed in. In case of a bag shape, bag and bag-holder geometry and flow valves act to enhance target cell isolation.

The substance may be any substance desired to enrich or remove from a sample. For example the substance may be a prokaryote or eukaryote cell, preferably a mammalian cell, cell organelle, bacteria, virus, protein, prion, peptide, nucleic acid, or toxin, such as endotoxin.

The sample may be any sample in which it is desired to enrich or remove one or more components, such as a blood sample.
In a second aspect, the invention relates to a method for separation, such as cell separation, comprising: a) suspending a sample in suspending media, b) sampling the sample in a device as described above, c) allowing specific substances in said sample to interact with the affinity ligands and bind thereto; and d) separating the remainder of the sample from the bound substances. The method may comprise a further step e) centrifugation before, during or after step c).

In a negative selection process, the bound substances are discarded while, in a positive selection process, the bound substances are recovered. Recovery could be effected in several ways including (a) having an inlet on the bag which allows washing with a buffer whose pH, tonicity, polarity or other physical properties cause release of bound target cells or other substances, (b) likewise washing with a buffer containing a competitive inhibitor of binding or similar added substance which effects release of bound cells or other substances, (c) use of more complex

methods such as tethering ligands to the bag surface via polymers which are responsive to pH, temperature, light, magnetic field, etc. Such polymers are well known in the general scientific literature.

Detailed description of the invention

According to the invention, affinity molecules are coupled to the inner surfaces of bags (and tubes) used when handling cells, optionally including centrifugally processing (e. g. blood or marrow) samples. This gives several advantages over standard approaches, including addition of (a) negative affinity selection capabilities to bags in which samples are stored prior to processing, or (b) positive or negative affinity selection capabilities to bags used in centrifugal processes.

Target cells such as CD34+ stem cells often comprise only a small fraction (1.2% to 0.1%) of a sample's total volume and since this typically represents a relatively small number at the microscopic level (e. g. on the order of millions) of small colloidal targets. As such they do not require relatively large (e. g., 10^{14}) numbers of ligands or adsorptive area. The same is true for other cell types (e. g. possible cancer cells in homologous bone marrow transplant in a lymphoma patient). For example, in a 250mL blood or other sample, half the volume (125 mL) is cells wherein about 1% (or 1.25 mL) is target cells. If this represents 100 million target cells per mL it is necessary to bind approximately 125 million cells. In a typical situation the target cells are spherical with equivalent diameter of 15 microns presenting a surface adsorbed area of approximately 200 square microns. This requires about 250 x 10^8 square microns. One square cm is 1 x 10^8 square microns. This means that a sample bag of approximately 10 cm x 20 cm offers, on both internal sides, 400 cm^2 or 1.6 times the necessary surface area. The above calculation assumes the bag surface is completely smooth. However surface micro-roughness (resulting from normal manufacturing) could more than double its surface area so that it could offer 3 to 4 times the required surface area. Manufacturing could be modified to enhance surface roughness so as to afford 10 times the needed surface area. Tethering more than one ligand to a bag surface location, via a polymer, could also be used to increase ligand density.

Some common approaches involve tethering the ligands or other affinity groups to the surface via polymer tether which allows for enhanced ligand-target interaction. One preferred method which has been fully described in the literature involves tethering via use of poly(ethylene glycol) [PEG] polymers which are chemically functionalized at both polymer ends. Such difunctional PEGs are FDA approved and biocompatible, afford good surface wetting (necessary for cell containing liquids to have contact with wall localized ligands), and offer good tether mobility to the ligands.

Difunctional and other suitable PEGs are commercially available. Various functional groups are available including those which couple via normal biological linkages such as amide linkages.

Various bag and affinity substance linking chemistries and approaches may be employed. These include many water based reactions. Furthermore PEG modified substances tend to be very soluble and offer reduced surface interactions making removal of nonlinked substances straightforward with water washing. In addition PEGs and many PEG-modified substances such as proteins and drug delivery colloids (liposomes) are FDA approved for internal use. Other polymers which might be used as tethers include various other polymers or copolymers or block copolymers containing "ethoxy" groups; as well as various polyacids and polysaccharides. Examples include dextran, agarose, polylactic acid, polyglycolic acid, ethylhydroxyethylcellulose, starch polymers; or combinations of such substances. Such tethers can be of any MW between 100 and several million, and carry one or more affinity substances. In some cases PEG or other tethers carrying affinity substances might be mixed with similar molecules carrying no such substances.

It should be noted that if tethered affinity substances are used then there are two basic approaches involving:
(i) attachment of the tether to the wall followed by tether interaction with affinity substance.
(ii) attachment of the affinity substance to the tether followed by attachment of the tether modified affinity substance to the wall.

By way of nonlimiting example, suppose that statistically it takes ten affinity ligands or less to favorably interact with and securely bind one cell (or other target such as a bacterium). Then based on the above numbers the ligands need to be present at a surface density of approximately 1 ligand per 20 square microns. The surface chemistry literature is replete with various techniques to readily achieve this and higher ligand densities on a variety of surfaces. Thus, such literature is replete with many examples of ways to increase surface area and ligand densities via the use of various easily applied biocompatible polymeric coatings and polymeric tethers.

To avoid a large diffusion distance for cells to be sampled by the surface, plastic baffles or other structural features made from the same bag material may be provided. In some cases it may be desired to concentrate the target cells of interest in a small physical volume or specific location. Again use of baffles may solve this; especially if the baffled section is only in a small part of the

bag near an outlet only used to target collection. In this case it is important to control location of affinity interaction to a specific location in a bag, tube or container.

The use of baffles added to either bag regions or in tubes may afford several advantages and simplifications over other approaches such as (a) allowing free washing of entire sample, (b) concentration, (c) elimination of clogging related to column or filtration units with possible loss of clots into sample, (d) in bags elimination of several outlet ports with need for controlled mechanical manipulation, (e) in case of modified outlet tubes; the ability to transfer target isolate in tubes which could be readily detached from the bags, (f) modification to suit various centrifuge and other products (e. g. simply attaching modified tubes to syringes used for taking samples from patients). In some cases addition of ligands to tubes or baffled tubes leading into bags or other containers might allow some affinity processing of samples as they are loaded into the containers prior to their being stored or further processed. In such cases this processing may enhance sample storage or other uses.

Experimental part

The examples below are only given in illustrative purposes and should not be construed as limiting for the present invention as defined in the claims.

The invention involves four basic steps:

a. Covalent or noncovalent activation of the bag surface
b. Covalent or noncovalent coupling of affinity ligands, macromolecules or other substances.
c. Coupling of target substance to affinity ligands
d. Removal of non-coupled substances (typically via washing)

In some positive selection applications a fifth step may also be involved

e. Removal of bound target substances

Example 1: Coupling of cell ligands to bags

A cell handling bag is provided and the surface in a specific outlet is activated for coupling of affinity ligand by adsorbing a high MW polymer such as polyethyleneimine (N.L. Burns, K. Emoto, K. Holmberg, J. Van Alstine, J.M. Harris, Biomaterials, 19, 423-440, 1998). The bag is oriented with the target region down and a few mls of 1% (w/w) polyethyleneimine of high MW (e. g. 1000000) are added for creation of a polyamine coated region.

The polyamine surface is then used for affinity molecule (e. g. Protein A, Mab, aptamer, lectin) attachment followed by "neutralizing" the reactive and positive charged surface with amine reactive PEG1000-NHS. Alternatively it might be modified with PEG difunctional reagent to which affinity molecules are then covalently attached. As another alternative it might be reacted with affinity molecules pre-modified with PEGs or other polymer tethers containing free amine reactive groups which react with the bag surface. If done under the proper conditions of bulk phase pH (e. g. neutral) then the above tethering reactions can be controlled as basic pH catalysed coupling will only occur at the amine coated surface where local amine concentration is so high as to control linking (K. Emoto, J. M. Harris, J. M. Van Alstine, Langmuir, 14, 2722-2729, 1998).

Example 2: Cell separation in bags

Here three examples are described of the use of ligand modified bags according to the invention.

In all of the cases noted a cell sample is added to a ligand modified bag in suspending media which may or may not mean density gradient media. The bag is then, optionally, gently agitated to promote greater cell to bag surface contact, and effect target cell binding. The sample is then processed in a normal manner (e. g. centrifugation). Cells interacting with the surface are held back in the bag.

a. Negative selection of peripheral or cord blood samples for stem cells

When processing peripheral or cord blood samples for stem cells one may wish to reduce or remove platelets or certain subpopulations of lymphocytes or other WBCs. Examples would be if the sample to be stored (for future therapeutic use by the donor or other patient) contains cells which might lyse on storage and negatively impact the efficacy of the sample. Another example would be if the sample is to be used for direct transplantation. In the case of homologous transplantation (say to promote post surgical healing) one might wish to remove platelets whose activation during the cell processing could negatively affect transplant efficacy. In the case of a blood sample pre-treated by apheresis or some other method (e. g. chemical lysis of lymphocytes), the platelets or other cells referred to might be residual cells which were not removed by the pre-treatment. In the case of heterologous transplantation one may wish to remove WBCs which trigger graft versus host disease (GVHD).

According to the present invention the cell sample is suspended in suspending medium and inserted into a cell handling device of the invention. Thereafter, the cell sample is allowed to react

with the affinity ligands on the inside of the device. Optionally, density based separation is combined with the affinity separation resulting in a more robust separation (and hence target cell enrichment). In this case the bag is centrifuged with the sample suspended in DGM or other density media resulting in a concentration of the target cells or other cells or other substances (bacteria, virus) at specific locations inside the bag where they may or may not interact with bag surface localized affinity ligands. Cells trapped on the bag and related tube surfaces via affinity interactions are discarded together with the disposable bags. Note that this allows for use of bags where more than one type of affinity ligand is localized at different locations in the bag. Of course in some cases one might wish to have a mixture of different affinity ligands (to different cells or other substances) randomly distributed over the inner bag or tube surfaces.

b. Negative selection of bone marrow samples for stem cells

In this case it is desired to remove any sample contaminating lymphocytes or macrophages (whose densities may be similar to that of the target stem cells) as well as, perhaps, some more differentiated progenitor cells. For cancer treatment bone marrow samples are used for homologous transplantation following whole body radiation. The samples need to be purified of both potential cancer cells (which could be much less than 1% of cells in the sample) and any cells which might give rise to GVHD.

The cell sample is suspended in suspending medium and inserted into a cell handling device of the invention. Thereafter, the cell sample is allowed to react with the affinity ligands on the inside of the device. Optionally, a density separation is combined with the affinity separation in which case the sample is suspended in density gradient media. Cells trapped on the bag and related tube surfaces via affinity interactions are discarded together with the disposable bags.

c. Positive selection of peripheral blood samples for stem cells

In this case the inside of the bag is coated with ligands to targeted stem cells in a certain region of the bag forming a ligand dense region. The cell sample is processed via centrifugation in Ficoll Paque™ or other density media in the bag resulting in concentration of the white blood cell fraction including stem and other cells in the ligand dense region. Affinity interaction localizes the target cells on the bag surface.

Cells trapped on the bag and related tube surfaces via affinity interactions are recovered by washing the sample bag from unbound substances. The target cells are then released by known methods (see above).

CLAIMS

1. Sample handling device for separating one or more substance(s) from a sample, comprising a flexible container with affinity ligands attached on the inside of the container, wherein said affinity ligands are specific for said substance(s).

2. Sample handling device according to claim 1, wherein the affinity ligands are antibodies, fractions of antibodies, proteins, glycoproteins, glycolipids, nucleic acids, aptamers, lectins, lipids or combinations thereof.

3. Sample handling device according to claim 2, wherein the substances are cells.

4. Sample handling device according to claim 3, wherein the cells are stem cells and the affinity ligands are ligands directed against stem cell related surface antigens or molecules.

5. Sample handling device according to one or more of the above claims, wherein the container is adopted for centrifugation.

6. Sample handling device according to one or more of the above claims, wherein the container is a blood sampling bag.

7. Sample handling device according to one or more of the above claims, wherein the sample is suspended in density media or density gradient media (DGM).

8. Sample handling device according to one or more of the above claims, wherein the inner wall of the container is provided with a coating comprising affinity ligands.

9. Sample handling device according to one or more of the above claims, wherein the container comprises inlet(s) provided with affinity ligands on the inside which may be the same of different from those in the container.

10. Sample handling device according to one or more of the above claims, wherein the inner surface of the container and/or inlet(s) comprises baffles to enhance the surface area for ligand binding.

11. Sample handling device according to one or more of the above claims, wherein ligands on the inner surfaces of the bag and related conduits are coated either randomly or non-randomly, i.e. at specific locations, with one or more affinity substances.

12. A method for separation, comprising the following steps: a) suspending a sample in suspending media, b) sampling the sample in a device according to one or more of claim 1-11, c) allowing specific substances in said sample to interact with the affinity ligands and bind thereto; and d) separating the remainder of the sample from the bound substances.

13. A method according to claim 12 comprising a further step e) centrifugation before, during or after step c).

14. Method for cell separation according to claim 12 or 13, comprising a further step of detaching the substances bound in step c).

15. Use of the sample handling device according to one or more of the claims 1-11 for cell separation.

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Application No: GB0723877.7 **Examiner:** Dr Jonathan Corden

Claims searched: 1-15 **Date of search:** 4 April 2008

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-6, 12-15	WO 90/04019 A1 (BAXTER INTERNATIONAL) see page 2 lines 25-31 and page 4 line 28 to page 5 line 7 especially
X	1-4, 8, 12-15	WO 00/68689 A1 (PROLINX) see abstract, page 6 lines 2-4 and claims especially
A	-	US 4722790 A (CAWLEY et al) see abstract and whole document
A	-	US 2002/0164825 A1 (CHEN) see claim 6 especially
A	-	US 5236604 A (SHERWOOD MEDICAL) see abstract especially
A	-	EP 1221342 A2 (BECTON DICKINSON) see whole document
A	-	EP 0588964 A1 (AMERSHAM) see whole document

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

G1B

Worldwide search of patent documents classified in the following areas of the IPC

B01D; B01L; G01N

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, wwwINTERNET

International Classification:

Subclass	Subgroup	Valid From
G01N	0001/10	01/01/2006
B01L	0003/00	01/01/2006
G01N	0001/00	01/01/2006
G01N	0033/49	01/01/2006