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(54) **Title:** METHODS AND DEVICES FOR THE NEUTRALIZATION OF HEPARIN IN A SAMPLE

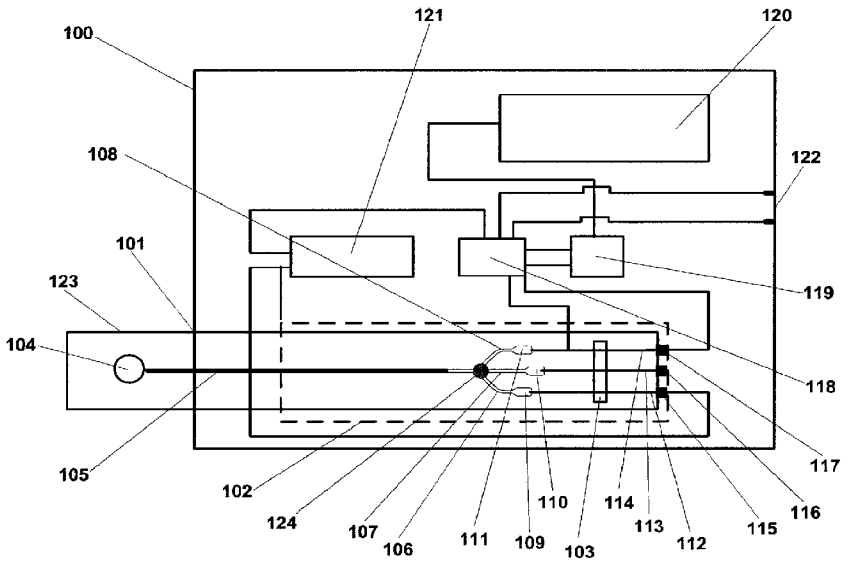


Fig. 1

(57) **Abstract:** This invention relates to methods and devices for neutralizing the effects of heparin in whole blood, plasma, or another biological sample, for the purpose of performing a clinical diagnostic test based on blood coagulation pathways without the interfering effect of heparin. This invention also pertains to performing such a test on a point of care device.

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METHODS AND DEVICES FOR THE NEUTRALIZATION OF HEPARIN IN A SAMPLE

BACKGROUND OF THE INVENTION

[0001] Heparin is a negatively charged polymeric molecule ranging in molecular weight from 3 to 40 kDa. It is commonly used therapeutically as an anticoagulant to reduce the risk of blood clotting in various coagulation disorders and during surgical procedures such as coronary bypass, coronary intervention, and heart valve implantation. Its biological mechanism of action involves binding to antithrombin (AT) and thereby amplifying the ability of AT to inhibit the function of other coagulation enzymes, particularly thrombin and factor Xa. Heparin, as unfractionated heparin or low molecular weight heparin (LMWH), may be used as the sole anticoagulant in therapy or during surgery, but is often used together with other anticoagulants such as warfarin (Coumadin®), aspirin or platelet inhibitors.

[0002] Anticoagulation therapy requires striking a balance between administering an effective amount of anticoagulant and simultaneously keeping the risk of bleeding low. For this reason, patients who are on anticoagulation therapy benefit greatly from being able to accurately monitor the coagulation properties of their blood. Convenient and reliable tests to allow monitoring the coagulation properties of blood are thus highly desirable. Several tests have been established to measure coagulation including the prothrombin time test (PT), the activated clotting time test (ACT), the activated partial thromboplastin time test (aPTT), and platelet function tests such as platelet aggregometry, VerifyNow, and the Dade PFA-100. More recently, point of care versions of some of these tests have been developed. Point of care tests allow more rapid turnaround time of test results, enable more convenient and frequent testing to help assure that patients are in the therapeutic range, and could reduce the number of inconvenient trips to a care provider.

[0003] In tests where coagulation is the endpoint for the measurement of particular coagulation factors or pathways, the presence of heparin in the test sample can interfere with test results due to its interactions with clotting pathway proteins.

[0004] For example, the PT test may be used to monitor the coagulation of patients taking a vitamin K antagonist (VKA). VKAs may interfere with synthesis of factors in the extrinsic coagulation pathway and thereby reduce the risk of unwanted clotting. VKA is typically a longer term anticoagulant, and its effects may continue after shorter term anticoagulants such as heparin are discontinued and decrease in physiological circulation. Because of this, it may be important to determine the specific effect of VKA-induced inhibition of coagulation as opposed to the activity of other anticoagulants. For example, in the treatment of venous thrombosis, or when anticoagulant therapy is administered after mechanical heart valve replacement, patients may receive heparin at the same time as VKA. In such situations, heparin may prolongs the PT such that the inhibition of coagulation caused by VKA treatment can be overestimated. In another example, heparin can also affect platelets, such that the response of platelets to certain agonists may not provide an accurate indication of platelet function. Because of the importance of obtaining accurate measurements of specific blood coagulation properties, reliable removal or quantitative reduction of the heparin content of a sample would be highly desirable.

SUMMARY OF THE INVENTION

[0005] In general, the invention provides devices, methods, test strips and kits for measuring the properties of a sample comprising heparin.

following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0014] **Figure 1** illustrates a device and test strips of the present invention.

[0015] **Figures 2 A-D** illustrate exemplary configurations of test strips according to the present invention.

[0016] **Figures 3 A-K** illustrate exemplary localization of heparin neutralizing reagent(s) on test strips according to the present invention.

[0017] **Figures 4 A-C** illustrate a method of preparing test strips of the present invention.

[0018] **Figure 5** describes the results of measurements of prothrombin time in the presence and absence of heparin neutralizing reagent.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention encompasses a device, test strips, methods and kits for measuring the properties of a sample comprising heparin.

[0020] In one embodiment, the measurements to be perfomed on a sample are coagulation or lysis assays. Such assays include, but are not limited: the prothrombin time (PT), the activated clotting time (ACT), the thrombin clotting time (TCT) or the auto hemolysis test (AHT). Specific examples of tests that can be used in the measurement areas are disclosed in more detail below.

[0021] The PT test was first described by Quick in 1935. The PT test measures the tissue factor–induced coagulation time of blood or plasma. It is used as a screening test to evaluate the integrity of the extrinsic coagulation pathway and is sensitive to coagulation factors II, V, VII, and X. A prolonged clotting time suggests the presence of an inhibitor to, or a deficiency in, one or more of the coagulation factors of the extrinsic pathway. The PT time can also be prolonged for patients on anticoagulant therapy, for example, coumarin drug therapy, or for those with vitamin K deficiency or liver dysfunction. Thus the PT test can provide an assessment of the extrinsic coagulation pathway. PT test results can be converted to International Normalized Ratio (INR) values according to the following formula:

[0022] $INR = (PT\ ratio)^{ISI}$, and PT ratio=Patient's PT/Mean Normal PT, where ISI is the International sensitivity index.

[0023] A device used to test PT may include, within one or more of its measurement areas, reagents appropriate for measuring PT, such as thromboplastin (e.g., recombinant or purified).

[0024] The ACT test is used primarily to monitor a patient's coagulation status in connection with clinical procedures that involve the administration of high doses of heparin or other anticoagulants (e.g., coronary bypass surgery and percutaneous transluminal coronary angioplasty). Prolongation of ACT is directly proportional to the concentration of heparin or other anticoagulants in the blood. A device used to test ACT may include within one or more of its measurement areas reagents that use appropriate for measuring ACT, such as kaolin or celite.

[0025] The TCT test measures the rate of a patient's clot formation compared to that of a normal plasma control. The TCT test can be used as an aid in the diagnosis of disseminated intravascular coagulation (DIC) and liver disease. Thus, a device of the invention may include, within one or more of its measurement areas, reagents that are appropriate for measuring TCT, such as thrombin.

[0026] Platelet function analysis measures the functionality of platelets in citrated platelet-rich plasma in response to certain agonists or activating agents. In platelet aggregometry, for example, platelet function in response

[0006] In some embodiments, the invention provides a test strip comprising at least one channel connecting at least one sample measurement area to at least one sample input area, wherein a heparin neutralizing reagent is deposited within at least one of the channels. The test strip can contain one, two, three or more sample measurement areas. In a particular embodiment, a test strip is provided according to the invention containing three sample measurement areas, each connected by channels to a sample input area, wherein a heparin neutralizing reagent is deposited within at least one of the channels.

[0007] In yet other embodiments, the heparin neutralizing reagent is deposited outside of the sample measurement area. In still other embodiments, the heparin neutralizing reagent is deposited in a junction region connecting two or more channels. Alternatively, the heparin neutralizing reagent is deposited within a channel connecting a sample input region and a junction region linking two or more additional channels. In another embodiment, the heparin neutralizing reagent is deposited within the sample input area.

[0008] In some embodiments of the present invention, at least one of the sample measurement areas comprises electrodes allowing a DC or AC potential to be applied to the sample. In a particular embodiment, a test strip is provided comprising two sample measurement areas, wherein one of the sample measurement areas is used for quality control purposes. An alternative embodiment provides for a test strip comprising three sample measurement areas, wherein two of the sample measurement areas are used for quality control purposes.

[0009] In other embodiments, the invention provides for test strips comprising a heparin neutralizing reagent and a reagent for increasing blood viscosity, wherein the heparin neutralizing reagent is located proximal to the reagent for increasing blood viscosity. The test strips described herein can be used in combination with a device to measure the properties of a sample comprising heparin. In some embodiments, the test strips of the invention are configured to measure a coagulation curvea time course of coagulation of a sample. In a specific embodiment, the test strips are configured to measure prothrombin time.

[0010] The present invention also provides a method for measuring blood properties by contacting a blood sample on a test strip with a heparin neutralizing reagent and subsequently contacting the sample with reagents for increasing blood viscosity. In some embodiments, the reagent for increasing blood viscosity is thromboplastin. In a related embodiment, the reagent for increasing blood viscosity is recombinant tissue factor.

[0011] The invention is also directed to a method for measuring blood properties by contacting a blood sample with a heparin neutralizing reagent and subsequently contacting the sample with reagents that initiate blood coagulation, followed by measurement of an electrical property of the sample. In some embodiments, the method will include measurement of one of the properties selected from the group consisting of impedance, resistance or capacitance. In other embodiments, a prothrombin time test (PT) or activated clotting test (ACT) is performed. In yet other embodiments, the sample is smaller than 20 μL.

INCORPORATION BY REFERENCE

[0012] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the

to an added agonist is measured by the aggregation of platelets to form a thrombus, said measurement being made by optical or electrical means.

[0027] Samples suitable for measurement according to the invention comprise whole blood or citrated blood samples. Samples may be extracted from a subject in various ways known to one skilled in art, including without limitation by an ear prick, a finger prick, and by drawing blood from a vein. Suitable samples may be collected by a health care worker, such as a nurse or medical technician, the patient, or others. The volume of collected sample may vary. In some embodiments, the volume collected may be as low as about 1, 2, 5, 10, 15, 20, 25 μL. In other embodiments, the volume may be as high as about 100, 250, 1000, 2000, 5000, or 10000 μL.

[0028] In some embodiments, the devices herein are used to measure the ability of a sample to coagulate or lyse. In such embodiments, a portion of the sample (e.g. plasma or whole blood) is contacted with a channel connecting a sample measurement area to a sample input area, and a heparin neutralizing reagent deposited within the channel. Once the sample reaches the measurement area that includes one or more reagent compositions that activate the intrinsic and/or extrinsic coagulation cascade, the time until clot formation is measured. In some embodiments, the heparin neutralizing reagent is deposited outside of said sample measurement area. The reagent composition(s) may comprise thromboplastin, which can be of human and/or recombinant origin. In particular embodiments, at least two sample measurement areas are provided. In embodiments where at least two sample measurement areas are provided, one or more of the sample measurement areas may be used as a quality control. Sample measurement areas may also comprise electrodes. The device may be configured to measure an electrical property. In some embodiments, the electrical property is impedance, resistance or capacitance. The sample tested in the device may be smaller than about 3, 10, 15, 20, or 25 μL. The device may be configured to measure a time course of coagulation. The device may also be configured to perform a blood test, such as a prothrombin time test. In some embodiments, the heparin neutralizing reagent comprises polycationic reagents, enzymes, metal salts, polypeptides or synthetic molecules. In related embodiments, the heparin neutralizing reagent comprises polybrene, polylysine, heparinase, protamine, calixarene, zinc salt or copper salt. The device may be configured to provide a response within about 5 minutes from initiation of coagulation. The device may comprise a test strip which is disposable.

[0029] In any of the embodiments herein, different coagulation or lysis promoting reagents may be provided within different measurement areas to allow for the simultaneous measurement of two or more different coagulation times. For example, in some embodiments PT assays might be carried out simultaneously each in a different measurement area. In some cases the PT test is in a delayed measurement area and the other is in a non-delayed measurement area. Typically, the reagent compositions for the specified test are applied to the test strip during the manufacturing process of the test strip and using various types of micro-dispensing techniques which include, but is not limited to, ink jet, striper and sprayer deposition methods, or dip coating, and air dried in situ during the manufacturing process.

[0030] In some cases, a sample may be assayed for the thrombolytic effect of a drug, and lysis of a blood sample is monitored. The effect of plasminogen activators can be monitored by assaying the rate of coagulation of a sample from a patient receiving a thrombolytic drug, such as urokinase or tPA. The clotting or lysis time of the patient sample may then be compared to the clotting or lysis time of pooled normal plasma or whole blood to provide a standard measurement of the patient's hemostatic status.

[0031] In some cases, a sample is collected from a patient before the patient is administered a drug (e.g. urokinase or tPA) and after drug administration. A difference in coagulation level, lysis, presence or absence or amount of an analyte in a sample can be determined using the devices herein. This can be used to determine effectiveness of drug treatment.

[0032] The present invention provides devices used to measure the properties of a sample comprising heparin. A representative example of the devices of the present invention is illustrated in **Fig. 1**. The device includes a housing **100** having an inlet port **101** for receiving a disposable fluidic test strip **123** for at least partial insertion therethrough. The test strip **123** is preferably disposable after performing the desired assay. Test strips can include any assay strip, cartridge, adapted and configured to receive a fluid sample and support one or more reagents and electrodes as described herein, as well as any other device suitable to achieve that purpose available to a person of skill in the art. Additionally the test strips and/or the device itself may assume any convenient geometric shape as long as the electronics and chemistry described herein are cost effectively contained with acceptable performance.

[0033] In one embodiment, the device is configured such that all of the components are mounted in the interior space of the housing **100**, including a power supply **121** to conduct the assay. Optionally, the device may provide a plug for an AC or DC adaptor. In this configuration, the test strip **123** is adapted and configured to be inserted into the device and is positioned in thermal proximity to a heater **102** which is used to warm the sample on the test strip to a target temperature. The target temperature can be pre-determined and can be above room temperature, i.e., greater than 35°C. Any conventional heater of the appropriate size and heating capacity for the anticipated sample size is suitable. Preferably, the heater is mounted between or below a substrate such as aluminum or other thermally conductive material for efficient and uniform transfer of heat to the test strip. Preferably, the temperature is maintained at 37°C so that the test results can readily compared to other standardized test results without interpolation.

[0034] A temperature sensor **103** is mounted in proximity to the detection or sampling area **109**, **110**, **111** where the sample is applied or transported to in order to detect the temperature of the system and provide ambient temperature information for calibration adjustment at temperature extremes. It is suitable to locate the temperature sensor **103** anywhere in or on the device. For example, the temperature sensor **103** may be located on the test strip.

[0035] The power supply **121** has a lead from its negative pole connected to one side of an electrode pair **115**, **116**, and **117** and a lead from its positive pole for delivering electric potential to the electrodes and detection of the impedance or resistance signal, which is relayed by electrical connection to an analog to digital converter **118** and display **120**. A processor and memory component **119** is connected to the analog to digital converter **118** and the display **120**. External ports **122** are connected to the analog to digital converter **118** for receiving assay calibration information, interfacing with a computer, or downloading test results. The distal end of the receiver electrodes **112**, **113**, and **114** that are in contact with corresponding contact pads **115**, **116**, and **117** of the measuring device which provide connection to the processor **119** and the power source **121**. The connection between the electrodes **112**, **113**, and **114** and the contact pads **115**, **116**, and **117** is made when the test strip **123** is inserted into the inlet port **101**.

[0036] The processor **119** can be any common or custom integrated circuit with memory. The power supply **121** can be any convenient device including, but not limited to, a battery, AC or DC adapter, or a solar cell. The display **120** preferably is a liquid crystal device LCD or any conventional, inexpensive display device. The number size in the display should be sufficiently large to allow most people to read the assay value, even if

they have poor vision. The display height can be about 2 cm. The number of digits in the display can be anywhere from 1 to about 10 digits, however, a 3 to 5 digit display is usually sufficient. In addition to showing the assay result, the display may show messages relating to the assay result or processing or give error messages.

[0037] The converter **118** can include a multiplexer to integrate the signal from the electrodes and provide the digital signal to the processor **119**. The processor can be used to count the time required for the integral to reach a fixed voltage comparator threshold. The time is proportional to the average signal over the sampling period.

[0038] The device can be of any convenient size with the optimal dimensions determined by several factors including convenience of use to the consumer. Preferably, the device has a volume range of about 5 cm^3 to about 500 cm^3.

[0039] As described above, the inserted test strip **123** is positioned in proximity of a heater **102**. Thus, when in use, the sample is maintained at a target temperature. Alternatively, a heater can be included within the test strip. The temperature of the sample is maintained constant by signaling from a temperature sensor **103** to the heater. The sensor is mounted in proximity to the detection area of the test strip, or it may be mounted anywhere on the device, and it provides temperature information for calibrating the device and ensuring that the assays are conducted at a predetermined constant temperature to eliminate interassay temperature variations.

[0040] In one embodiment described in **Fig. 1**, a sample is applied to the sample input area **104** of the test strip **123** and is transported by capillary action through the microfluidic channel **105** to the junction area **124**, where it contacts a heparin neutralizing reagent. The sample further travels through the branch channels **106**, **107** and **108** by to the measurement areas **109**, **110**, and **111**. The volume of blood that is applied to the test strip can be between 5 and 20 μL, or between 10 and 15 μL. Sample flow is stopped within each of the measurement area(s) by air vents **109**, **110**, and **111** that act as stop junctions.

[0041] Within the measurement area, the fluid sample reacts with the reagent(s) in the measurement area and bridges the electrodes, thereby changing the impedance, resistance or capacitance across the electrodes and signaling the change to the measuring device, thereby initiating the assay. The electrodes are adapted to receive and pass a predetermined signal into the sample. The electrodes generate an electrical signal corresponding to the impedance of the sample. For example, the change in impedance is signaled through the distal end of the receiver electrodes **112**, **113**, and **114** that are in contact with corresponding contact pads **115**, **116**, and **117** of the measuring device to an analog to digital converter **118**, which integrates the signal from the electrodes and conveys it to a processor and memory component **119**, and a display **120**. The device is driven by a source **121** that can be any power source such as a battery, AC or DC adaptor, or a solar powered cell.

[0042] Thus a test strip of the device can comprise a heparin neutralizing reagent and a reagent for inducing blood coagulation wherein the heparin neutralizing reagent is located in a different location than the reagent for inducing blood coagulation.

[0043] The reagent for inducing blood coagulation may be thromboplastin, including human and/or recombinant thromboplastin. The test strip may comprise one channel or more than one channel. The heparin neutralizing reagent may be located at a junction formed by the channels, or it may be located near a junction formed by said channels. In some embodiments, the heparin neutralizing reagent comprises polycationic reagents, enzymes, metal salts, polypeptides or synthetic molecules. In related embodiments,

the heparin neutralizing reagent comprises polybrene, polylysine, heparinase, protamine, calixarene, zinc salt or copper salt. The test strip may be configured to measure a time course of coagulation. In come embodiments, the test strip is configured to provide a response within about 5 minutes from initiation of coagulation.

[0044] Electrical properties that can be measured by the devices herein include, but are not limited to, electrochemical and electrical conductivity, and impedance. Impedance can be reflective of the change in viscosity or coagulation or lysis of a sample. Thus, coagulation or lysis of a sample can be measured as a function of a change in impedance, or it may also be measured as a function of a change in the electrolytic conductance of the sample. Electrolytic conductance is a measure of the ability of electrolytes in a solution to carry an electrical current by virtue of the mobility of the ions in the solution.

[0045] Reagents in the measurement areas can comprise any combination of cations, salts, lipids, phosphatides, sulfatides, proteins or nucleic acids. In one embodiment, at least one of the measurement areas comprises a reagent for inducing blood coagulation. In a preferred embodiment, at least one of the measurement areas comprises thromboplastin. The thromboplastin used in the present invention can be of mammalian or bacterial origin, and can be purified from a natural source or obtained through recombinant methods. Coagulation or lysis can also be measured through the quantitation of the activity or activities of a specific coagulation factor or factors of interest. Such activity can be measured through a physical properties of the sample, such as viscosity, optical extinction, electrical impedance, electrical capacitance, or electrical conductivity. The activity of one or more specific coagulation factors can be measured by detectable reaction processes or products that are caused or generated by the factors of interest. Such reaction products may be detected by a measurable label, whether the label be one that is measured through optical extinction, optical fluorescence, optical dichroism, optical polarization, fluorescence polarization, luminescence, paramagnetism, electrochemical properties, electrical impedance, electrical conductance, or electrical capacitance.

[0046] The present invention provides for test strips to be used for measuring the properties of samples comprising heparin. In some embodiments, illustrated in **Figures 2A-D**, the test strips comprise a sample input area **201** in fluid connection (**202**) with a junction region **227** comprising a heparin neutralizing reagent. Additional microfluidic channels link the junction area **227** with the sample measurement areas **207**, **208** and **209**. The test strips may be configured to have one, two, or more measurement areas. Each measurement area also contains a detector for detecting an electrical property of the sample comprising a driver and a receiver electrode. The driver electrodes **217**, **223** and **225** deliver an electric potential to the measurement areas **206**, **207**, and **208**, and the receiver electrodes **218**, **224**, and **226** deliver an electrical signal reflective of the the electrical properties, such as impedance or conductance, of the sample present in the measurement areas to a measuring device that translates the impedance signal into a digital output. In one embodiment, an AC electric potential is applied to the electrodes through the fluid sample and measured signal is impedance.

[0047] A number of heparin neutralizing reagents can be used to carry out the present invention. Reagents suitable for use include substances that directly bind, precipitate, immobilize or degrade heparin, or alternatively interfere with the binding of heparin to blood clotting proteins. Heparin neutralizing reagents can be used alone or in combination to carry out the described invention.

[0048] One category of heparin neutralizing reagents includes enzymes and other compounds that degrade heparin. This category comprises natural and recombinant heparinases of various origins, e.g. mammalian or

bacterial origin. Examples of such heparinases include heparinase I, II, and III. Alternatively, polycationic reagents may be used as heparin neutralizing reagents. Polycationic reagents include positively charged compounds or polymers. The cationic residues of such reagents can be tertiary or quaternary amino residues, urea, guanidine, histidine or other positively charged moieties, such as polybrene and polylysine. Other heparin inactivating reagents suitable for use according to the invention comprise natural or synthetic polypeptides and their analogs. Reagents in this category include, but are not limited to, natural or recombinant protamine or protamine analogs. Yet another category of heparin neutralizing reagents encompasses synthetic molecules such as calixarene, calixarene analogs and derivatives thereof. These can be used as heparin neutralizing reagents according to the present invention. Furthermore, metal salts such as zinc and copper salts may be used to inactivate heparin in a sample.

[0049] The heparin neutralizing reagents may be present in solid form, or as a semisolid gel, a powder, a liquid solution, or liquid suspension. Heparin neutralizing reagents may also be layered on top of another surface or may be shaped in the form of a filter or membrane contacting a sample. The reagent may be deposited in liquid or gel form by spraying, droplet deposition, brushing, layering, or blotting onto a substrate. The amount of the heparin neutralizing reagent will vary depending on type of molecule and charge density. The amount can range from 0.1 to 10 micrograms for a sufficient amount to neutralize therapeutic amounts of heparin in an approximately 15 microliter sample of whole blood. Typical maximum therapeutic levels of heparin in plasma are approximately 1 IU/mL unfractionated heparin or 2 anti-Xa units/mL low molecular weight heparin, and whole blood levels are usually about half of these concentrations. A person skilled in the art will know how to derive an appropriate amount of heparin neutralizing reagent based on the expected concentration of heparin in the samples to be measured. Suitable heparin neutralizing reagents of the invention additionally include substrates functionalized or associated with heparin neutralizing reagents. Such substrates can be any solid matter amenable for carrying the heparin neutralizing reagent, including but not limited to non-porous or porous media, hollow or solid fibers, polymeric membranes or beads. Any of the heparin neutralizing reagents noted previously may be used to functionalize the substrate.

[0050] Neutralization of heparin in a sample according to the present invention can be complete or incomplete, and the extend of neutralization will vary based on the specific desired goal of each embodiment. In some applications, it may be desirable to remove most or all of the heparin present in the sample, such as for prothrombin time tests performed on blood samples. In other instances, partial removal is preferred, for example if the goal is to measure the response of a sample to a known quantity of heparin through quantitative removal of heparin from the sample.

[0051] **Figure 3** illustrates several configurations for the location of the heparin neutralizing reagent deposited within the test strips. **Figs. 3A-D** and **Fig. 3I-J** show the heparin neutralizing reagent positioned proximal to a junction formed by the microfluidic channels on the test strip. **Fig. 3A** shows the heparin inactivating reagent **301** deposited along the microfluidic channel between the sample input area and the channel junction area. Alternatively, as described in **Figs. 3B-D**, the heparin neutralizing reagent **301** can be deposited along the channel between the junction area and each sample measurement area. One, two or all of the channels leading from the junction to the sample measurement areas can contain heparin neutralizing reagents. Another embodiment described in **Fig. 3E** shows the heparin neutralizing reagent **301** located within the sample input area. In yet another embodiment **(Fig. 3F)** the heparin neutralizing reagent **301** is positioned within the channel junction area. The invention is applicable to test strips containing one or two

sample chambers as shown in **Figs. 3G, H**, or to test strips in which the length, volume or position of individual channels or sample chambers is different (**Figs. 3I-K**).

[0052] The invention is also applicable to embodiments comprising bibulous or porous structures. In one such embodiment, a bibulous membrane may comprise assay reagents in one part of the membrane and additional heparin neutralizing reagents in the same or a different area of the membrane. Reagents, including assay reagents and heparin neutralizing reagents, may be deposited on the membrane, adsorbed to the membrane, impregnated into the membrane, or otherwise associated with the membrane. In a related embodiment, the heparin neutralizing reagent is deposited on one face or close to one face of the membrane, while other assay reagents are deposited on the opposite face or close to the opposite face of the membrane.

[0053] One method for fabricating a test strip for measuring electrical properties of a heparin-neutralized sample such as a blood sample is shown in **Fig. 4**. In this example, the test strip can be produced as a laminate that preferably comprises three layers **401**, **402** and **403**, as shown in the embodiment depicted in **Fig. 4A**. Layer **401** is the top layer; layer **402** is the middle layer, and layer **403** is the bottom electrode layer. The assembled strip **400** (**Figure 4B**) is obtained by first assembling the top layer **401** and middle layer **402** to form a top-middle layer **404** (illustrated in **Figure 4C**), and then combining the top-middle layer **404** with the electrode layer **403**.

[0054] As illustrated in **Figure 4A**, the fluidic path **405**, which transports the sample from the sample well **406** through the microfluidic feeder **407** and branch channels **408** to the measurement areas **409** of the assembled strip, can be formed by microfabricating a cutout on a two-sided adhesive middle layer **402**. The top layer **401** which is made of nonporous material is perforated to provide a circular opening **410** that aligns with the portion of the microfludic path **405** that forms the sample well **406** in the assembled test strip **400**. Additional cutouts of the top layer **401** can be made to provide the air vents **411**, which act as stop junctions during use of the strip. The top layer houses the reagents or compositions that enable the coagulation or lysis of a sample during use of the test strip. The reagents are deposited in the reagent areas **412** that are below the cut of the air vent, and on the side of the top layer that is adjacent to the middle layer. Additionally, the heparin neutralizing reagents are deposited on reagent area **416**. The adhesive is removed from the a first side of the middle layer **402** that is adjacent to the top layer **401**, and the top **401** and middle layer **402** are assembled to form the top-middle layer **404** (**Figure 4C**). The electrodes are formed on the electrode layer **403** by depositing a suitable inert conductor using a pump or any other means of deposition, such as vacuum or sputtering. Electrodes may also be produced by the printing of an ink using methods known in the art, such as screen printing. Suitable conductor materials that can be used to form the electrodes include but are not limited to silver, carbon, gold or platinum. The electrodes have a proximal end **413** and a distal end **414**, which respectively span the region of the electrode layer **403** that is complementary to that defining the measurement area **409** and the signaling edge **415** of the assembled test strip **400**. The adhesive is removed from the second side of the middle layer **402** of the top-middle layer assembly **404**, and is adhered to the electrode layer **403** to form the assembled strip.

[0055] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the

scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Example 1. Construction of a test strip for measuring prothrombin time.

[0056] A test strip is constructed comprising a sample input area linked through a capillary channel to a junction region. The junction region is connected via three separate capillary channels to three independent sample measurement chambers. Test strips were produced with deposition of 70 nanoliters of a reagent containing the heparin-neutralizing reagent in the channel in the region indicated as Fig. 4B (**407, 416**). The deposited reagent contained 0.32 micrograms of hexadimethrine bromide (HMB) as the heparin neutralizing reagent, 0.021 micrograms of sodium chloride, and 0.7 micromolar sorbitol for facilitating resolubilization of the reagent. The deposited reagent was dried on the strip by evaporation.

Example 2. Measurement of prothrombin time.

[0057] The test strip of Example 1 is inserted into an INRatio™ PT/INR monitor (Hemosense™). Non-anticoagulated venous whole blood is obtained from a human subject taking a vitamin K antagonist. The obtained blood is applied to the test strip. The blood is drawn into the test strip by capillary action where it first mixes with the polybrene deposited in the junction of the test strip, and subsequently mixes with reagents that initiate coagulation in the measurement chambers. The monitor performs the PT test and two quality control tests (normal and therapeutic) simultaneously, and determines whether the controls are within preset limits. If they are, strip integrity is verified, and the monitor reports the PT test result. If they are not, the monitor displays an error message. The test results are displayed in less than 2 minutes as an INR or as PT seconds and INR.

[0058] **Example 3. Measurement of prothrombin time in the presence and absence of heparin neutralizing reagent.**

[0059] An experiment is performed to measure prothrombin time on test strips with and without a heparin neutralizing reagent. The general method and test strip of Example 2 is used. Five types of test strips are tested, two without heparin neutralizing reagent (Control Strips Lot 1 and Control Strips Lot 2) and three with heparin neutralizing reagent ("Strips with HMB" Lot 1, Lot 2 and Lot 3). (**Fig. 5**) Blood samples without heparin and blood samples containing 1.32 u/ml of a low-molecular weight heparin (Fragmin™) are analyzed. On the control strips, the measured prothrombin time (PT) is about 25 to 30 seconds with no heparin added to the sample. When heparin is added, the prothrombin time is highly elevated (see Fig. 5, control strips). On test strips containing heparin neutralizing agent the prothrombin times of blood with and without heparin are essentially the same (see **Fig. 5**, "Strips with HMB").

WHAT IS CLAIMED:

1. A test strip comprising:

a channel connecting a sample measurement area to a sample input area; and

a heparin neutralizing reagent deposited within said channel.

2. The test strip of claim 1, wherein said heparin neutralizing reagent is deposited outside of said sample measurement area.

3. The test strip of claim 1, wherein said sample measurement area comprises thromboplastin.

4. The test strip of claim 3, wherein said thromboplastin is recombinant thromboplastin.

5. The test strip of claim 4, wherein said recombinant thromboplastin is recombinant human thromboplastin.

6. The test strip of claim 1, wherein said test strip comprises at least two sample measurement areas.

7. The test strip of claim 5, wherein at least one of said sample measurement areas is used as a quality control.

8. The test strip of claim 1, wherein the sample measurement area comprises electrodes.

9. The test strip of claim 1, wherein a sample used in said test strip is smaller than about 20 μL.

10. The test strip of claim 1, wherein said test strip is configured to measure a time course of coagulation.

11. The test strip of claim 1, wherein said test strip is configured to perform a blood test.

12. The test strip of claim 11, wherein said test strip is configured to perform a prothrombin time test.

13. The test strip of claim 1, wherein said heparin neutralizing reagent is selected from the group consisting of polycationic reagents, enzymes, metal salts, polypeptides and synthetic molecules.

14. The test strip of claim 13, wherein said heparin neutralizing reagent is selected from the group consisting of polybrene, polylysine, heparinase, protamine, calixarene, zinc salt and copper salt.

15. The test strip of claim 1, wherein said test strip is configured to provide a response within about 5 minutes from initiation of coagulation.

16. The test strip of claim 1, wherein said test strip is disposable.

17. The test strip of claim 1, wherein said test strip is configured to measure an electrical property.

18. The test strip of claim 17, wherein said electrical property is chosen from the group consisting of impedance, resistance and capacitance.

19. A test strip comprising a heparin neutralizing reagent and a reagent for inducing coagulation, wherein said heparin neutralizing reagent is located in a different location than the reagent for inducing coagulation.

20. The test strip of claim 19, wherein said reagent for increasing blood viscosity is thromboplastin.

21. The test strip of claim 20, wherein said reagent for increasing blood viscosity is recombinant human thromboplastin.

22. The test strip of claim 21, wherein said reagent for inducing coagulation is recombinant human thromboplastin

23. The test strip of claim 19, wherein said test strip comprises a channel.

24. The test strip of claim 19, wherein said test strip comprises more than one channel.

25. The test strip of claim 24, wherein said heparin neutralizing reagent is located at a junction formed by said channels.

26. The test strip of claim 24, wherein said heparin neutralizing reagent is located near a junction formed by said channels.

27. The test strip of claim 19, wherein said heparin neutralizing reagent is selected from the group of polycationic reagents, enzymes, metal salts, polypeptides or synthetic molecules.

28. The test strip of claim 27, wherein said heparin neutralizing reagent is selected from the group consisting of polybrene, polylysine, heparinase, protamine, calixarene, zinc salt and copper salt.

29. The test strip of claim 19, wherein said test strip is configured to measure a time course of coagulation.

30. The test strip of claim 29, wherein said test strip is configured to provide a response within about 5 minutes from initiation of coagulation.

31. A method of measuring a blood sample property, comprising:

on a test strip, contacting said blood sample with a heparin neutralizing reagent; and

subsequently contacting said blood sample on said test strip with additional reagents to initiate blood clotting.

32. The method of claim 31, wherein said blood sample is less than about 20 μL.

33. The method of claim 31, wherein one of said additional reagents comprises thromboplastin.

34. The method of claim 33, wherein said thromboplastin is recombinant.

35. The method of claim 31, further comprising measuring a time course of coagulation for said sample.

36. The method of claim 31, further comprising determining the prothrombin time for said sample.

37. The method of claim 31, wherein said heparin neutralizing reagent is a polycationic reagent.

38. The method of claim 37, wherein said heparin neutralizing reagent is polybrene.

39. The method of claim 31, wherein said heparin neutralizing reagent is an enzyme.

40. The method of claim 39, wherein said heparin neutralizing reagent is heparinase.

41. The method of claim 31, wherein said test strip is configured to provide a response within about 5 minutes from initiation of coagulation.

42. The method of claim 31, wherein said test strip is disposable.

43. The method of claim 31, further comprising contacting said sample with a pair of electrodes.

44. The method of claim 31, wherein said test strip is configured to measure an electrical property of said sample.

45. The method of claim 43 ,wherein said electrical property is chosen from the group consisting of impedance, resistance or capacitance.

46. A method of measuring blood properties, comprising:

contacting a blood sample with a heparin neutralizing reagent;

subsequently contacting said blood sample with additional reagents; and

measuring an electrical property of said blood sample.

47. The method of claim 46, wherein said electrical property is chosen from the group consisting of impedance, resistance and capacitance.

48. The method of claim 46, wherein said heparin neutralizing reagent is a polycationic reagent.

49. The method of claim 48, wherein said heparin neutralizing reagent is polybrene.

50. The method of claim 46, wherein said heparin neutralizing reagent is an enzyme.

51. The method of claim 50, wherein said heparin neutralizing reagent is heparinase.

52. The method of claim 46, wherein one of said additional reagents comprises thromboplastin.

53. The method of claim 52, wherein said thromboplastin is recombinant thromboplastin.

54. The method of claim 46, further comprising measuring prothrombin time of said sample.

55. The method of claim 46, wherein said blood sample is less than about 20 μL.

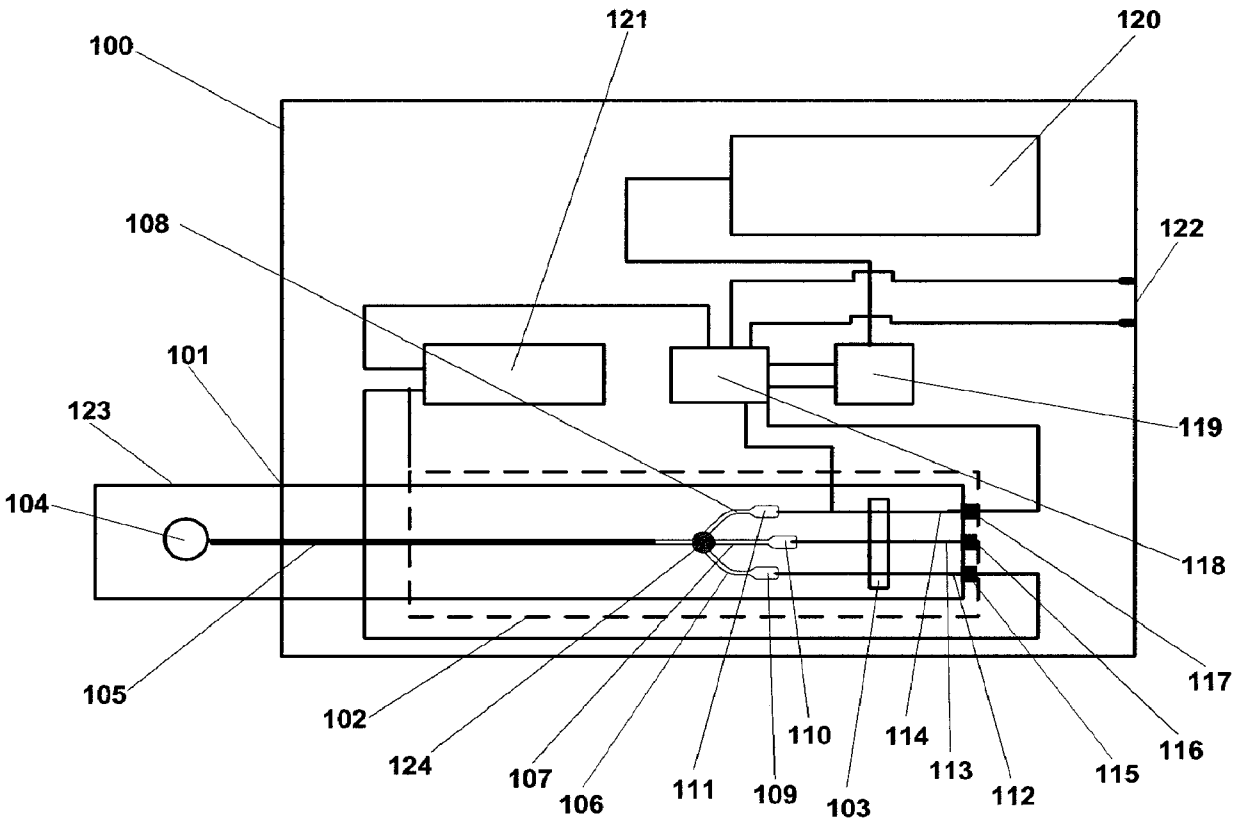

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Fig. 1

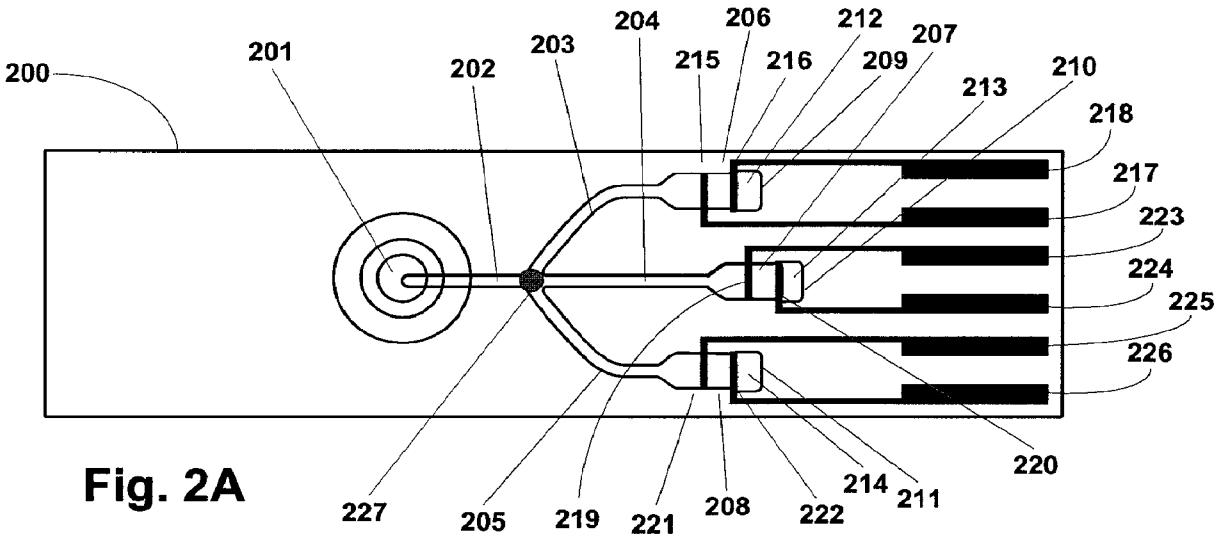

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Fig. 2A

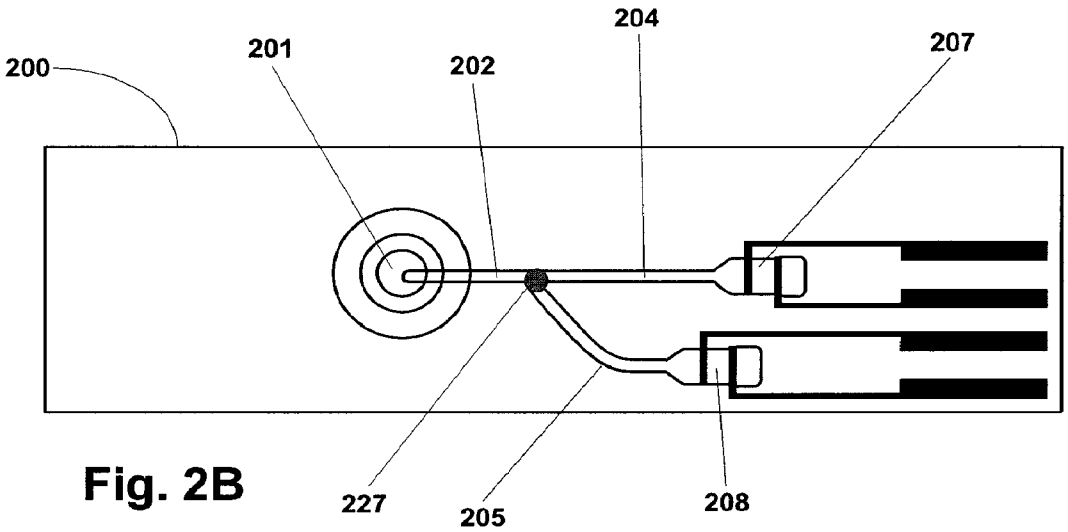

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Fig. 2B

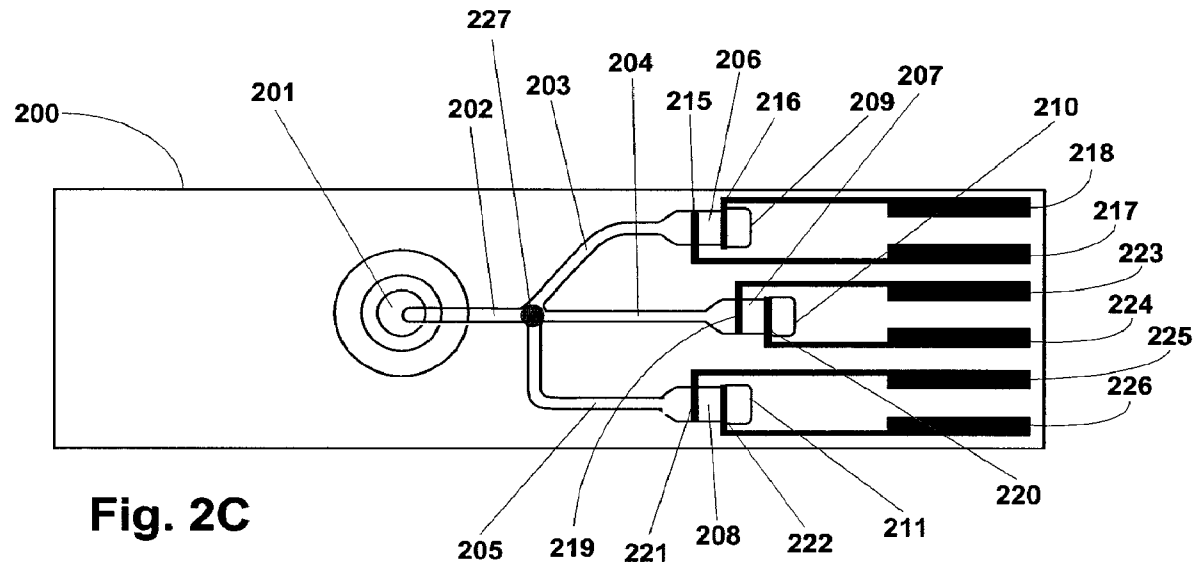

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Fig. 2C

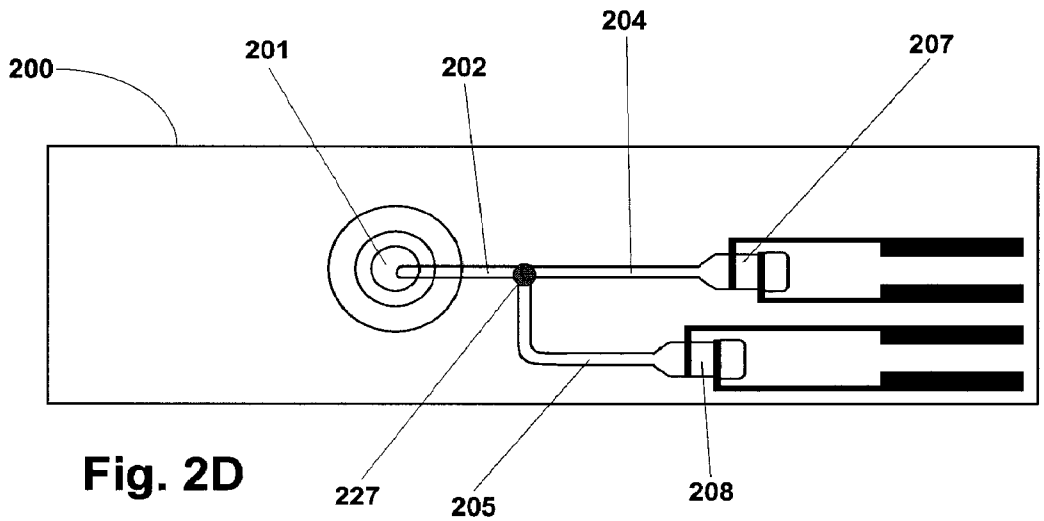

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Fig. 2D

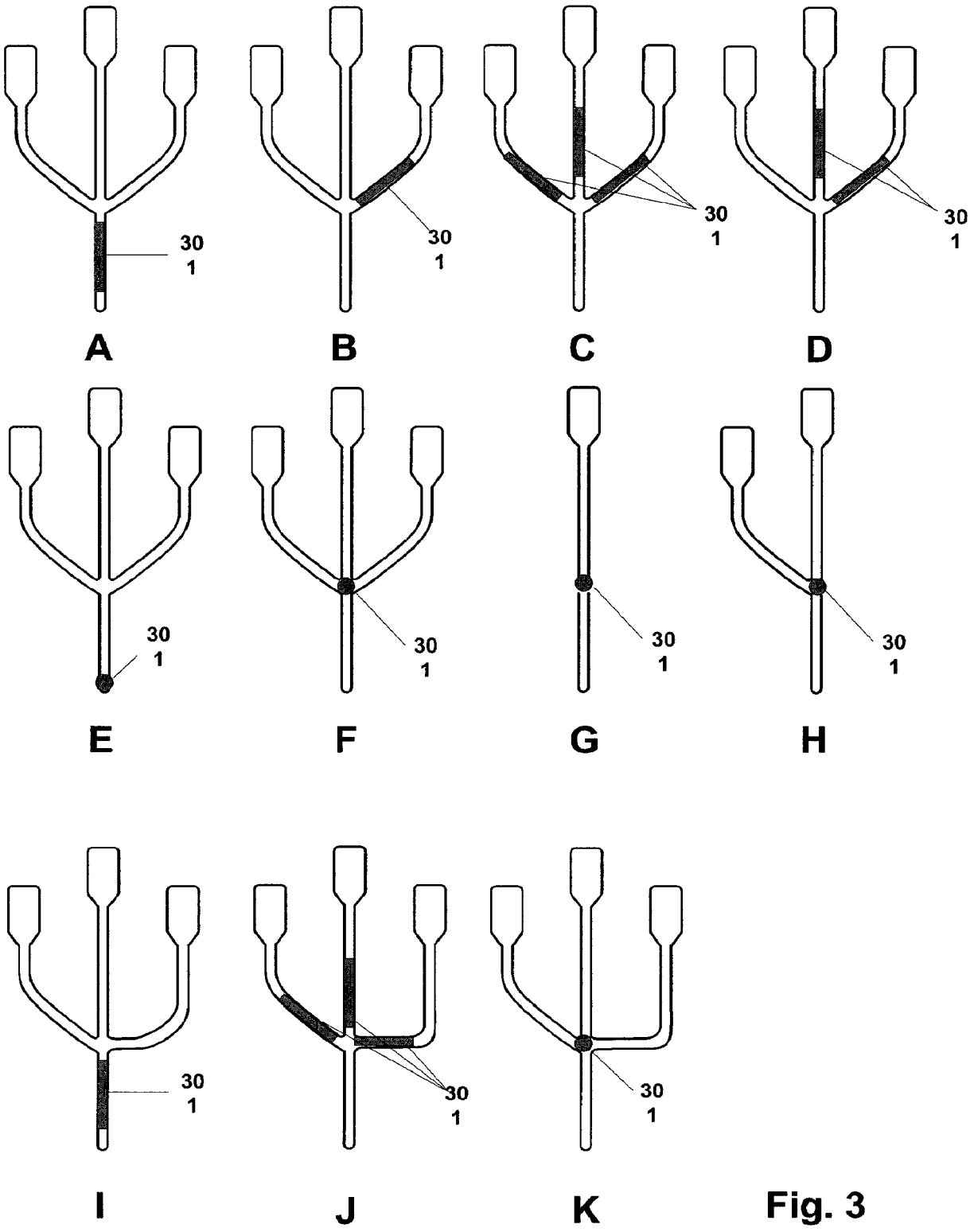


Fig. 3

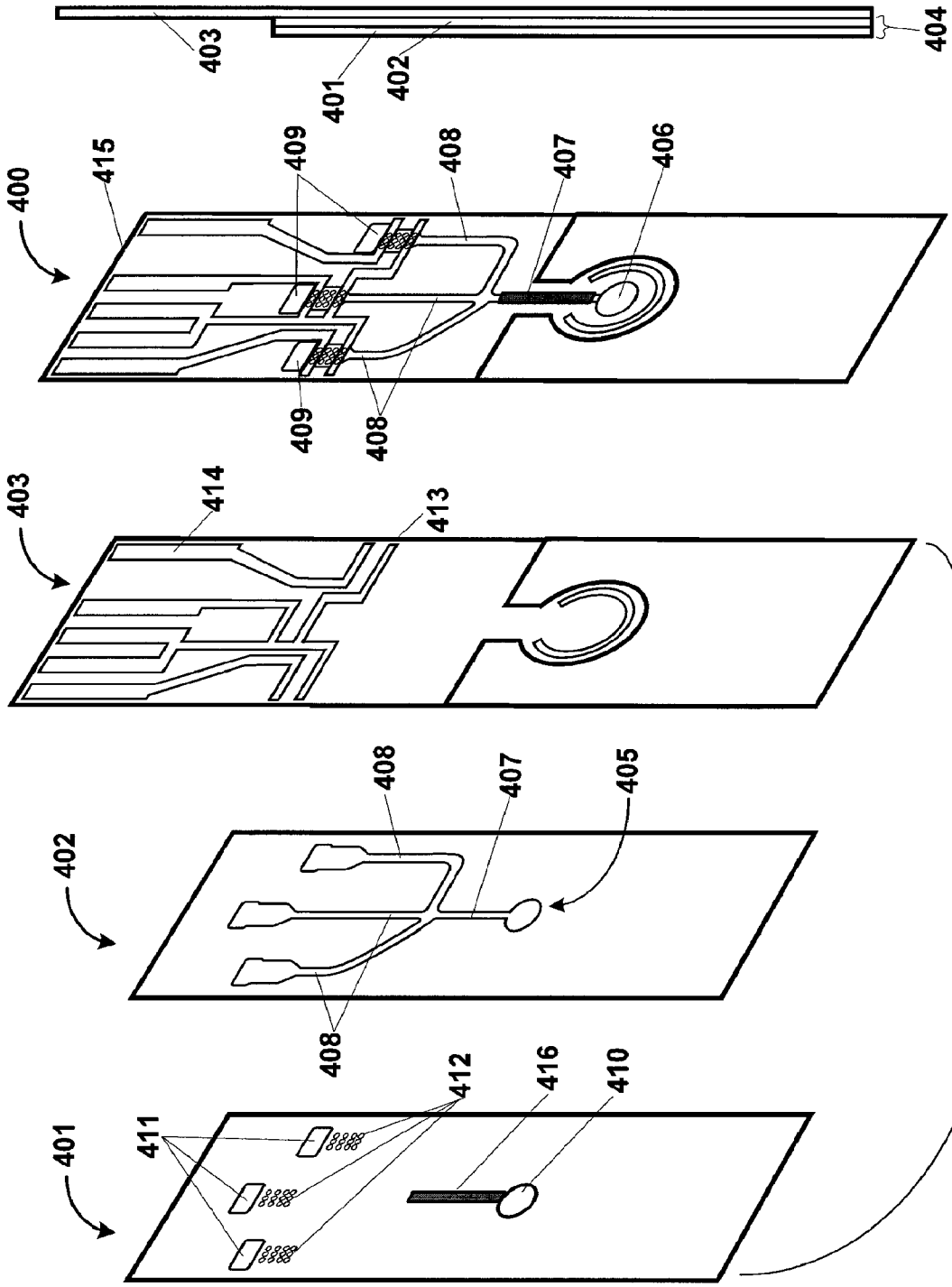


Fig. 4C

Fig. 4B

Fig. 4A

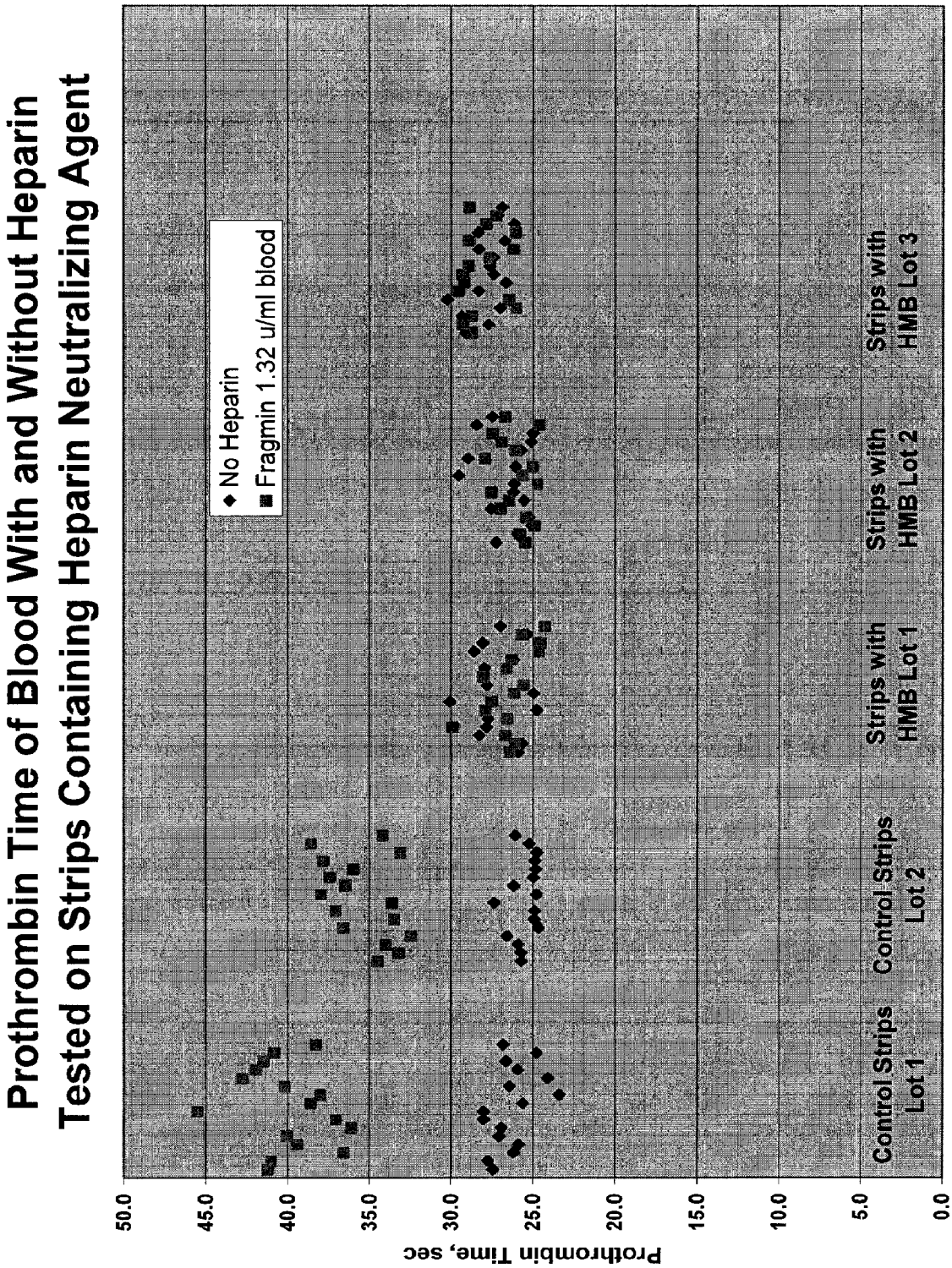

Prothrombin Time of Blood With and Without Heparin
Tested on Strips Containing Heparin Neutralizing Agent
No Heparin
Fragmin 1.32 u/ml blood
Prothrombin Time, sec
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Control Strips Lot 1
Control Strips Lot 2
Strips with HMB Lot 1
Strips with HMB Lot 2
Strips with HMB Lot 3

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/85745

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/00 (2009.01)
USPC - 422/73
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)
422/73

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
73/64.41; 324/449, 324/722; 422/73

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST (PGPB, USPT, USOC, EPAB, JPAB); Google
Test strip, blood, coagulate, coagulation, thromboplastin, heperin, neutralize, neutralizer, polybrene, polylysine, heparinase, protamine, calixarene, zinc, copper, salt, electrode, prothrombine, and PT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0082372 A1 (Ghai et al.) 12 April 2007 (12.04.2007), para [0009], [0048]-[0049], [0062]-[0063], [0082], [0102]-[0104], and [0118]	1-3, 9-15, 19-20, 23-33, and 35-41 --- 4-8, 16-18, 21-22, 34, and 42-55
Y	US 2006/0035298 A1 (Hill et al.) 16 February 2006 (16.02.2006), para [0083]	4-5, 21-22, 34 and 53
Y	US 2005/0233466 A1 (Wright et al.) 20 October 2005 (20.10.2005), para [0024] and [0036]	6-7, 16, and 42
Y	US 2006/0016701 A1 (Qin et al.) 26 January 2006 (26.01.2006), para [0104]	8, 17-18, and 43-55

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2009 (14.01.2009)	28 JAN 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)