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(54) Title: METHOD FOR SEPARATING RED BLOOD CELLS FROM BLOOD SAMPLES AND USE THEREOF

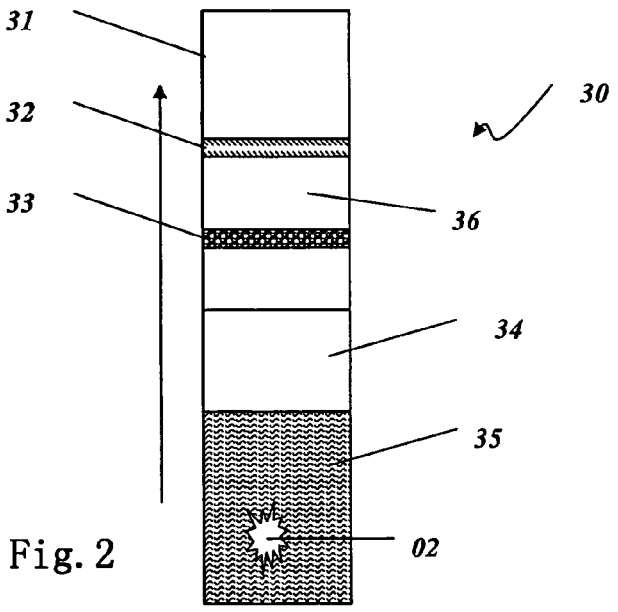

Fig. 2

(57) Abstract: A method for separating red blood cells from a blood sample and use thereof, comprises: a) contacting the blood sample with a receptor capable of binding to fibrins in the blood sample; and b) subsequently separating the fibrin-binding receptor from the blood sample. Through employing the method, inhibition of the flow of blood samples caused by red blood cells could be removed, and background interference produced by red blood cells could be reduced. The method and apparatus thereof do not adversely affect detection results.

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METHOD FOR SEPARATING RED BLOOD CELLS FROM BLOOD SAMPLES AND USE THEREOF

Technical Field

The present invention relates to a method and apparatus for separating red blood cells from a whole blood sample. More particularly, the present invention relates to a detection apparatus that employs the method and apparatus of separating red blood cells through separating fibrins from a whole blood sample, thereby reducing the interference effects of the red blood cells on the detection.

Background

Modern clinical diagnostic determinations are routinely carried out on blood samples. Unfortunately, red blood cells interfere with many diagnostic determinations. Thus, red blood cells must first be removed from a whole blood sample before tests or assays can be carried out on the blood sample. On the other hand, in chromatography assay devices, particularly in chromatographic immunoassay devices, red blood cells could impede fluid flow which is necessary for reactions to occur on these devices. For these reasons and others, many assay methodologies are carried out on plasma or serum which must first be separated from a whole blood sample.

Many known technique exists for separating red blood cells from a whole blood sample. Centrifuge is a conventional method. There are also methods disclosed in U.S Patent Nos. 5939331, 6673629, 4594327, 4678757, 5558834, and 6818180, and in U.S. Patent Publication Nos. 2006/0029923 and 2004/0202783. In general, these methods mainly employ physical and chemical processes to separate red blood cells from

plasma, most of them are costly, complicated, and may result in incomplete separation of red blood cells. Moreover, the existing techniques are not suitable for whole blood samples that have been disposed for so long that coagulation has occurred. Particularly, coagulated blood samples may cause non-specific binding or high backgrounds in chromatography immunoassay devices, resulting in losing sensitivity of the assay. Furthermore, they may block chromatography supports and inhibit flow of plasma or serum on the supports, rendering the assay invalid. This could be fatal particularly for devices that are required to give detection results in a very short period. Therefore, there is a need to have a reagent as well as an apparatus and method comprising the reagent for separating red blood cells from a whole blood sample, while the apparatus and method do not adversely affect the detection system.

Summary of the Invention

In order to solve the problem mentioned above, the present invention provides a reagent for separation of red blood cells form a whole blood sample and applications of the reagent. By using the reagent, red blood cells can be separated from a whole blood sample effectively.

One aspect of the present invention relates to a method of separating red blood cells from a whole blood sample. Specifically, the method comprises contacting a whole blood sample with a receptor capable of binding to fibrins in the blood sample, and subsequently removing the fibrin-binding receptor from the blood sample. The method may also comprise a receptor capable of binding to red blood cells. The method may further comprise providing a support, which is equipped with either the fibrin-binding receptor alone or both the fibrin-binding receptor and the red blood cell-binding receptor, and having the blood sample flowing therethrough. Preferably, the support is a chromatography support.

Another aspect of the present invention relates to an apparatus for separating red blood cells from a whole blood sample, which comprises a support equipped with a receptor capable of binding to fibrins in the blood sample. The support may be also equipped with a receptor capable of binding to red blood cells in the blood sample. Preferably, the support is a chromatography support.

Another aspect of the present invention relates to a detection apparatus for detecting an analyte in a whole blood sample, which comprises a support having a sample receiving region and a detection region located downstream of the sample receiving region, wherein the support upstream of the detection region is equipped with a receptor capable of binding to fibrins in the blood sample. The sample receiving region may be the region where the fibrin-binding receptor is immoblized. The sample receiving region may be also equipped with a receptor capable of binding to red blood cells. Furthermore, the support upstream the detection region may further comprise a labeled substance that can be carried away by the flowing of the blood sample, while the detection region may be equipped with a specific binding molecule. Preferably, the support is a chromatography support.

Another aspect of the present invention relates to a method for detecting an analyte in a whole blood sample, the method comprises providing a chromatography support comprising a sample receiving region, wherein the sample receiving region is equipped with a receptor capable of binding to fibrins, allowing flowing therethrough of a blood sample and measuring content of the analyte in the blood sample. The support may further comprise a receptor capable of binding to red blood cells, and may comprise a detection region in the downstream region of the support for

detecting the analyte.

In all of above embodiments, receptors capable of binding to fibrins comprise anti-fibrin antibodies, while receptors capable of binding to red blood cells comprise anti-red blood cell antibodies; and said antibodies comprise monoclonal antibody, polyclonal antibodies, and antibody fragments.

The invention disclosed herein provides many advantages. It effectively removes red blood cells from a whole blood sample, no matter the sample is fresh or already has coagulation occurred, thereby prolonging the usable life of the blood sample. In addition, apparatus that employs receptors capable of binding to fibrins for separating red blood cells from a blood sample or detecting an analyte in the blood sample also has a prolonged usable life.

Brief Description of the Figures

Fig. 1 depicts a specific embodiment of the present invention. A detection apparatus 10 comprises a filtering layer 11 for samples receiving thereon and passing therethrough, and a reaction layer 12. The reaction layer is equipped with a substrate for reduction reaction and a handle 13. The filtering layer is equipped with polyclonal anti-fibrin antibodies and polyclonal anti-red blood cell antibodies. After applying a whole blood sample 01 onto the sample receiving layer 11, the anti-fibrin antibodies and the anti-red blood cell antibodies attached on the layer 11 work in a coordinated manner to intercept the red blood cells, retaining them on the filtering layer and allowing the plasma to pass through to the reaction layer 12. If an analyte such as blood sugar is presented in the sample, color change could be directly observed at the bottom surface of the reaction layer 12.

Fig. 2 depicts a preferred specific embodiment of the present invention. In the detection apparatus 30, the sample receiving region 35 is made of glass fibers; the detection region 36 is made of nitrocellulose membrane, and locates downstream of the sample receiving region; the label holding region 34 locates between the sample receiving pad 35 and the detection region 36. The detection region comprises a test line 33, which is equipped with specific molecules capable of binding to an analyte, and a control line 32, which is used as a control to validate the detection. The glass fibers in the sample receiving region are treated with polyclonal anti-fibrin antibodies and polyclonal anti-red blood cell antibodies. The sample receiving region 35, the label holding region 34, and the detection region 36 are all connected between each other so that a fluid sample may flow from the sample receiving region 35 to the detecting region 36 for further reaction.

Reference Numbers: detecting devices 10, 30; blood samples 01, 02; filtering layer 11; reaction layer 12; handling 13; sample receiving pad 35; label holding pad 34; test line 33; control line 32; detection region 36; and water absorbent filter paper 31.

Detailed Description of the Invention

Below are detailed descriptions of structures related to the present invention and definitions of technical phrases used therein.

Detection

As used here, "detection" means testing or assaying the existence of a substance or a material, such as but not limited to, a chemical substance, an organic compound, an inorganic compound, a metabolic product, a drugs or a drug metabolite, an organic tissue or a metabolite of organic tissues, nucleic acid, protein or polymer; in addition, it also means testing

and assaying the content of a substance or material. Further, assay used here includes immune assay, chemical assay, enzyme assay and the like.

Fibrins and fibrin-binding receptors

Fibrinogen is the earliest found coagulation factor. It has a shape of a stretched ellipse, and is a dimer formed by two set of three polypeptides linked to each other through disulfide bonds (i.e., one pair of alpha chains, one pair of beta chains, and one pair of gamma chains), with a molecular weight of 340,000 dalton. Fibrinogen is synthesized in the liver and then enters the plasma and presents in form of dissolved. There is about 0.3 g of fibrinogen per 100 ml of human plasma. Fibrin, however, is a highly-insoluble protein multimer and is a crystal with a fine-needle shape. The conversion of fibrinogen to fibrin is the fundamental change in the blood coagulation process, which has to undergo three stages: (1) hydrolysis of the fibrinogen: each of the two alpha chains and two bate chains of the fibrinogen molecule has a peptide bond broken under the effect of thrombin, resulting in release of two pairs of small polypeptides (i.e., fibrinopeptide A and fibrinopeptide B, having a total molecular weight of about 9000 dalton) and formation of the fibrin monomer. Thus, the molecular weight of the fibrin monomer is smaller than that of the fibrinogen molecule. (2) Aggregation of fibrin monomers: In the presence of Ca^{2+}, a number of fibrin monomers are aggregated and polymerized to form a soluble fibrin polymer. (3) Formation of the clots: In the presence of thrombin, Factor IIIa, and Ca^{2+}, the alpha chains of fibrin monomers cross link with each other to form an interlacing fibrous network, thereby turning fibrin monomers into insoluble fibrin polymers, trapping red blood cells in the fibrous network and converting the original sol-like blood to gel-like blood coagulation.

Surprisingly, it has been found that removing fibrins from whole

blood samples with receptors capable of binging to fibrins can effectively separate red blood cells in the blood samples. Specifically, when the fibrin-binding receptors are used in coordination with receptors capable of binging to red blood cells, the separation of the red blood cells is more efficient and completed. Furthermore, such a technique of removing red blood cells works well no matter the blood sample is fresh or has coagulation occurred.

Separation method

The present invention, on the one hand, relates to a method of separating red blood cells form a whole blood sample. Specifically, the method comprises contacting a whole blood sample with a receptor capable of binding to fibrins in the blood and subsequently removing the fibrin-binging receptor from the blood sample. In a specific embodiment, anti-fibrin antibodies are used as receptors to bind to or capture fibrin monomers or polymers in blood samples, wherein the anti-fibrin antibodies can be polyclonal antibodies, monoclonal antibody or antibody fragments. The receptors can also be other naturally synthesized or artificially synthesized receptors capable of binding to fibrins. The said binding can be "specific" or "non-specific," and the receptors are deemed to be capable of binding to fibrins as long as they are able to bind to or capture fibrins in blood samples. One relatively simple example comprises contacting a whole blood sample with anti-fibrin antibodies, then separating red blood cells in the blood sample by separating the anti-fibrin antibodies in the blood sample. A more preferable embodiment comprises first contacting a whole blood sample with anti-red blood cell antibodies, then contacting the blood sample with anti-fibrin antibodies, and then separating red blood cells in the sample by separating the anti-fibrin antibodies in the blood sample. Yet another embodiment comprises contacting a whole blood sample with anti-fibrin antibodies and anti-red blood cell antibodies

simultaneously, then separating the red blood cells by separating the anti-fibrin antibodies and anti-red blood cell antibodies in the sample simultaneously. The phrase "separating" used herein means that the anti-fibrin antibodies are separated or removed from the blood sample directly or indirectly by means of physical or chemical manner. For example, an antibody against the fibrin-binding receptor can directly be used as a means for separating the fibrin-binding receptor, or other methods can be used.

Support and separating apparatus comprising said support

In another embodiment, the method comprises providing a support equipped with a receptor capable of binding to fibrins, and passing a whole blood sample therethrough. In addition, the invention also relates to an apparatus separating the red blood cells from a whole blood sample, comprising a support equipped with a receptor capable of binding to fibrins. The support may be composed of or consists of a water absorbent material, which is also referred to as "chromatography support," or a non-absorbent material.

Non-absorbent material includes, but not limited to, plastics, glass, ceramic, and other metallic materials. For example, receptors capable of binding to fibrins, such as antibodies, can first be fixed onto a plastic surface, contacting a whole blood sample with the receptors on the plastic surface, fibrins in the blood sample will then be captured by the receptors fixed on the plastic surface, and thus be separated from the blood sample, resulting in the red blood cells being separated from the sample, too. In a preferable embodiment, such a non-absorbent material is provided wherein antibodies against red blood cells and antibodies against fibrins are both fixed onto the material, and then a whole blood sample is allowed to pass through the surface of the non-absorbent material.

On the other hand, the support may be a "chromatography support", wherein the "chromatography support" refers to any suitable porous and absorbent materials or capillary materials. These materials should be absorbent inherently whereon fluid can flow due to capillary action. They can be, for example, filter paper, glass cellulose, polyester film, nylon film, nitrocellulose membrane, acetic acid cellulose, natural material (such as cotton) fabrics and synthetic material (nylon) fabrics, porous gels, and the like. In a preferred embodiment using the support that supports fluid flowing, fibrin-binding receptors are treated onto the support such that they cannot be carried away by blood sample due to fluid flow. When a whole blood sample is applied to the support, fibrin molecules in the blood sample are bound to the receptors and are congregated on the support, thus being separated from the blood sample due to the continuing flow of the blood samples. In yet another preferred embodiment, the support may also be equipped with one or several receptors capable of binding to red blood cells. These receptors can congregate red blood cells in a whole blood sample. The red blood cell-binding receptors may be located upstream of the fibrin-binding receptors, or they can be at the same position as that of the fibrin-binding receptors, or at other positions. How to treat said receptors onto said supports is a common knowledge of the field. In addition, it will be appreciated by one skilled in the art that a support can be made of a single material or more than one materials (for example, different portions, regions, or layers can be made of different materials), so long as the multiple layers are in fluid flow contact with one another so that fluid sample can pass between these materials. After receiving fluid samples, these supports can allow the samples to pass therethrough in different manners, for example, perpendicularly, as shown in Fig. 1, horizontally, as shown in Fig. 2, or any other manners.

The reason why separating fibrins in a whole blood sample can separate or remove red blood cells in the sample may be as follows: fibrin-binding receptors, such as anti-fibrin antibodies, may congregate fibrin molecules through binding to them and thereby form a fibrous network, in which fibrin molecules cross link with one another and are capable of trapping red blood cells in the samples; thus, if the fibrin-binding receptors in the sample are separated, red blood cells in the sample are indirectly separated. In another preferred embodiment, when receptors capable of binding to red blood cells, such as antibodies against red blood cell surface antigens, are also present, such red blood cell-binding receptors can gather individual red blood cells to form a "red blood cell aggregation", and the said fibrous network formed by fibrin-binding receptors and fibrin molecules, if present, can easily trap these "red blood cell aggregations" such that the red blood cells cannot be easily carried away by the flow of blood samples; consequently, red blood cells in blood samples can be separated more efficiently. In a particularly preferred embodiment, the receptors capable of binding to fibrins are polyclonal antibodies that are treated and fixed on the chromatography support; in addition, said chromatography support is also equipped and fixed with polyclonal antibodies against red blood cell surface antigens. When a whole blood sample is applied to the chromatography support, the anti-red blood cell antibodies equipped on the support bind to or capture the red blood cells in the sample to form "red blood cell aggregations," and the anti-fibrin antibodies equipped on the support bind to or capture fibrins in the sample to form a fibrin molecule network. These fibrin molecule network can easily trap the "red blood cell aggregations" that have greater volume than that of individual red blood cells, thus impeding red blood cells from flowing continuously more effectively and separating red blood cells from the blood sample more effectively. The method for separating red blood cells through separating fibrin-binding receptors equipped on the

support can be achieved by directly fixing the receptors on the support, or by other indirect means of separating fibrins. For example, antibodies against anti-fibrin antibodies (referred to as secondary antibodies against anti-fibrin antibodies) are fixed on a support, mixing and reacting anti-fibrin antibodies with a whole blood sample first, then applying the mixed solution to a support equipped with the secondary antibodies against anti-fibrin antibodies. Said secondary antibodies capture said anti-fibrin antibodies, thereby capturing fibrins indirectly. Likewise, antibodies against anti-red blood cell antibodies (referred to as secondary antibodies against ant-red blood cell antibodies) are fixed on a support to capture red blood cells in a blood sample indirectly. In a preferable embodiment, ant-fibrin antibodies and anti-red blood cell antibodies are directly treated onto a support.

Detection apparatus

Another aspect of the present invention relates to a detection apparatus for detecting an analyte in a whole blood sample. The apparatus comprises a support comprising a sample receiving region and a detection region located downstream of the sample receiving region, and the support upstream of the detection region is equipped with receptors capable of binding to fibrins in a blood sample. Preferably, the support is a chromatography support, and the sample receiving region is equipped with receptors capable of binding to fibrins in blood samples. The object of the present invention can be achieved by using any detection system, preferably a chromatographic immunoassay system, include but not limited to, horizontal flow system, vertical flow system, and test bar. The descriptions of some known detection systems are given below.

In general, on a chromatographic reagent test strip, there are at least one sample receiving region and one detection region, wherein the

detection region comprises certain specific binding molecules or chemical substances through which whether an analyte exists in a sample or its content in the sample can be determined. The specific binding molecule can be an antibody or antibody fragment. The binding molecule can directly or indirectly bind to the analyte, or it can bind to a moiety associated with the analyte. That moiety can be an immunological epitope on the analyte itself, or on a reagent bound to the analyte (e.g., a reagent that bound to the analyte as it passed through the label holding region). When a fluid sample to be detected is applied to the sample receiving region, it migrates along the chromatography support due to capillary action and thus, through binding reaction (such as immunological reaction) occurred in the detection region, allows accumulation of labeled substances (labeled or conjugated with labels) that are treated and arranged on the chromatography support beforehand and can be carried away and flowing along with the fluid sample. The accumulating action is in direct proportion (such as double antibody sandwich) or inverse proportion (competitive method) with the existence or content of the analyte in the sample, and by measuring the existence or the content of the labeled substance, the existence or the content of the analyte in the sample can be indirectly determined. Of course, reactions occurred in the detection region can also be a color reaction between substrates having pure chemical properties, such as oxidization-reduction reaction and the like, and the existence or the content of the analyte in the sample then can be determined through measuring the intensity of the color shown in the detection region. Common detection apparatus applying this principle includes blood sugar reagent test strip and the like, such as that described in U.S. Patent No. 6,818,180. In a specific embodiment, sample receiving region is located at the same position as where the labeled substance is located, but preferably upstream of the labeled substances (the direction of the movement of a sample caused by capillary action is called

"downstream" and the opposite is called "upstream"). When contacting a fluid sample (suspected to contain an analyte of interest) with the sample receiving region, due to capillary action, the fluid sample flows downstream together with the analyte along the chromatography support. Said analyte is generally a compound, which can bind to a detecting molecule that is treated and fixed on the detection region in a particular manner. For example, an analyte is an antigen, the labeled substance is an antibody against the antigen and the detecting molecule fixed on the detection region is another antibody against the antigen. The apparatus and methods for detecting the existence of an analyte in a blood sample through applying the above principles are already in the prior art and are not the focus of the present invention. The present invention, however, is intended to be used with any blood samples, including serum and plasma, but is preferably used with a blood sample containing red blood cells, e.g., a whole blood sample.

The labeled substances used here are substances, e.g. antibodies or antigens, provided or conjugated with labels. The "label" can be any suitable label that provides a detectable signal. For example, the label can be a sol particle, a fluorescent molecule, a chemiluminescent molecule, a metal or alloy (e.g., colloidal gold), or a sac, in particular a liposome containing a visible dye. Also useful are hydrophobic sols, which hydrophobic organic dyes or pigments are insoluble in water or soluble only to a very limited extent. The label can also be polymer particles, such as colored polystyrene particles (e.g., spherically shaped), or colored particles (e.g., dextran bead). Other useful particulate labels include ferritin, phycoerythrins or other phycobili-proteins, precipitated or insoluble metals or alloys, fungal, algal, or bacterial pigments or derivatives such as bacterial chlorophylls, or other plant materials. In certain embodiments, the labeled substance positioned upstream of the

detection region can bind (directly or indirectly) to the analyte of interest, thereby labeling the analyte of interest with a detectable label as it flows through the support.

In various embodiments the reagent bound to the analyte can be an antibody, a fragment or portion of an antibody, an antibody (or fragment thereof) derived from a species different from the antibody affixed to the analyte binding area, or another member of a specific binding pair, for example, avidin, streptavidin, or biotin, which itself can be bound to a moiety bound to the analyte. "Antibody" used here refers to an immunoglobulin, whether natural or partially or wholly synthetically produced. The term also includes derivatives thereof which maintain specific binding ability. The term also covers any protein having a binding domain which is homologous or largely homologous to an immunoglobulin binding domain. These proteins may be derived from natural sources, or partly or wholly synthetically produced. An antibody may be monoclonal or polyclonal. The antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, IgG, and IgE. An "antibody fragment" is any derivative or portion of an antibody which is less than full-length. The antibody fragment can retain at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, scFv, Fv, dsFv diabody, and Fd fragments.

The antibody fragment may be produced by any means. For instance, the antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, the antibody fragment may be wholly or partially synthetically produced. The antibody fragment may optionally

be a single chain antibody fragment. Alternatively, the fragment may comprise multiple chains which are linked together, for instance, by disulfide linkages. The fragment may also optionally be a multimolecular complex. A functional antibody fragment will typically comprise at least about 50 amino acids and more typically will comprise at least about 200 amino acids.

Before detecting an analyte of interest in a blood sample, the red blood cells in the sample are preferably removed if the detection is to work with a desired sensitivity. Therefore, according to the present invention, a receptor capable of binding to fibrins in the blood sample is treated on a chromatography support, preferably in its sample receiving region. The reason for the fibrin-binding receptor being treated in the sample receiving region of the chromatography support is preferable is that once the blood sample is applied onto the sample receiving region, such an arrangement allows the red blood cells being removed from the blood sample effectively, because it eliminates or at least reduces to a minimum of the interference of the red blood cells on the succeeding flow of the plasma or serum along the support, thereby keeping the flowing from being affected; meanwhile, it reduces the background interference. Fig. 2, for example, describes a preferred specific embodiment of the present invention. In the detection apparatus 30, the sample receiving region 35 is made of glass fibers; the detection region 36 is made of nitrocellulose membrane, and is located downstream of the sample receiving region; the label holding region 34 is located between the sample receiving region 35 and the detection region 36. The detection region comprises a test line 33, which is equipped with specific binding molecules, and a control line 32, which is used as a control to validate the detection. The glass fibers in the sample receiving region are treated with polyclonal anti-fibrin antibodies and polyclonal anti-red blood cell antibodies. The sample receiving

region 35, the label holding region 34, and the detection region 36 are all connected so that fluid may flow from the sample receiving region 35 to the detection region 36 for further reaction.

Fig. 1 depicts another preferably specific embodiment of the present invention. Detecting apparatus 10 comprises a filtering layer 11 for samples receiving and passing therethrough and a reaction layer 12. The reaction layer is equipped with a substrate for reduction reaction and a handle 13. The filtering layer is equipped with polyclonal anti-fibrin antibodies and polyclonal anti-red blood cell antibodies. After applying a whole blood sample 01 onto the sample receiving layer 11, the anti-fibrin antibodies and the anti-red blood cell antibodies attached on the filtering layer 11 work in a coordinated manner to intercept red blood cells in the samples, thereby retaining the red blood cells on the filtering layer and allowing the plasma to pass through to the reaction layer 12. If an analyte such as blood sugar is presented in the sample, color change can be directly observed at the bottom surface of the reaction layer 12. Applications of the method described in the present invention relating to removing red blood cells from a whole blood sample should not be limited to the above specific embodiments. It can be used in any other detection systems. For example, it can be used in the detection apparatus described in Fig. 1 of the U.S. Patent No. 6818180. Specifically, it can be applied to the surface layer 5 of the porous membrane 1 for separating a whole blood sample applied through hole 21. It will be appreciated by a person skilled in the art that the method or apparatus of the present invention can be used in combination with any other methods, structures, and reagents in the prior art for separating red blood cells. For example, it can be combined with the structure and reagent described in U.S. Patent No. 6818180.

Detection method

Another aspect of the present invention relates to a method for detecting an analyte in a whole blood sample. The method preferably applies to the said chromatography support apparatus. Specifically, the method comprises providing a chromatography support comprising a sample receiving region, wherein the sample receiving region is equipped with receptors capable of binding to fibrins in blood samples; passing a blood sample through the sample receiving region and then detecting whether an analyte presents in the blood sample. Preferably, the sample receiving region is equipped with antibodies capable of binding to red blood cells. Preferably, downstream of the sample receiving region comprises a detection region, which is fixed with a specific binding molecule, and allowing the samples passed through the sample receiving region passing through the detection region.

The following examples will further illustrate the present invention but should not be construed, in any case, as limiting its scope.

Examples

Example 1: Application of separation red blood cells from blood samples by anti-fibrin antibodies in CTI (Cardiac Troponin I) detection

The polyclonal anti-fibrin antibodies (Cat. No. G11135796J162) used in the present experiment were obtained from Beijing Sanboyuanzhi Biotechnology Co., Ltd. (Dongbeiwang South Rd., No. 26, Haidian District, Beijing); the monoclonal anti-red blood cell antibody (Cat. No. ME060822-1-0419) and the polyclonal anti-red blood cell antibodies (Cat. No. Q05920-1113) were obtained from Cenclonn Company (Gudang Economic Park, No. 198, Tianmushan Rd, Hangzhou, Zhejiang Province). Except for the parts with additional remarks, CTI detection reagent test strips used in the present experiment were prepared through methods

known in the prior art. The preparation and assembly of the reagent test strip are further described herein by reference to Fig. 2.

1. Configuration and preparation of the sample receiving pad 35

The sample receiving pad 35 is made of glass fibers. Such glass fiber pads were first treated with Tris buffer; subsequently, they were subjected to different treatments, according to the following description:

1) Glass fiber pads treated with only polyclonal anti-fibrin antibodies (100 glass fiber pads in total, 20 in each treatment): 100 glass fiber pads were evenly divided into five groups. Polyclonal anti-fibrin antibodies prepared at different dilution ratios, namely, 1:50, 1:100, 1:200, 1:300 and 1:400, were used to treat each of the five groups of glass fiber pads, respectively, thereby obtaining five groups of glass fiber pads, each of which had differently diluted anti-fibrin antibodies attached: Treatment A (1:50), Treatment B(1:100), Treatment C(1:200), Treatment D(1:300), and Treatment E(1:400).

2) Glass fiber pads treated with both anti-red blood cell polyclonal antibodies and polyclonal anti-fibrin antibodies (50 glass fiber pads in total, 10 in each group): Each of the treatments A, B, C, D, and E were further evenly divided into two subgroups; thus each subgroup comprised 10 glass fiber pads. One of these two subgroups in each treatment (i.e. A, B, C, D, and E) was further treated with polyclonal anti-red blood cell antibodies (1:10 diluted), thereby obtaining five new groups of glass fiber pads (10 glass fiber pads in each group), each of which had attached two kinds of antibodies, which were named as: Treatment G = 1:50 (anti-fibrin) + 1:10 (anti-RBC); Treatment H = 1:100 (anti-fibrin) + 1:10 (anti-RBC), Treatment I = 1:200 (anti-fibrin) + 1:10 (anti-RBC), Treatment J = 1:300 (anti-fibrin) + 1:10 (anti-RBC), Treatment K = 1:400 (anti-fibrin) +1:10 (anti-RBC) .

3) Glass fiber pads treated with only polyclonal anti-red blood cell

antibodies (1:10 diluted): Another group of 10 untreated glass fiber pads (with only Tris-buffer) were selected and treated with polyclonal anti-red blood cell antibodies (1:10 diluted), thereby obtaining a new group named as Treatment F (10 glass fiber pads).

All these treated glass fiber pads were then dried at 37°C in an oven and stored for further use.

2. Preparation of the label holding pad 34

Rabbit monoclonal anti-human CTI antibodies and mouse polyclonal anti-human IgG antibodies were conjugated by a commonly used colloidal gold solution having an average particle size around 20-40 nm, and then were sprayed homogeneously onto a polyester membrane by a device. The membrane was further dried at 37°C in an oven and stored for further use.

3. Preparation of nitrocellulose membrane 36

By using a microprocessor-controlled micro-injector, first polyclonal goat anti-mouse IgG antibodies (1.3 mg/ml) were dispensed and positioned onto a nitrocellulose membrane as a control line (C line) 32 (for test validation) and then mouse anti-human CTI monoclonal antibodies (4.0mg/ml) were dispensed and positioned onto the nitrocellulose membrane as a test line (T line) 33 (for analyte binding). Both antibodies were dispensed at a rate of 1.1 μl/cm. Upon completion, the coated nitrocellulose membrane was immediately dried at 45°C (for two hours) in order for the attached antibodies to be fixed on the membrane.

4. Assembly of the reagent test strip

The CTI detection reagent test strips were assembled according to the schematic illustration as shown in Fig 2. The sample receiving pad 35

(one of the aforementioned treated glass fiber pads) was positioned overlapping the label holding pad 34, then the label holding pad 34 overlapping the nitrocellulose membrane 36 at its another end (the end away from the sample receiving pad), and subsequently the nitrocellulose membrane 36 was overlapped by a absorbent filter paper 31 positioned at the distal end of the test strip, thereby forming a reagent test strip as shown in Fig 2.

5. Preparation of blood samples

20 whole blood samples (collected for more than 7 days, stored at 2-8°C) were prepared for further use.

6. Test procedure

100 μl of whole blood sample was dropped onto the sample receiving pad of the reagent test strip, and the time for the C line to appear on the reagent test strip was recorded. The time for the C line to appear indicated the flowing rate of the blood sample liquid. The C line had to appear within 5 minutes of the sample introduction, and the sample had to run through the nitrocellulose membrane 36 to reach the absorbent filter paper 31 within 10 minutes of the sample introduction. Otherwise, the reagent test strip was considered as unqualified.

Treatments F and J were used to determine the reagent test strips' sensitivity and specificity, respectively. Positive preparations were prepared from CTI-negative (experimentally confirmed) whole blood samples so that the CTI concentrations in the preparations could be manipulated to be 0.5ng/ml, and their testing results were recorded. In order to determine the specificity, 20 standard CTI-negative (experimentally confirmed) whole blood samples were used as negative preparations in the test. Those strips showed red background were also

deemed as unqualified.

7. Results

Table 1. Times for the C line to appear

Time / No.	Times for the C line to appear										
	A	B	C	D	E	F	G	H	I	J	K
1	5'15"	4'45"	4'40"	4'10"	4'35"	4'30"	4'	3'45"	3'45"	3'	3'30"
2	5'45"	4'30"	5'10"	4'15"	5'20"	3'40"	5'10"	4'50"	4'45"	3'15"	4'30"
3	4'45"	5'10"	4'20"	3'45"	4'15"	4'30"	3'40"	3'40"	3'50"	2'20"	3'20"
4	6'20"	5'45"	5'25"	5'20"	5'30"	4'50"	4'30"	3'40"	3'50"	2'35"	3'10"
5	3'50"	3'40"	3'50"	3'20"	3'50"	3'45"	3'30"	3'	3'20"	2'45"	3'20"
6	6'20"	5'45"	5'15"	4'10"	5'10"	4'45"	4'30"	4'20"	4'30'	3'30"	5'30"
7	5'20"	5'15"	5'20"	5'10"	5'25"	5'10"	4'30"	4'10"	4'	3'40"	3'50"
8	4'	3'45"	3'30"	3'	3'40"	3'40"	3'20"	3'10"	3'20"	2'45"	4'30"
9	6'	5'55"	5'30"	5'30"	4'50"	4'40"	2'20"	3'50"	3'	2'10"	3'
10	5'15"	5'20"	5'10"	5'30"	5'15"	3'40"	3'20"	3'	3'	3'	3'

Note: the results are underlined when the reagent test strips tested were deemed as unqualified either because the time for the C line to appear was more than 5 minutes, or because the sample had not run through the nitrocellulose membrane within 10 minutes.

Table 2. Percentages of unqualified products

		Treatments										
		A	B	C	D	E	F	G	H	I	J	K
		70%	60%	60%	40%	50%	30%	10%	10%	10%	0%	10%

Table 3. Sensitivity Tests

Results / No.	Treatment F	Treatment J
1	+	+
2	+	+
3	+	+

Note: CTI-positive preparations were used to verify the sensitivity; "+" indicates a positive test result was obtained.

Table 4. Specificity Test

Results / No.	Treatment F	Treatment J
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
13	-	-
14	-	-
15	-	-
16	-	-
17	-	-
18	-	-
19	-	-
20	-	-

Note: CTI-negative whole blood samples were used to test the specificity; "-" indicates a negative test result was obtained.

8. Conclusions

The above results suggest that anti-fibrin antibodies are able to separate red blood cells from blood samples, thereby greatly reducing the chance of coagulated blood samples blocking chromatography supports and greatly enhancing the efficiency of the blood separation; meanwhile, it appears that anti-fibrin antibodies have no disadvantageous effect on the

detection itself. Moreover, if anti-fibrin antibodies are to be used in combination with anti-red blood cell antibodies, their advantageous effects on blood separation would be more obvious.

Example 2: Application of separation red blood cells from blood samples by anti-fibrin antibodies in PSA (Prostate Specific Antigen) detection

The antibodies used in this experiment were the same as used in experiment 1. Compared with experiment 1, the construction of the reagent test strip used herein resembled that of the strip used in experiment 1, except that the designed analyte was different and, accordingly, the treatments of the sample receiving pad of the strip were different. The preparation and assembly of the reagent test strip used in the present experiment are again described herein by reference to Fig. 2.

1. Configuration and preparation of the sample receiving pad 35

The sample receiving pad 35 was made of glass fibers. Such glass fiber pads were first treated with Tris buffer. Subsequently, they were subjected to the following different treatments:

1) Treatment 1 (Glass fiber pads treated with both polyclonal anti-fibrin antibodies and polyclonal anti-red blood cell antibodies): Glass fiber pads were treated with diluted polyclonal anti-fibrin antibodies (diluted in 1:300) and diluted polyclonal anti-red blood cell antibodies (diluted in 1:10).

2) Treatment 2 (Glass fiber pads treated with polyclonal anti-red blood cell antibodies): Glass fiber pads were treated with diluted polyclonal anti-red blood cell antibodies (diluted in 1:10).

3) Control treatment CK (Glass fiber pads treated with monoclonal anti-red blood cell antibody): Glass fiber pads were treated with diluted monoclonal anti-red blood cell antibody (1:75 diluted).

All treated glass fiber pads were then dried at 37°C in an oven and stored for further use.

2. Preparation of label holding pad 34

Rabbit monoclonal anti-human PSA antibodies and mouse polyclonal anti-human IgG antibodies were conjugated by a commonly used colloidal gold solution having an average particle size around 20-40 nm, and then were sprayed homogeneously onto a polyester membrane by a device. The membrane was further dried at 37°C in an oven and stored for further use.

3. Preparation of nitrocellulose membrane 36

By using a microprocessor-controlled micro-injector, first polyclonal goat anti-mouse IgG (1.3 mg/ml) antibodies were dispersed and position onto a nitrocellulose membrane as a control line (C line) 32 (for test validation), then monoclonal mouse anti-human PSA antibody (4.0mg/ml) was dispensed and positioned onto the nitrocellulose membrane as a test line (T line) 33 (for analyte binding). Both antibodies were dispensed at a rate of 1.1 μl/cm. Upon completion, the coated nitrocellulose membrane was immediately dried at 45°C (for two hours) in order for the attached antibodies to be fixed on the membrane.

4. Assembly of the reagent test strip

The PSA detection reagent test strips were assembled according to the schematic illustration as shown in Fig 2. The sample receiving pad 35 was positioned to overlap the label holding pad 34, then the label holding pad 34 overlapped the nitrocellulose membrane 36 at its another end (the end away from the sample receiving pad), and finally the nitrocellulose membrane 36 was overlapped by a absorbent filter paper 31

positioned at the distal end of the test strip, thereby forming a reagent test strip as shown in Fig 2.

5. Preparation of blood samples

20 whole blood samples (collected for more than 7 days, stored at 2-8°C) were prepared for further use.

6. Test procedure

40 μl of whole blood sample, together with 40 μl whole blood phosphate buffered saline that was used to facilitate the migration of the blood sample, were dropped on the sample receiving pad. The time for the C line to appear was recorded. The time for the C line to appear indicated the flowing rate of the blood sample liquid. The C line had to appear within 3 minutes of the sample introduction, and the sample had to run through the nitrocellulose membrane 36 to reach the absorbent filter paper 31 within 5 minutes of the sample introduction. Otherwise, the reagent test strip was considered as unqualified.

Control treatment CK and Treatment 1 were used to determine the product's sensitivity and specificity, respectively. Positive preparations were prepared from PSA-negative (experimentally confirmed) whole blood samples so that the PSA concentrations in the preparations could be manipulated to be 2ng/ml, 4ng/ml, 10ng/ml, and 20ng/ml, respectively, and their testing results were recorded. In order to determine the specificity, 20 standard PSA-negative (experimentally confirmed) whole blood samples were used as negative preparations in the test.

7. Results

Table 1. Time for the C line to appear in Treatment CK (CK) and Treatment 2 (T2) (in seconds):

	1	2	3	4	5	6	7	8	9	10	Qualified rate
CK	-*	-	72	60	-	-	-	-	-	-	20%
T2	-	163	74	134	-	136	-	-	124	-	50%

Note: "-" indicates that the blood sample could not pass the C line within 3 minutes; "*" indicates that the blood sample could not run through the nitrocellulose membrane within 5 minutes.

Table 2. Time for the C line to appear in Treatment 1(T1) and Treatment 2 (T2) (in seconds):

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Average Time
T2	110	-	170	90	129	100	100	92	67	81	116	148	149	87	124
T1	103	80	120	96	63	82	84	122	67	64	105	140	116	84	94

Note: "-" indicates that the blood sample could not pass the C line within 3 minutes.

Table 3. Sensitivity comparison between Treatment 1 (T1) and Treatment CK (CK):

	PSA concentration of Preparations							
	2ng/ml		4ng/ml		10ng/ml		20ng/ml	
CK	G2	G2	G4	G4-5	G5-6	G6	G7	G7
T1	G2	G2	G4-5	G4-5	G6	G6	G7	G7

Note: PSA-positive whole blood samples were used; "G+number" indicates the color intensity of the T line/Test line (a bigger number indicates a more intense color).

Table 4. Comparison of specificity between Treatment 1 (T1) and Treatment CK (CK):

	Negative Preparations	Clinical Preparations
CK	G1 (100%)	G1 (100%)
T1	G1 (100%)	G1 (100%)

Note: Two types of preparations were used here. Standard PSA-negative (experimentally confirmed) whole blood samples were tested, and 100% of the results, both in CK and T1 groups, were negative (the color intensity of the test lines was G1). Clinically collected whole blood samples (which were not experimentally confirmed PSA-negative samples and could contain PSA) were also used, and all the results, both in CK and T1 groups, appeared to be all negative (the color intensity of the test line was G1), too.

8. Conclusions

The above results suggest that anti-fibrin antibodies are able to separate red blood cells from blood samples, thereby greatly reducing the chance for coagulated blood samples blocking chromatography supports and greatly enhancing the efficiency of blood separation; meanwhile, it appears that anti-fibrin antibodies have no disadvantageous effect on the detection itself. Moreover, if anti-fibrin antibodies are to be used in combination with anti-red blood cell antibodies, their advantageous effect on blood separation would be more obvious.

Example 3: Accelerated stability test

Treatments F and J from experiment 1 were also used to perform an accelerated stability test to determine the longest effective time of the products.

1. Methods

Products from Treatments F and J were exposed at a temperature of 55℃. Products subjected to this high temperature treatment were then used to perform sensitivity and specificity tests according to the date schedule listed in the following table 3-1. 10 samples from each treatment (Treatment F and Treatment J) were used for each of the test performed,

respectively. For each test, 100μl of whole blood sample was dropped onto the sample receiving pad, and the time for the C line to appear was subsequently recorded.

Table 1. Time for stability test and scheduled test date

Temperature	Day	Day	Day	Day	Day	Day	Day
	0*	7	14	21	30	35	40
55℃	X	X	X	X	X	X	X

Results at 0 day:

Table 2-1. Results from Treatment F

No.	CTI-negative Preparations	Positive Preparations (with 0.5ng/ml CTI)	Positive Preparations (with 5ng/ml CTI)	Time for the C line to appear
1	-	3+	5.5+	4'15"
2	-	3+	5.5+	3'45"
3	-	3+	6+	4'15"
4	-	3.5+	5.5+	3'15"
5	-	3+	6+	3'10"
6	-	3+	5.5+	3'35"
7	-	3.5+	6+	4'35"
8	-	3+	5.5+	2'40"
9	-	3+	6+	4'35"
10	-	3+	6+	4'15"

Note: Hereinafter, "+" indicates that the result was positive; "-" indicates that the result was negative; the number before "+" indicates the color intensity of the resulted test line (i.e., 3 in "3+" indicates that the color intensity of the resulted test line was G3).

Table 2-2. Results from Treatment J:

No.	CTI-negative Preparations	Positive Preparations (with 0.5ng/ml CTI)	Positive Preparations (with 5ng/ml CTI)	Time for the C line to appear
1	-	3+	5.5+	3'25"
2	-	3.5+	5.5+	3'15"
3	-	3+	6+	4'
4	-	3.5+	5.5+	2'45"
5	-	3+	5.5+	3'10"
6	-	3+	5.5+	3'05"
7	-	3.5+	6+	3'45"
8	-	3+	5.5+	2'30"
9	-	3+	6+	3'45"
10	-	3+	6+	3'20"

Table 3. Results at the 7th day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+,3+,3.5+	3+,3+,3+
Positive Preparations (with 5ng/ml CTI)	6+,6+,6+	6+,6+,6+
Time for the C line to appear	4'20", 3'35", 4'50"	3'35", 2'45", 3'20"

Table 4. Results at the 14th day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+, 3.5+, 3.5+	3+, 3+, 3.5+
Positive Preparations (with 5ng/ml CTI)	6.5+, 6+, 6+	6+, 6+, 6+
Time for the C line to appear	4'50", 4'25", 4'45"	3'30", 3'15", 3'30"

Table 5. Results at the 21st day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+, 3.5+, 3.5+	3+, 3+, 3.5+
Positive Preparations (with 5ng/ml CTI)	6.5+, 6+, 6+	6+, 6+, 6+
Time for the C line to appear	5'20", 4'45", 4'10"	3'40", 3'10", 3'35"

Table 6. Results at the 30th day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+, 3.5+, 3.5+	3+, 3+, 3.5+
Positive Preparations (with 5ng/ml CTI)	6.5+, 6+, 6+	6+, 6+, 6+
Time for the C line to appear	6'20", 4'35", 4'25"	3'50", 3'25", 3'45"

Table 7. Results at the 35th day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+, 3.5+, 3.5+	3+, 3+, 3.5+
Positive Preparations (with 5ng/ml CTI)	6.5+, 6+, 6+	6+, 6+, 6+
Time for the C line to appear	5'10", 5'15", 4'50"	2'55", 2'35", 2'50"

Table 8. Results at the 40th day:

	F	J
CTI-negative Preparations	-, -, -	-, -, -
Positive Preparations (with 0.5ng/ml CTI)	3+, 3.5+, 3.5+	3+, 3+, 3.5+
Positive Preparations (with 5ng/ml CTI)	6.5+, 6+, 6+	6+, 6+, 6+
Time for the C line to appear	5'10", 4'25", 5'50"	3'15", 2'55", 3'35"

2. Conclusions

It is obvious from the above results that when the sample receiving pad is equipped with anti-fibrin antibodies, the effective lifetime of the products can be as long as 2 years; meanwhile, the time for the blood sample to reach C line is reduced.

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Claims

1. A method for separating red blood cells from a blood sample, which comprises:

a) contacting the blood sample with a receptor capable of binding to fibrins in the blood sample; and

b) subsequently separating said fibrin-binding receptor from the blood sample.

2. The method according to claim 1, further comprising contacting the blood sample with a receptor capable of binding to red blood cells in the blood sample.

3. The method according to claim 2, wherein said fibrin-binding receptor and said red blood cell-binding receptor are fixed on a support supporting fluid flowing therethrough.

4. The method according to any of claims 1-3, wherein said receptors comprise: a) monoclonal antibody, or b) polyclonal antibodies, or c) fragment of a) or b).

5. A method for separating red blood cells from a blood sample, which comprises providing a chromatography support and allowing flowing therethrough of the blood sample, wherein said chromatography support is equipped with a receptor capable of binding to fibrins in the blood sample.

6. The method according to claim 5, wherein said chromatography support is further equipped with a receptor capable of binding to red blood cells in the blood sample.

7. The method according to any of claims 5-6, wherein said receptors comprise: a) monoclonal antibody, or b) polyclonal antibodies, or c) fragment of a) or b).

8. An apparatus for separating red blood cells from a blood sample, which comprises a support equipped with a receptor capable of binding to fibrins in the blood sample.

9. The apparatus according to claim 8, wherein said support is further equipped with a receptor capable of binding to red blood cells in the blood sample.

10. The apparatus according to any of claims 8-9, wherein said support is a chromatography support.

11. The apparatus according to any of claims 8-9, wherein said receptors comprise: a) monoclonal antibody, or b) polyclonal antibodies, or c) fragment of a) or b).

12. A detection apparatus for detecting an analyte in a blood sample, which comprises a support having a sample receiving region and a detection region located downstream of said sample receiving region, wherein the support upstream of said detection region is equipped with a receptor capable of binding to fibrins in the blood sample.

13. The apparatus according to claim 12, wherein said support is a chromatography support.

14. The apparatus according to claim 13, wherein said fibrin-binding

receptor is located in the sample receiving region.

15. The apparatus according to claim 14, wherein said sample receiving region is further equipped with a receptor capable of binding to red blood cells in the blood sample.

16. The apparatus according to any of claims 12-15, wherein said receptors comprise: a) monoclonal antibody, or b) polyclonal antibodies, or c) fragment of a) or b).

17. The apparatus according to claim 14, wherein the support downstream of said sample receiving region further comprises a labeled substance that can be carried away and flow along with the blood sample.

18. The apparatus according to claim 14, wherein said detection region is equipped with a specific binding molecule.

19. A method of detecting an analyte in a blood sample, which comprises: providing a chromatography support, allowing flowing therethrough of the blood sample, and measuring content of the analyte in the blood sample; wherein said chromatography support is equipped with a receptor capable of binding to fibrins in the blood sample.

20. The method according to claim 19, wherein said support further comprises a receptor capable of binding to red blood cells in the blood sample.

21. The method according to any of claims 19-20, wherein said receptors comprise: a) monoclonal antibody, or b) polyclonal antibodies, or c) fragment of a) or b).

22. The method according to claim 19, wherein there further comprises a detection region in the downstream region of said chromatography support for detecting said analyte in the blood sample.

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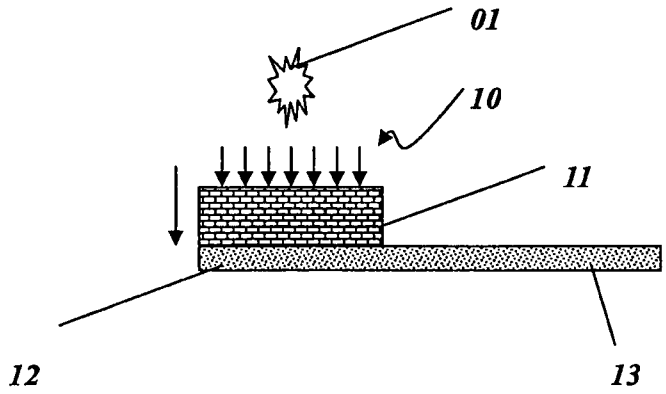

Fig. 1

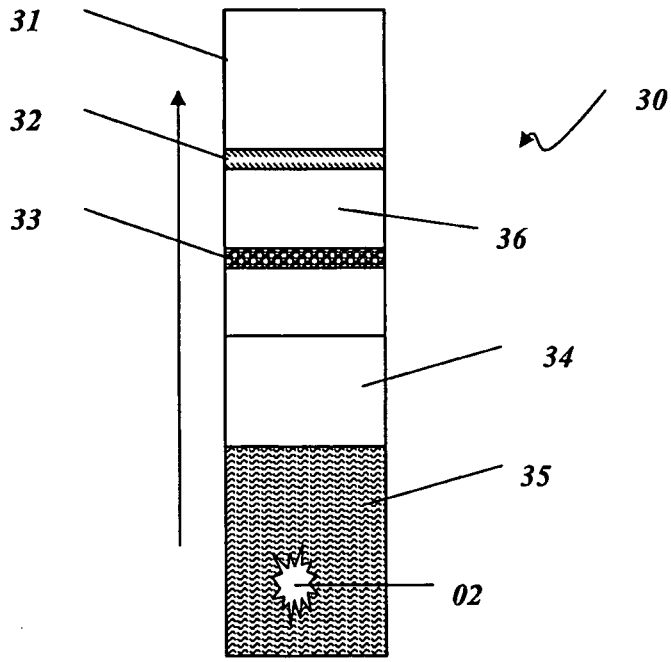

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/001137

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: G01N33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNPAT, WPI, EPODOC: fibrin, red blood cell, erythrocyte, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN1243251A(ZHENGZHOU BRANCH CO HUAMEI BIOLOGICAL EN) 02 Feb.2000(02.02.2000), see the whole document	1-22
A	US6890728B(MEDTRONIC INC) 10 May 2005(10.05.2005), see the whole document	1-22
A	US5849178A(BRISTOL-MYERS SQUIBB CO) 15 Dec.1998(15.12.1998), see the whole document	1-22

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 Jan.2010 (05.01.2010)	**21 Jan. 2010 (21.01.2010)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer WANG, Lihua Telephone No. (86-10)62085676

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2009/001137

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1243251A	2000-02-02	NONE	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/001137

According to International Patent Classification (IPC) or to both national classification and IPC:

G01N33/49 (2006.01) i

G01N33/48 (2006.01) i

G01N33/543 (2006.01) i

G01N33/53 (2006.01) i