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(54) **ASSAY METHODS, ASSAY CARTRIDGES, AND ASSAY DEVICES INCLUDING MAGNETIC PARTICLE DE-AGGREGATION**

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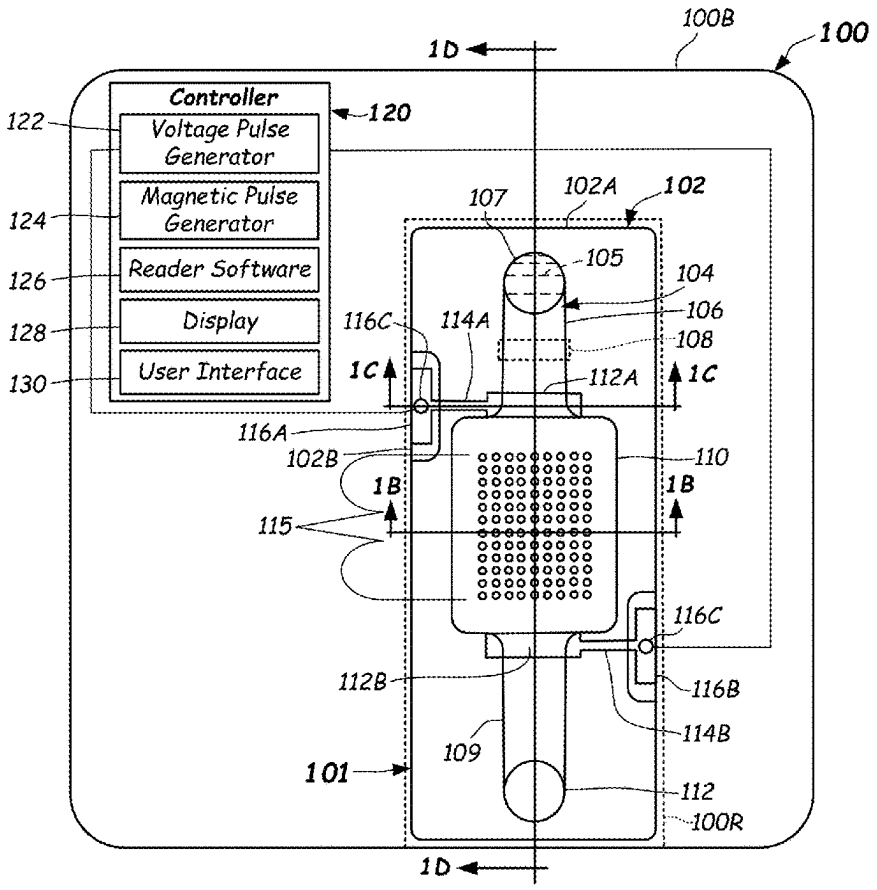
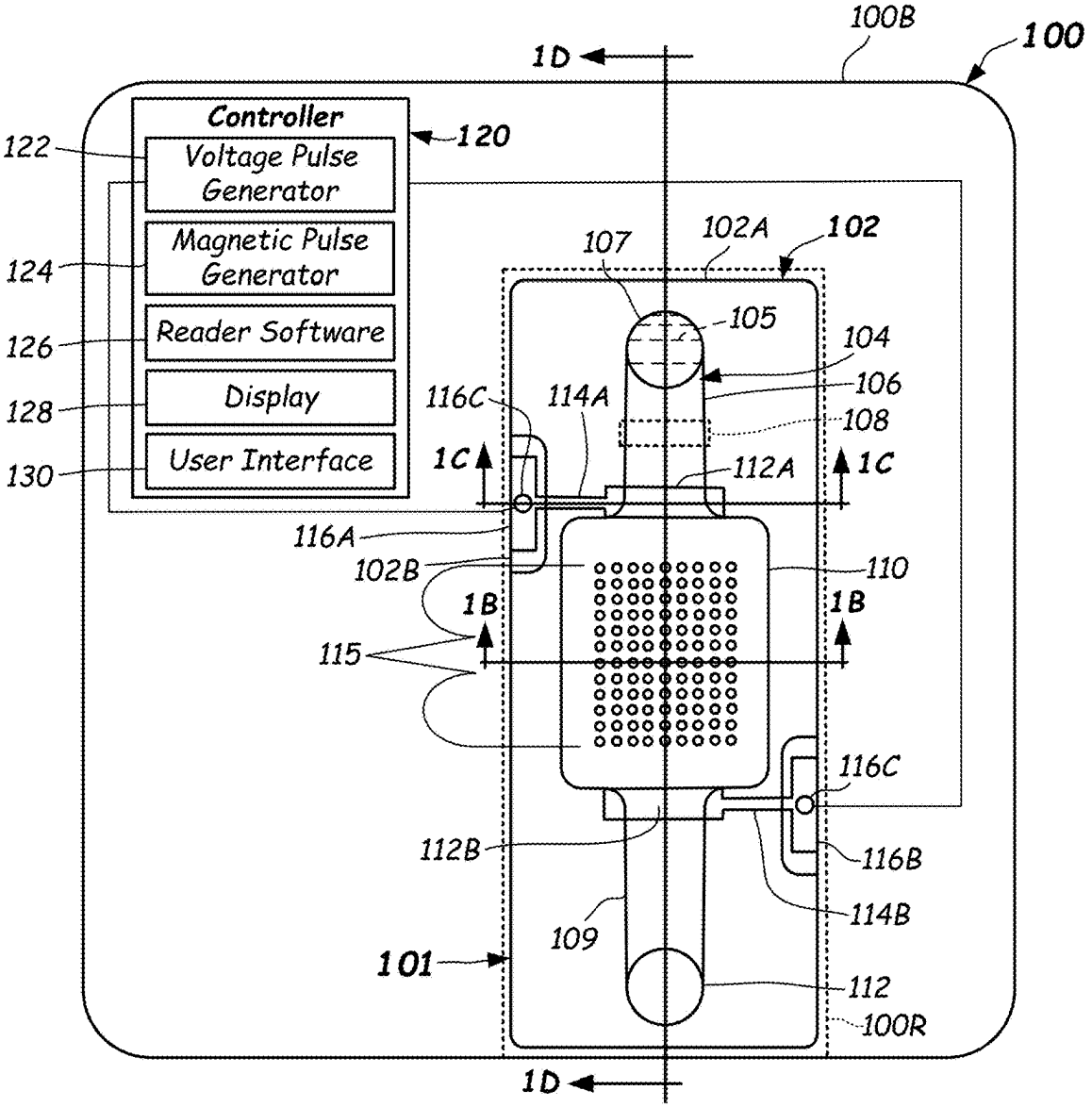
(57) **ABSTRACT**

An assay cartridge enabling improved measurement sensitivity of a target component (Troponin I or T) in the presence of high amount of albumin in a test sample. The assay cartridge includes a cartridge body including a sample inlet adapted to receive a patient sample and a reaction chamber coupled to the sample inlet, magnetic particles contained in the reaction chamber comprising a first antibody with a specific affinity towards the target component, electrodes provided at a location configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber, and a sensor surface of the reaction chamber comprising a second antibody with a specific affinity towards the target component. Methods of quantifying a target component (e.g., a Troponin component) in immunoassay testing are provided, as are assay devices adapted to quantify a target component (e.g., Troponin I or T).

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100
1D
Controller
120
122
Voltage Pulse Generator
124
Magnetic Pulse Generator
126
Reader Software
128
Display
130
User Interface
107
102A
102
105
104
106
108
116C
114A
1C
112A
116A
102B
110
1B
115
116C
112B
116B
114B
109
101
112
100R
1D

FIG. 1A

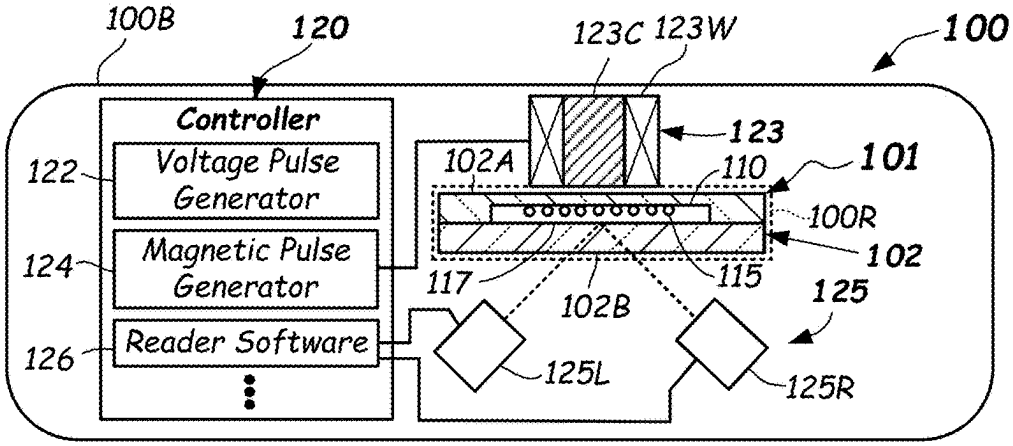

100B
120
123C
123W
100
Controller
122
Voltage Pulse Generator
124
Magnetic Pulse Generator
126
Reader Software
123
102A
110
101
100R
102
117
115
102B
125
125L
125R

FIG. 1B

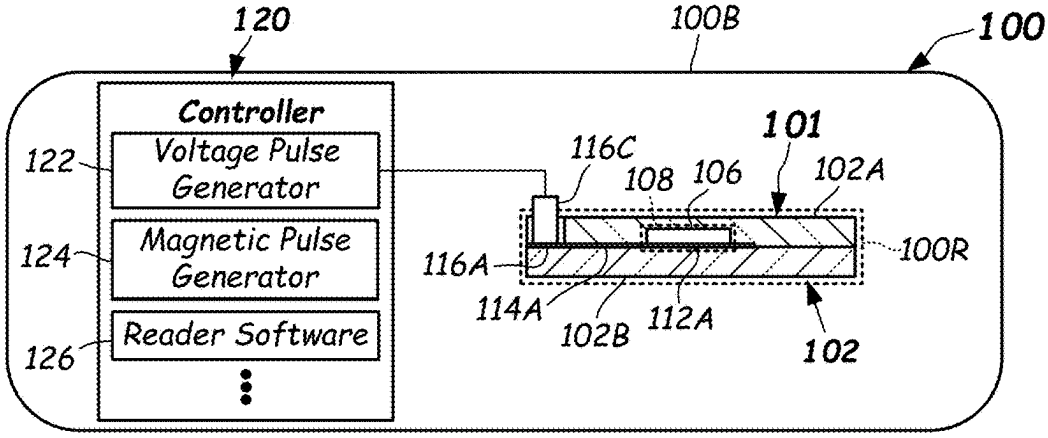


FIG. 1C

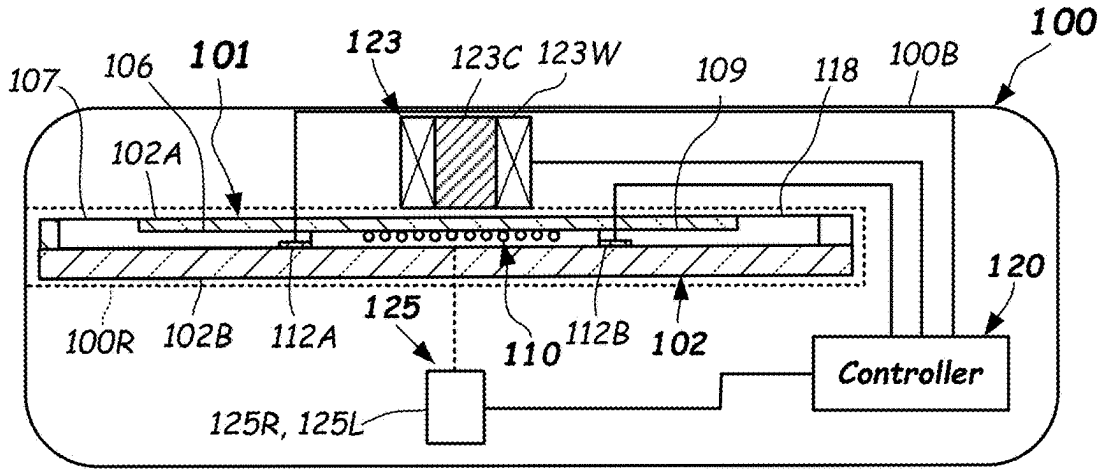
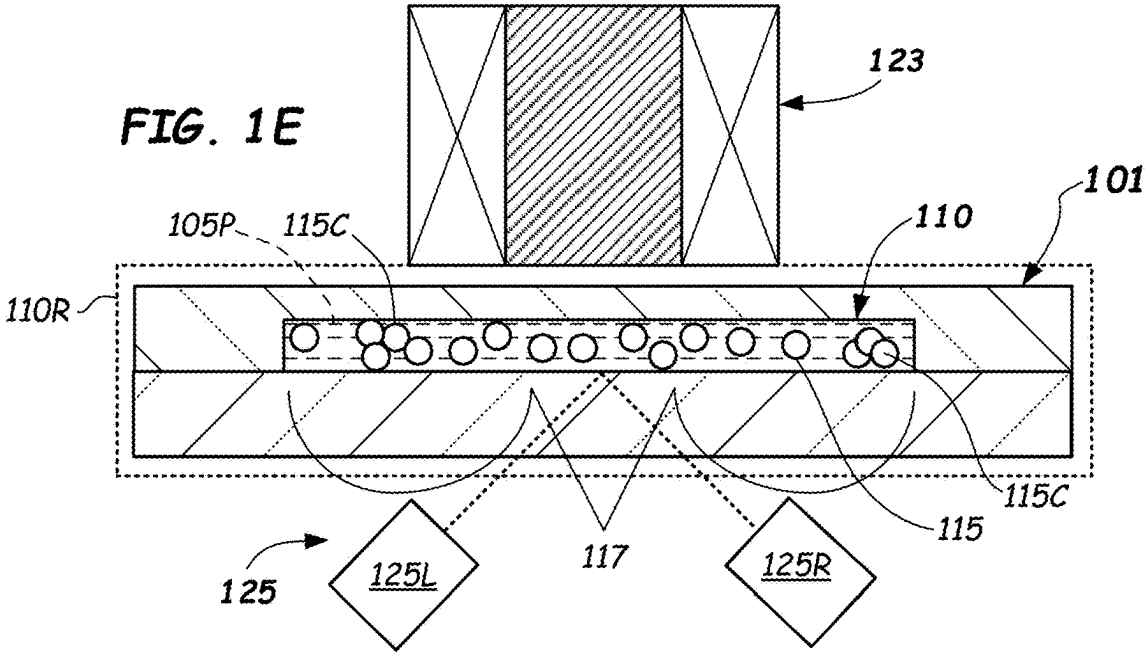


FIG. 1D

FIG. 1E



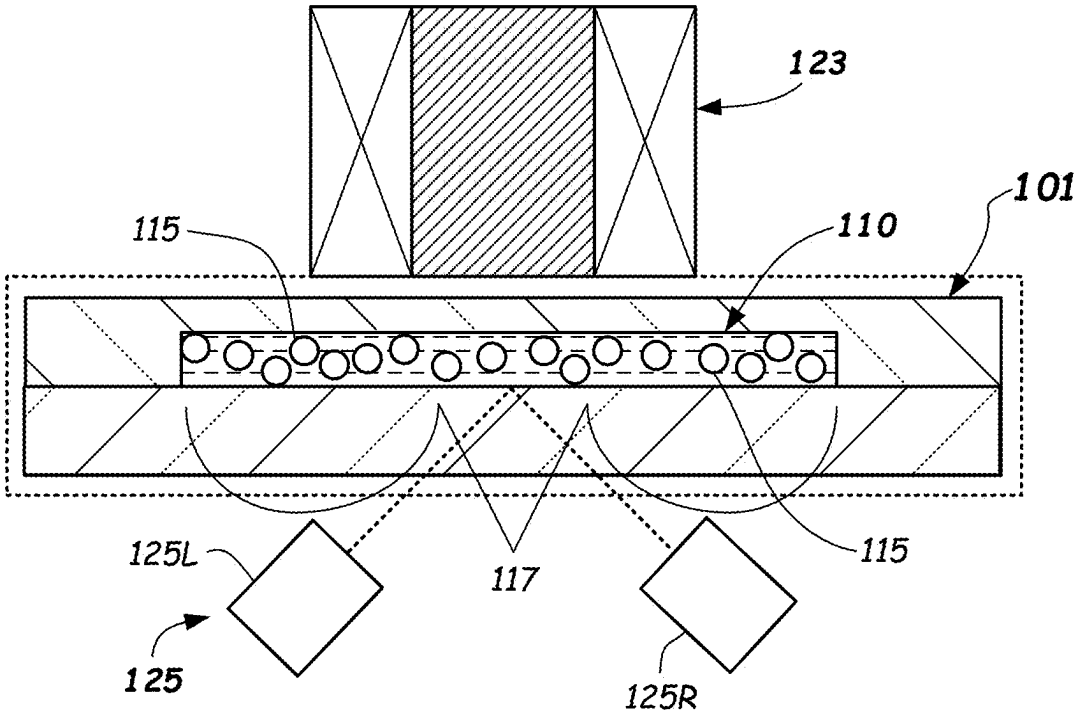

123
101
115
110
125L
117
115
125
125R

FIG. 1F

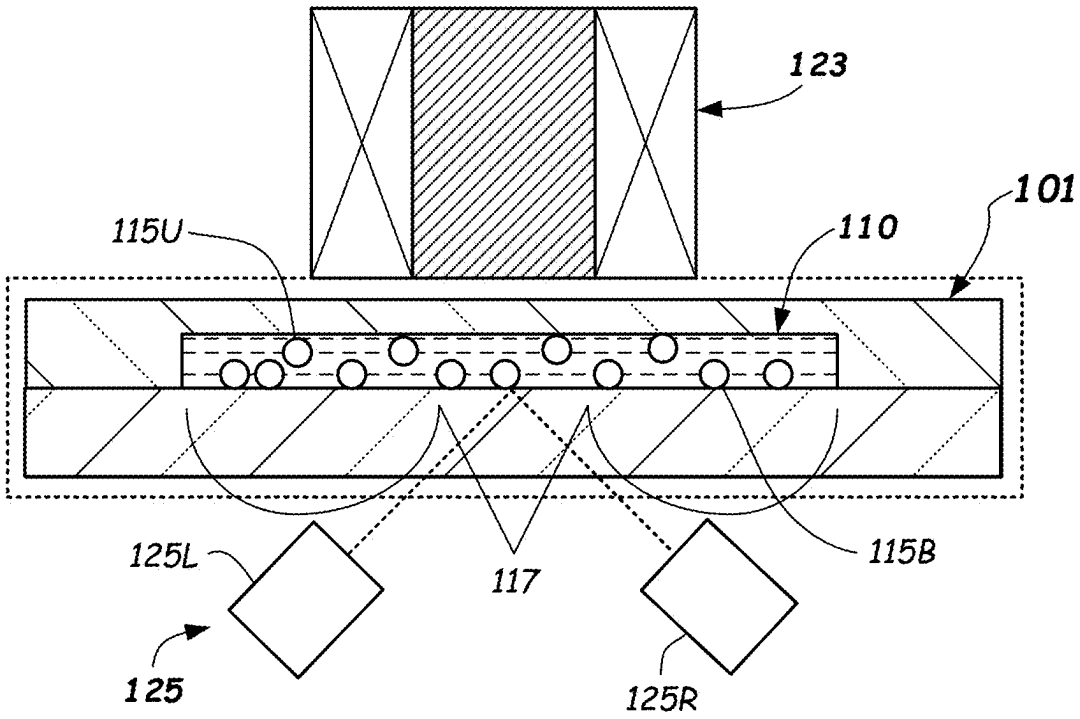

123
101
115U
110
125L
117
115B
125
125R

FIG. 1G

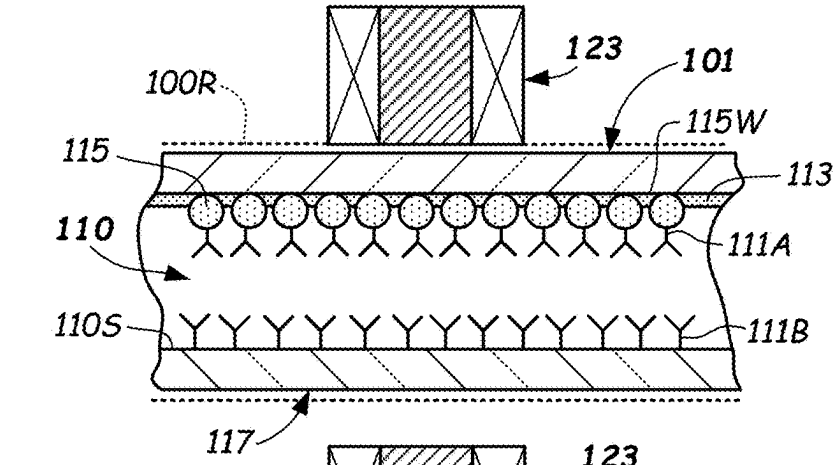


FIG. 1H

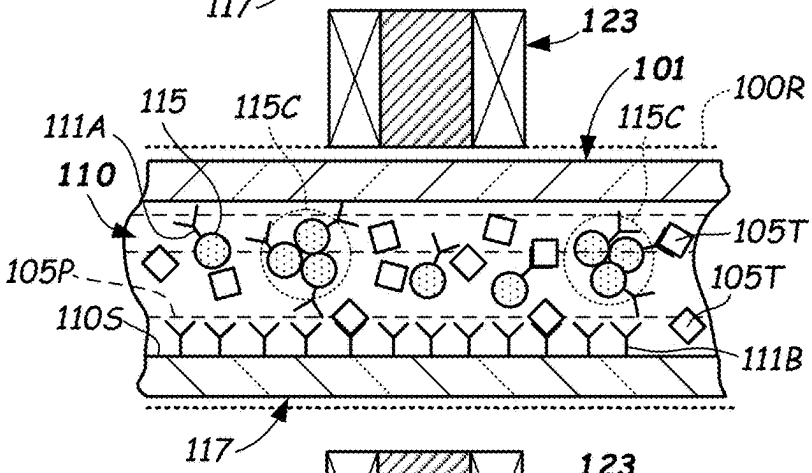


FIG. 1I

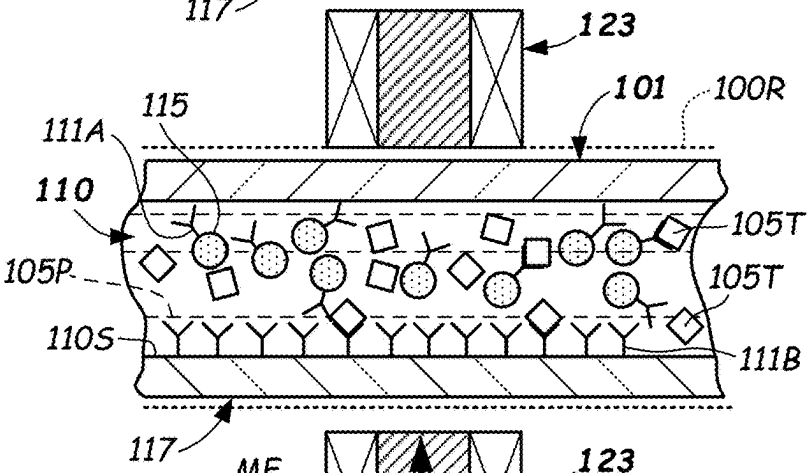


FIG. 1J

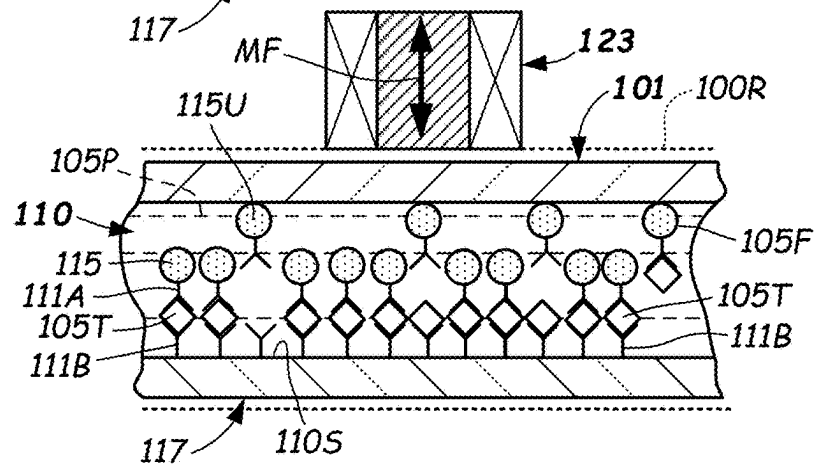


FIG. 1K

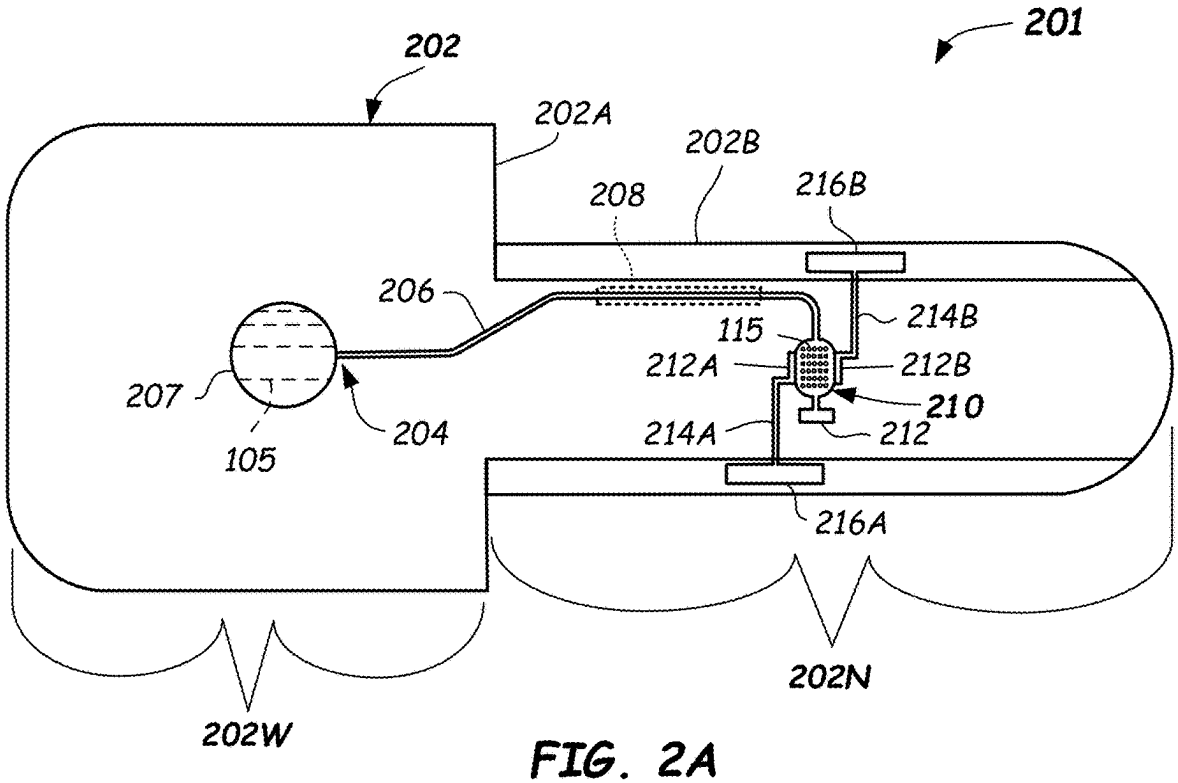

201
202
202A
202B
208
216B
206
214B
115
212A
212B
210
207
204
214A
212
105
216A
202N
202W

FIG. 2A

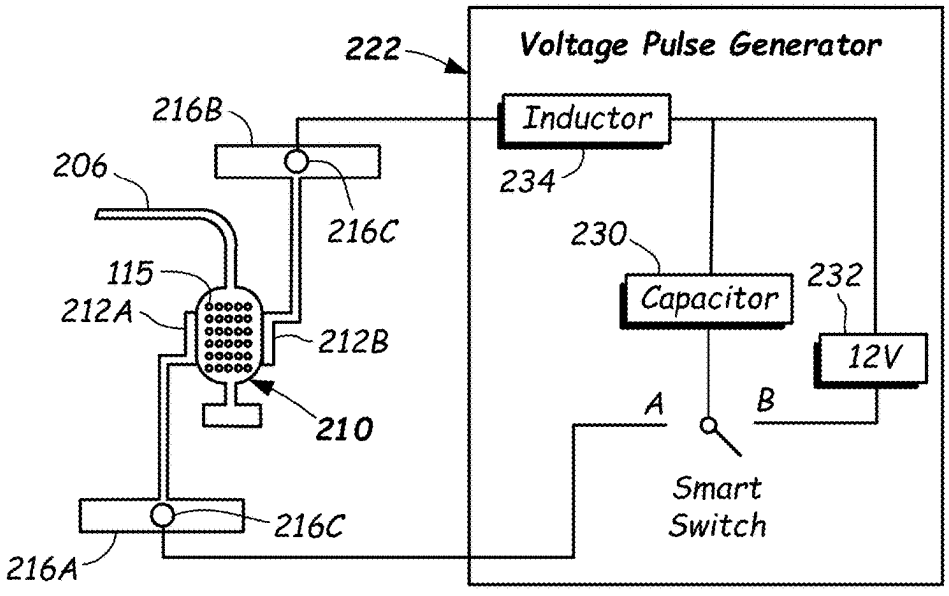

222
Voltage Pulse Generator
Inductor
234
216B
206
216C
230
115
Capacitor
232
212A
212B
12V
210
A
B
Smart
Switch
216C
216A

FIG. 2B

300A

Control 1 50x

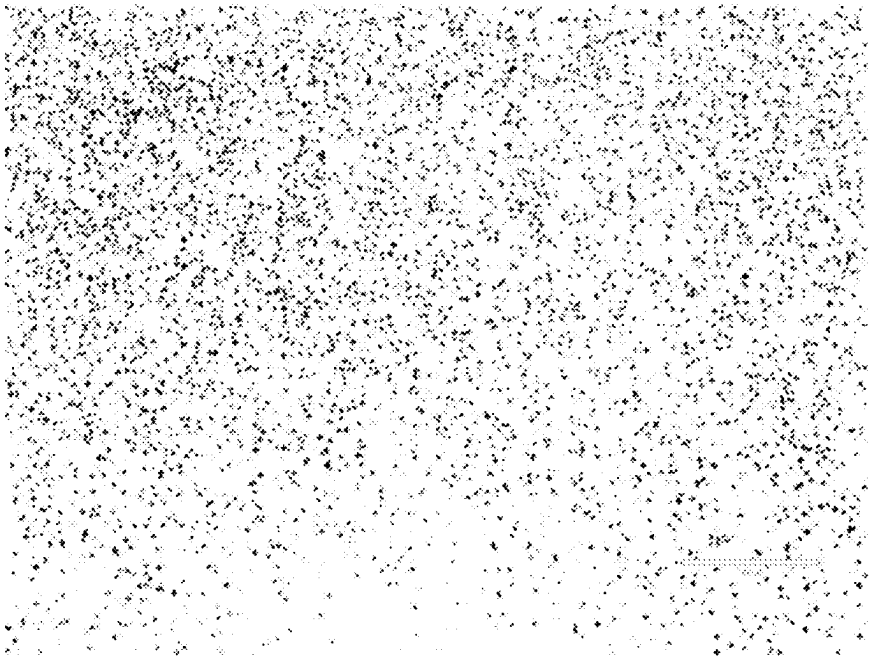


FIG. 3A

300B

Experimental 1 50x

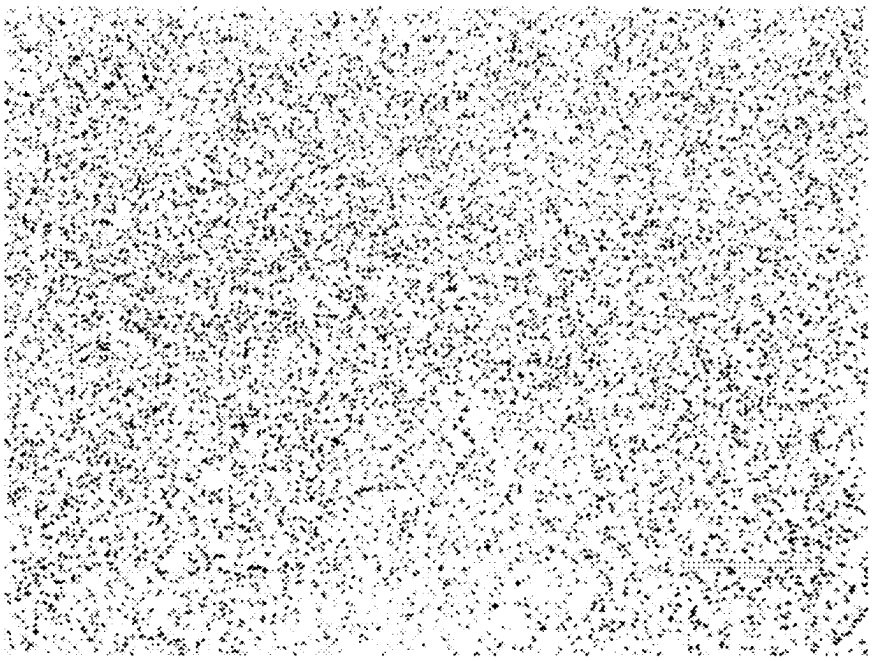


FIG. 3B

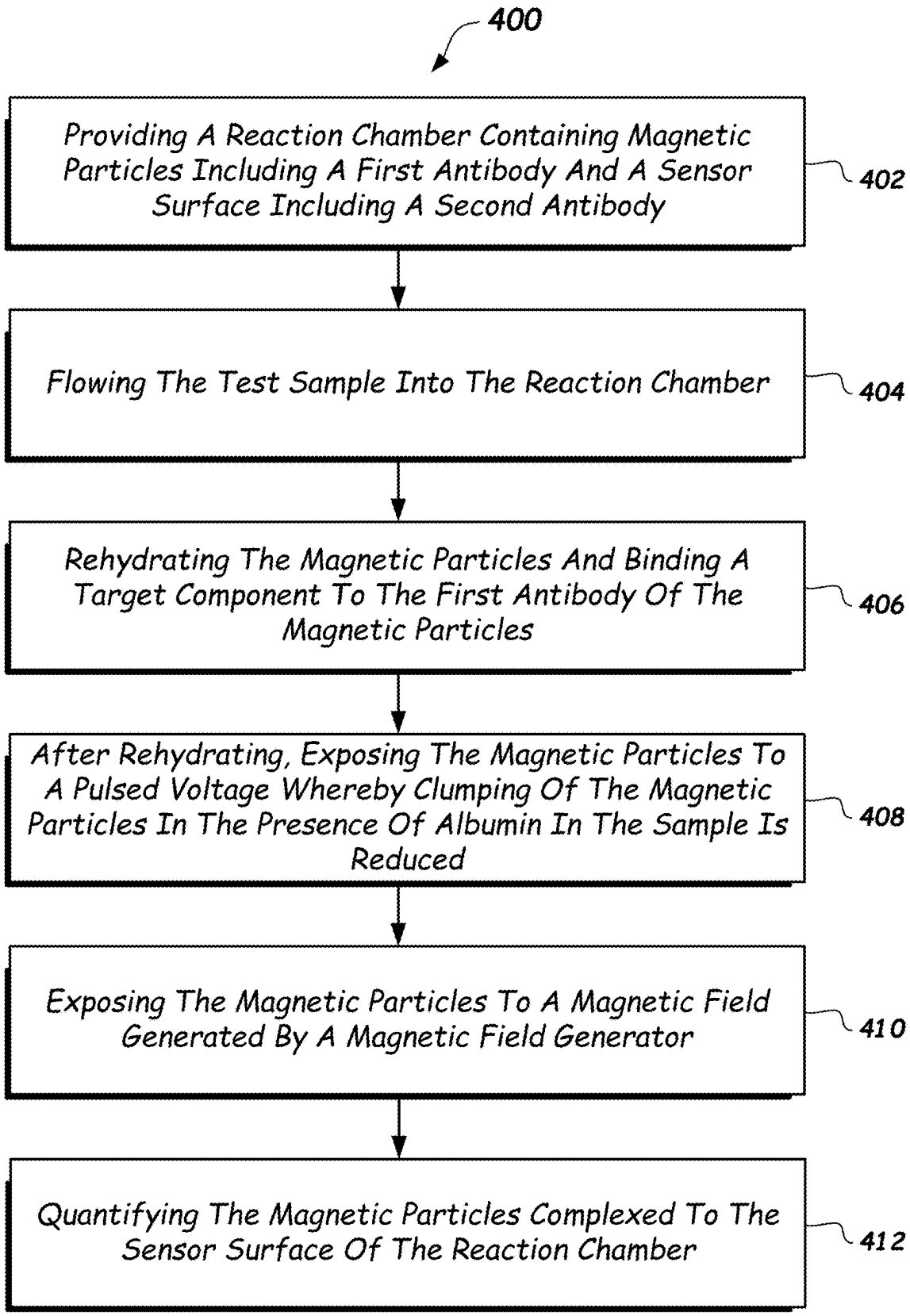

400
Providing A Reaction Chamber Containing Magnetic Particles Including A First Antibody And A Sensor Surface Including A Second Antibody
402
Flowing The Test Sample Into The Reaction Chamber
404
Rehydrating The Magnetic Particles And Binding A Target Component To The First Antibody Of The Magnetic Particles
406
After Rehydrating, Exposing The Magnetic Particles To A Pulsed Voltage Whereby Clumping Of The Magnetic Particles In The Presence Of Albumin In The Sample Is Reduced
408
Exposing The Magnetic Particles To A Magnetic Field Generated By A Magnetic Field Generator
410
Quantifying The Magnetic Particles Complexed To The Sensor Surface Of The Reaction Chamber
412

FIG. 4

ASSAY METHODS, ASSAY CARTRIDGES, AND ASSAY DEVICES INCLUDING MAGNETIC PARTICLE DE-AGGREGATION

FIELD

[0001] The present disclosure relates to methods, assay cartridges, and assay devices configured to measure a target component (e.g., a Troponin component) in a test sample.

BACKGROUND

[0002] An immunoassay that is configured to measure cardiac Troponin (e.g., Troponin I or Troponin T) can provide a tool for diagnosing myocardial infarction, for example. While most contemporary assays provide adequate diagnostic performance, increased sensitivity of high sensitivity assays introduced into clinical practice provide the potential to further shorten intervals between blood draws and/or the time needed to detect significant Troponin elevations. Furthermore, high sensitivity cardiac Troponin assays offer improvements for predicting major adverse cardiovascular events. Such high sensitivity assays can measure Troponin concentrations in 50%=−100% of healthy individuals and therefore allow for the distribution of Troponin values within a healthy cohort to be measured, patient's baseline troponin levels to be monitored, and clinicians to be alerted to deteriorating cardiac conditions. These high sensitivity assays may aid in predicting a patient's risk of future adverse events and for guiding and monitoring corresponding adjustments to therapeutic interventions. Such high-sensitivity cardiac Troponin I assays, for example, can detect levels above 14 ng/l, which can signal the likelihood of heart damage or a heart attack.

[0003] In some cases, a condition of the test sample may reduce the sensitivity of such high sensitivity Troponin assays. This disclosure is directed at assay methods, biosensor apparatus (assay cartridges), and assay devices that may increase the sensitivity of such assays configured to measure a target component, such as a cardiac Troponin component, when such conditions are encountered.

SUMMARY

[0004] This disclosure is directed, in one aspect, to a biosensor configured as an assay cartridge that is capable of measuring a target component, such as a Troponin component.

[0005] In another embodiment, a method of quantifying a target component (e.g., a Troponin component such as Troponin I or Troponin T, for example) in a test sample is provided. The method of quantifying the target component comprises providing a reaction chamber containing magnetic particles including a first antibody and a sensor surface including a second antibody, flowing the test sample into the reaction chamber, rehydrating the magnetic particles and binding a target component to the first antibody of the magnetic particles, and after rehydration, exposing the magnetic particles to a pulsed voltage whereby clumping of the magnetic particles in the presence of albumin in the test sample is reduced.

[0006] In an apparatus embodiment, an assay cartridge adapted for measuring a target component (e.g., a Troponin component) in immunoassay testing is provided. The assay cartridge comprises a cartridge body including a sample inlet adapted to receive a patient sample and a reaction chamber coupled to the sample inlet, magnetic particles contained in the reaction chamber comprising a first antibody with a specific affinity towards the target component, electrodes provided at a location configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber, and a sensor surface of the reaction chamber comprising a second antibody with a specific affinity towards the target component.

[0007] In another apparatus embodiment, an assay device configured to improve sensitivity of immunoassay testing of a target component (e.g., Troponin I or Troponin T) is provided. The assay device comprises a device body including a cartridge receiver, an assay cartridge received in the cartridge receiver, the assay cartridge comprising a cartridge body including an inlet channel adapted to receive a test sample and a reaction chamber therein, magnetic particles having a first antibody with a specific affinity towards the target component provided in the reaction chamber, electrodes provided at locations configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber, and a voltage pulse generator coupled to the electrodes and configured to provide one or more voltage pulses to the electrodes.

[0008] Still other aspects, features, and advantages of this disclosure may be readily apparent from the following description and illustration of a number of example embodiments, including the best mode contemplated for carrying out the disclosure. This disclosure may also be capable of other and different embodiments, and its several details may be modified in various respects, all without departing from the scope of the disclosure. It is to be understood that embodiments of the methods described herein are capable of operation in other sequences than described or illustrated herein. This disclosure is intended to cover all modifications, equivalents, and alternatives falling within the scope of the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The drawings, described below, are for illustrative purposes, may be exaggerated in size, and thus are not necessarily drawn to scale. Any reference signs in the claims shall not be construed as limiting the claim scope. Accordingly, the drawings and descriptions thereof are to be regarded as illustrative in nature, and not as restrictive. The drawings are not intended to limit the scope of the disclosure in any way.

[0010] FIG. **1**A illustrates a top schematic view of a first embodiment of an assay cartridge installed in an assay device according to one or more embodiments of the disclosure.

[0011] FIG. **1**B illustrates a cross-sectioned end view of a first embodiment of an assay cartridge installed in an assay device taken along section **1**B-**1**B of FIG. **1**A according to one or more embodiments of the disclosure.

[0012] FIG. **1**C illustrates another cross-sectioned end view of a first embodiment of an assay cartridge installed in an assay device taken along section **1**C-**1**C of FIG. **1**A according to one or more embodiments of the disclosure.

[0013] FIG. **1**D illustrates a cross-sectioned side view of a first embodiment of an assay cartridge installed in an assay device taken along section **1**D-**1**D of FIG. **1**A according to one or more embodiments of the disclosure.

[0014] FIG. **1**E illustrates an enlarged cross-sectioned end view taken through a reaction chamber of an assay cartridge

illustrating re-hydration and clumping of magnetic particles in a test sample according to one or more embodiments of the disclosure.

[0015] FIG. **1**F illustrates an enlarged cross-sectioned end view taken through the reaction chamber of an assay cartridge illustrating breaking up of the clumping of magnetic particles after application of pulsed voltage across the reaction chamber according to one or more embodiments of the disclosure.

[0016] FIG. **1**G illustrates an enlarged cross-sectioned end view taken through the reaction chamber of an assay cartridge illustrating separation of unbound magnetic particles from magnetic particles having a target component complexed to a sensor surface after application of a magnetic field to the reaction chamber according to one or more embodiments of the disclosure.

[0017] FIG. **1**H illustrates an enlarged partial cross-sectioned end view taken through a reaction chamber of an assay cartridge, prior to insertion of the test sample, functionally and structurally depicting one embodiment including magnetic particles having a first antibody affixed to a surface of the reaction chamber and a sensor surface including a second antibody bound thereto according to one or more embodiments of the disclosure.

[0018] FIG. **1**I illustrates an enlarged partial cross-sectioned end view taken through a reaction chamber of an assay cartridge after insertion of the test sample including a target component to be quantified, functionally and structurally depicting an embodiment where magnetic particles are clumped in the presence of a high amount of albumin according to one or more embodiments of the disclosure.

[0019] FIG. **1**J illustrates an enlarged partial cross-sectioned end view taken through a reaction chamber of an assay cartridge after application of a pulsed voltage, functionally and structurally depicting an embodiment where the magnetic particle clumping in the presence of a high amount of albumin is reduced (eliminated entirely as shown) according to one or more embodiments of the disclosure.

[0020] FIG. **1**K illustrates an enlarged partial cross-sectioned end view taken through a reaction chamber of an assay cartridge after application of a magnetic field to the reaction chamber, functionally and structurally depicting an embodiment where magnetic particles having the target component bound thereto being moved in order to complex to a second antibody provided on a sensor surface according to one or more embodiments of the disclosure.

[0021] FIG. **2**A illustrates a schematic top view of a second embodiment of an assay cartridge including electrodes, which when energized, are adapted to break up magnetic particle clumping and provide improved quantification of a target component in a test sample according to one or more embodiments of the disclosure.

[0022] FIG. **2**B illustrates a voltage pulse generator circuit that can be coupled to an assay cartridge including electrodes according to one or more embodiments of the disclosure.

[0023] FIGS. **3**A and **3**B illustrate pictorial results (at **50**X) of particle counts before (FIG. **3**A—control) and after (FIG. **3**B) application of a pulsed voltage to a test sample including a high volume of albumin therein illustrating the higher particle count as well as improved, more homogenous, particle distribution according to one or more embodiments of the disclosure.

[0024] FIG. **4** illustrates a flow chart of a method of quantifying a target component (e.g., Troponin I or Troponin T) in a test sample according to one or more embodiments of the disclosure.

DETAILED DESCRIPTION

[0025] This disclosure is directed at improving the sensitivity of assays, such as cardiac Troponin assays, where the patient sample contains a high level of albumin therein. In one aspect of the disclosure, it was discovered by the inventors that high levels of albumin contained in the patient sample can affect the sensitivity of the assay (e.g., a Troponin assay) conducted using a test sample derived from the patient sample. Normal albumin levels range from 3.4 to 5.4 g/dL in whole blood. When the albumin level is high (i.e., provided in an amount greater than 5.4 g/dL in whole blood), clumping and agglomeration of the magnetic label (e.g., magnetic particles) utilized in the Troponin assay for binding the target component (e.g., Troponin I or Troponin T, for example) can occur. Where albumin is present in the patient sample **105** in a quantity greater than 10 g/dL even greater extent of clumping can occur, and especially when albumin is present in the patient sample **105** in a quantity greater than 100 g/dL in whole blood. This clumping can result in a lesser portion of the respectively clumped magnetic particles being available for complexing the target component (e.g., Troponin I or Troponin T) to a sensor surface thus reducing the sensitivity of target component detection.

[0026] In one or more embodiments, clumping in the assay (e.g., a Troponin assay) can be minimized by exposing the test sample (e.g., plasma) that has rehydrated magnetic particles therein to one or more pulses of an electric voltage potential (V). In some embodiments, a reaction chamber containing magnetic particles that have been rehydrated within plasma derived from the patient sample (e.g., whole blood) are exposed to the voltage potential (V). In some embodiments, the reaction chamber containing magnetic particles that have been rehydrated within the test sample (e.g., plasma) is subjected to successive voltage pulses, such as two or more pulses of electrical voltage potential (V) to facilitate breaking up of clumps of magnetic particles that can occur when a high amount of albumin is present in the patient sample.

[0027] Further details of the method enabling improved sensitivity of such assays (e.g., Troponin assays) when the patient sample contains high levels of albumin are described with reference to FIGS. **1**A-**4** herein. Assay cartridges enabling improved measurement sensitivity of the target component (e.g., Troponin I or Troponin T) in the presence of such high albumin levels in the patient sample are also described herein, as well as assay devices utilizing such assay cartridges.

Definitions

[0028] The following definitions are provided solely to aid in the Understanding of this disclosure. These definitions should not be construed to have a scope less than understood by a person of ordinary skill in the art.

[0029] "Assay" as used herein means an analysis or examination to determine a presence and/or quantity of a target component of a substance, such as a biological fluid sample from a patient.

[0030] "Assay cartridge" as used herein means a cartridge structure that is receivable in a receiver of an assay device.

[0031] "Assay device" as used herein means an apparatus configured to carry out an assay, such as a point-of-care instrument adapted to test for a Troponin component.

[0032] "Target component" as used herein means a specific component sought to be quantified that is contained in a test sample to be analyzed.

[0033] "Troponin I and Troponin T" are subunit of the troponin complex (Tn) that is used to diagnose cardiovascular disease and myocardial infarction.

[0034] "Sensor surface" as used herein refers to a surface to which antibodies are coupled and that allows the detection of magnetic particles complexed to such surface.

[0035] "Rehydration" as used herein means the constituents of the assay, such as magnetic particles and dry reagents, have been assimilated into the test sample as a rehydrated test sample.

[0036] "Clump" as used herein means a plurality of particles that are adhered to one another and that are coalesced into an agglomerated particle mass.

[0037] FIGS. **1**A-**1**D schematically illustrates a first embodiment of a biosensor configured as an assay cartridge **101** received in an assay device **100**. The assay cartridge **101** includes a body **102** of suitable material providing a configuration including at least one sample inlet **104** that can be configured to receive a patient sample **105** of biological material that has been obtained from a patient via a pinprick or otherwise. The patient sample **105** may be whole blood and may be deposited into a well **107** formed in the body **102**. An inlet channel **106** formed in the body **102** provides a flow passageway for the patient sample **105** to flow from the sample inlet **104** towards a reaction chamber **110**.

[0038] In the depicted embodiment, a filter **108** can be provided in the inlet channel **106** between the sample inlet **104** and the reaction chamber **110**, or even within the well **107** in some embodiments. The filter **108**, when the patient sample **105** is whole blood, operates to remove cellular components such as red blood cells and other solid portions of the whole blood and leaves only the plasma as a test sample **105**P to flow into the reaction chamber **110**. Thus, the inlet channel **106** is coupled to the reaction chamber **110** and is configured to flow the test sample **105**P into the reaction chamber **110**, wherein the inlet channel **106** can be formed as a capillary channel.

[0039] However, in some embodiments, the whole blood may be separated into cellular components and plasma by centrifugation and only the plasma alone may be deposited directly into the well **107** as the test sample **105**P. In this embodiment where the test sample **105**P is plasma deposited into the well **107**, the filter **108** is not needed, and would not be present. In this embodiment, the test sample **105**P may flow through the inlet channel **106** to the reaction channel **110** via capillary action.

[0040] Again referring to FIGS. **1**A-**1**D, the assay device **100**, comprises a device body **100**B, such as a molded plastic body, including a cartridge receiver **100**R that is configured to receive the assay cartridge **101** therein. The cartridge receiver **100**R can be a port or other opening into which the assay cartridge **101** can be inserted or placed. In some embodiments, the cartridge receiver **100**R can include a door (not shown) that is opened to allow the insertion of the cartridge **101** after which the door may be closed.

[0041] The assay cartridge **101** includes the cartridge body **102**. The cartridge body **102** may be made of two or more body parts of material that can be adhered or otherwise bonded or fastened together. The connection can be by any suitable mechanism, such as through the use of an adhesive. For example, one body part, such as first body part **102**A may include recessed portions formed therein to aid in construction of the inlet channel **106**, reaction chamber **110**, as well as an outlet channel **109**. The well **107** may be formed as a hole passing fully through the first body part **102**A and its interaction with a surface of the second body part **102**B. The first body part **102**A and second body part **102**B can cooperate and engage one another to collectively form the well **107**, sample inlet **104**, inlet channel **106**, reaction chamber **110**, outlet channel **109**, and outlet **118**. The outlet **118** may also be formed as a hole passing fully through the first body part **102**A and its interaction with a surface of the second body part **102**B. Optionally, a vent may be provided that is connected to the outlet channel **109**. Other suitable configurations can be used to form the reaction chamber **110** and inlet channel **106**, such as including recesses only in the second body part **102**B, or in both of the first body part **102**A and the second body part **102**B.

[0042] In the depicted embodiment, the second body part **102**B may be highly transparent and may be a planar body at least in the area of the reaction chamber **110**. The first body part **102**A may be transparent or translucent, or even opaque in some embodiments, for example. The first body part **102**A and second body part **102**B may be made of glass, plastic, or other suitable moldable or formable materials. The second body part **102**B should be as optically transparent as possible when optical methods are used to quantify the target component **105**T after the assay reaction is completed, such as transparent glass, acrylic, polystyrene, styrene-acrylonitrile, polycarbonate, polyethylene terephthalate, and combinations thereof.

[0043] By way of example, the reaction chamber **110** may be about 1.5 mm long×1.0 mm wide and 0.25 mm in height and may include dried reagent therein to be described later herein. The inlet channel **106** and the outlet channel **109** may be appropriately sized to facilitate liquid capillary action. For example, the inlet channel **106** and outlet channel **109** may include cross-sectional area of about 0.125 mm^2, for example. Other suitable channel and reaction chamber sizes may be used.

[0044] In order to facilitate quantification of the target component **105**T present in the test sample **105**P, magnetic particles **115** can be utilized as a target component binding agent. The target component **105**T may be a Troponin component, such as Troponin I (cTnI), although other target components, such as Troponin T (cTnT) may instead be quantified. Other target components that are cardiac markers may also be quantified. Other target components may benefit from the anti-aggregating properties achieved by the embodiments described herein

[0045] The magnetic particles **115** can have attached thereto, such as through a suitable coating or application process, a first antibody **111**A with a specific affinity towards binding to the target component **105**T (e.g., Troponin I or Troponin T). Also provided in the reaction chamber **110** is a second antibody **111**B that can be coupled to a sensor surface **110**S of the reaction chamber **110**. The sensor surface **110**S may be provided as part of a quantification window **117** through which detection and quantification of

the target component **105**T may be accomplished. The sensor surface **110**S can be located proximate to the quantification window **117** of the reaction chamber **110**, such as on one side thereof and may be approximately the same size as the quantification window **117**.

[0046] The sensor surface **110**S can be a specially derivatized surface to which molecules, more particularly antibodies (or functional fragments thereof) can be bound. Examples of suitable sensor surfaces **110**S can include, glass, plastic, an organic crystal or an inorganic crystal (e.g., silicon), an amorphous organic material, or an amorphous inorganic material (e. g. silicon nitride, silicon oxide, silicon oxynitride, aluminum oxynitride, aluminum oxide). Other suitable surfaces can be used.

[0047] In some embodiments, the quantification of the target component **105**T can be performed optically. Such optical detection can be performed by detecting a quantity of magnetic particles **115** that are complexed to the sensor surface **110**S, such as by frustrated total internal reflection (FTIR). In yet other embodiments, the magnetic particles **115** complexed to the sensor surface **110**S can be detected by other optical means.

[0048] In some embodiments, the first antibody **111**A can be a monoclonal antibody, such as a monoclonal anti-troponin I antibody. Optionally, the first antibody **111**A can be a monoclonal antibody, such as a monoclonal anti-troponin T antibody. The monoclonal antibody according to the present disclosure can also include Fab fragments from the monoclonal antibody, aptamers, affibodies, scFv fragments, and any other single epitope binding moiety known to the person of ordinary skill in the art.

[0049] In some embodiments, monoclonal antibodies **111**A are coupled to the magnetic particles **115** so as to allow manipulation of the labeled antibody via a suitable magnetic field generator provided in the assay device **100** that is operable with the assay cassette **101**. For example, the assay device **100** may include a magnetic field generator **123**, which may comprise any suitable form of an electromagnet or moveable magnet. Magnetic field generator **123** may include a core **123**C and a winding **123**W of sufficient size and number of winds to generate, when energized via a signal from a magnetic pulse generator **124**, a sufficient magnetic field MF to allow manipulation and movement of the magnetic particles **115** through the test sample **105**P. Such magnetic particles may be manipulated as described in U.S. Pat. No. 9,720,003, for example, to maximize forming of complexes to the sensor surface **110**S.

[0050] In some embodiments, the second antibody **111**B can be a polyclonal antibody such as a polyclonal anti-Troponin I antibody and can be coupled to the sensor surface **110**S. For example, goat polyclonal anti-Troponin I antibody was found to be quite effective. The use of goat polyclonal antibodies can lead to optimized assay results. The polyclonal antibody according to the present disclosure can also include Fab fragments from polyclonal antibodies, and any group of binding moieties with variable structure known to the person of ordinary skill in the art.

[0051] In some embodiments, the antibodies according to the present disclosure are directed at amino acid sequence 30-110, or even 80-110, of the Troponin I molecule. This is a generally stable part of the molecule. Preferably the epitopes chosen do not overlap with known regions for heparin binding, which may result in interference from heparinized patient samples.

[0052] In some embodiments, the monoclonal anti-Troponin I antibody can be selected from the group comprising clones: a34500 (binding to amino acid 87-91 of Troponin I), 81-7 (a34780 (binding to amino acid 136-154 of Troponin I), a34650 (binding to amino acid 41-49 of Troponin I), 267 (ab 14530, binding to amino-acid 169-184 of Troponin I), 16A11 (a24460, binding to amino-acid 87-91 of Troponin I), 19C7 (a19615, binding to amino-acid 41-49 of Troponin I), 560 (binding to amino acid 83-93 of cTnI), or combinations thereof. The use of these clones can lead to improved selectivity in the magnetic immunoassay according to the disclosure.

[0053] Certain procedures can be used to provide the antibodies on the magnetic particles that are used in the Troponin quantification assay. For example, an Ademtech protocol, known to a person of ordinary skill in the art can be used to coat the magnetic particles **115** with the first antibody **111**A. In this protocol, monoclonal antibody (e.g., first antibody **111**A) at a concentration of, for example, 20 μg antibody per mg of magnetic particle **115** can be coupled to carboxylated magnetic particles in the presence of EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide). Other suitable methods for coupling the first antibody **111**A comprising the monoclonal antibody to the magnetic particles **115** may be used.

[0054] The magnetic particles **115**, which are sometimes referred to as magnetic labels, can be provided in the reaction chamber **110** prior to receiving the test sample **105**T therein. For example, the magnetic particles **115** may be provided on one or more walls **110**W of the reaction chamber **110**, such as shown in FIG. **1**H. The magnetic particles **115** may be adhered to a wall by any suitable binding material **113** in some embodiments, such as by any material that is non-interfering with the assay. For example, the binding material **113** may be a sugar and protein solution that includes the magnetic particles **115**, and which is applied to the wall of the reaction chamber **110** and then dried. Other suitable binding materials may be used.

[0055] In addition to the magnetic particles **115**, the dry reagents can be provided at a suitable location in the reaction chamber **110**. The dry reagents include the buffer components necessary for conducting the assay. The components of the dry reagents can be deposited and dried individuality at different locations in the reaction chamber **110** or together at the same location. The dry reagents can be deposited via several drying techniques including lyophilization, for example. Lyophilization prevents the formation of crystals and allows the reagents to be dried to an amorphous glassy state that is readily re-dispersed and rehydrated upon the addition of the test sample **105**P (e.g., plasma) into the reaction chamber **110**.

[0056] The sensor surface **110**S as shown in FIG. **1**H-**1**K according to some embodiments of the present disclosure can be functionalized with the second antibody **111**B, which can comprise the polyclonal antibody. Polyclonal antibodies are preferred as the degrees of freedom for binding of Troponin I, especially when bound to the monoclonal anti-Troponin I antibody of the magnetic particles **115** are limited. One advantage is a consequence of the variability in orientation of polyclonal antibodies immobilized on a sensor surface **110**S due to the different antibody types of a polyclonal. Further advantages can arise by increasing the amount of suitable binding sites by using polyclonal antibodies directed to different epitopes leading to optimized

assay results. Due to favorable assay sensitivity, polyclonal goat antibodies specific for Troponin I or Troponin T can be used.

[0057] In another embodiment, an additional antibody specific for Troponin I, or a mixture of several antibodies specific for Troponin I can be coated on the sensor surface **110**S. Preferably the additional antibody is a monoclonal antibody. In another embodiment, the sensor surface **110**S can be coated with at least two, even more preferably at least three, different monoclonal anti-Troponin I antibodies or a mixture of monoclonal troponin I antibodies. This also leads to formation of a "polyclonal" antibody on the sensor surface **110**S as receptors, as binding sites for more than one epitope are present. This situation functionally resembles coating with a polyclonal antibody and thus is considered a polyclonal antibody herein.

[0058] The polyclonal antibody **111**B can, for example, be coated onto the sensor surface **110**S by any suitable process. For example, the second antibody **111**B (e.g., polyclonal antibody) can be provided on the sensor surface **110**S by inkjet printing a plurality of drops (e.g., of approx. 2 nL each) at a desired concentration (e.g., of about 150 μg/mL). Other suitable drop sizes and concentrations may be used. Printing antibody pattern features such as dots, squares, text, fine lines, or combinations thereof can be used. Optionally, the second antibody **111**B may be bound to the sensor surface **110**S by other processes such as microcontact printing, immersion coating (in a bulk solution), and/or drop coating (from a nanopipette or micropipette).

[0059] In more detail, the magnetic particles **115** may be attached to a wall **110**W, such as to a wall located opposite from the sensor surface **110**S. Optionally, the magnetic particles **115** may be attached to other walls, such as to the side walls of the reaction chamber **110** or even to the sensor surface **110**S itself along with the second antibody **111**B. Attachment may be by way of any suitable binding material **113**, as previously discussed. In some embodiments, the magnetic particles **115** can have a size (approximate diameter) of about 200 nm to 1000 nm. Particles in this size range allow for optimal assay conditions and detection.

[0060] In more detail, the assay cartridge **101** includes electrodes **112**A, **112**B that are provided at locations configured to be in contact with the test sample **105**P containing the magnetic particles **115** in the reaction chamber **110**. This allows for a desired pulsed voltage to be applied to the test sample **105**P containing the magnetic particles **115** in the reaction chamber **110**. The electrodes **112**A, **112**B may be made from any suitable electrically conductive material, such as silver, copper, gold, or other conductive metals or materials. For example, the electrodes **112**A, **112**B may be provided on opposite sides of the reaction chamber **110**, such as proximate the inlet channel **106** and outlet channel **109**, as shown. Optionally, the electrodes **112**A, **112**B may be provided on other opposite sides of the reaction chamber **110**, such as is shown in FIG. **2**A.

[0061] The electrodes **112**A, **112**B may be connected to contact pads **116**A, **116**B that are exposed so to be able to be contacted by electrical contacts **116**C (FIG. **1**C) that are part of the assay device **100**. Electrical contacts **116**C may be pogo type or other suitable spring-loaded contacts, for example. The contact pads **116**A, **116**B may be interconnected to the electrodes **112**A, **112**B by conductor paths **114**A, **114**B. The electrodes **112**A, **112**B, conductor paths **114**A, **114**B, and contact pads **116**A, **116**B may be formed by any suitable method, such as by any coating, masked deposition or plating method, such as vapor deposition, laser-induced transfer deposition, electroless plating, immersion coating, sputtering, gilding, electroplating, and the like, or otherwise adhering a formed conductive member made up of the electrodes **112**A, **112**B, conductor paths **114**A, **114**B, and contact pads **116**A, **116**B, or combinations thereof.

[0062] A voltage pulse generator **122**, which may be included as part of a controller **120**, or otherwise provided, may be electrically coupled to the electrodes **112**A, **112**B through the contact pads **116**A, **116**B and conductor paths **114**A, **114**B. The voltage pulse generator **122** can be configured to provide one or more voltage pulses to the electrodes **112**A, **112**B. This exposes the test sample **105**P containing rehydrated magnetic particles **115** and rehydrated reagents to one or more pulses of voltage potential V. Thus, clumping **115**C is effectively reduced and may be minimized or even eliminated.

[0063] The applied voltage potential V between the respective electrodes **112**A, **112**B may have an initial magnitude of greater than 150 V in some embodiments, and an initial magnitude greater than 300 V in other embodiments. The pulsed voltage V may be applied once, and in some embodiments, more than once. The pulsed voltage magnitude may decay to some extent during its duration of exposure after its initial provision. For example, the exposing of the test sample **105**P including rehydrated magnetic particles **115** to the pulsed voltage V can comprise providing at least two voltage pulses in succession to break up clumping **115**C of the magnetic particles **115** in the presence of albumin in the test sample **105**P. For example, each pulse can have a time duration of greater than 2 milliseconds. In some embodiments where multiple pulses are successively applied, each of the at least two pulsed voltages can have a duration of greater than 2 milliseconds, but the magnitude of the voltage V may fall off (be reduced) somewhat during each duration. In some embodiments, the magnitude of the initial voltage pulse V may be different (e.g., lower) in successive pulses that are applied. Thus, each pulse may be of a different amplitude and/or duration. The voltage pulse should be low enough in magnitude and applied in a short enough duration so as to not cause cell lysis.

[0064] By way of example, and not by limitation, the voltage pulse generator **122** can include any suitable circuitry having the ability to generate a desired voltage pulse profile. For example, FIG. **2**B illustrates one possible configuration of a voltage pulse generator **222** that can be used as the voltage pulse generator for the FIG. **1**A-**1**D embodiment. This embodiment uses a smart switch that can be toggled between A and B as programmed to charge and discharge a capacitor **230** using a DC voltage source **232** such as a 12V battery, for example. An inductor **234** may be included to control the resonant frequency. Suitable values of capacitance can range from 25 μF to 1500 μF and from 2 mH to 9 mH for the inductor **234**, for example. A designed resistance can be used in the circuit to provide damping to the pulses, such as critical damping.

[0065] FIG. **2**A illustrates another embodiment of assay cartridge **201** that is configured to conduct an assay in an assay device **100**, such as assay device **100** in order to quantify a target component **105**T, such as a Troponin component (Troponin I or Troponin T). The assay cartridge **201** includes all the elements of the FIG. **1**A-**1**D embodiment, except that the body **202** includes a key shape, having

a wider width portion **202**W and a narrower width portion **202**N. The wider width portion **202**W can have formed therein the well **207**, inlet **204**, and part of the inlet channel **206**. The narrower width portion **202**N can include the remainder of the inlet channel **206**, the reaction chamber including the magnetic particles **115** and dry reagents therein. Further, the narrower width portion **202**N can include a vent **212**. The electrodes **212**A, **212**B, conductor paths **214**A, **214**B, and contact pads **216**A, **216**B may be provided on the first body part **202**A by any suitable method, such as the methods described herein. The second body part **202**B may include recesses therein forming, in combination with the first body portion, the well **207**, inlet channel **206**, reaction chamber **210**, and vent **212**. As before, a filter **208** may be included to allow only the test sample **105**P (e.g., plasma) to flow into the reaction chamber **210** when whole blood is deposited in the well **207** as the patient sample **105**. As is shown in FIG. **2**B, the contact pads **216**A, **216**B may be connected to the voltage pulse generator **222** by any suitable electrical contacts **216**C as described herein.

[0066] To better understand the various operations that are undertaken during the process of conducting the assay, the following is offered with reference to FIGS. **1**H-**1**K. According to some embodiments, the assay uses two different antibodies, a first antibody **111**A (e.g., a monoclonal anti-Troponin I or anti-Troponin T antibody) coupled to the magnetic particles **115** (the magnetic labels) and a second antibody **111**B (e.g., a polyclonal anti-Troponin I or anti-Troponin T antibody) coupled to the sensor surface **110**A proximate the quantification window **117**. The location of the first antibody **111**A and the second antibody **111**B may be switched in some embodiments. For example, the first antibody **111**A may be a polyclonal anti-Troponin I or anti-Troponin T antibody and may be coupled to the magnetic particles **115** (the magnetic labels), and the second antibody **111**B may be a monoclonal anti-Troponin I or anti-Troponin T antibody that is coupled to the sensor surface **110**S.

[0067] FIG. **1**H shows a schematic depiction of a portion of the reaction chamber **110** embodied in an assay cartridge **101** according to embodiments of the disclosure. In FIG. **1**I a test sample **105**P potentially comprising the target component **105**T (e.g., Troponin I or Troponin T) in the presence of albumin is introduced into the reaction chamber **110** and this operates to rehydrate the magnetic particles **115** and the dried reagents that are present in the reaction chamber **110**. In the presence of high amounts of albumin, the magnetic particles **115** can aggregate and produce clumps **115**C in the reaction chamber **110**, which are unwanted.

[0068] FIG. **1**J illustrates the application of the pulsed voltage V (e.g., one or more voltage pulses) and that the clumps **115**C previously present in FIG. **1**I are broken up and reduced, minimized, or even substantially eliminated. The exposure of the test sample **105**T including the clumped magnetic particles **115**C to the pulsed voltage V can involve one and preferably more than one successive pulse of a voltage potential V applied between the electrodes **112**A, **112**B. The simple presence of the magnetic particles **115** in the test sample **105**P may facilitate coupling and binding of some of the target component **105**T present in the test sample **105**P to the magnetic particles **115** as shown schematically in FIG. **1**J. In particular, the target component **105**T, if present, can come into contact with the first antibody **111**A and becomes coupled to the magnetic particles **115**. The test sample **105**P additionally comes into contact with the second antibody **111**B coupled to a sensor surface **110**S. The first antibody **111**A and second antibody **111**B are selected such that the analyte of interest (the target component **105**T, e.g., Troponin I or Troponin T) can be bound to both the first antibody **111**A and the second antibody **111**B simultaneously, and thus form a complex on the sensor surface **110**S as best shown in FIG. **1**K. In particular, if the target component **105**T is present in the sample **105**P, complexes made up of the magnetic particle with first antibody **111**A, the target component **105**T, and the second antibody **111**B will be formed on the sensor surface **110**S.

[0069] Following the exposing the test sample **105**P in the reaction chamber **110** to the pulsed voltage, the magnetic particles **115** can be exposed to various magnetic fields MF from a magnetic field generator **123** to assist in moving unbound magnetic particles **115**U away from the sensor surface **110**S and/or moving them towards the sensor surface **100**S and also assisting in the formation of complexes by binding the target component **105**T bound to the first antibody **111**A to the second antibody **111**B at a sensor surface **110**S. In particular, to enhance and maximize the formation of such complexes, the magnetic field MF may be applied to the reaction chamber **110** as shown in FIG. **1**K. The magnetic field MF may be reversed in flux direction one or more times to facilitate movement (at least translation) of the magnetic particles **115** within the test sample **105**P towards and away from the sensor surface **110**S so as to aid in forming a maximum number of complexes. This can involve alternating the direction of a magnetic field MF perpendicular to the sensor surface **110**S as described in U.S. Pat. No. 9,720,003, or optionally parallel to the sensor surface **110**S or a combination of different fields with different orientations, such as are described in WO2007129275.

[0070] After sufficiently long time, as determined through experimentation, and reversals allowing the formation of substantial amounts of complexes, a sufficient magnetic field MF can be applied by the magnetic field generator **123** in a negative direction away from the sensor surface **110**S so that any unbound magnetic particles **115**U (unbound to a target component **105**T) can be moved away from the sensor surface **110**S, such as to a location directly adjacent to the wall opposite from the sensor surface **110**S as shown in FIGS. **1**G and **1**K. Further, the application of the magnetic field MF in the negative direction will also remove any magnetic particles **115**B that may be bound to a target component **105**T, but that are not specifically complexed to the sensor surface **110**S via the target component **105**T and second antibody (so called bound yet free-floating target components **105**F) as shown in FIG. **1**K.

[0071] The final magnetic field MF should be of a magnitude low enough so as to not remove any of the previously formed complexes. One example actuation scheme consists of about 1 minute incubation of the test sample **105**T causing rehydrating the dry reagents and magnetic particles **115** followed by about 4 minutes of pulsed actuation and about 10 second removal of unbound magnetic particles **115**U and any bound yet free-floating target components **105**F with the magnetic field generator **123**.

[0072] Following removal of any unbound magnetic particles **115**U and/or any bound yet free-floating target components **105**F away from the sensor surface **110**S, the amount of the target component **105**T (e.g., Troponin I or Troponin T) that is present and complexed to the sensor

surface **110**S can be determined by any suitable detection method carried out by reader software **126**. The assay results may be displayed on a suitable display **128** of the assay device **100**. The assay device **100** may further include any suitable user interface **130** for initiating the assay, retrieving assay results, and/or otherwise manipulating data.

[0073] For example, referring to FIGS. **1**A-**1**G, detection may be achieved by any suitable detection system **125** that cooperates with the sensor surface **110**S in some embodiments. For example, one or more optical sensors can be located in the vicinity of (e.g., under) the quantification window **117**, allowing optical detection of magnetic particles **115**, which are the complexed to the sensor surface **110**S. However, detection and quantification of the concentration of the target component **105**T in a test sample **105**P with different detection techniques is also possible.

[0074] Suitable surface materials and linking chemistries are known to the person of ordinary skill in the art, and are described for instance in "Diagnostic Biosensor Polymers," by A. M. Usmani and N. Akmal, American Chemical Society, 1994 Symposium Book Series 556, Washington D.C., USA, 1994, in "Protein Architecture, Interfacing Molecular Assemblies and Immobilization Biotechnology," edited by Y. Lvov and H. Mhwald (Marcel Dekker, New York, 2000), in "The immunoassay Handbook" by David Wild (Nature Publishing Group, London, 2001, ISBN 1-56159-270-6), and in the "Handbook of Biosensors and Electronic Noses. Medicine, Food and the Environment" by Kress-Rogers (ISBN 0-8493-8905-4). Supports for coupling proteins to coated and uncoated plastic and glass supports are also disclosed in Angenendt et al. (2002*; Anal Biochem.* 309, 253-260).

[0075] Detection means suitable for use in the assay methods and assay devices of the present disclosure comprise any detection system **125** capable of detecting a relevant signal such as, but not limited to, an optical signal (reflection, absorption, scattering, fluorescence, chemiluminescence, RAMAN, FTIR, or the like). Active labels that may be coupled to the magnetic particles **115** are known to persons of ordinary skill in the art and include fluorescein dyes, such as 5- (and 6-) carboxy-4',5'-dichloro-2',7'-dimethoxy fluorescein, 5-carboxy-2',4',5',7'-tetrachlorofluorescein and 5-carboxyfluorescein, rhodamine dyes such as 5- (and 6-) carboxy rhodamine, 6-carboxytetramethyl rhodamine and 6-carboxyrhodamine X, phthalocyanines such as methyl, nitrosyl, sulphonyl and amino phthalocyanines, azo dyes, azomethines, cyanines and xanthines such as the methyl, nitro, sulphano and amino derivatives, and succinylfluoresceins. Other suitable active labels that can be coupled to the magnetic particles **115** are fluorophores from the group of cyanine dimers and monomers, such as TOTO, YOYO, TO-PRO, Cy3, Cy5, Cy5.5, Cy7, etc., or dyes such as LCRed 705, and can be used as the fluorescent dye.

[0076] Depending on the nature of the signal to be detected, the detection system can be an integral part of the sensor surface **110**S or can allow the detection of the presence of magnetic labels on the sensor surface **110**S. In one example, radioactive labels, such as luminescent or fluorescent labels, are embedded in or otherwise attached to the magnetic particles **115** that are used. Excitation of the fluorescent labels can be done using an irradiation source, such as a focused laser beam or via evanescent field excitation allowing optical detection of such radioactive labels. Detection can be done in any suitable way, such as by using confocal detection or using a high-NA lens. The use of fluorescent labels enables multiplexing by using different fluorophores, which can differ in excitation and/or emission wavelengths, and thus can allow detection of different target components at once.

[0077] As mentioned above the detection of magnetic particles **115** complexed at a sensor surface **110**S can be measured by any direct or indirect method known in the art. Particular detection methods described herein can be based on optical properties of the magnetic particles **115**, such as detection with frustrated total internal reflection (FTIR).

[0078] As shown in FIGS. **3**A and **3**B, particle distribution images **300**A, **300**B of a sensor surface **110**S illustrate experimental results using a particle counter that are shown respectively using a control sample **300**A (without application of the pulsed voltage to the test sample **105**P with high levels of albumin) in FIG. **3**A, and then an experimental sample **300**B that included exposing the test sample **105**P with high levels of albumin to pulsed voltage as shown in FIG. **3**B. Each image is shown at 50× magnification. The images and particle count results illustrate that a large amount of the clumping was reduced over the control (with no voltage pulse applied) based not only on increased particle counts, but also noting that the mean size of the magnetic particles **115** measured was reduced by approximately 6.0% to 7.8% when the pulsed voltage was applied.

[0079] As best shown and described with reference to FIG. **4**, a method **400** of quantifying a target component (e.g., Troponin I or Troponin T) in a test sample **105**P is provided according to another embodiment of the disclosure. The method **400** comprises, in block **402**, providing a reaction chamber (e.g., reaction chamber **110**) containing magnetic particles (e.g., magnetic particles **115**) including a first antibody (e.g., first antibody **111**A) and a sensor surface (e.g., sensor surface **110**S) including a second antibody (e.g., second antibody **111**B).

[0080] The method **400** further comprises, in block **404**, flowing the test sample (e.g., test sample **105**P) into the reaction chamber (e.g., reaction chamber **110**), followed by, in block **406**, rehydrating the magnetic particles (e.g., magnetic particles **115**) and binding a target component **105**T (e.g., Troponin I or Troponin T) to the first antibody **111**A of the magnetic particles (e.g., magnetic particles **115**).

[0081] Additionally, the method **400** further comprises, in block **408**, after rehydration in block **406**, exposing the magnetic particles (e.g., magnetic particles **115**) to a pulsed voltage V whereby clumping (e.g., clumps **115**C) of the magnetic particles (e.g., magnetic particles **115**) in the presence of albumin in the test sample (e.g., test sample **105**P) is reduced.

[0082] Following the exposure of the magnetic particles (e.g., magnetic particles **115**) to the pulsed voltage V, the magnetic particles (e.g., magnetic particles **115**) now including a lesser amount of clumping **115**C may be exposed to a magnetic field MF generated by a magnetic field generator (e.g., magnetic field generator **123**).

[0083] The application of the magnetic field MF provided by the magnetic field generator **123** in a direction towards the sensor surface **110**S can provide actuation forces to the magnetic particles **115** carrying the first antibodies **111**A during the assay to ensure optimized contact with the sensor surface **110**S. In some embodiments, the magnetic field MF can be pulsed, such as by reversing the magnetic flux direction. Magnetic particles **115** can be manipulated in

different ways to optimize contact with the immobilized second antibodies **111**B provided on the sensor surface **110**S.

[0084] The reversal of the magnetic field MF can facilitate removal of any magnetic particle **115** from the sensor surface **110**S that has no target component **105**T bound to the first antibody **111**A (e.g., monoclonal anti-troponin I or T antibody), or that may have a target component **105**T bound to the first antibody **111**A, but is not complexed to the sensor surface **110**S. The magnetic particles **115** that have a target component **105**T bound to the first antibody **111**A and wherein that target component **105**T is also bonded to the sensor surface **110**S by the second antibody **111**B form complexes that remain and thus can be quantified to provide an amount of the target component **105**T in the test sample **105**P. Several magnetic field reversals may be used to maximize the amount of complexes including the target component on the sensor surface **110**S.

[0085] In some embodiments, during the step of binding the target component to the first antibody of the magnetic particles, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in keeping unbound magnetic particles away from the sensor surface **110**S. This prevents early binding to the secondary antibody which would otherwise prevent performing a reference signal measurement at the sensor surface.

[0086] In some embodiments, following the exposing of the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in moving magnetic particles towards the sensor surface **110**S and binding the target component bound to the first antibody to the second antibody at a sensor surface.

[0087] In some embodiments, after rehydrating the magnetic particles and before the exposing to the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in binding target components attached to the first antibody to the secondary antibody at the sensor surface, whereby the clumping of the magnetic particles due to the magnetic fields is reduced.

[0088] In some embodiments, the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles to capture to the target component via the first antibody, whereby the clumping of the magnetic particles due to the magnetic fields is reduced, and thereby any loss in target component capture efficiency caused by the clumping

[0089] In some embodiments, the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles with target components bound to the first antibody, to bind the captured target component to the second antibody at the sensor surface, whereby the clumping of the magnetic particles due to the magnetic fields is reduced, and thereby any loss in binding efficiency of captured target component to the second antibody caused by the clumping. This allows in particular to increase the use of magnetic actuation and enables to apply stronger field strengths and/or to use prolonged periods with actuation since undesired clumping is overcome. The increased use of magnetic fields allows to further speed up the binding processes and thereby enables faster and/or more sensitive assay results.

[0090] In some embodiments, the pulsed voltage is applied every 5, 10, 30, 60, and/or 120 seconds.

[0091] In some embodiments, the magnetic field MF may be applied substantially simultaneously with one or more pulses of the voltage V to minimize clumping and further aid in maximizing the formation of complexes to the sensor surface **110**S. Following application of the magnetic field MF, the results of the assay of the target component **105**T can be detected. In particular, in block **412**, quantifying the magnetic particles **115** complexed to the sensor surface **110**S of the reaction chamber **110** can be accomplished by any of the methods described herein. In some embodiments, the assay cartridge **101** is suitable for optical detection of the magnetic particles **115** complexed to the sensor surface **110**S by a detection system **125**, such as by FTIR where a range of wavelengths in the infrared region that are absorbed (or reflected) by a material are measured.

[0092] FTIR involves using, as shown in FIG. **1A-1**G, a source **125**L, such as an illumination source (e.g., an infrared (IR) source projected onto the quantification window **117** and thus onto the magnetic particles **115** complexed to the sensor surface **110**S. In some embodiments, the projection can be provided along the quantification window through the use of one or more prisms. The test sample's absorbance of the infrared light energy at various wavelengths can be measured by receiver **125**R and recorded. This data may be used to determine a quantity of magnetic particles **115** complexed to the sensor surface **110**S, which can be substantially directly proportional to the quantity of the target component **105**T present in the test sample **105**P. Other suitable optical quantification methods may be used.

[0093] The following is a list of non-limiting illustrative embodiments disclosed herein:

1. A method of quantifying a target component in a test sample, comprising:
   - **[0094]** providing a reaction chamber containing magnetic particles including a first antibody and a sensor surface including a second antibody;
   - **[0095]** flowing the test sample into the reaction chamber;
   - **[0096]** rehydrating the magnetic particles and binding a target component to the first antibody of the magnetic particles; and
   - **[0097]** after rehydration, exposing the magnetic particles to a pulsed voltage whereby clumping of the magnetic particles in the presence of albumin in the test sample is reduced.
2. The method of the first illustrative embodiment, wherein the target component is Troponin I (cTnI) or Troponin T (cTnT).
3. The method of any one of the illustrative embodiments 1 to 2, wherein the magnetic particles are provided with a monoclonal anti-Troponin antibody comprising the first antibody, and/or wherein the first antibody is a monoclonal anti-Troponin antibody.
4. The method of any one of the illustrative embodiments 1 to 3, comprising a sensor surface, wherein the sensor surface is located proximate to a quantification window of the reaction chamber and the sensor surface is provided with a polyclonal anti-Troponin antibody comprising a second antibody.
5. The method of any one of the illustrative embodiments 1 to 4, wherein the pulsed voltage comprises a magnitude greater than 150 V.

6. The method of any one of the illustrative embodiments 1 to 5, wherein the pulsed voltage comprises a magnitude greater than 300 V.
7. The method of any one of the illustrative embodiments 1 to 6, wherein the exposing of the pulsed voltage comprises providing at least two voltage pulses to break up the clumping of the magnetic particles.
8. The method of the illustrative embodiment 7, wherein each of the at least two voltage pulses have a duration of greater than 2 milliseconds.
9. The method of any one of the illustrative embodiments 1 to 8, wherein during the step of binding the target component to the first antibody of the magnetic particles, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in keeping unbound magnetic particles away from the sensor surface **110**S.
10. The method of any one of the illustrative embodiments 1 to 9, wherein following the exposing of the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in moving magnetic particles towards the sensor surface **110**S and binding the target component bound to the first antibody to the second antibody at a sensor surface.
11. The method of any one of the illustrative embodiments 1 to 10, wherein following the exposing of the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in moving unbound magnetic particles away from the sensor surface **110**S.
12. The method of any one of the illustrative embodiments 1 to 11, wherein after rehydrating the magnetic particles and before the exposing to the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in binding target components attached to the first antibody to the secondary antibody at the sensor surface, whereby the clumping of the magnetic particles due to the magnetic fields is reduced.
13. The method of any one of the illustrative embodiments 1 to 12, wherein the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles to capture to the target component via the first antibody.
14. The method of any one of the illustrative embodiments 1 to 13, wherein the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles with target components bound to the first antibody, to bind the captured target component to the second antibody at the sensor surface.
15. The method of any one of the illustrative embodiments 1 to 14, wherein the pulsed voltage is applied every 5, 10, 30, 60, and/or 120 seconds.
16. The method of any one of the illustrative embodiments 1 to 15, comprising an inlet channel configured to flow the test sample to the reaction chamber, wherein the inlet channel is a capillary channel.
17. The method of the illustrative embodiment 1 to 16, comprising a filter configured to allow substantially only test sample to pass through to the reaction chamber.
18. The method of any one of the illustrative embodiments 1 to 17, wherein the albumin is present in a sample from which the test sample is derived in a quantity greater than 5.4 g/dL in whole blood.
19. The method of the illustrative embodiment 18, wherein the albumin is present in a in a quantity greater than 10 g/dL in whole blood.
20. The method of the illustrative embodiment 18, wherein the albumin is present in a quantity greater than 100 g/dL in whole blood.
21. An assay cartridge, comprising:

[0098] a cartridge body including a sample inlet adapted to receive a sample and a reaction chamber coupled to the sample inlet;

[0099] magnetic particles contained in the reaction chamber comprising a first antibody with a specific affinity towards a target component;

[0100] electrodes provided at a location configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber; and

[0101] a sensor surface of the reaction chamber comprising a second antibody with a specific affinity towards the target component.

22. The assay cartridge of the illustrative embodiment 21, wherein the target component comprises Troponin I or Troponin T.
23. The assay cartridge of any one of the illustrative embodiments 21 to 22, further comprising a first electrode positioned on a first side of the reaction chamber and a second electrode positioned on a second side of the reaction chamber.
24. The assay cartridge of any one of the illustrative embodiments 21 to 23, further comprising a filter configured to retain red blood cells and allow the test sample to pass through the filter into the reaction chamber.
25. The assay cartridge of any one of the illustrative embodiments 21 to 24, wherein the magnetic particles are provided with a monoclonal antibody comprising the first antibody.
26. The assay cartridge of any one of the illustrative embodiments 21 to 25, further wherein a sensor surface proximate a quantification window of the reaction chamber is provided with a polyclonal antibody comprising the second antibody.
27. An assay device, comprising:

[0102] a device body including a cartridge receiver;

[0103] an assay cartridge received in the cartridge receiver, the assay cartridge comprising a cartridge body including an inlet channel adapted to receive a test sample and a reaction chamber therein, magnetic particles having a first antibody with a specific affinity towards a target component provided in the reaction chamber, and electrodes provided at locations configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber; and

[0104] a voltage pulse generator coupled to the electrodes and configured to provide one or more voltage pulses to the electrodes.

[0105] While this disclosure is susceptible to various modifications and alternative forms, specific method, assay cartridge, and assay device embodiments have been shown by way of example in the drawings and are described in detail herein. It should be understood, however, that the particular method, assay cartridges, and assay devices disclosed herein are not intended to limit the disclosure or the claims.

What is claimed is:

1. A method of quantifying a target component in a test sample, comprising:

providing a reaction chamber containing magnetic particles including a first antibody and a sensor surface including a second antibody;

flowing the test sample into the reaction chamber;

rehydrating the magnetic particles and binding a target component to the first antibody of the magnetic particles; and

after rehydration, exposing the magnetic particles to a pulsed voltage whereby clumping of the magnetic particles in a presence of albumin in the test sample is reduced.

2. The method of claim **1**, wherein the target component is Troponin I or Troponin T.

3. The method of claim **1**, wherein the first antibody is a monoclonal anti-Troponin antibody.

4. The method of claim **1**, comprising a sensor surface, wherein the sensor surface is located proximate to a quantification window of the reaction chamber and the sensor surface is provided with a polyclonal anti-Troponin antibody comprising a second antibody.

5. The method of claim **1**, wherein the pulsed voltage comprises a magnitude greater than 150 V.

6. The method of claim **1**, wherein the pulsed voltage comprises a magnitude greater than 300 V.

7. The method of claim **1**, wherein the exposing of the pulsed voltage comprises providing at least two voltage pulses to break up the clumping of the magnetic particles.

8. The method of claim **7**, wherein each of the at least two voltage pulses have a duration of greater than 2 milliseconds.

9. The method of claim **1**, wherein during the step of binding the target component to the first antibody of the magnetic particles, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in keeping unbound magnetic particles away from the sensor surface **110**S.

10. The method of claim **1**, wherein following the exposing of the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in moving magnetic particles towards the sensor surface **110**S and binding the target component bound to the first antibody to the second antibody at a sensor surface.

11. The method of claim **1**, wherein following the exposing of the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in moving unbound magnetic particles away from the sensor surface **110**S.

12. The method of claim **1**, wherein after rehydrating the magnetic particles and before the exposing to the pulsed voltage, the magnetic particles are exposed to a magnetic field MF from a magnetic field generator to assist in binding target components attached to the first antibody to the secondary antibody at the sensor surface, whereby the clumping of the magnetic particles due to the magnetic fields is reduced.

13. The method of claim **1**, wherein the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles to capture the target component via the first antibody.

14. The method of claim **1**, wherein the pulsed voltage is applied intermittently while exposing the magnetic particles to a magnetic field MF from a magnetic field generator to assist the magnetic particles with target components bound to the first antibody, to bind the captured target component to the second antibody at the sensor surface.

15. The method of claim **14**, wherein the pulsed voltage is applied every 5, 10, 30, 60, and/or 120 seconds.

16. The method of claim **1**, comprising an inlet channel configured to flow the test sample to the reaction chamber, wherein the inlet channel is a capillary channel.

17. The method of claim **16**, comprising a filter configured to allow substantially only test sample to pass through to the reaction chamber.

18. The method of claim **1**, wherein the albumin is present in a sample from which the test sample is derived in a quantity greater than 5.4 g/dL in whole blood.

19. The method of claim **18**, wherein the albumin is present in a in a quantity greater than 10 g/dL in whole blood.

20. The method of claim **18**, wherein the albumin is present in a quantity greater than 100 g/dL in whole blood.

21. An assay cartridge, comprising:

a cartridge body including a sample inlet adapted to receive a sample and a reaction chamber coupled to the sample inlet;

magnetic particles contained in the reaction chamber comprising a first antibody with a specific affinity towards a target component;

electrodes provided at a location configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber; and

a sensor surface of the reaction chamber comprising a second antibody with a specific affinity towards the target component.

22. The assay cartridge of claim **21**, wherein the target component comprises Troponin I or Troponin T.

23. The assay cartridge of claim **21**, further comprising a first electrode positioned on a first side of the reaction chamber and a second electrode positioned on a second side of the reaction chamber.

24. The assay cartridge of claim **21**, further comprising a filter configured to retain red blood cells and allow the test sample to pass through the filter into the reaction chamber.

25. The assay cartridge of claim **21**, wherein the magnetic particles are provided with a monoclonal antibody comprising the first antibody.

26. The assay cartridge of claim **21**, further wherein a sensor surface proximate a quantification window of the reaction chamber is provided with a polyclonal antibody comprising the second antibody.

27. An assay device, comprising:

a device body including a cartridge receiver;

an assay cartridge received in the cartridge receiver, the assay cartridge comprising a cartridge body including an inlet channel adapted to receive a test sample and a reaction chamber therein, magnetic particles having a first antibody with a specific affinity towards a target component provided in the reaction chamber, and electrodes provided at locations configured to be in contact with a rehydrated test sample containing the magnetic particles in the reaction chamber; and

a voltage pulse generator coupled to the electrodes and configured to provide one or more voltage pulses to the electrodes.

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