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(12) United States Patent
Lowery, Jr. et al.**(10) Patent No.: US 8,563,298 B2**
(45) Date of Patent: Oct. 22, 2013**(54) NMR SYSTEMS AND METHODS FOR THE RAPID DETECTION OF ANALYTES****(75) Inventors:** **Thomas Jay Lowery, Jr.**, Belmont, MA (US); **Mark John Audeh**, Brighton, MA (US); **James Franklin Chepin**, San Diego, CA (US); **Rahul Dhanda**, Needham, MA (US); **Lori Anne Neely**, Reading, MA (US); **Charles William Rittershaus**, Malden, MA (US)**(73) Assignee:** **T2 Biosystems, Inc.**, Lexington, MA (US)**(*) Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 104 days.**(21) Appl. No.:** **12/910,594****(22) Filed:** **Oct. 22, 2010****(65) Prior Publication Data**

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(57) ABSTRACT

This invention features systems and methods for the detection of analytes, and their use in the treatment and diagnosis of disease.

19 Claims, 44 Drawing Sheets

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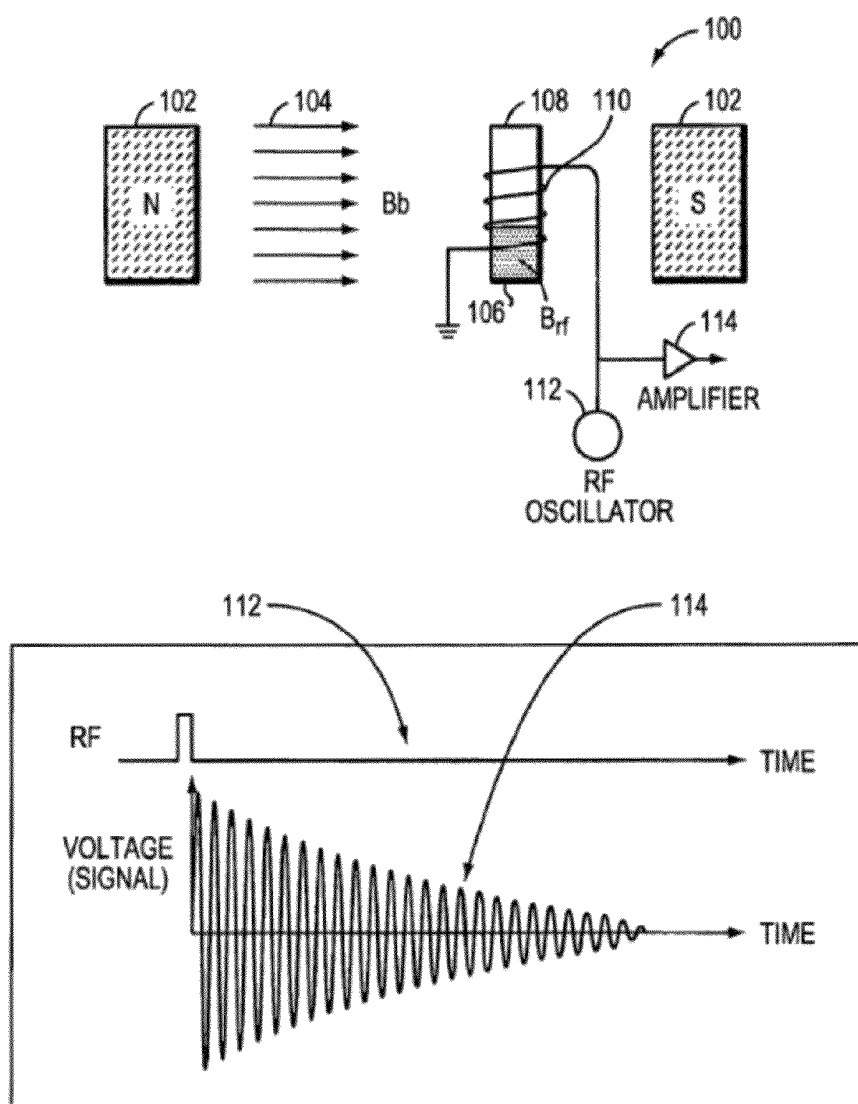
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Figure 1



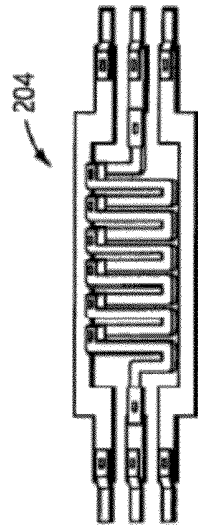


FIG. 2C

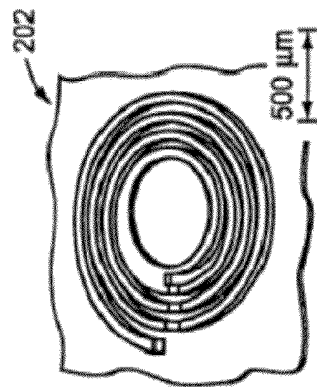


FIG. 2B

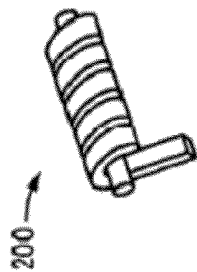


FIG. 2A

Figure 2

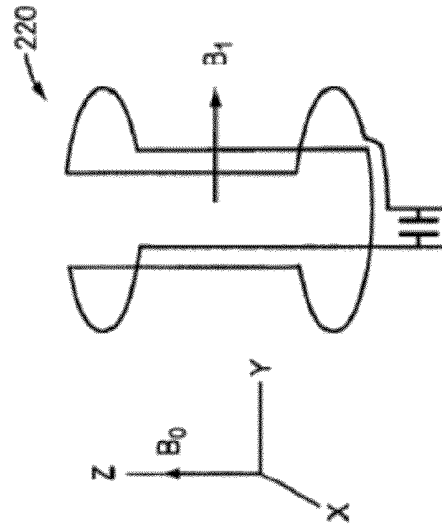


FIG. 2E

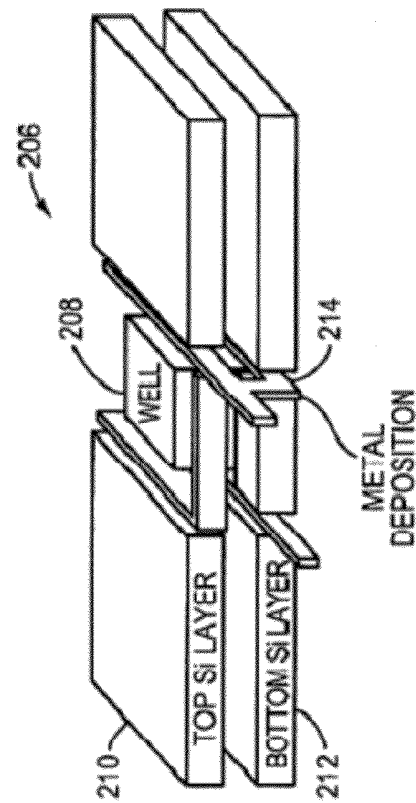
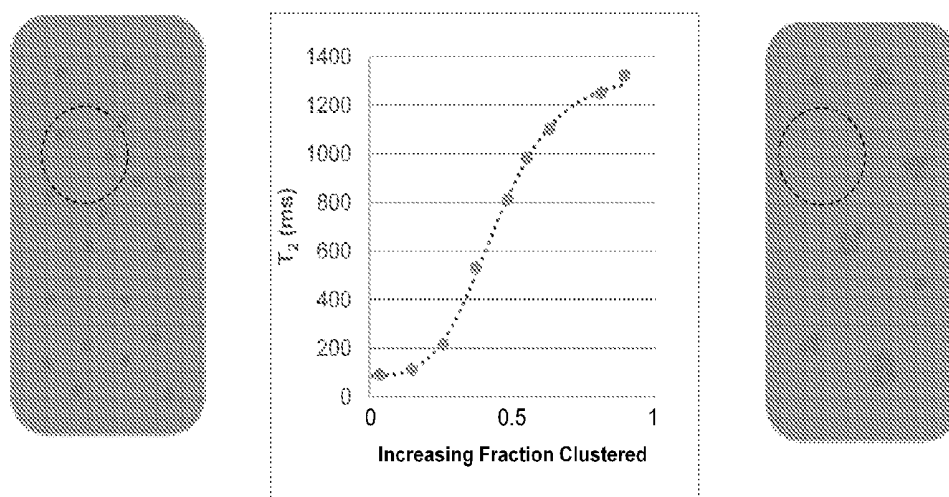


FIG. 2D

Figure 3



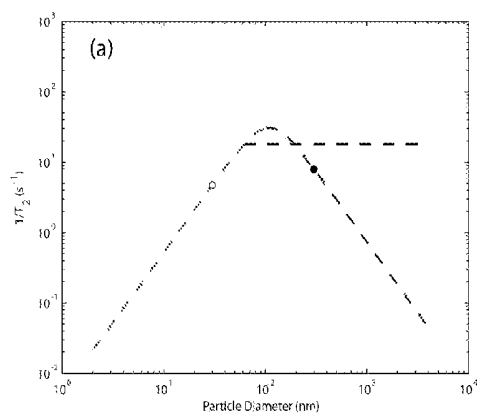


Figure 4A

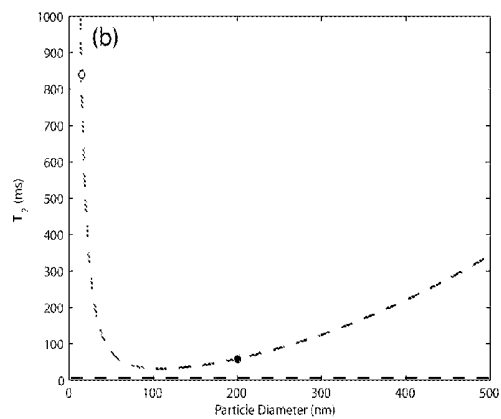


Figure 4B

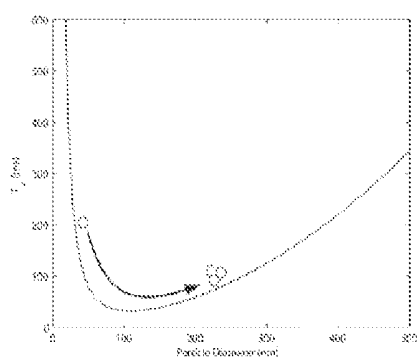


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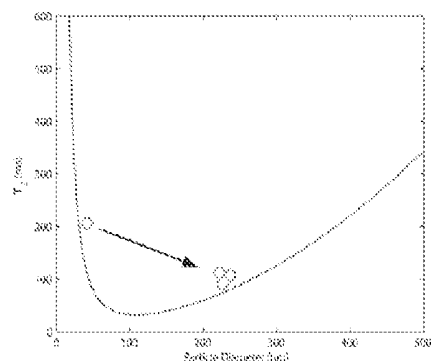


Figure 4D

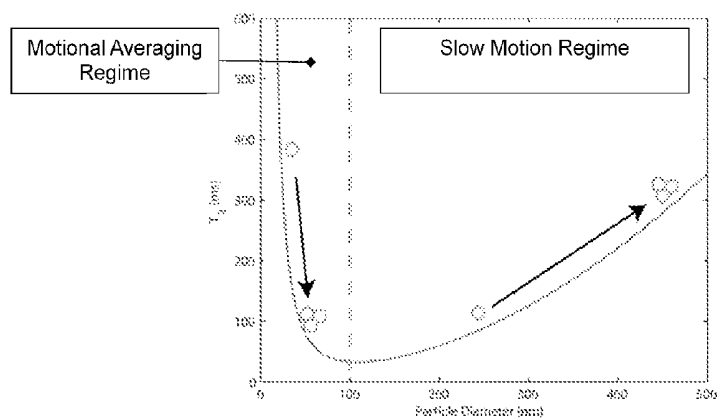


Figure 4E

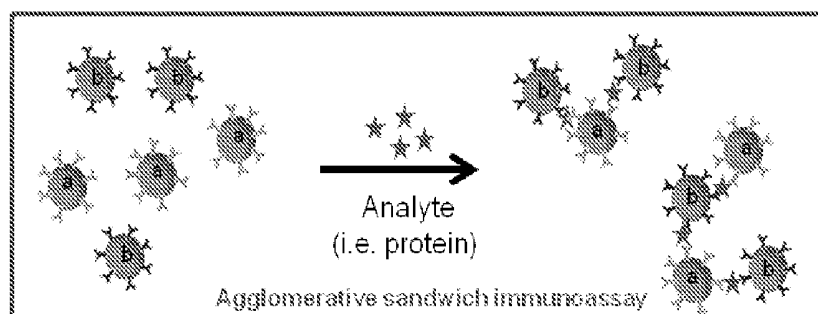


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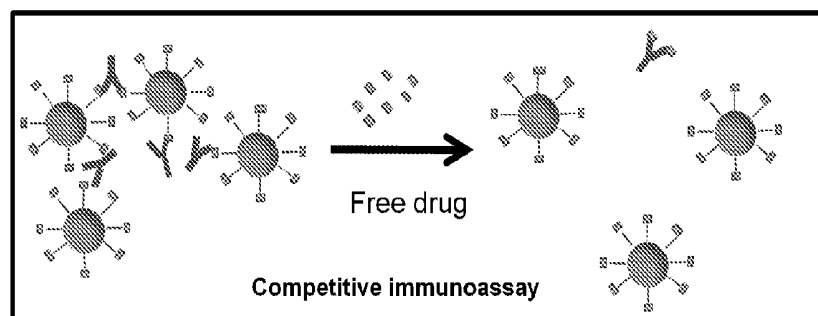


Figure 5B

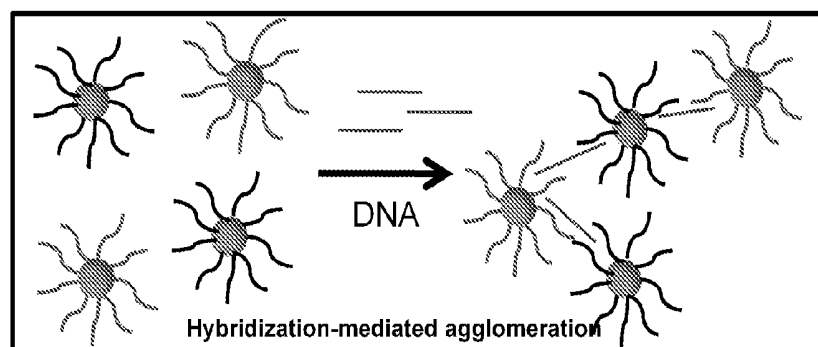
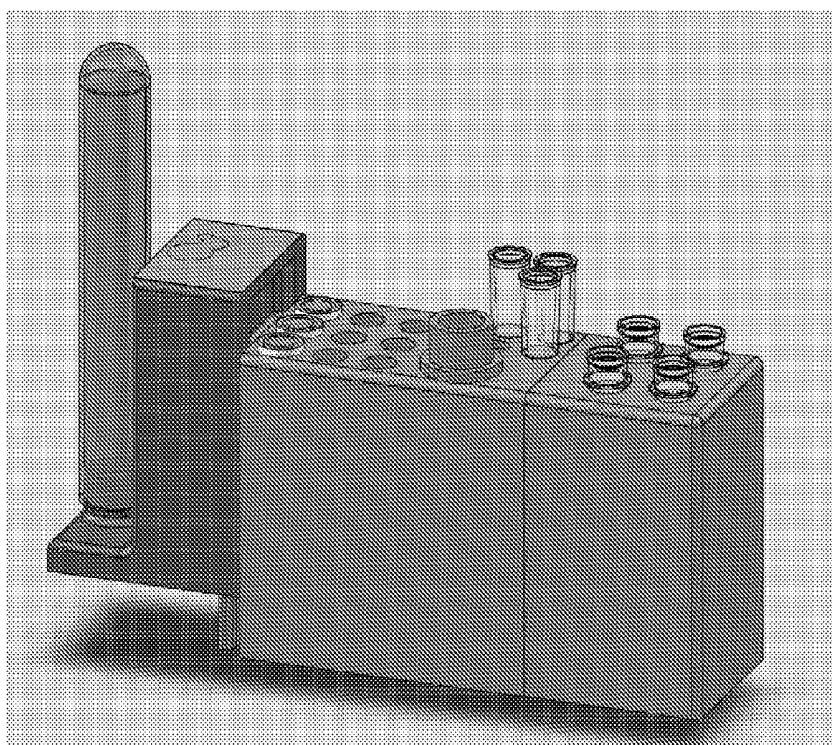


Figure 5C

Figure 6



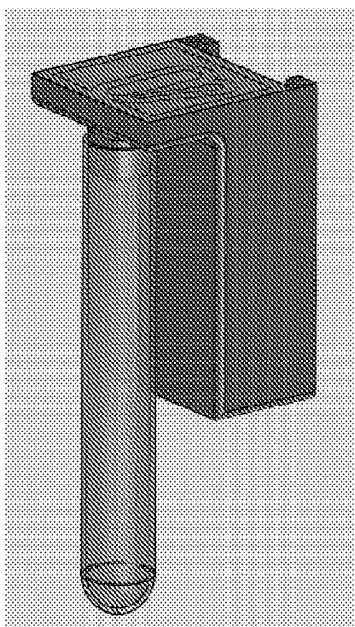


Figure 7A

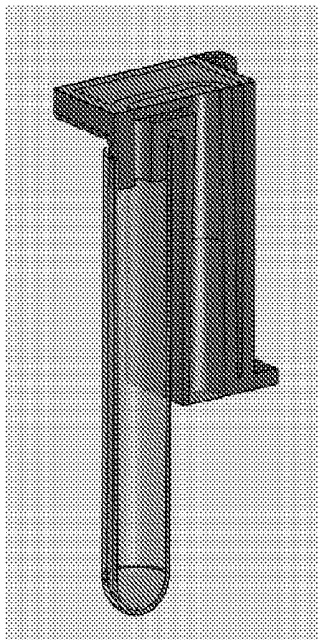


Figure 7B

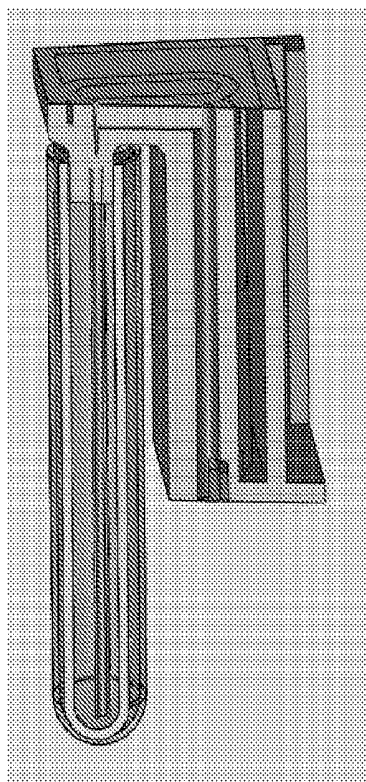
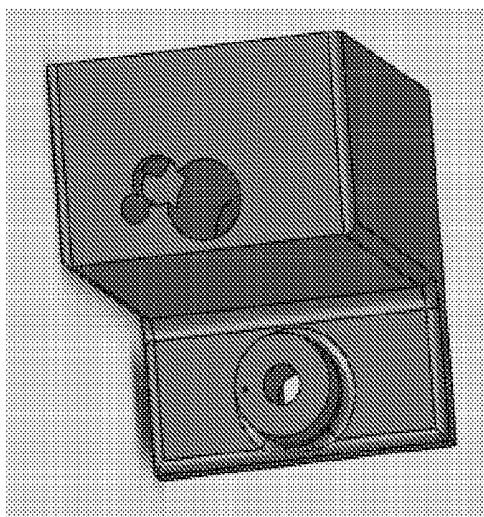


Figure 7C

Figure 8



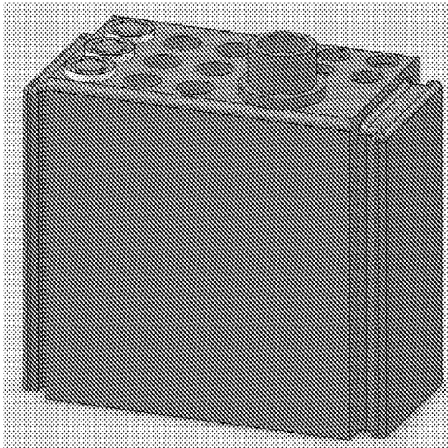


Figure 9A

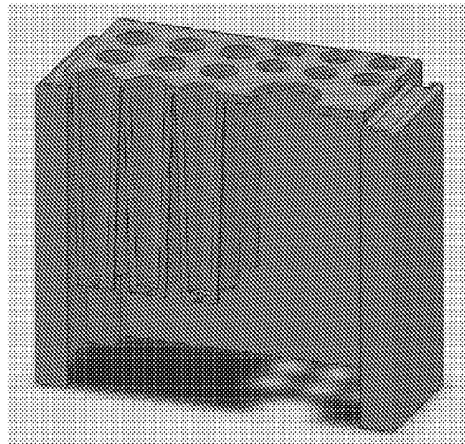


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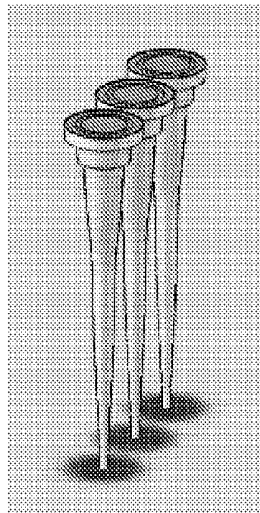


Figure 9C

Figure 10

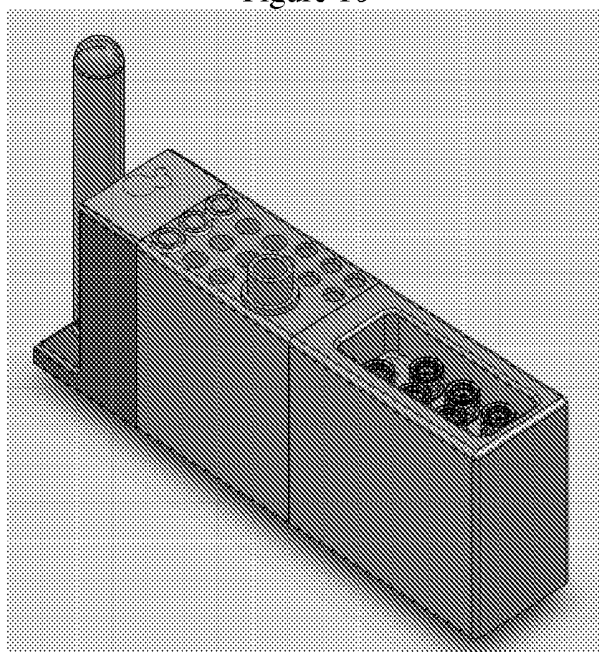


Figure 11

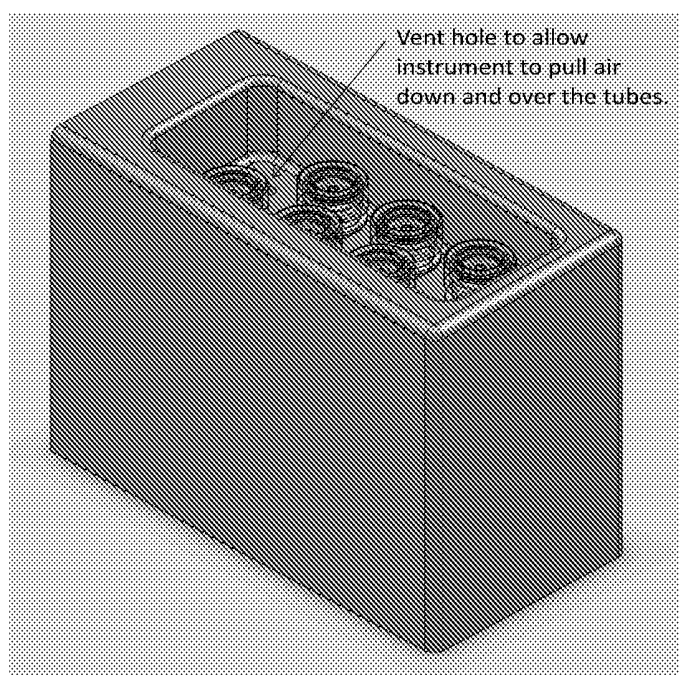
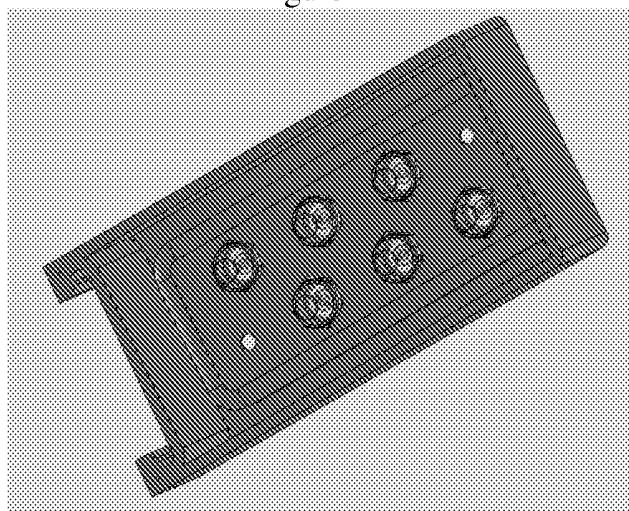


Figure 12



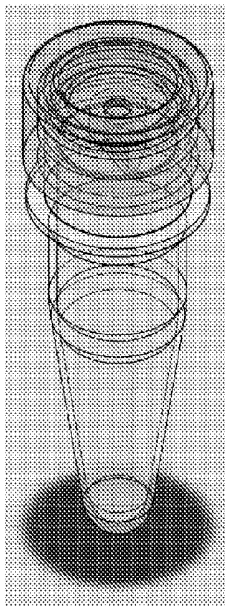


Figure 13A

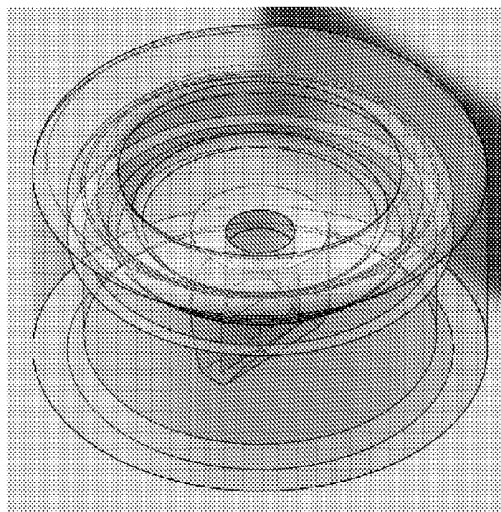


Figure 13B

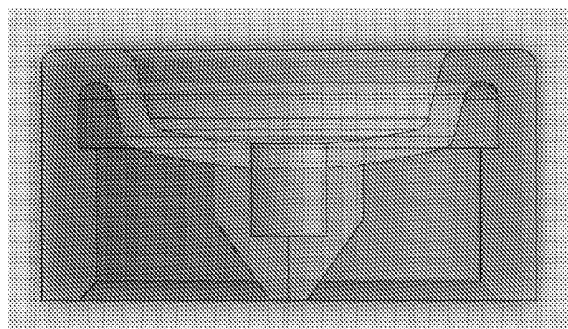


Figure 13C

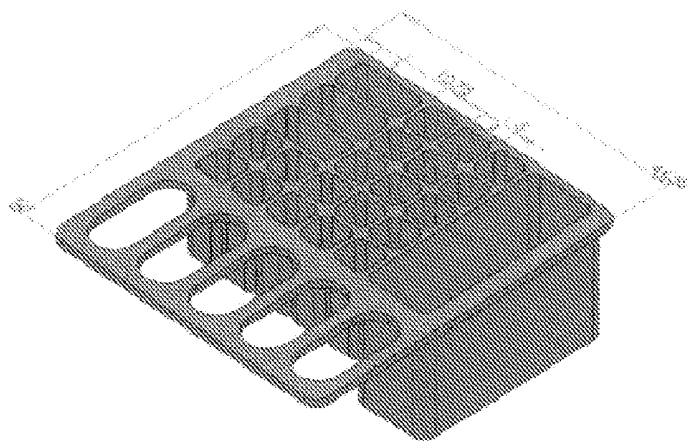


Figure 14A

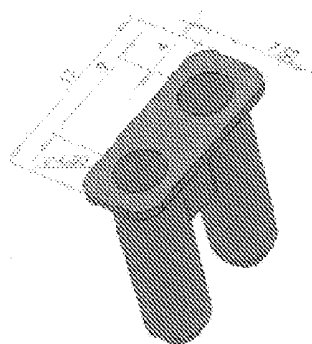


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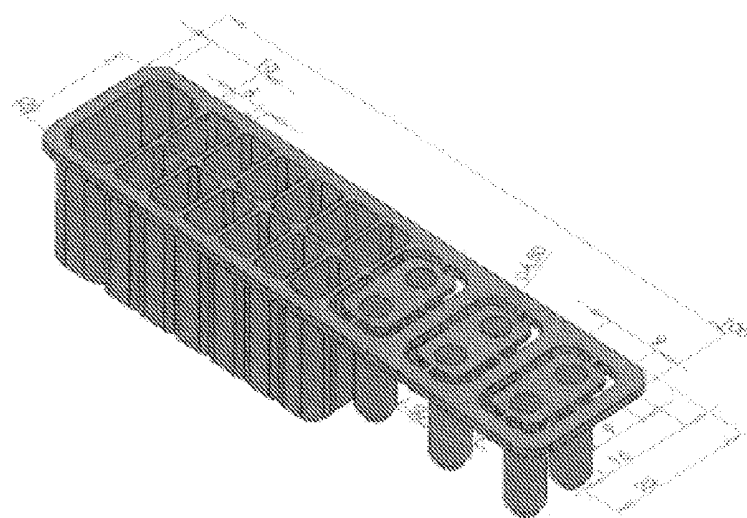


Figure 14C

Figure 15

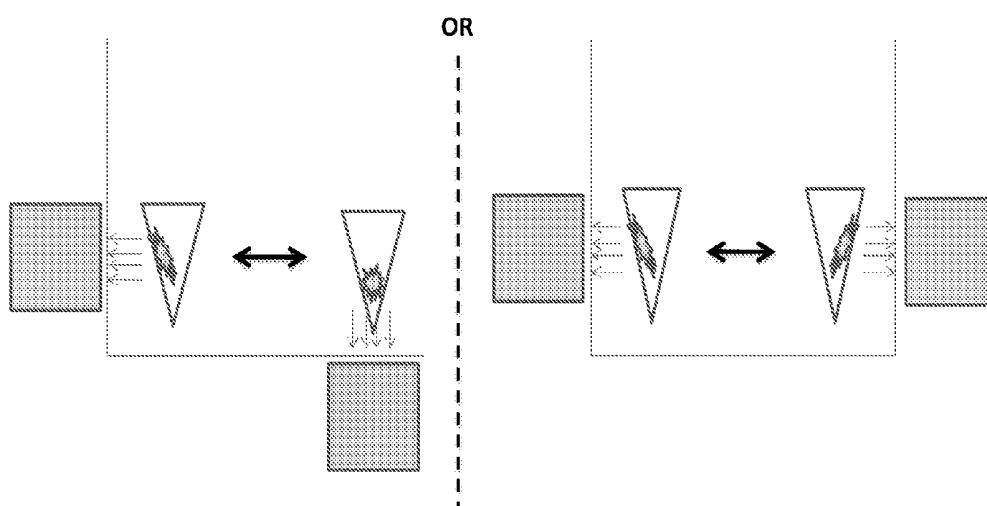


Figure 16

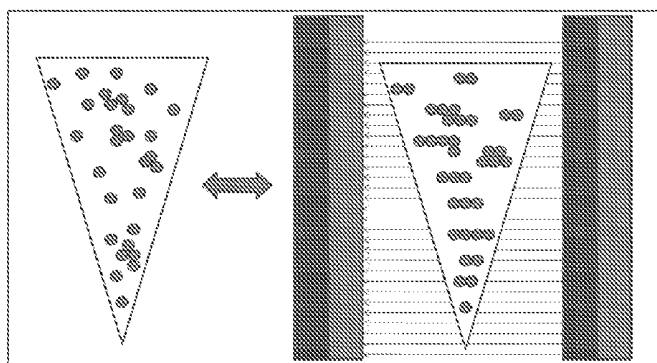
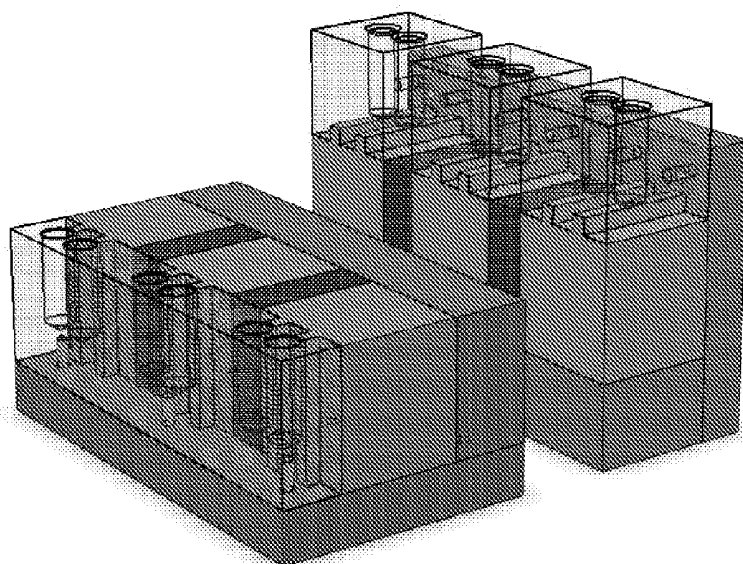


Figure 17



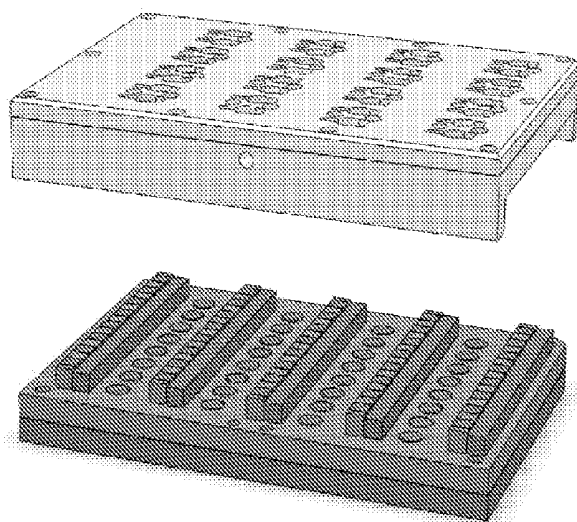


Figure 18A

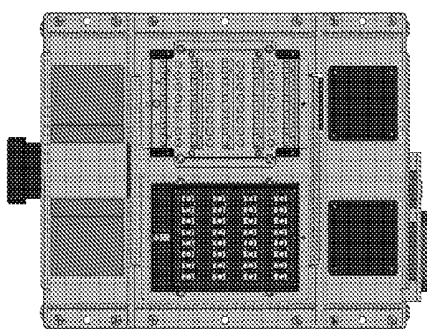


Figure 18B

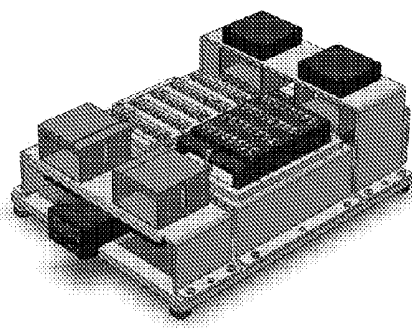


Figure 18C

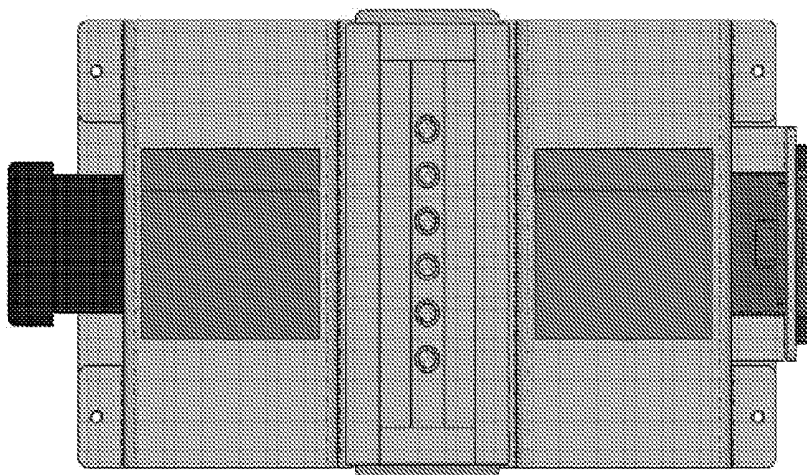


Figure 19A

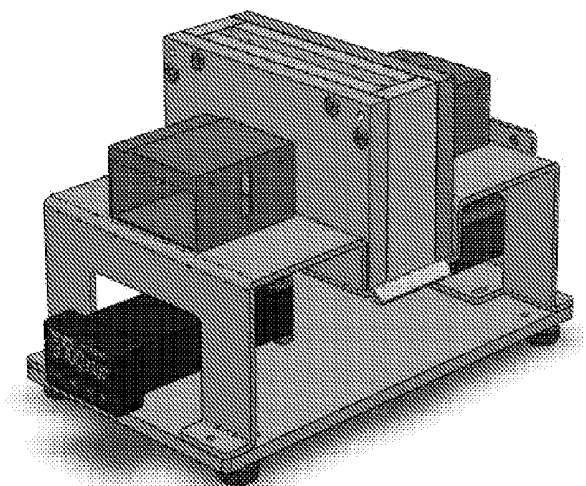


Figure 19B

Figure 20

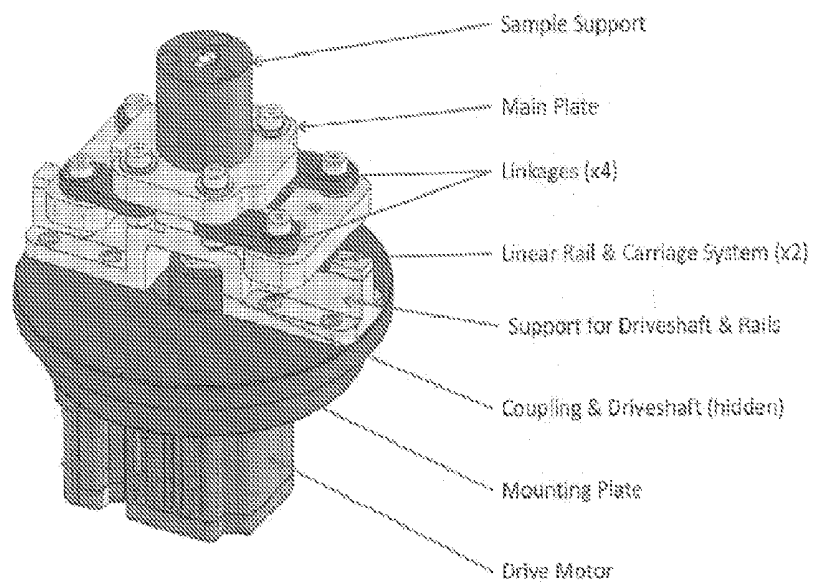
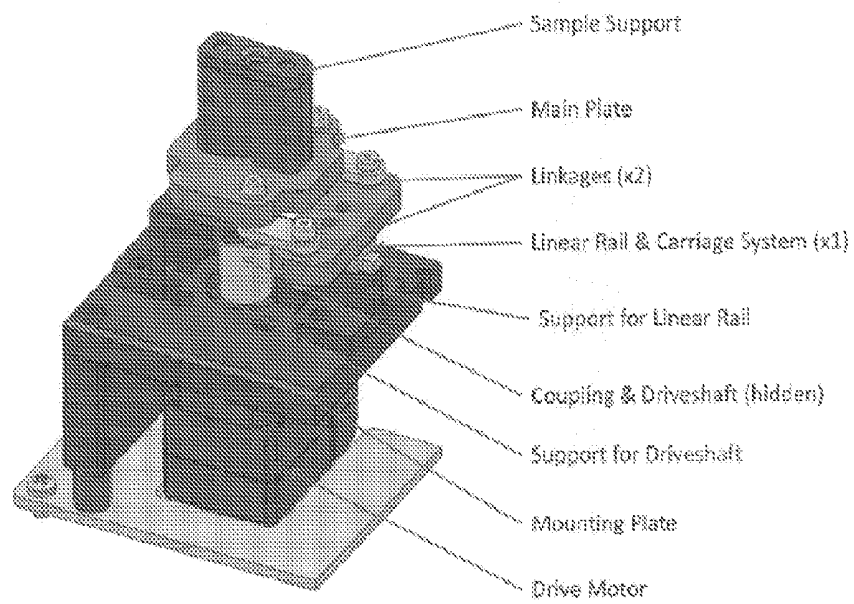


Figure 21



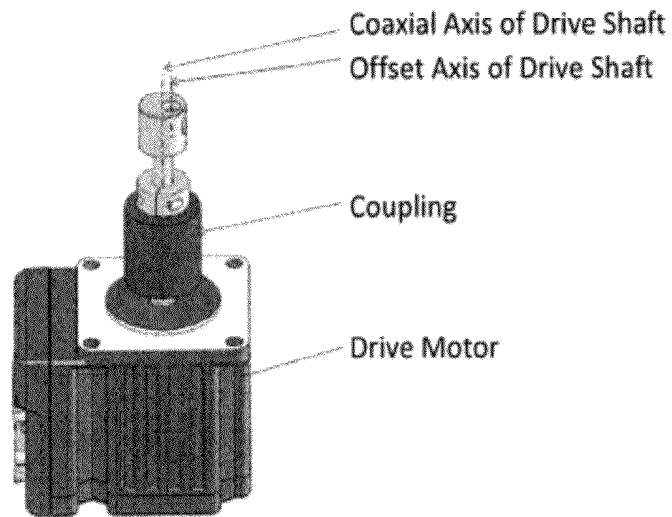


Figure 22A

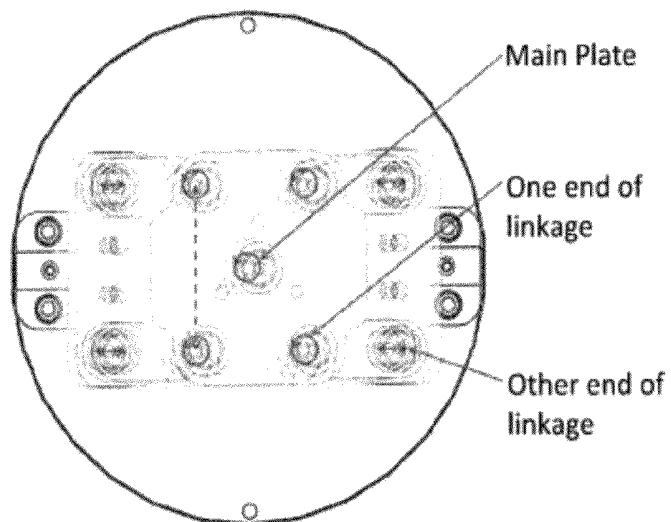


Figure 22B

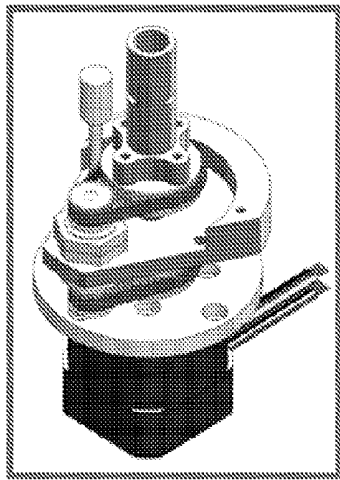


Figure 23A

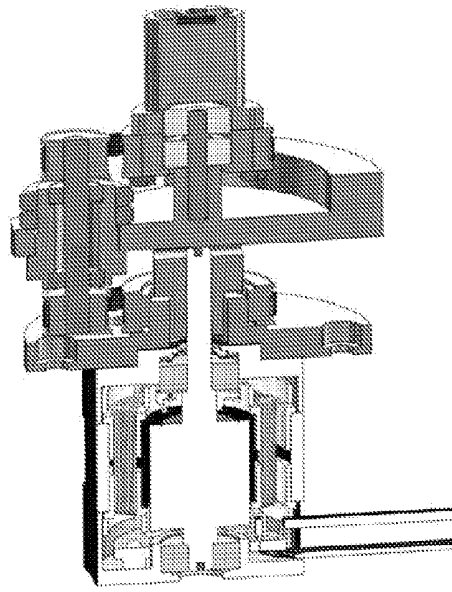


Figure 23B

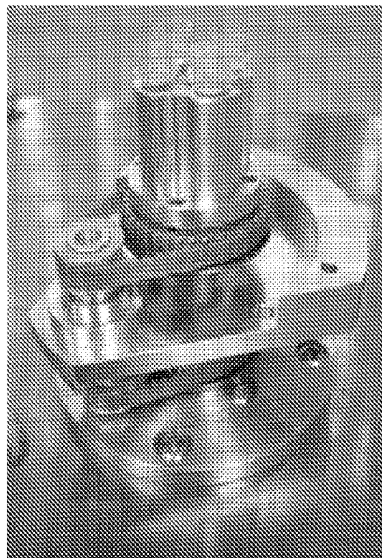
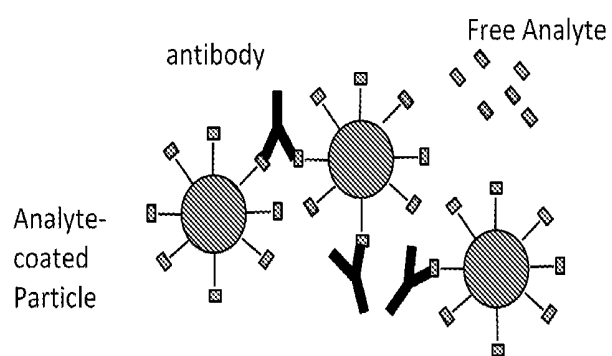


Figure 23C

Figure 24



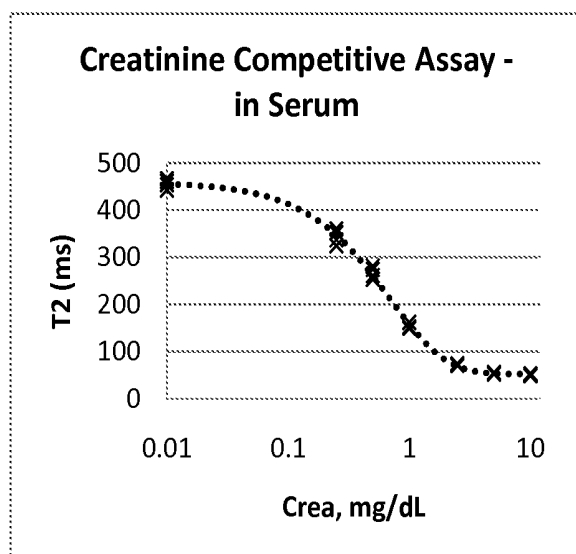


Figure 25A

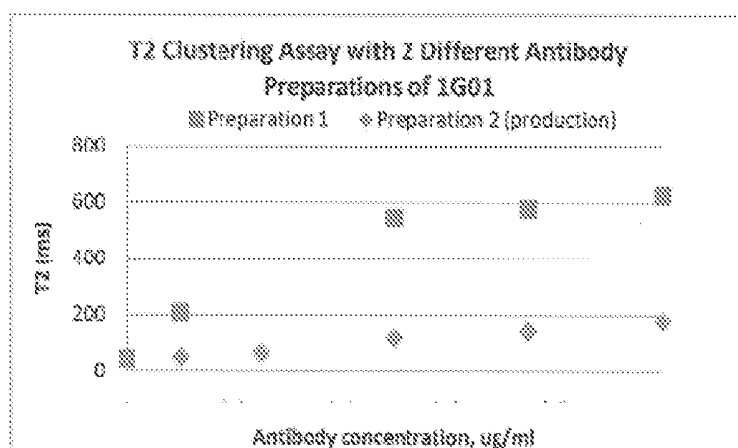


Figure 25B

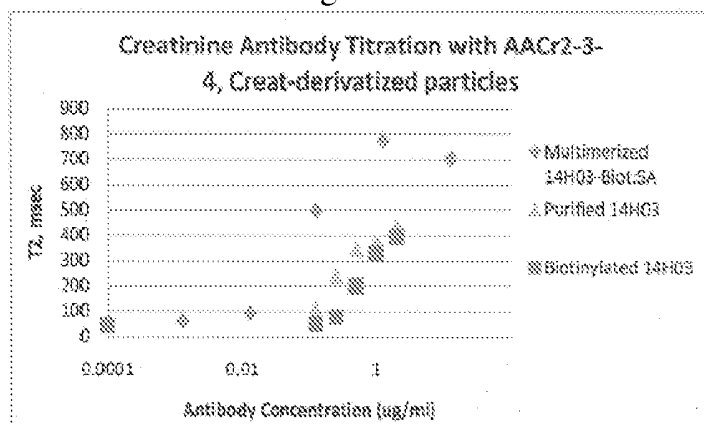


Figure 25 C

Figure 26

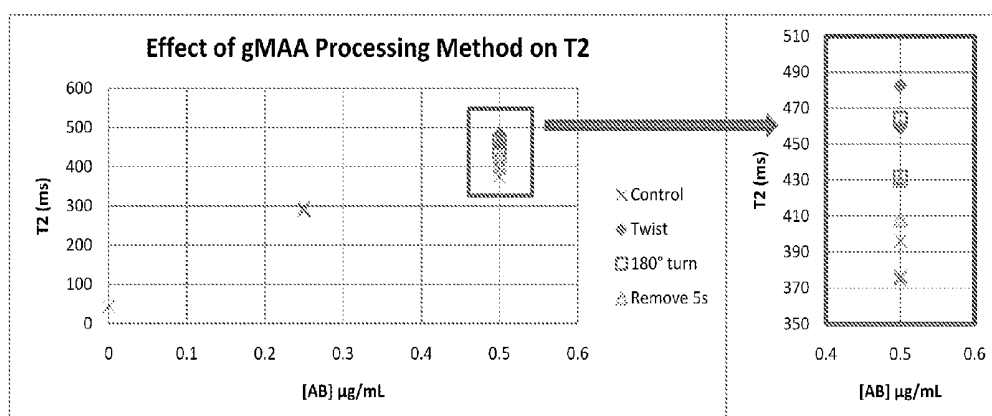


Figure 27

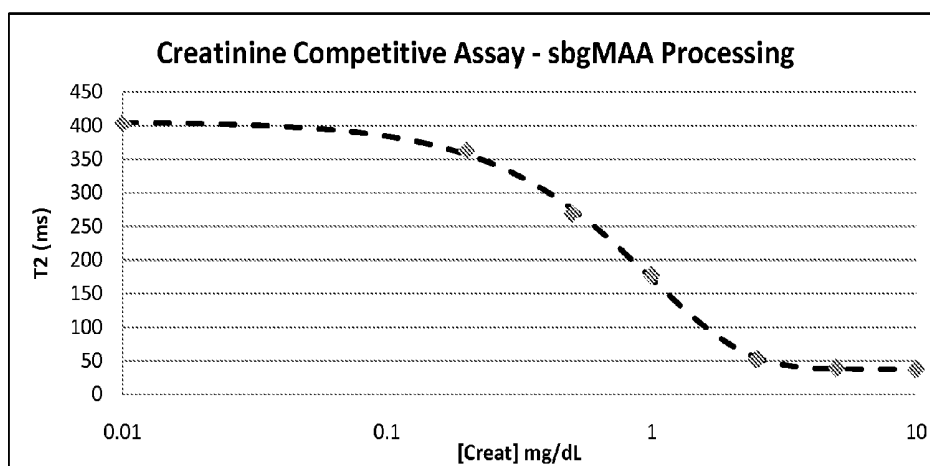


Figure 28

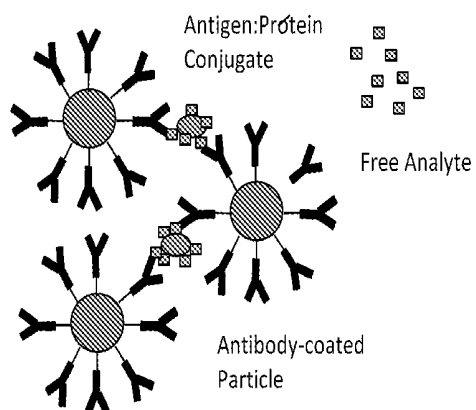
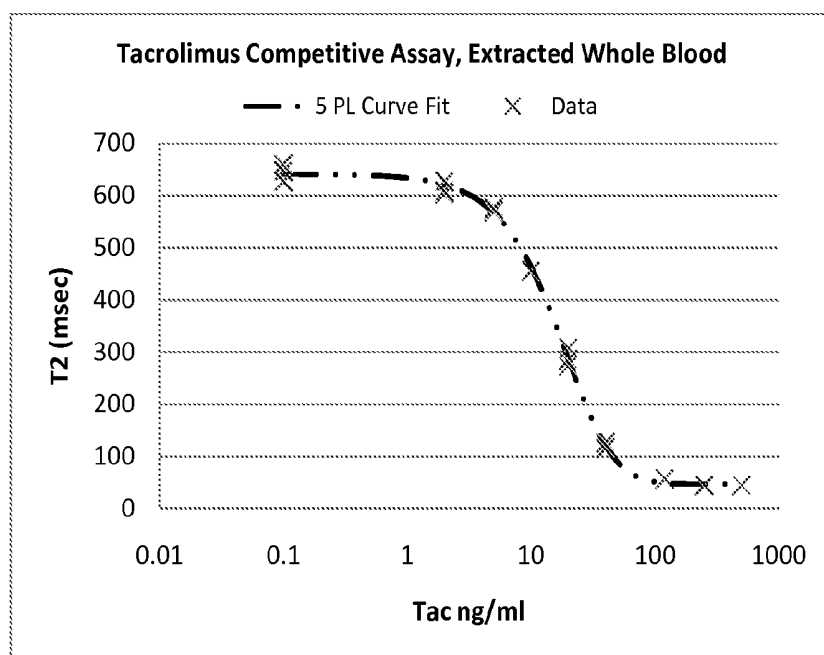


Figure 29



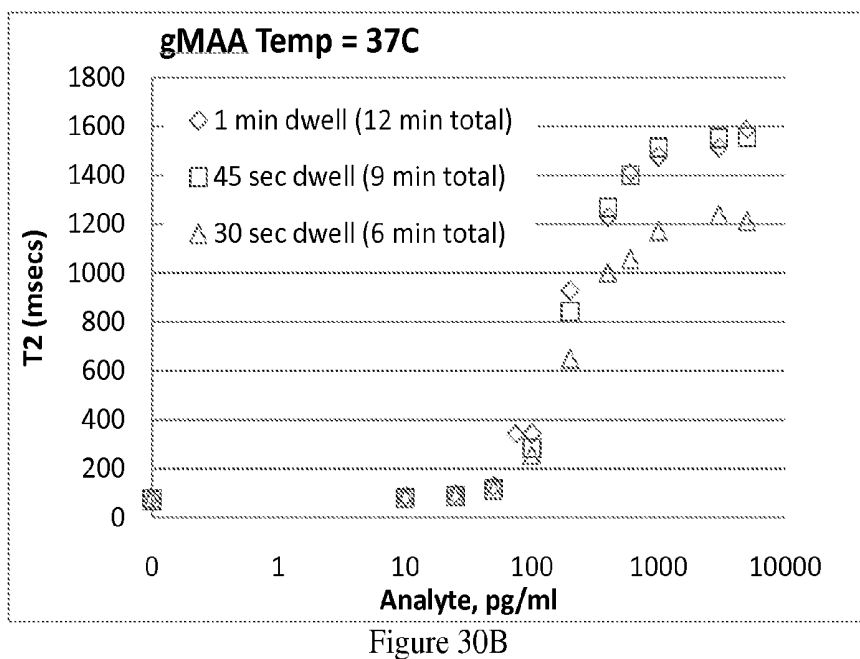
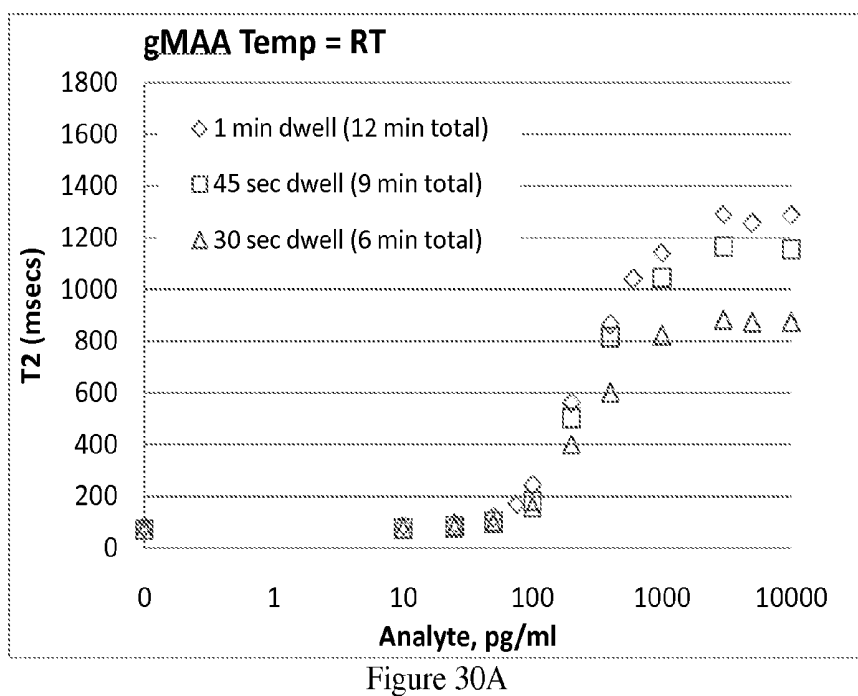


Figure 31

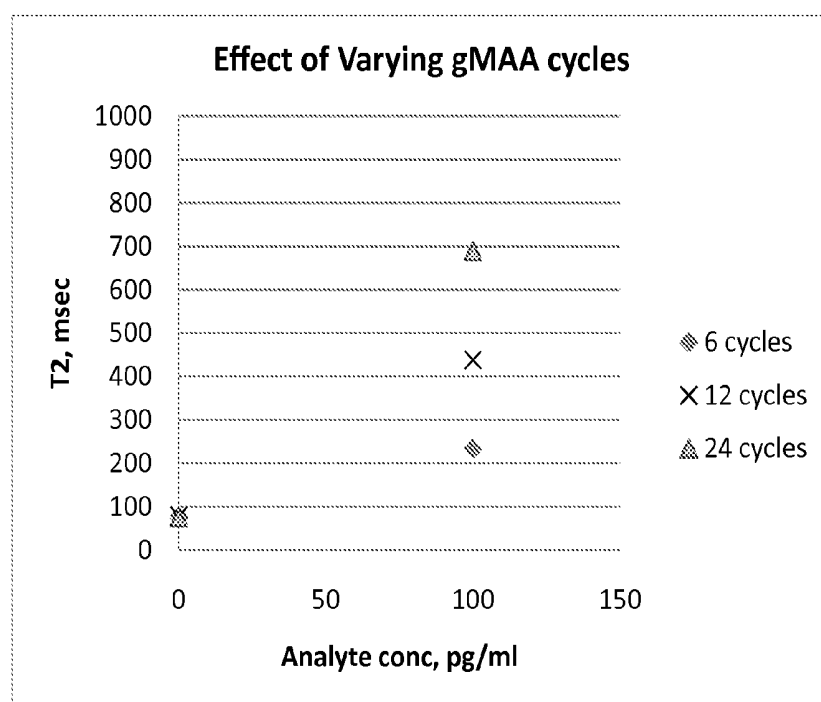


Figure 32

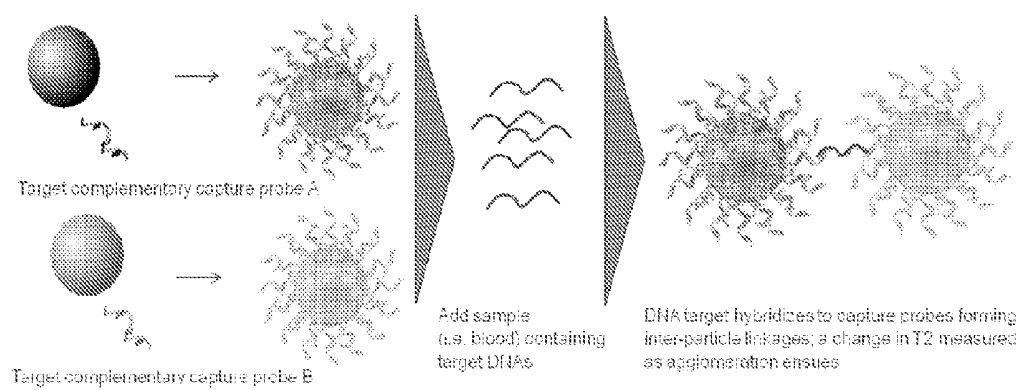


Figure 33

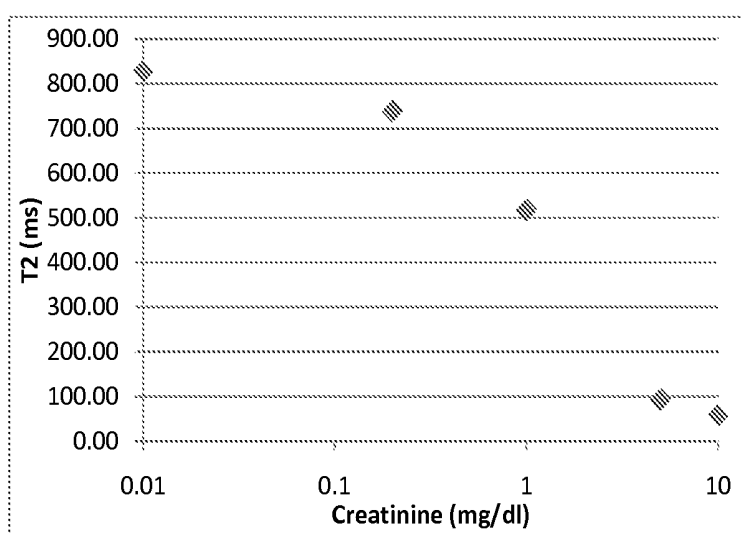


Figure 34

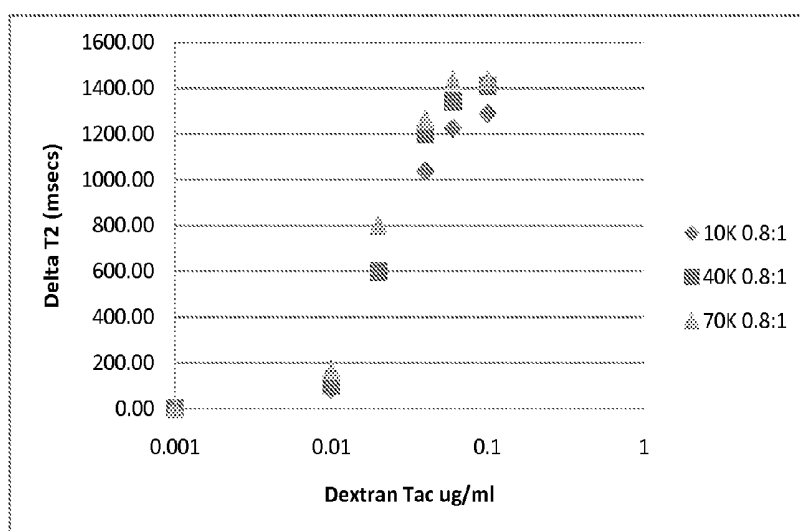


Figure 35

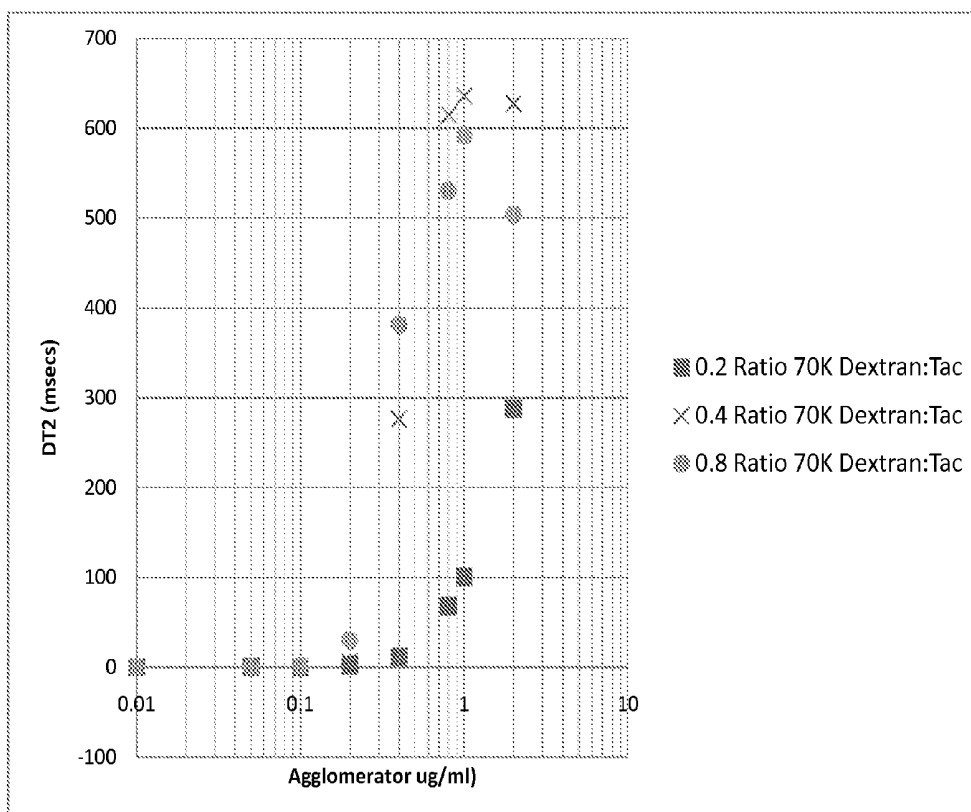


Figure 36

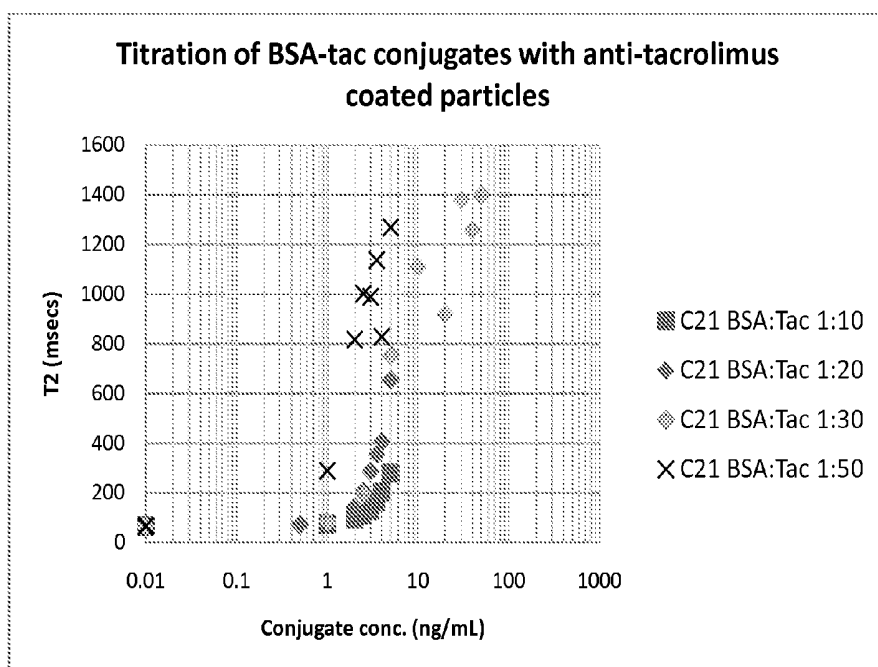


Figure 37

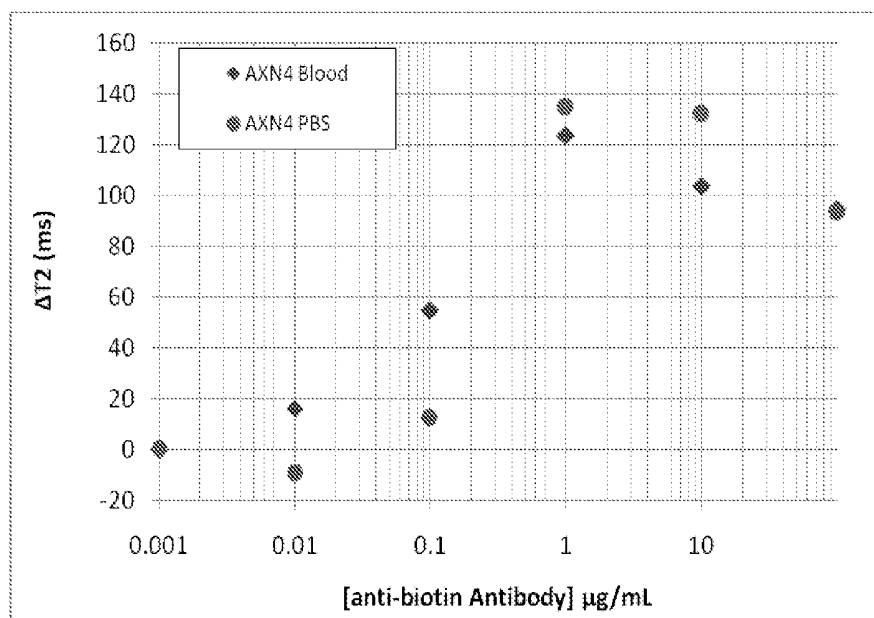


Figure 38

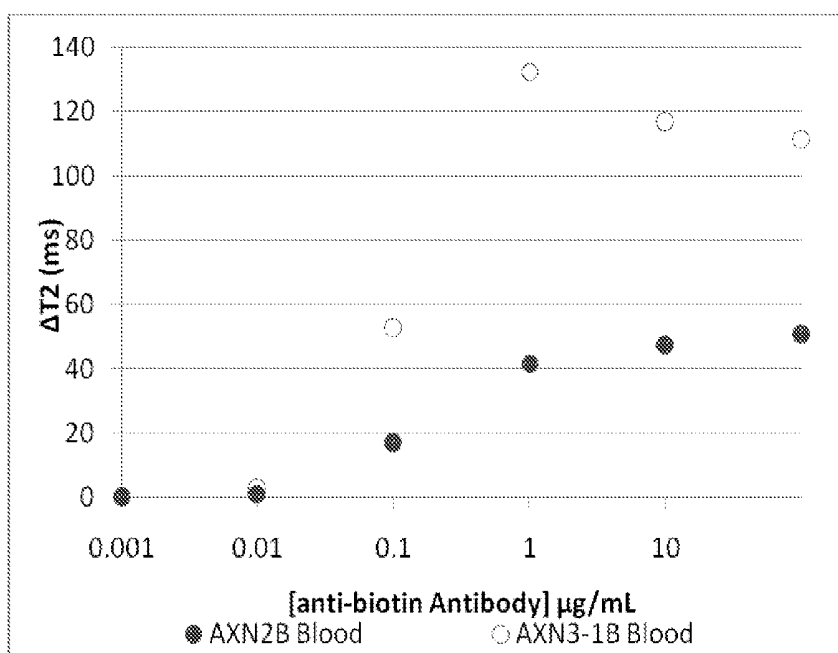
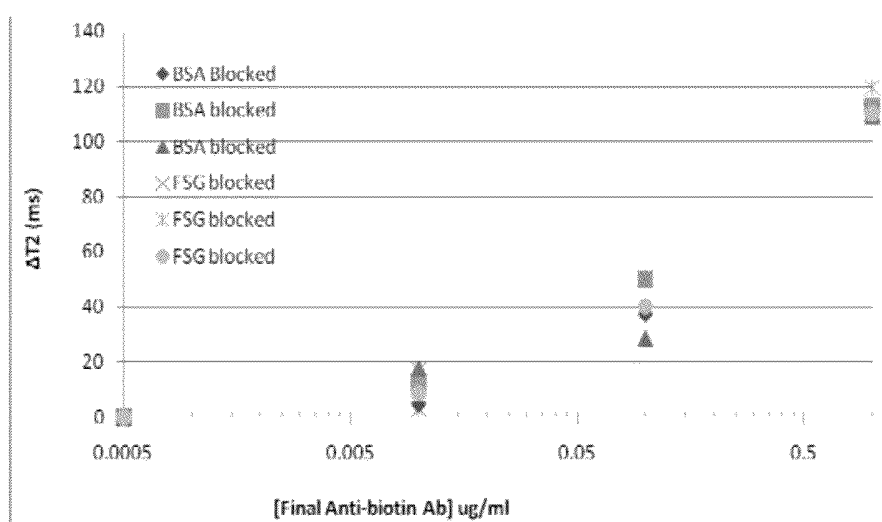
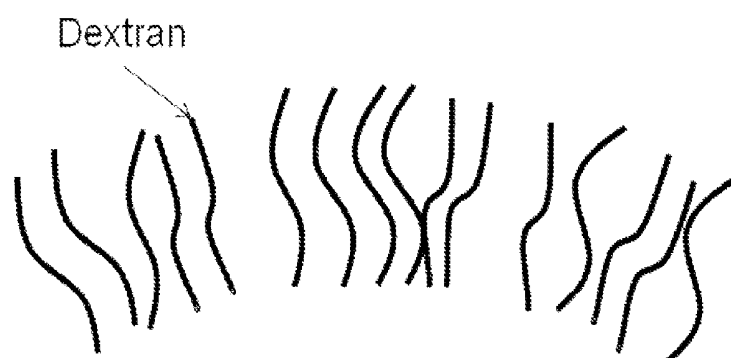


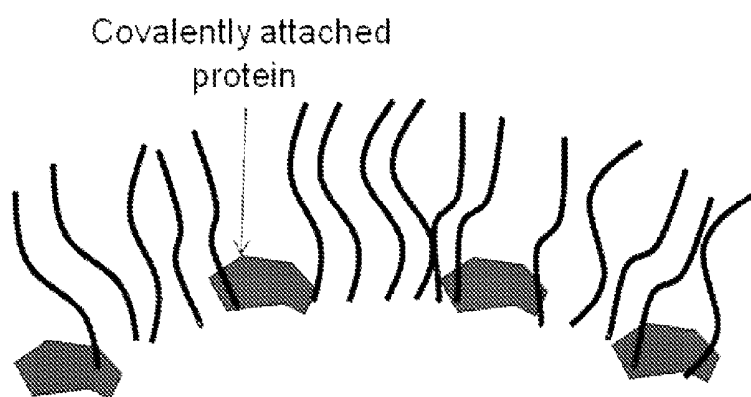
Figure 39





Particle Surface before protein block

Figure 40A



Particle Surface after protein block

Figure 40B

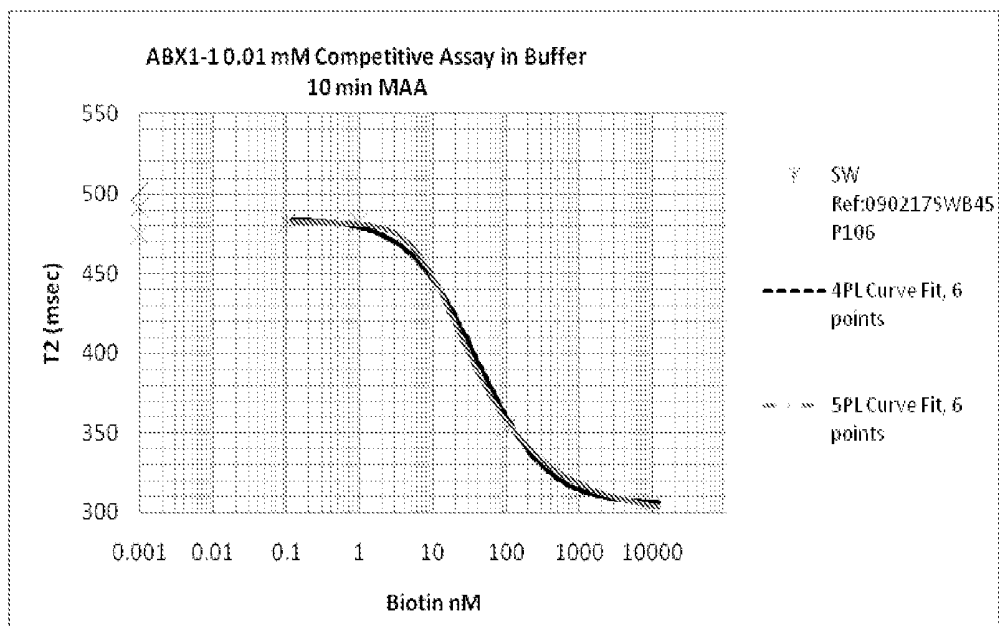


Figure 41A

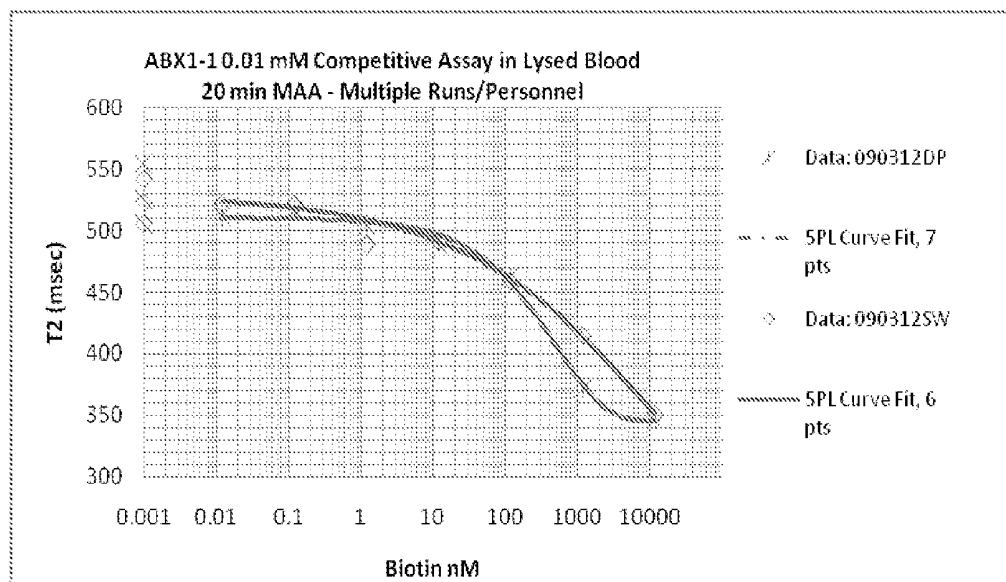
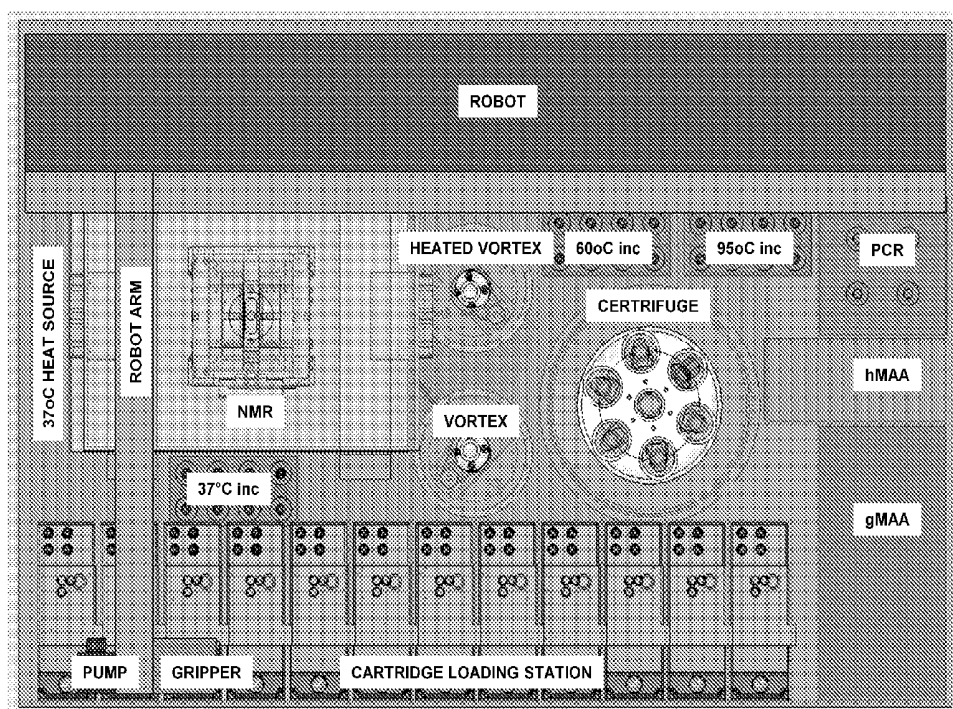


Figure 41B

Figure 42



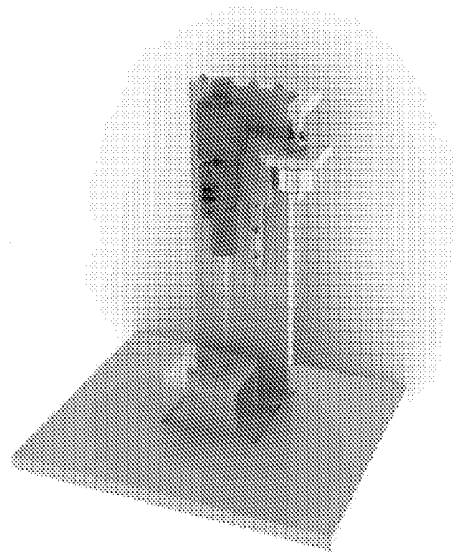


Figure 43A

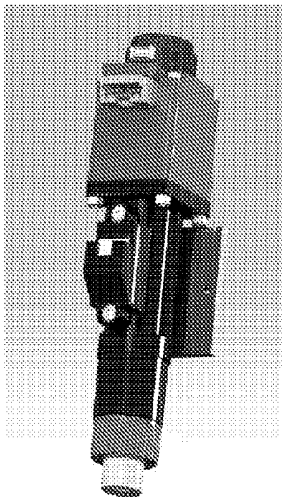


Figure 43B

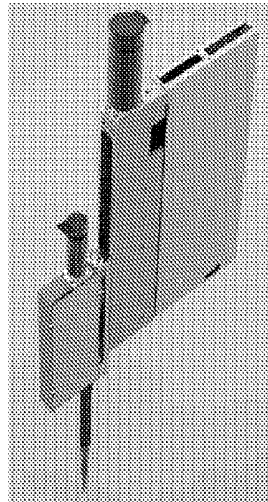


Figure 43C

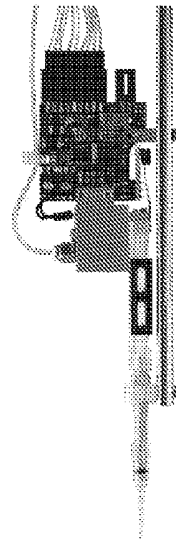


Figure 43D

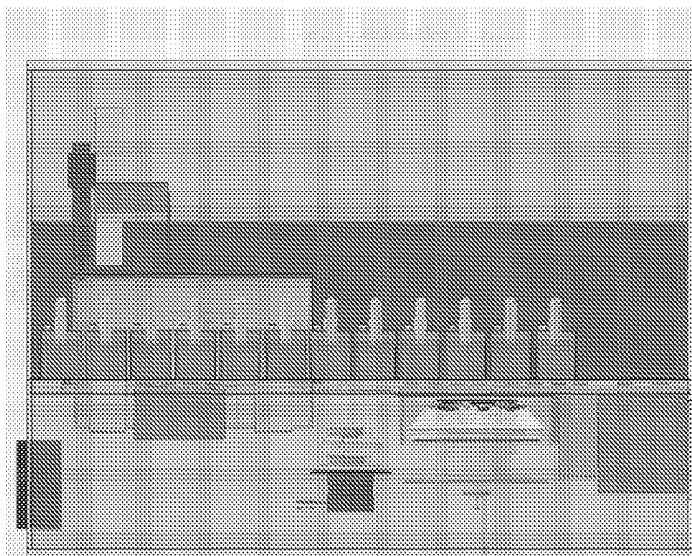
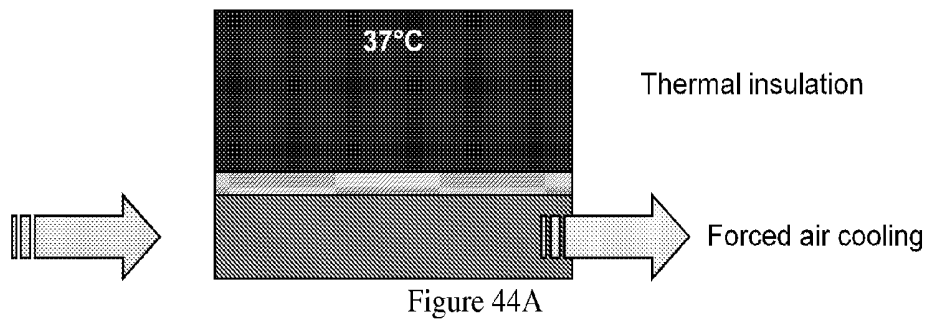


Figure 44B

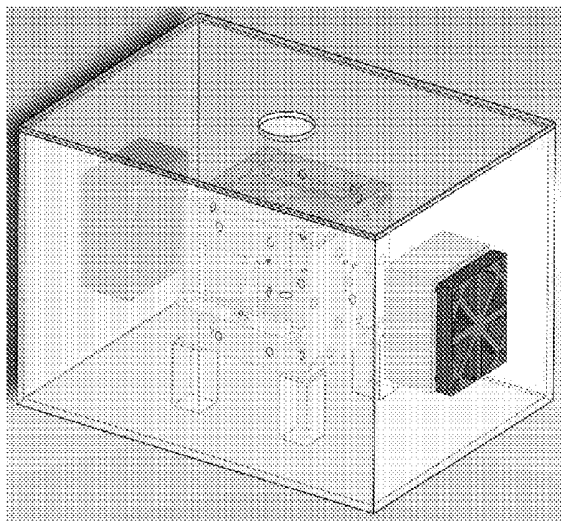


Figure 45A

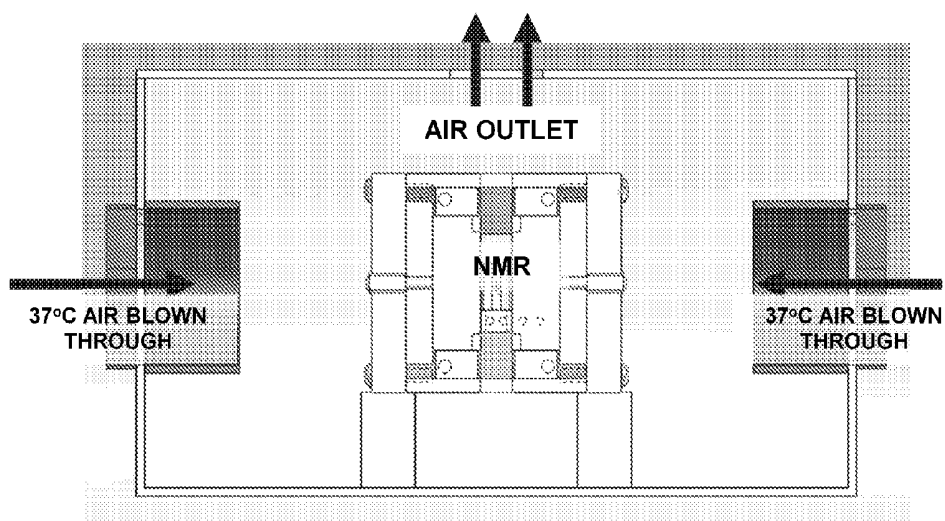


Figure 45B

NMR SYSTEMS AND METHODS FOR THE RAPID DETECTION OF ANALYTES

BACKGROUND OF THE INVENTION

This invention features assays and devices for the detection of analytes, and their use in the treatment and diagnosis of disease.

Magnetic sensors have been designed to detect molecular interactions in a variety of media, including biofluids, food products, and soil samples, among other media. Upon target binding, these sensors cause changes in properties of neighboring water molecules (or any solvent molecule with free hydrogens) of a sample, which can be detected by magnetic resonance (NMR/MRI) techniques. Thus, by using these sensors in a liquid sample, it is possible to detect the presence, and potentially quantify the amount, of an analyte at very low concentration. For example, small molecules, DNA, RNA, proteins, carbohydrates, organisms, metabolites, and pathogens (e.g., viruses) can be detected using magnetic sensors.

In general, magnetic sensors are magnetic particles that bind or otherwise link to their intended molecular target to form clusters (aggregates). It is believed that when magnetic particles assemble into clusters and the effective cross sectional area becomes larger (and the cluster number density is smaller), the interactions with the water or other solvent molecules are altered, leading to a change in the measured relaxation rates (e.g., T_2 , T_1 , T_2^*), susceptibility, frequency of precession, among other physical changes. Additionally, cluster formation can be designed to be reversible (e.g., by temperature shift, chemical cleavage, pH shift, etc.) so that "forward" or "reverse" (competitive and inhibition) assays can be developed for detection of specific analytes. Forward (clustering) and reverse (declustering) types of assays can be used to detect a wide variety of biologically relevant materials. The MRS (magnetic resonance switch) phenomenon was previously described (see U.S. Patent Publication No. 20090029392).

Many diagnostic assays require sensitivity in the picomolar or subpicomolar range. In such assays an equally low concentration of paramagnetic particles is employed. As a result, the binding events leading to cluster formation can become a rate-limiting step in the completion of the assay as the collision frequency of antigens, paramagnetic particles, and partially formed clusters is low in this concentration range (see Baudry et al., Proc Natl Acad Sci USA, 103:16076 (2006)). The current detection of infectious agents, nucleic acids, small molecules, biowarfare agents and organisms, and molecular targets (biomarkers) or the combination of molecular and immunoassay targets usually requires up-front sample preparation, time to analyze the sample, and single tests for each of the individual analytes. There is a need for a rapid, commercially-realizable NMR-based analyte detection device suitable for use with magnetic nanosensors having four unique features and qualities: 1) little to no sample preparation, 2) multiplex detection across multiple molecular types, 3) rapid acquisition of diagnostic information, and 4) accurate information for point-of-care clinical decision making.

SUMMARY OF THE INVENTION

The invention features systems and methods for the detection of analytes.

The invention features a system for the detection of creatinine, tacrolimus, and *Candida*, the system including: (a) a first unit including (a1) a permanent magnet defining a mag-

netic field; (a2) a support defining a well for holding a liquid sample including magnetic particles and the creatinine, tacrolimus, and *Candida* and having an RF coil disposed about the well, the RF coil configured to detect signal produced by exposing the liquid sample to a bias magnetic field created using the permanent magnet and an RF pulse sequence; and (a3) an electrical element in communication with the RF coil, the electrical element configured to amplify, rectify, transmit, and/or digitize the signal; and (b) a second unit including a removable cartridge sized to facilitate insertion into and removal from the system, wherein the removable cartridge is a modular cartridge including (i) a plurality of reagent modules for holding one or more assay reagents; and (ii) a plurality of detection module including a detection chamber for holding a liquid sample including the magnetic particles and the creatinine, tacrolimus, and *Candida*, wherein the plurality of reagent modules includes (i) a first population of magnetic particles having a mean diameter of from 150 nm to 699 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from 500 to 699 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and creatinine antibodies conjugated to their surface; (ii) a multivalent binding agent bearing a plurality of creatinine conjugates designed to form aggregates with the first population of magnetic particles in the absence of creatinine; (iii) a second population of magnetic particles having a mean diameter of from 150 nm to 699 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from 500 to 699 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and tacrolimus antibodies conjugated to their surface; (iv) a multivalent binding agent bearing a plurality of tacrolimus conjugates designed to form aggregates with the second population of magnetic particles in the absence of tacrolimus; (v) a third population of magnetic particles have a mean diameter of from 700 nm to 1200 nm (e.g., from 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), a T_2 relaxivity per particle of from 1×10^9 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$, and having a first probe and a second probe conjugated to their surface selected to form aggregates in the presence of a *Candida* nucleic acid, the first probe operative to bind to a first segment of the *Candida* nucleic acid and the second probe operative to bind to a second segment of the *Candida* nucleic acid.

In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg .) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold. In another

embodiment, the liquid sample includes from 1×10^6 to 1×10^{13} of the magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter).

The invention further features a method for detecting the presence of a pathogen in a whole blood sample, the method including: (a) providing a whole blood sample from a subject; (b) mixing the whole blood sample with a hypotonic solution to produce disrupted red blood cells; (c) following step (b), separating intact cells from the disrupted red blood cells and reconstituting the intact cells to form an extract; (d) combining the extract with beads to form a mixture and agitating the mixture to form a lysate; (e) placing the lysate of step (d) in a detection tube and amplifying a target nucleic acid in the lysate to form an amplified lysate solution including the target nucleic acid, wherein the target nucleic acid is characteristic of the pathogen to be detected; (f) following step (e), adding to the detection tube from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the amplified lysate solution (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 700 nm to 1200 nm (e.g., from 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), and have a first probe and a second probe conjugated to their surface, the first probe operative to bind to a first segment of the target nucleic acid and the second probe operative to bind to a second segment of the target nucleic acid, wherein the magnetic particles form aggregates in the presence of the target nucleic acid; (g) placing the detection tube in a device, the device including a support defining a well for holding the detection tube including the magnetic particles and the target nucleic acid, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (h) exposing the sample to a bias magnetic field and an RF pulse sequence; (i) following step (h), measuring the signal from the detection tube; and (j) on the basis of the result of step (i), detecting the pathogen. In certain embodiments, steps (a) through (i) are completed within 4 hours (e.g., within 3.5 hours, 3.0 hours, 2.5 hours, or 2 hours). In another embodiment, step (i) is carried out without any prior purification of the amplified lysate solution (i.e., the lysate solution is unfractionated after it is formed). In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the lysate further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μ g to 100 μ g (e.g., 40 μ g to 60 μ g, 50 μ g to 70 μ g, 60 μ g to 80 μ g, or 80 μ g to 100 μ g), of one or more proteins per milligram of the magnetic particles. The lysate can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold.

The invention features a method for measuring the concentration of creatinine in a liquid sample, the method including: (a) contacting a solution with (i) magnetic particles to produce a liquid sample including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 1200 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, 500 to 700 nm, 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and creatinine antibodies conjugated to their surface, and (ii) a multivalent binding agent bearing a plurality of creatinine conjugates designed to form aggregates with the magnetic particles in the absence of creatinine; (b) placing the liquid sample in a device, the device including a support defining a well for holding the liquid sample including the magnetic particles, the multivalent binding agent, and the creatinine, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (c) exposing the sample to a bias magnetic field and an RF pulse sequence; (d) following step (c), measuring the signal; and (e) on the basis of the result of step (d), determining the concentration of creatinine in the liquid sample. In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μ g to 100 μ g (e.g., 40 μ g to 60 μ g, 50 μ g to 70 μ g, 60 μ g to 80 μ g, or 80 μ g to 100 μ g) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold.

The invention further features a method for measuring the concentration of tacrolimus in a liquid sample, the method including: (a) contacting a solution with (i) magnetic particles to produce a liquid sample including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 1200 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, 500 to 700 nm, 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and tacrolimus antibodies conjugated to their surface, and (ii) a multivalent binding agent bearing a plurality of tacrolimus conjugates designed to form aggregates with the

magnetic particles in the absence of tacrolimus; (b) placing the liquid sample in a device, the device including a support defining a well for holding the liquid sample including the magnetic particles, the multivalent binding agent, and the tacrolimus, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (c) exposing the sample to a bias magnetic field and an RF pulse sequence; (d) following step (c), measuring the signal; and (e) on the basis of the result of step (d), determining the concentration of tacrolimus in the liquid sample. In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold.

The invention features a method for detecting the presence of a *Candida* species in a liquid sample, the method including: (a) lysing the *Candida* cells in the liquid sample; (b) amplifying a nucleic acid to be detected in the presence of a forward primer and a reverse primer, each of which is universal to multiple *Candida* species to form a solution including a *Candida* amplicon; (c) contacting the solution with magnetic particles to produce a liquid sample including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 700 nm to 1200 nm (e.g., from 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), a T_2 relaxivity per particle of from 1×10^9 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and have a first probe and a second probe conjugated to their surface, the first probe operative to bind to a first segment of the *Candida* nucleic acid and the second probe operative to bind to a second segment of the *Candida* amplicon, wherein the magnetic particles form aggregates in the presence of the *Candida* amplicon; (d) placing the liquid sample in a device, the device including a support defining a well for holding the liquid sample including the magnetic particles and the *Candida* amplicon, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (e) exposing the sample to a bias magnetic field and an RF pulse sequence; (f) following step (e), measuring the signal; and (g) on the basis of the result of step (f), determining whether the *Candida* species was present in the sample. In certain embodiments, the magnetic particles are substantially

monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold. The forward primer can include, for example, the sequence 5'-GGC ATG CCT GTT TGA GCG TC-3' (SEQ ID NO. 1). The reverse primer can include, for example, the sequence 5'-GCT TAT TGA TAT GCT TAA GTT CAG CGG GT-3' (SEQ ID NO. 2). In certain embodiments, (i) the *Candida* species is *Candida albicans*, the first probe includes the oligonucleotide sequence 5'-ACC CAG CGG TTT GAG GGA GAA AC-3' (SEQ ID NO. 3), and the second probe includes the oligonucleotide sequence 5'-AAA GTT TGA AGA TAT ACG TGG TGG ACG TTA-3' (SEQ ID NO. 4); (ii) the *Candida* species is *Candida krusei*, the first probe includes the oligonucleotide sequence: 5'-CGC ACG CGC AAG ATG GAA ACG-3' (SEQ ID NO. 5), and the second probe includes the oligonucleotide sequence: 5'-AAG TTC AGC GGG TAT TCC TAC CT-3' (SEQ ID NO. 6); (iii) the *Candida* species is *Candida glabrata*, the first probe includes the oligonucleotide sequence: 5'-CTA CCA AAC ACA ATG TGT TTG AGA AG-3' (SEQ ID NO. 7), and the second probe includes the oligonucleotide sequence: 5'-CCT GAT TTG AGG TCA AAC TTA AAG ACG TCT G-3' (SEQ ID NO. 8); (iv) the *Candida* species is *Candida parapsilosis* or *Candida tropicalis*, the first probe includes the oligonucleotide sequence: 5'-AGT CCT ACC TGA TTT GAG GTCTNIT-IndAA-3' (SEQ ID NO. 9), and the second probe includes the oligonucleotide sequence: 5'-CCG NITIndGG GTT TGA GGG AGA AAT-3' (SEQ ID NO. 10). In certain embodiments, steps (a) through (h) are completed within 4 hours (e.g., within 3.5 hours, 3.0 hours, 2.5 hours, or 2 hours). In particular embodiments, the magnetic particles include two populations, a first population bearing the first probe on its surface, and the second population bearing the second probe on its surface. In another embodiment, the magnetic particles are a single population bearing both the first probe and the second probe on the surface of the magnetic particles.

In a related aspect, the invention features a method for assisting the specific agglomeration of magnetic particles in a liquid sample, the method including: (i) providing a liquid sample including one or more analytes and the magnetic particles, wherein the magnetic particles have binding moieties on their surfaces, the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; (ii) exposing the liquid sample to a magnetic field; (iii) removing the liquid sample from the magnetic field; and (iv) repeating step (ii).

The invention further features a method for assisting the specific agglomeration of magnetic particles in a liquid sample by (i) providing a liquid sample including one or more

analytes and the magnetic particles, wherein the magnetic particles have binding moieties on their surfaces, the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; (ii) applying a magnetic field gradient to the liquid sample for a time sufficient to cause concentration of the magnetic particles in a first portion of the liquid sample, the magnetic field gradient being aligned in a first direction relative to the liquid sample; (iii) following step (ii), applying a magnetic field to the liquid sample for a time sufficient to cause concentration of the magnetic particles in a second portion of the liquid sample, the magnetic field being aligned in a second direction relative to the liquid sample; and (iv) optionally repeating steps (ii) and (iii). In certain embodiments, the angle between the first direction and the second direction relative to the liquid sample is between 0° and 180° (e.g., from 0° to 10°, 5° to 120°, 20° to 60°, 30° to 80°, 45° to 90°, 60° to 120°, 80° to 135°, or from 120° to 180°).

The invention features a method for assisting the specific agglomeration of magnetic particles in a liquid sample by (i) providing a liquid sample including one or more analytes and the magnetic particles, wherein the magnetic particles have binding moieties on their surfaces, the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; (ii) applying a magnetic field gradient to the liquid sample for a time sufficient to cause concentration of the magnetic particles in a first portion of the liquid sample; (iii) following step (ii), agitating the liquid sample; and (iv) repeating step (ii). In certain embodiments, step (iii) includes vortexing the liquid sample, or mixing the sample using any method described herein.

The invention also features a method for assisting the specific agglomeration of magnetic particles in a liquid sample by (i) providing a liquid sample including one or more analytes and the magnetic particles, wherein the magnetic particles have binding moieties on their surfaces, the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; and (ii) exposing the liquid sample to a gradient magnetic field and rotating the gradient magnetic field about the sample, or rotating the sample within the gradient magnetic field. The sample can be rotated slowly. In certain embodiments, the sample is rotated at a rate of 0.0333 Hz, or less (e.g., from 0.000833 Hz to 0.0333 Hz, from 0.00166 Hz to 0.0333 Hz, or from 0.00333 Hz to 0.0333 Hz). In other embodiments, the method further includes (iii) following step (ii), agitating the liquid sample; and (iv) repeating step (ii).

In any of the above methods for assisting specific agglomeration step (ii) can be repeated from 1 to 100 times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times, from 10 to 20 times, or from 80 to 100 times). In particular embodiments, the one or more magnets providing the magnetic field gradient within the liquid sample have a maximum field strength of from 0.01 T to 10 T (e.g., from 0.01 T to 0.05 T, 0.05 T to 0.1 T, 0.1 T to 0.5 T, 0.5 T to 1 T, 1 T to 3 T, or from 3 T to 10 T) and wherein the gradient magnetic field varies from 0.1 mT/mm to 10 T/mm across the liquid sample (e.g., from 0.1 mT/mm to 0.5 mT/mm, 0.3 mT/mm to 1 mT/mm, 0.5 mT/mm to 5 mT/mm, 5 mT/mm to 20 mT/mm, 10 mT/mm to 100 mT/mm, 100 mT/mm to 500 mT/mm, 500 mT/mm to 1 T/mm, or from 1 T/mm to 10 T/mm). In certain embodiments of any of the above methods for assisting specific agglomeration, step (ii) includes applying the magnetic field gradient to the liquid sample for a period of from 1 second to 5 minutes (e.g., from 1 to 20 seconds, from 20 to 60 seconds, from 30

seconds to 2 minutes, from 1 minutes to 3 minutes, or from 2 minutes to 5 minutes). In particular embodiments, (i) the liquid sample includes from 1×10^5 to 1×10^{15} of the one or more analytes per milliliter of the liquid sample (e.g., from 1×10^5 to 1×10^6 , 1×10^6 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{12} , or 1×10^{11} to 1×10^{15} analytes per milliliter); (ii) the liquid sample includes from 1×10^6 to 1×10^{13} of the magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter); (iii) the magnetic particles have a T_2 relaxivity per particle of from 1×10^4 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^4 to 1×10^7 , 1×10^6 to 1×10^{10} , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} or from 1×10^{11} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$); (iv) the magnetic particles have an average diameter of from 150 nm to 1200 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, 500 to 700 nm, 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm); (v) the magnetic particles are substantially monodisperse; (vi) the magnetic particles in the liquid sample exhibit nonspecific reversibility in the absence of the one or more analytes and multivalent binding agent; (vii) the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran); (viii) the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof; and/or (ix) the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles.

The invention features a system for the detection of one or more analytes, the system including: (a) a first unit including (a1) a permanent magnet defining a magnetic field; (a2) a support defining a well for holding a liquid sample including magnetic particles and the one or more analytes and having an RF coil disposed about the well, the RF coil configured to detect a signal by exposing the liquid sample to a bias homogeneous magnetic field created using the permanent magnet and an RF pulse sequence; and (a3) one or more electrical elements in communication with the RF coil, the electrical elements configured to amplify, rectify, transmit, and/or digitize the signal; and (b) one or more second units including (b1) a permanent magnet adjacent a first sample position for holding a liquid sample and configured to apply a first gradient magnetic field to the liquid sample. The one or more second units can further include a second permanent magnet adjacent a second sample position for holding a liquid sample and configured to apply a second gradient magnetic field to the liquid sample, the second magnetic field aligned to apply a gradient magnetic field to the sample from a direction different from the direction of the first field gradient, and a means for moving a liquid sample from the first sample position to the second sample position. In certain embodiments, the one or more second units is incapable of measuring a signal (e.g., incapable of measuring an NMR relaxation rate), and/or lacks an RF coil, or a means for producing an RF pulse. In certain embodiments, the angle between the first direction and the second direction relative to the liquid sample is between 0° and 180° (e.g., from 0° to 10°, 5° to 120°, 20° to 60°, 30° to 80°, 45° to 90°, 60° to 120°, 80° to 135°, or from 120° to

180°). The system can further include a sample holder for holding the liquid sample and configured to move the liquid sample from the first position to the second position. In particular embodiments, the system includes an array of the one or more second units for assisting the agglomeration of an array of samples simultaneously. The system can include a cartridge unit, an agitation unit, a centrifuge, or any other system component described herein. For example, the system can further include (c) a third unit including a removable cartridge sized to facilitate insertion into and removal from the system and having a compartment including one or more populations of magnetic particles having binding moieties on their surfaces, wherein the binding moieties are operative to alter an aggregation of the magnetic particles in the presence of the one or more analytes. In particular embodiments, the removable cartridge is a modular cartridge including (i) a reagent module for holding one or more assay reagents; and (ii) a detection module including a detection chamber for holding a liquid sample including magnetic particles and one or more analytes, wherein the reagent module and the detection module can be assembled into the modular cartridge prior to use, and wherein the detection chamber is removable from the modular cartridge. The modular cartridge can further include an inlet module, wherein the inlet module, the reagent module, and the detection module can be assembled into the modular cartridge prior to use, and wherein the inlet module is sterilizable. In another embodiment, the system can further include a system computer with processor for implementing an assay protocol and storing assay data, and wherein the removable cartridge further includes (i) a readable label indicating the analyte to be detected, (ii) a readable label indicating the assay protocol to be implemented, (iii) a readable label indicating a patient identification number, (iv) a readable label indicating the position of assay reagents contained in the cartridge, or (v) a readable label including instructions for the programmable processor.

The invention further features a system for the detection of one or more analytes, the system including: (a) a first unit including (a1) a permanent magnet defining a magnetic field; (a2) a support defining a well for holding a liquid sample including magnetic particles and the one or more analytes and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using the permanent magnet and an RF pulse; and (a3) one or more electrical elements in communication with the RF coil, the electrical elements configured to amplify, rectify, transmit, and/or digitize the signal; and (b) a second unit including a removable cartridge sized to facilitate insertion into and removal from the system, wherein the removable cartridge is a modular cartridge including (i) a reagent module for holding one or more assay reagents; and (ii) a detection module including a detection chamber for holding a liquid sample including the magnetic particles and the one or more analytes, wherein the reagent module and the detection module can be assembled into the modular cartridge prior to use, and wherein the detection chamber is removable from the modular cartridge. The modular cartridge can further include an inlet module, wherein the inlet module, the reagent module, and the detection module can be assembled into the modular cartridge prior to use, and wherein the inlet module is sterilizable. In certain embodiments, the system further includes a system computer with processor for implementing an assay protocol and storing assay data, and wherein the removable cartridge further includes (i) a readable label indicating the analyte to be detected, (ii) a readable label indicating the assay protocol to be implemented, (iii) a readable label indicating a patient

identification number, (iv) a readable label indicating the position of assay reagents contained in the cartridge, or (v) a readable label including instructions for the programmable processor. The system can include a cartridge unit, an agitation unit, a centrifuge, or any other system component described herein.

The invention features an agitation unit for the automated mixing of a liquid sample in a sample chamber, including a motor for providing a rotational driving force to a motor shaft coupled to a drive shaft, the driveshaft having a first end coupled to the motor shaft and a second end coupled to a plate bearing a sample holder for holding the sample chamber, the draft shaft including a first axis coaxial to the motor shaft, and a second axis that is offset and parallel to the motor shaft, such that the second axis of the driveshaft, the plate, and the sample holder are driven in an orbital path, wherein the motor includes an index mark for positioning the sample chamber in a predetermined position following the mixing or a sensor which tracks the sample's position throughout its path.

The invention features a system for the detection of one or more analytes, the system including: (a) a first unit including (a1) a permanent magnet defining a magnetic field; (a2) a support defining a well for holding a liquid sample including magnetic particles and the one or more analytes and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using the permanent magnet and an RF pulse sequence; and (a3) one or more electrical elements in communication with the RF coil, the electrical elements configured to amplify, rectify, transmit, and/or digitize the signal; and (b) a second unit for the automated mixing of a liquid sample in a sample chamber, including a motor for providing a rotational driving force to a motor shaft coupled to a drive shaft, the driveshaft having a first end coupled to the motor shaft and a second end coupled to a plate bearing a sample holder for holding the sample chamber, the draft shaft including a first axis coaxial to the motor shaft, and a second axis that is offset and parallel to the motor shaft, such that the second axis of the driveshaft, the plate, and the sample holder are driven in an orbital path, wherein the motor includes an index mark for positioning the sample chamber in a predetermined position following the mixing or a sensor which tracks the sample's position throughout its path.

In certain embodiments, the system further includes a robotic arm for placing the sample chamber in, and removing the sample chamber from, the agitation unit.

The invention further features a system for the detection of one or more analytes, the system including: (a) a first unit including (a1) a permanent magnet defining a magnetic field; (a2) a support defining a well for holding a liquid sample including magnetic particles and the one or more analytes and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using the permanent magnet and an RF pulse sequence; and (a3) one or more electrical elements in communication with the RF coil, the electrical elements configured to amplify, rectify, transmit, and/or digitize the signal; and (b) a centrifuge including a motor for providing a rotational driving force to a drive shaft, the drive shaft having a first end coupled to the motor and a second end coupled to a centrifuge rotor bearing a sample holder for holding the sample chamber, wherein the motor includes an index mark for positioning the sample chamber in a predetermined position following the centrifuging of the sample or a sensor which tracks the sample's position throughout its path.

The invention further features a system for the detection of one or more analytes, the system including: (a) a disposable sample holder defining a well for holding a liquid sample and having an RF coil contained within the disposable sample holder and disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using the permanent magnet and an RF pulse sequence, wherein the disposable sample holder includes one or more fusible links; and (b) an MR reader including (b1) a permanent magnet defining a magnetic field; (b2) an RF pulse sequence and detection coil; (b3) one or more electrical elements in communication with the RF coil, the electrical elements configured to amplify, rectify, transmit, and/or digitize the signal; and (b4) one or more electrical elements in communication with the fusible link and configured to apply excess current to the fusible link, causing the link to break and rendering the coil inoperable following a predetermined working lifetime. In certain embodiments, the electrical element in communication with the RF coil is inductively coupled to the RF coil.

The invention features a method for detecting the presence of an analyte in a liquid sample, the method including: (a) contacting a solution with magnetic particles to produce a liquid sample including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 699 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from 500 to 699 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and binding moieties on their surface, the binding moieties operative to alter aggregation of the magnetic particles in the presence of the analyte or a multivalent binding agent; (b) placing the liquid sample in a device, the device including a support defining a well holding the liquid sample including the magnetic particles, the multivalent binding agent, and the analyte, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (c) exposing the sample to a bias magnetic field and an RF pulse sequence; (d) following step (c), measuring the signal; and (e) on the basis of the result of step (d), detecting the analyte. In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold. For example, the

analyte can be creatinine, the liquid sample can include a multivalent binding agent bearing a plurality of creatinine conjugates, and the magnetic particles can include a surface decorated with creatinine antibodies. In another embodiment, the analyte can be tacrolimus, the liquid sample can include a multivalent binding agent bearing a plurality of tacrolimus conjugates, and the magnetic particles can include a surface decorated with tacrolimus antibodies. In particular embodiments of the method, step (d) includes measuring the T_2 relaxation response of the liquid sample, and wherein increasing agglomeration in the liquid sample produces an increase in the observed T_2 relaxation rate of the sample.

The invention features a method for detecting the presence of an analyte in a liquid sample, the method including (a) contacting a solution with magnetic particles to produce a liquid sample including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 700 nm to 1200 nm (e.g., from 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), a T_2 relaxivity per particle of from 1×10^9 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and have binding moieties on their surface, the binding moieties operative to alter an aggregation of the magnetic particles in the presence of the analyte; (b) placing the liquid sample in a device, the device including a support defining a well holding the liquid sample including the magnetic particles, the multivalent binding agent, and the analyte, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (c) exposing the sample to a bias magnetic field and an RF pulse sequence; (d) following step (c), measuring the signal; and (e) on the basis of the result of step (d), detecting the presence or concentration of an analyte. In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a polymeric scaffold. For example, the

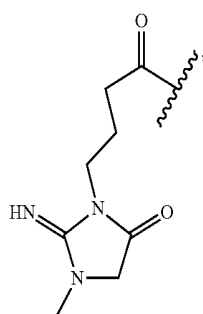
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the analyte can be tacrolimus, the liquid sample can include a multivalent binding agent bearing a plurality of tacrolimus conjugates, and the magnetic particles can include a surface decorated with tacrolimus antibodies. In particular embodiments of the method, step (d) includes measuring the T_2 relaxation response of the liquid sample, and wherein increasing agglomeration in the liquid sample produces an increase in the observed T_2 relaxation rate of the sample.

The invention features a multivalent binding agent including two or more creatinine moieties covalently linked to a scaffold. In certain embodiments, the multivalent binding agent is a compound of formula (I):

(A)_n-(B)

wherein (A) is

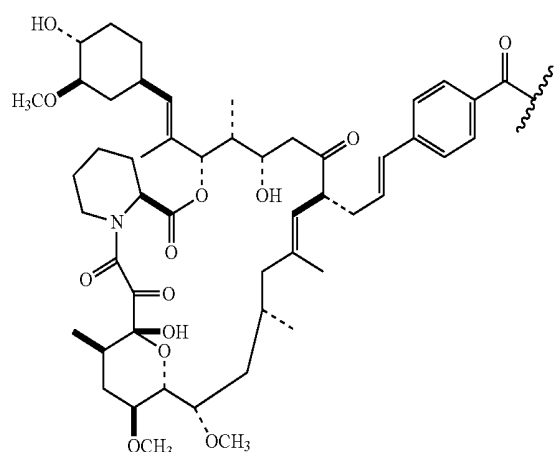


(B) is a polymeric scaffold covalently attached to each (A), and n is an integer from 2 to 50.

The invention features a multivalent binding agent including two or more tacrolimus moieties covalently linked to a scaffold. In certain embodiments, the multivalent binding agent is a compound of formula (II):

(A)_n-(B)

wherein (A) is



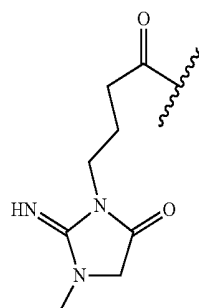
(B) is a polymeric scaffold covalently attached to each (A), and n is an integer from 2 to 50.

The invention features a solution including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the solution (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 600 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from

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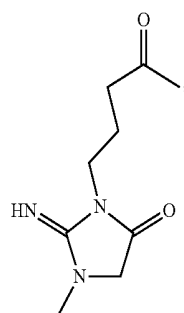
500 to 600 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and a surface bearing creatinine conjugate (A):

(A)



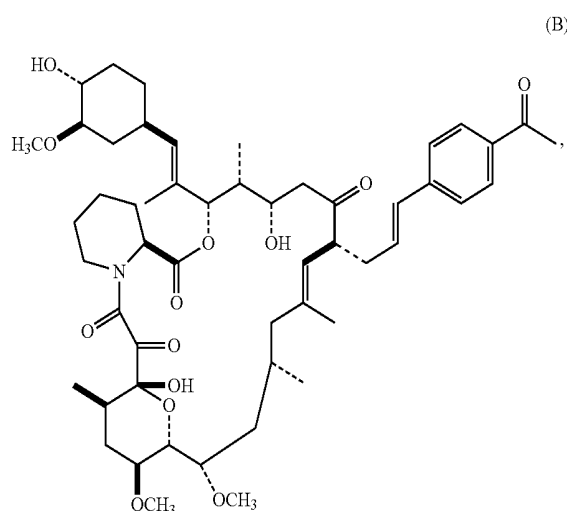
The invention further features solution including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the solution (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 600 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from 500 to 600 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and a surface bearing antibodies having affinity for the creatinine conjugate:

(B)



wherein (B) is a polymeric scaffold.

The invention features a solution including from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the solution (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 600 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, or from 500 to 600 nm), a T_2 relaxivity per particle of from 1×10^8 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$), and a surface bearing antibodies having affinity for the tacrolimus conjugate:



wherein (B) is a polymeric scaffold.

In an embodiment of any of the above solutions, (i) the magnetic particles are substantially monodisperse; (ii) the magnetic particles exhibit nonspecific reversibility in plasma; (iii) the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran); (iv) the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof; and/or (iv) the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The solutions can be used in any of the systems or methods described herein.

The invention features a method of monitoring one or more analytes in a liquid sample derived from a patient for the diagnosis, management, or treatment of a medical condition in a patient, the method including (a) combining with the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , or 1×10^{10} to 1×10^{13} magnetic particles per milliliter), wherein the magnetic particles have a mean diameter of from 150 nm to 1200 nm (e.g., from 150 to 250, 200 to 350, 250 to 450, 300 to 500, 450 to 650, 500 to 700 nm, 700 to 850, 800 to 950, 900 to 1050, or from 1000 to 1200 nm), and a T_2 relaxivity per particle of from 1×10^8 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$ (e.g., from 1×10^8 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{10} , 1×10^9 to 1×10^{11} , or from 1×10^{10} to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$), and wherein the magnetic particles have binding moieties on their surfaces, the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; (b) placing the liquid sample in a device, the device including a support defining a well for holding the liquid sample including the magnetic particles and the one or more analytes, and having an RF coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence; (c) exposing the sample

to the bias magnetic field and the RF pulse sequence; (d) following step (c), measuring the signal; (e) on the basis of the result of step (d), monitoring the one or more analytes; and (f) using the result of step (e) to diagnose, manage, or treat the medical condition. In one embodiment, the one or more analytes include creatinine. In another embodiment, the patient is immunocompromised, and the one or more analytes include an analyte selected from bacteria, fungi, viruses, antibiotic agents, antifungal agents, and antiviral agents (e.g., the one or more analytes can include *Candida* spp., tacrolimus, fluconazole, and creatinine). In still another embodiment, the patient has cancer, and the one or more analytes are selected from anticancer agents, and genetic markers present in a cancer cell. The patient can have, or be at risk of, an infection, and the one or more analytes include an analyte selected from bacteria, fungi, viruses, antibiotic agents, antifungal agents, and antiviral agents. The patient can have an immunoinflammatory condition, and the one or more analytes include an analyte selected from antiinflammatory agents and TNF-alpha. The patient can have heart disease, and the one or more analytes can include a cardiac marker. The patient can have HIV/AIDS, and the one or more analytes can include CD3, viral load, and AZT. In certain embodiments, the method is used to monitor the liver function of the patient, and wherein the one or more analytes are selected from albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, bilirubin, alpha fetoprotein, lactate dehydrogenase, mitochondrial antibodies, and cytochrome P450. For example, the one or more analytes include cytochrome P450 polymorphisms, and the ability of the patient to metabolize a drug is evaluated. The method can include identifying the patient as a poor metabolizer, a normal metabolizer, an intermediate metabolizer, or an ultra rapid metabolizer. The method can be used to determine an appropriate dose of a therapeutic agent in a patient by (i) administering the therapeutic agent to the patient; (ii) following step (i), obtaining a sample including the therapeutic agent or metabolite thereof from the patient; (iii) contacting the sample with the magnetic particles and exposing the sample to the bias magnetic field and the RF pulse sequence and detecting a signal produced by the sample; and (iv) on the basis of the result of step (iii), determining the concentration of the therapeutic agent or metabolite thereof. The therapeutic agent can be an anticancer agent, antibiotic agent, antifungal agent, or any therapeutic agent described herein. In any of the above methods of monitoring, the monitoring can be intermittent (e.g., periodic), or continuous. In certain embodiments, the magnetic particles are substantially monodisperse; exhibit nonspecific reversibility in the absence of the analyte and multivalent binding agent; and/or the magnetic particles further include a surface decorated with a blocking agent selected from albumin, fish skin gelatin, gamma globulin, lysozyme, casein, peptidase, and an amine-bearing moiety (e.g., amino polyethyleneglycol, glycine, ethylenediamine, or amino dextran). In particular embodiments, the liquid sample further includes a buffer, from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In still other embodiments, the magnetic particles include a surface decorated with 40 μg to 100 μg (e.g., 40 μg to 60 μg , 50 μg to 70 μg , 60 μg to 80 μg , or 80 μg to 100 μg) of one or more proteins per milligram of the magnetic particles. The liquid sample can include a multivalent binding agent bearing a plurality of analytes conjugated to a poly-

meric scaffold. The method for monitoring can include any of the magnetic assisted agglomeration methods described herein.

In any of the systems and methods of the invention, the liquid sample can include from 1×10^8 to 1×10^{10} magnetic particles having a mean diameter of from 100 nm to 350 nm, a T_2 relaxivity per particle of from 5×10^8 to $1 \times 10^{10} \text{ mM}^{-1} \text{ s}^{-1}$, and binding moieties on their surfaces (e.g., antibodies, conjugated analyte), the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent.

The invention features a removable cartridge sized to facilitate insertion into and removal from a system of the invention, wherein the removable cartridge includes one or more chambers for holding a plurality of reagent modules for holding one or more assay reagents, wherein the reagent modules comprise (i) a chamber for holding from 1×10^8 to 1×10^{10} magnetic particles having a mean diameter of from 100 nm to 350 nm, a T_2 relaxivity per particle of from 5×10^8 to $1 \times 10^{10} \text{ mM}^{-1} \text{ s}^{-1}$, and binding moieties on their surfaces (e.g., antibodies, conjugated analyte), the binding moieties operative to alter the specific aggregation of the magnetic particles in the presence of the one or more analytes or a multivalent binding agent; and (ii) a chamber for holding a buffer including from 0.1% to 3% (w/w) albumin (e.g., from 0.1% to 0.5%, 0.3% to 0.7%, 0.5% to 1%, 0.8% to 2%, or from 1.5% to 3% (w/w) albumin), from 0.01% to 0.5% nonionic surfactant (e.g., from 0.01% to 0.05%, 0.05% to 0.1%, 0.05% to 0.2%, 0.1% to 0.3%, 0.2% to 0.4%, or from 0.3% to 0.5% nonionic surfactant), or a combination thereof. In one embodiment, the magnetic particles and buffer are together in a single chamber within the cartridge.

The terms "aggregation," "agglomeration," and "clustering" are used interchangeably in the context of the magnetic particles described herein and mean the binding of two or more magnetic particles to one another, e.g., via a multivalent analyte, multimeric form of analyte, antibody, nucleic acid molecule, or other binding molecule or entity. In some instances, magnetic particle agglomeration is reversible.

By "analyte" is meant a substance or a constituent of a sample to be analyzed. Exemplary analytes include one or more species of one or more of the following: a protein, a peptide, a polypeptide, an amino acid, a nucleic acid, an oligonucleotide, RNA, DNA, an antibody, a carbohydrate, a polysaccharide, glucose, a lipid, a gas (e.g., oxygen or carbon dioxide), an electrolyte (e.g., sodium, potassium, chloride, bicarbonate, BUN, magnesium, phosphate, calcium, ammonia, lactate), a lipoprotein, cholesterol, a fatty acid, a glycoprotein, a proteoglycan, a lipopolysaccharide, a cell surface marker (e.g., CD3, CD4, CD8, IL2R, or CD35), a cytoplasmic marker (e.g., CD4/CD8 or CD4/viral load), a therapeutic agent, a metabolite of a therapeutic agent, a marker for the detection of a weapon (e.g., a chemical or biological weapon), an organism, a pathogen, a parasite (e.g., a protozoan or a helminth), a protist, a fungus (e.g., yeast or mold), a bacterium, an actinomycete, a cell (e.g., a whole cell, a tumor cell, a stem cell, a white blood cell, a T cell (e.g., displaying CD3, CD4, CD8, IL2R, CD35, or other surface markers), or another cell identified with one or more specific markers), a virus, a prion, a plant component, a plant by-product, algae, an algae by-product, plant growth hormone, an insecticide, a man-made toxin, an environmental toxin, an oil component, and components derived therefrom. Additional exemplary analytes are described herein. The term analyte further includes components of a sample that are a

direct product of a biochemical means of amplification of the initial target analyte, such as the product of a nucleic acid amplification reaction.

As used herein, "linked" means attached or bound by covalent bonds, non-covalent bonds, and/or linked via Van der Waals forces, hydrogen bonds, and/or other intermolecular forces.

The term "magnetic particle" refers to particles including materials of high positive magnetic susceptibility such as paramagnetic compounds, superparamagnetic compounds, and magnetite, gamma ferric oxide, or metallic iron.

As used herein, "nonspecific reversibility" refers to the colloidal stability and robustness of magnetic particles against non-specific aggregation in a liquid sample and can be determined by subjecting the particles to the intended assay conditions in the absence of a specific clustering moiety (i.e. an analyte or an agglomerator). For example, nonspecific reversibility can be determined by measuring the T_2 values of a solution of magnetic particles before and after incubation in a uniform magnetic field (defined as $<5000 \text{ ppm}$) at 0.45 T for 3 minutes at 37° C . Magnetic particles are deemed to have nonspecific reversibility if the difference in T_2 values before and after subjecting the magnetic particles to the intended assay conditions vary by less than 10% (e.g., vary by less than 9%, 8%, 6%, 4%, 3%, 2%, or 1%). If the difference is greater than 10%, then the particles exhibit irreversibility in the buffer, diluents, and matrix tested, and manipulation of particle and matrix properties (e.g., coating and buffer formulation) may be required to produce a system in which the particles have nonspecific reversibility. In another example, the test can be applied by measuring the T_2 values of a solution of magnetic particles before and after incubation in a gradient magnetic field 1 Gauss/mm-10000 Gauss/mm.

As used herein, the term "NMR relaxation rate" refers to a measuring any of the following in a sample T_1 , T_2 , $T_{1\rho}$, $T_{2\rho}$, and T_2^* . The systems and methods of the invention are designed to produce an NMR relaxation rate characteristic of whether an analyte is present in the liquid sample. In some instances the NMR relaxation rate is characteristic of the quantity of analyte present in the liquid sample.

By "pulse sequence" or "RF pulse sequence" is meant one or more radio frequency pulses to be applied to a sample and designed to measure, e.g., certain NMR relaxation rates, such as spin echo sequences. A pulse sequence may also include the acquisition of a signal following one or more pulses to minimize noise and improve accuracy in the resulting signal value.

As used herein, the term "signal" refers to an NMR relaxation rate, frequency shift, susceptibility measurement, diffusion measurement, or correlation measurements.

As used herein, reference to the "size" of a magnetic particle refers to the average diameter for a mixture of the magnetic particles as determined by microscopy.

As used herein, the term "substantially monodisperse" refers to a mixture of magnetic particles having a polydispersity in size distribution as determined by the shape of the distribution curve of particle size in light scattering measurements. The FWHM (full width half max) of the particle distribution curve less than 25% of the peak position is considered substantially monodisperse. In addition, only one peak should be observed in the light scattering experiments and the peak position should be within one standard deviation of a population of known monodisperse particles.

By " T_2 relaxivity per particle" is meant the average T_2 relaxivity per particle in a population of magnetic particles.

As used herein, "unfractionated" refers to an assay in which none of the components of the sample being tested are

removed following the addition of magnetic particles to the sample and prior to the NMR relaxation measurement.

It is contemplated that units, systems, methods, and processes of the claimed invention encompass variations and adaptations developed using information from the embodiments described herein. Throughout the description, where units and systems are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are units and systems of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps. It should be understood that the order of steps or order for performing certain actions is immaterial, unless otherwise specified, so long as the invention remains operable. Moreover, in many instances two or more steps or actions may be conducted simultaneously.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram of an NMR unit for detection of a signal response of a sample to an RF pulse sequence, according to an illustrative embodiment of the invention.

FIGS. 2A-2E illustrate micro coil geometries which can be used in NMR (for excitation and/or detection); designs include, but are not limited to a wound solenoid coil (FIG. 2A), a planar coil (FIG. 2B), a MEMS solenoid coil (FIG. 2C), a MEMS Helmholtz coil (FIG. 2D), and a saddle coil (FIG. 2E), according to an illustrative embodiment of the invention. Three dimensional lithographic coil fabrication of well characterized coils used in MR detection is also established and can be used for these applications, Demas et al. "Electronic characterization of lithographically patterned microcoils for high sensitivity NMR detection" J Magn Reson 200:56 (2009).

FIG. 3 is a drawing depicting an aggregation assay of the invention. The magnetic particles (dots) are coated with a binding agent (i.e., antibody, oligo, etc) such that in the presence of analyte, or multivalent binding agent, aggregates are formed. The dotted circles represent the diffusion sphere or portion of the total fluid volume that a solution molecule may experience via its diffusion during a T_2 measurement (the exact path traveled by a water molecule is random, and this drawing is not to scale). Aggregation (right hand side) depletes portions of the sample from the microscopic magnetic non-uniformities that disrupt the water's T_2 signal, leading to an increase in T_2 relaxation.

FIGS. 4A-4E are a series of graphs depicting the dependence of transverse relaxivity (R_2) on particle diameter and particle aggregation. FIG. 4A is a graph depicting the motional averaging regime (light line, left side); the R_2 ($1/T_2$) measured by a CPMG sequence increases as particle size increases because the refocusing pulses are ineffective to counteract the dephasing effects of the particles. As the system transitions to the visit limited regime (dark line, right side) the refocusing pulses begin to become effective and the R_2 decreases as particle size increases. For homogeneous magnetic fields, the R_2^* in the motional averaging regime matches the R_2 and the R_2^* reaches a constant value in the visit limited regime. In a homogenous field, when the R_2^* is less than the R_2 of either the motional averaging regime or visit limited regime the system is in the static dephasing

regime. The empty circle represents the R_2 of a solution of 100% dispersed particles (diameter=15 nm) and the solid circle represents a solution of 100% clustered particles (diameter=200 nm). This is an example of how to interpret these curves for clustering reactions. The conditions for this curve are 0.1 mM Fe, $\Delta\omega=8.85\times10^6$, $D=2.5\times10^{-5}$ m²/s, and $\tau_{CP}=0.25$ ms. FIG. 4B is a graph depicting the same light and dark curves plotted in terms of T_2 and diameter, on a linear scale. In this figure the black dashed line represents the T_2^* measured in a non-uniform magnetic field where T_2^* is always lower than T_2 and doesn't reflect the particle size. The data points are the same as well. FIG. 4C is a graph depicting the monodisperse clustering model and showing that T_2 will follow the curve as analyte is added because the average diameter of the population particles will cover all intermediate diameters between the initial and final states. FIG. 4D is a graph depicting the polydisperse model and showing that the T_2 will transition between the two points on this curve when particles form clusters of specific sizes. The response curve will be linear with regard to analyte addition, but non-linear with regard to volume fraction of clusters, because particles transition between state 1 and state 2. The slope of the response curve is directly proportional to the sensitivity of the assay. FIG. 4E is a graph showing the two regimes for particle aggregation and T_2 affects based on particle size and how clustering assays in the different regimes map onto the T_2 versus diameter curves (i) for the motional averaging regime T_2 decreases when particles cluster; and (ii) for the slow motion regime T_2 increases when particles cluster. Under the conditions shown in these models, the boundary between the two regimes is ca. 100 nm diameter particles. When small magnetic particles form aggregates under 100 nm in diameter, the result is a decrease in T_2 upon aggregate formation. When magnetic particles at or above 100 nm in diameter form larger aggregates, the result is an increase in T_2 upon aggregate formation.

FIGS. 5A-5C are drawings depicting different assay formats for the assays of the invention. FIG. 5A depicts an agglomerative sandwich immunoassay in which two populations of magnetic particles are designed to bind to two different epitopes of an analyte. FIG. 5B depicts a competitive immunoassay in which analyte in a liquid sample binds to a multivalent binding agent (a multivalent antibody), thereby inhibiting aggregation. FIG. 5C depicts a hybridization-mediated agglomerative assay in which two populations of particles are designed to bind to the first and second portions of a nucleic acid target, respectively.

FIG. 6 illustrates a modular cartridge concept in sections that can be packaged and stored separately. This is done, for example, so that the inlet module (shown elevated with inverted Vacutainer tube attached) can be sterilized while the reagent holding module in the middle is not. This allows the component containing reagents to be the only refrigerated component.

FIGS. 7A-7C depict a Vacutainer inlet module. FIG. 7A shows it in the inverted position after the user has removed the closure from the Vacutainer tube and placed the cartridge onto it. FIG. 7B shows the molded in path that the blood will follow out of the Vacutainer and into the sample loading region once the cartridge is turned right side up. The foil seal can be the bottom side of the channels, forming an inexpensively molded part with closed channels. FIG. 7C is a cutaway view showing the vent tube which allows air to enter into the vial as the blood leaves and fills the sample region.

FIG. 8 depicts the sample inlet module with the foil seal removed. On the top, one can see the small air inlet port to the left, the larger sample well in the center and a port which

connects them together. This port provides a channel through which air can flow once the foil seal is pierced. It also provides an overflow into the body of the module to allow excess blood to drain away and not spill over. This effectively meters the blood sample to the volume contained in the sample well.

FIGS. 9A-9C depict a reagent module. FIG. 9A depicts the module of the cartridge that is intended to hold reagents and consumables for use during the assay. On the left are sealed pre-dispensed aliquots of reagents. On the right is a 2 ml conical bottomed centrifuge tube that is used for initial centrifugation of the blood. The other holes can be filled as need with vials, microcentrifuge tubes, and pipette tips. FIG. 9B is a cutaway view of the reagent module showing the holders for the pre-aliquoted reagent tips, including the feature at the bottom into which the tips are pressed to provide a seal. FIG. 9C depicts three representative pipette tips into which reagents can be pre-dispensed, and then the backs sealed. The tips are pressed into the sample holder to provide a seal.

FIG. 10 depicts an alternative design of the modular cartridge, showing a detection module with a recessed well for use in assays that require PCR. Cross-contamination from PCR products is controlled in two ways. First, the seals that are on the detection tubes are designed to seal to a pipette tip as it penetrates. Second, the instrument provides air flow through the recessed well by means of holes in the well to ensure that any aerosol is carried down and does not travel throughout the machine.

FIG. 11 depicts a detection module of cartridge showing detection tubes and one of the holes used to ensure air flow down and over the tubes during pipetting to help prevent aerosol escape.

FIG. 12 depicts a bottom view of the detection module, showing the bottom of the detection tubes and the two holes used to ensure airflow. An optional filter can be inserted here to capture any liquid aerosol and prevent it from entering the machine. This filter could also be a sheet of a hydrophobic material like Gore-tex that will allow air but not liquids to escape.

FIGS. 13A-13C depict a detection tube. FIG. 13A is a view of the detection tube. The tube itself could be an off the shelf 200 microliter PCR tube, while the cap is a custom molded elastomer part that provides a pressure resistant duckbill seal on the inside and a first seal to the pipette tip from the top. The seal is thus a make-break type of seal, where one seal is made before the other is broken. FIG. 13B depicts the custom molded seal component. Note the circular hole into which the pipette tip is inserted and the duckbill seal below, which provides a second seal that resists pressure developed in the tube. FIG. 13C depicts the seal showing the duckbill at bottom and the hole at top.

FIGS. 14A-14C depict a cartridge for performing a multiplexed assay. FIG. 14A shows a reagent strip for the cartridge. The oval holes are the supports for the detection modules, and these are constructed separately and then placed into the holes. The detection wells could be custom designed or commercially available. FIG. 14B shows the detection module for the cartridge depicted in FIG. 14A. In this example, the detection module contains two detection chambers, but could contain any number of chambers as required by the assay and as the detection system (the MR reader) is designed to accept. FIG. 14C depicts an alternate footprint for the modular multiplexed cartridge. This cartridge includes 3 detection modules that are molded as part of the reagent strip, and these portions are popped out of the frame and individually processed at other units (i.e., the NMR unit and/or MAA unit) within the assay system.

FIG. 15 is a scheme depicting one embodiment of the cycling gradient magnetic assisted agglomeration (gMAA) method of the invention. Two magnets are placed in two positions such that if the sample tube is placed close to the a region of strong magnetic field gradient produced by the first magnet, the magnetic particles will be drawn towards the direction of the field gradient produced by the first magnet, the sample tube is then placed next to the second magnet producing a field gradient, and the magnetic particles are drawn to the direction of the field gradient produced by second magnet. The cycle can be repeated until the aggregation reaction reaches a steady state (as observed by the change in the NMR relaxation rate of the sample); a smaller number of cycles can be used as well. A single magnet used to produce a field gradient can also be used, while for cycling the sample tube can be moved relative to the magnetic field gradient.

FIG. 16 is a scheme depicting a homogenous magnetic assisted agglomeration (hMAA) setup. On the left hand side, the magnetic particles are shown as dots in a partially clustered state. When exposed to a homogeneous magnetic field, as depicted on the right hand side, clustering of the magnetic particles is promoted as the magnetic particles form chains along the direction of the field produced by the hMAA setup. On the right hand side, the two magnets are represented by bars, to depict the formation of a standard dipole field. hMAA can also be used to evaluate the nonspecific reversibility of a magnetic particle to assess its utility in an assay of the invention.

FIG. 17 depicts a gradient MAA unit configured to apply a gradient magnetic field to the side and to the bottom of a sample. The specific setup has magnets with a surface field of approximately 0.7 T, while the produced gradient is in the order of 0.25 T/mm. Similar gMAA units, covering a much bigger range of fields and gradients can be used.

FIGS. 18A-18C depict a gradient MAA unit configured to apply a gradient magnetic field to the side and to the bottom of an array of samples. FIG. 18A depicts the gMAA unit array of 32 bottom magnets and 40 side magnets (32 functional, 8 used to balance the stray magnetic fields seen by all sample), each with a field strength of about 0.5 T, used for assisting agglomeration in an array of samples simultaneously. FIG. 18B depicts a setup for the automation of the an automated gMAA unit wherein a plate gMAA along with a configuration for containing an array of samples is cycled between the bottom and side magnet positions by a robotic systems, within a temperature controlled array. The magnets are stationary, while the plate holding the sample tubes moves through a preset trajectory. An exemplary field strength on the surface of individual magnets is 0.4-0.5 T, with a gradient in the order of 0.1 T/mm.

FIGS. 19A-19B depict a top view (FIG. 19A) and side view (FIG. 19B) of a homogenous MAA unit configured to apply a homogenous magnetic field to an array samples. Field strengths from 0.2-0.7 T can be used with homogeneity from 500 to 5000 ppm over the sample tube region.

FIG. 20 is a drawing of a vortexer which includes the following components: (i) a sample support, (ii) a main plate, (iii) four linkages, (iv) linear rail and carriage system (x2), (v) a support for driveshaft and rails, (vi) coupling and driveshaft, (vii) a mounting plate, and (viii) a drive motor.

FIG. 21 is a drawing of a compact vortexer which includes the following components: (i) a sample support, (ii) a main plate, (iii) two linkages, (iv) linear rail and carriage system, (v) a support for linear rail, (vi) support for driveshaft, (vii) coupling and driveshaft, (viii) a mounting plate, and (ix) a drive motor.

FIGS. 22A and 22B depict portions of a vortexer. FIG. 22A is a drawing depicting the bottom portion (i.e., the drive motor, coupling, and drive shaft) of a vortexer of the invention. The motor includes an index mark that allows the motor to find a specific point in its rotation. These index marks are used to home the system, and ensure that the sample can be returned to a known position after mixing and allows the vortexer to be easily accessed by robotic actuators, and thus integrated into an automated system. In lieu of index marks, external home switches or position tracking sensors could be employed. Typically, the motor is a servo or stepper motor with an encoder. The coupling is not necessarily required and may allow for misalignment. Typically, the coupling is helical. The drive shaft can include one axis that is coaxial to the motor shaft and another axis that is offset from the motor shaft, which creates an orbital pattern. Typically, the offset is $\frac{1}{4}$ ", but this offset can be optimized for sample volume and geometry. FIG. 22B is a drawing depicting the guide mechanism of a vortexer of the invention. The main plate is connected to the offset axis of the drive shaft and is free to rotate. The plate follows the orbital path around and dictated by the motor shaft. One end of a linkage is connected to the main plate and is free to rotate. This end also follows an orbital path. The other end of a linkage is connected to the carriage of a linear rail system and is also free to rotate. This end follows a linear path. Having two linkages connected to one carriage defines a line in the main plate, which prevents the carriage from rotating around its own center. Four linkages can be used to balance the system.

FIGS. 23A-23C are a series of drawings depicting a vortexer utilizing a planetary belt drive. FIG. 23A is an overall view showing the vortexer configured for one large tube. FIG. 23B is a section view showing two tube holders for small tubes. FIG. 23C is an overall view of vortexer showing four tubes and a close up of planetary belt drive mechanism.

FIG. 24 is a drawing depicting the components of the creatinine competitive assay of Example 6. A magnetic particle decorated with creatinine is used in combination with a creatinine antibody to form an aggregating system. The creatinine present in a liquid sample competes with the magnetic particles for the antibody, leading to a reduction in aggregation with increasing creatinine concentration. The change in aggregation is observed as a change in the T_2 relaxation rate of the hydrogen nuclei in the water molecules of the liquid sample. By comparing the observed T_2 relaxation rate of the liquid sample to a standard curve, the concentration of creatinine is determined.

FIGS. 25A-25C are a series of graphs showing the response curve for creatinine competitive assays. FIG. 25A is a graph showing a standard curve for the creatinine competitive assay of Example 6 correlating the observed T_2 relaxation rate with the concentration of creatinine in the liquid sample. FIG. 25B shows the T_2 response of a creatinine-decorated particle with 2 different preparations of antibody. Preparation 1 is pre-production (with aggregated antibody) and Preparation 2 is production purified (no aggregated antibody present). FIG. 25C shows the T_2 response of a creatinine-decorated particle with unaggregated antibody, biotinylated antibody and deliberately multimerized antibody, and confirms the increased clustering ability of multi-valent agglomerating agents.

FIG. 26 is a graph showing the specific clustering achieved, as determined via T_2 relaxation rates, with various methods of gMAA as described in Example 10. In FIG. 26 (i) "control" is gMAA (magnet exposure+vortex, repeat) in which the relative position of the sample and the magnetic field direction are unchanged with each cycle; (ii) "twist" is gMAA (magnet

exposure+rotation within magnet, repeat) with rotating tube ca. 90° relative to the gradient magnet with each cycle; (iii) "180° turn" is gMAA (magnet exposure+remove tube from magnet, rotate, place back in magnet, repeat) with rotating tube ca. 180° relative to the gradient magnet with each cycle; and "remove 5 s" is removal of tube from magnet, 5 seconds rest (no rotation), repeat. The results show that the rate at which a steady state degree of agglomeration, and stable T_2 reading, is achieved is expedited by cycling between the two or more positions over a number of gMAA treatments. Further, field gradient combinations, cycling field (side or bottom) to null or side field to bottom, field (side or bottom) to vortex are also iterations that can be used for gMAA. Exposure or dwell times (either on the field or away), and number of cycles can be varied to optimize assisted aggregation for a specific assay (not shown).

FIG. 27 is graph showing the response curve for the creatinine competitive assay for samples processed with alternating side-bottom magnet gMAA as described in Example 11.

FIG. 28 is a drawing depicting the tacrolimus competitive assay architecture of Example 9.

FIG. 29 is a graph showing a standard curve for the tacrolimus competitive assay of Example 9 correlating the observed T_2 relaxation rate observed for a liquid sample with the concentration of tacrolimus in the liquid sample.

FIGS. 30A-30B are graphs depicting the degree to which gMAA assisted aggregation is dependent upon temperature and dwell time in the assay of Example 11. FIG. 30A is a graph showing that the degree of aggregation as determined by measuring the T_2 response of the sample is increased with increasing dwell time at room temperature. FIG. 30B is a graph showing that the degree of aggregation as determined by measuring the T_2 response of the sample is increased with increasing gMAA dwell time at 37°C . As shown in FIGS. 30A and 30B, increasing temperature and increasing dwell time enhance the extent of gMAA assisted aggregation as observed by changes in the observed T_2 .

FIG. 31 is a graph showing that the degree of aggregation as determined by measuring the T_2 response of the sample is increased with increasing the number of gMAA cycles in the assay of Example 13.

FIG. 32 is a drawing depicting the *Candida* agglomerative sandwich assay architecture of Example 14.

FIG. 33 is a graph depicting a creatinine inhibition curve (see Example 7) for using an antibody coated particle and an amino-dextran-creatinine multivalent binding agent to induce clustering by competing with any target analyte (creatinine) present in the sample to cause particle clustering. The binding agent used is a 40 kDa dextran with ~10 creatinines per dextran molecule.

FIG. 34 is a graph depicting the evaluation of Tac-dextran conjugates for clustering ability (see Example 8) by performing a titration. As observed, that increased molecular weight of Tac-dextran results in the improved T_2 signal.

FIG. 35 is a graph depicting the evaluation of Tac-dextran conjugates for clustering ability (see Example 8) by performing a titration. As observed, higher substitution improved T_2 signal.

FIG. 36 is a graph depicting the evaluation of Tac-BSA conjugates for clustering ability (see Example 8) by performing a titration similar to that used for the Tac-dextran conjugates. As observed, clustering performance varies with the tacrolimus substitution ratio.

FIG. 37 is a graph depicting the results of T_2 assays for detecting anti-biotin antibody using prepared magnetic particles in blood and PBS matrices as described in Example 1.

FIG. 38 is a graph depicting results of T_2 assays for detecting anti-biotin antibody using prepared magnetic particles with (open circle) and without (filled circle) a protein block as described in Examples 8 and 9.

FIG. 39 is a graph depicting results of T_2 assays for detecting anti-biotin antibody using prepared magnetic particles having a BSA block (dark filled diamond, square, triangle) or a FSG block (light gray X's and circle) as described in Example 2.

FIGS. 40A-40B are schematics of provided particle coatings.

FIGS. 41A-41B depict results of T_2 assays for detecting biotin in a competitive assay format described in Example 4. FIG. 41A depicts experimental results in buffer; while FIG. 41B depicts experimental results in lysed blood.

FIG. 42 is a sketch of a system of the invention including an NMR unit, a robotic arm, a hMAA unit, a gMAA unit, two agitation units, a centrifuge, and a plurality of heating blocks.

FIGS. 43A-43D are images depicting various fluid transfer units which can be used in the systems of the invention.

FIGS. 44A and 44B are sketches showing how a system of the invention can be designed to regulate the temperature of the working space.

FIGS. 45A and 45B are sketches depicting an NMR unit having a separate casing for regulation of the temperature at the site of the NMR measurement, and useful where tight temperature control is needed for precision of the measurement. The temperature control configuration depicted in this figure is one of many different ways to control temperature.

DETAILED DESCRIPTION

The invention features systems, devices, and methods for the rapid detection of analytes or determination of analyte concentration in a sample. The systems and methods of the invention employ magnetic particles, an NMR unit, optionally one or more MAA units, optionally one or more incubation stations at different temperatures, optionally one or more vortexer, optionally one or more centrifuges, optionally a fluidic manipulation station, optionally a robotic system, and optionally one or more modular cartridges. The systems, devices, and methods of the invention can be used to assay a biological sample (e.g., blood, sweat, tears, urine, saliva, semen, serum, plasma, cerebrospinal fluid (CSF), feces, vaginal fluid or tissue, sputum, nasopharyngeal aspirate or swab, lacrimal fluid, mucous, or epithelial swab (buccal swab), tissues, organs, bones, teeth, or tumors, among others). Alternatively, the systems, devices, and methods of the invention are used to monitor an environmental condition (e.g., plant growth hormone, insecticides, man-made or environmental toxins, nucleic acid sequences that are important for insect resistance/susceptibility, algae and algae by-products), as part of a bioremediation program, for use in farming plants or animals, or to identify environmental hazards.

The magnetic particles are coated with a binding moiety (i.e., antibody, oligo, etc.) such that in the presence of analyte, or multivalent binding agent, aggregates are formed. Aggregation depletes portions of the sample from the microscopic magnetic non-uniformities that disrupt the solvent's T_2 signal, leading to an increase in T_2 relaxation (see FIG. 3).

The T_2 measurement is a single measure of all spins in the ensemble, measurements lasting typically 1-10 seconds, which allows the solvent to travel hundreds of microns, a long distance relative to the microscopic non-uniformities in the liquid sample. Each solvent molecule samples a volume in the liquid sample and the T_2 signal is an average (net total signal) of all (nuclear spins) on solvent molecules in the sample; in

other words, the T_2 measurement is a net measurement of the entire environment experienced by a solvent molecule, and is an average measurement of all microscopic non-uniformities in the sample.

The observed T_2 relaxation rate for the solvent molecules in the liquid sample is dominated by the magnetic particles, which in the presence of a magnetic field form high magnetic dipole moments. In the absence of magnetic particles, the observed T_2 relaxation rates for a liquid sample are typically long (i.e., T_2 (water) \sim 2000 ms, T_2 (blood) \sim 1500 ms). As particle concentration increases, the microscopic non-uniformities in the sample increase and the diffusion of solvent through these microscopic non-uniformities leads to an increase in spin decoherence and a decrease in the T_2 value. The observed T_2 value depends upon the particle concentration in a non-linear fashion, and on the relaxivity per particle parameter.

In the aggregation assays of the invention, the number of magnetic particles, and if present the number of agglomerant particles remain constant during the assay. The spatial distribution of the particles change when the particles cluster. Aggregation changes the average "experience" of a solvent molecule because particle localization into clusters is promoted rather than more even particle distributions. At a high degree of aggregation, many solvent molecules do not experience microscopic non-uniformities created by magnetic particles and the T_2 approaches that of solvent. As the fraction of aggregated magnetic particles increases in a liquid sample, the observed T_2 is the average of the non-uniform suspension of aggregated and single (unaggregated) magnetic particles. The assays of the invention are designed to maximize the change in T_2 with aggregation to increase the sensitivity of the assay to the presence of analytes, and to differences in analyte concentration.

In designing magnetic relaxation switch (MRSw) biosensors, it is important to consider the relaxation mechanisms of the magnetic particles. First, in the case of superparamagnetic particles the solvent longitudinal and transverse relaxivities (defined as $R_1 = 1/T_1$ and $R_2 = 1/T_2$, respectively) are a function of particle size. Furthermore, R_2 and R_2^* (where $R_2^* = 1/T_2^*$, $R_2^* = R_2 + \Delta\omega_F$, where $\Delta\omega_F$ is dephasing due to field inhomogeneities) increase with particle diameter until about 100 nm, and then R_2 decreases with increasing particle size and the R_2^* reaches a plateau (see FIG. 4A). Superparamagnetic particles are typically divided into categories of strongly magnetized and weakly magnetized particles, based on the relative magnitude of the precession frequency difference between nuclei at the surface of the particle and nuclei distant from the particle, $\Delta\omega$, and the inter-echo delay of the CPMG detection sequence, τ_{CP} . $\Delta\omega$ is essentially a relative measure of the effect of the dipolar magnetic field generated by a superparamagnetic particle on the resonant frequency of hydrogen nuclei in adjacent water molecules. When the product $\Delta\omega\tau_{CP} > 1$ then the particles are classified as strongly magnetized and when $\Delta\omega\tau_{CP} < 1$ then the particles are classified as weakly magnetized. For typical relaxometers, τ_{CP} is no shorter than tens of microseconds, so $\Delta\omega$ must be less than 10^5 for the particles to be within the weakly magnetized regime. Most superparamagnetic particles used for MRSw assays have a surface dephasing $\Delta\omega$ of approximately 1×10^7 , therefore they are classified as strongly magnetized. This means that the inter-echo delay is always longer than the amount of dephasing that occurs at the surface of a particle.

Another characteristic of superparamagnetic particle solutions that is used to differentiate physical behavior is the diffusion time, or travel time, of water (τ_D) relative to the inter-echo time of the pulse sequence, τ_{CP} . Particle solutions

are in the long echo limit when the τ_D is significantly less than that τ_{CP} . τ_D can be determined by the relationship:

$$\tau_D = \frac{R^2}{D}, \quad (1)$$

where τ_D is the time it takes a water molecule to diffuse the distance of a particle radius, R , and D the diffusion constant of water, $10^{-9} \text{ m}^2/\text{s}$. τ_D can be thought of as the time it takes a water molecule to pass a hemisphere of a particle, or a flyby time. When τ_D is much larger than τ_{CP} , then the particle system is within the "short echo limit". Typical CPMG sequences have echo times on the order of hundreds of microseconds to several milliseconds. Therefore, the "short echo limit" cannot be approached unless the particle diameter approaches 1000 nm. The most common MRSw biosensors are within the "long echo limit" because the length of the inter-echo delays ($\tau_{CP} > 0.25 \text{ ms}$) is longer than the time it takes a water molecule to diffuse past the hemisphere of a particle (0.2-100 microseconds).

As the particle size of a solution of superparamagnetic particles at fixed iron concentration is increased there is an initial increase in R_2 , then a plateau and later decrease (FIG. 4A). The regime on the left hand side of the curve is been termed the motional averaging regime, the regime in the middle is been termed the static dephasing regime, and the regime on the right is been termed the visit limited, or slow motion regime. The boundaries between the motional averaging and visit limited regimes can be determined by generating plots such as that shown in FIG. 4A, or they can be determined by the relationship between $\Delta\omega$ and τ_D . If $\Delta\omega\tau_{CP} < 1$, then the system is in the motional averaging regime; if $\Delta\omega\tau_D > 1$, then the system is in the visit limited regime (also termed the slow motion regime). As the diameter of the particles increases in the motional averaging regime the refocusing echos in the CPMG pulse sequence cannot efficiently refocus the magnetization that has been dephased by the particles, hence the increase in R_2 (or decrease in T_2). In other words, the refocusing pulses cannot compensate for increased dephasing by larger particles. The flat region of the static dephasing regime is due to the R_2 being limited by R_2^* . The decreasing R_2 with increasing diameter in the visit limited regime results in the refocusing pulses being able to refocus the dephasing caused by the particles. Also apparent in FIG. 4A is that the R_2 in the slow motion regime exhibits a dependence on the inter-echo delay of the spin echo sequence.

In a homogenous magnetic field, one can determine which regime applies to a sample by comparing the R_2 to the R_2^* ; the two values are identical in the motional averaging or static dephasing regime and they are different in the visit limited regime. However, in cases of inhomogeneous fields, such as those present on benchtop and portable MR devices, the T_2^* is dominated by the field gradient. In fact, the measured T_2^* value is not indicative of the particle or particle cluster size state (FIG. 4B).

The shape of the R_2 response as particles agglomerated generally matches the expected trend for the increase in average particle size. The similarity between the R_2 of particle agglomerates and that of spherical particles suggests that one can equate particle aggregates and spherical shapes. Even though this assumption may seem to be in contradiction with the fractal nature of particle agglomerates, the shape of the particle aggregates observed by the magnetic resonance measurement is determined by the ensemble of diffusing water

molecules in solution, which can be approximated by the radius of hydration measured by light scattering.

The analytical models for R_2 can be applied to magnetic relaxation biosensors to aid in the design of biosensor assays. Conveniently, these models accurately predict the dependence of R_2 on parameters that a biosensor designer can control—iron concentration, temperature, magnetic susceptibility, and particle size. Additionally, these analytical models allow for predictive modeling of the dependence of T_2 relaxation on these parameters. Results are not entirely quantitative, but the general trends and response curves predicted by these models can be instructive. One useful model is the chemical exchange model for strongly magnetized spheres:

$$\frac{1}{T_2} = \frac{(4/9)V\tau_D(\Delta\omega_r)^2}{1 + (4/9)^2(\tau_D/\tau_{CP})^2\alpha^5} \quad (2)$$

$$\alpha = \left[\frac{\Delta\omega\tau_{CP}}{a + b\Delta\omega\tau_{CP}V} \right]^{1/3} \quad (3)$$

where $1/T_2$ is the transverse relaxivity, V the volume fraction of iron in solution, τ_D the diffusion, or flyby time, $\Delta\omega_r$ the frequency shift at the surface of a particle relative to bulk solution, τ_{CP} one half the inter-echo delay in a CPMG sequence, and a and b are derived constants ($a=1.34$ and $b=0.99$). Equations (2) and (3) can be used to generate a curve that describes the dependence of R_2 on particle sizes, as shown by the light and dark lines in FIGS. 4A and 4B (dark line on right side of the curve; light line on left side of the curve).

A modification of Equation 2 can be used to generate a plot that is more intuitive to an assay developer. This plot is in terms of T_2 and particle diameter with linear units rather than logarithmic units (FIG. 2). As discussed above, magnetic relaxation biosensor assays function due to a transition between dispersed and clustered states. For a given agglomerative assay, the measured T_2 can follow one of two pathways over the course of an analyte titration. The population of dispersed particles can cluster in a uniform manner leading to an increase in average particle size that is proportional to the amount of analyte that has been added. This type of agglomeration is termed the monodisperse model because it would lead to a monodisperse intermediate population of particles. In this case, T_2 would be expected to decrease as particle size increases as long as the system is within the motional averaging regime. As the system approaches and enters the visit limited regime the T_2 would increase with particle size (FIG. 4C).

A different type of agglomeration that may occur is one where the addition of analyte seeds the self-assembly of clusters, a process with energetics similar to crystal formation or fractal aggregation. For this model one would expect a preferred size for particle clusters that depended on the conditions of the solution. Systems that followed this model would exhibit polydisperse intermediate populations; one would find a mixture of particles with discrete sizes. Given two discrete populations, dispersed particles and clustered particles, the system would transition between the T_2 value of the starting monodisperse population of unclustered particles and the final T_2 value of the fully clustered particles. For both models, full titration may lead to a monodisperse solution of clustered particles. Although the exact energetics, kinetics, and thermodynamics of particle agglomeration will depend on characteristics of the assay system such as valency and binding affinities, these two models are instructive in under-

standing the dependencies and possible scenarios one may encounter during MRSw biosensor design.

There are two regimes for particle clustering and T_2 affects based on particle size (see FIG. 4D, the boundary is typically ca. 100 nm diameter particles). For any given assay of a liquid sample the particle count for 250 nm sized magnetic particles can be ca. 1×10^7 particles, whereas for 30 nm sized magnetic particles can be ca. 1×10^{13} . This is because the smaller particles have a lower relaxivity per particle (for the same type of material), resulting in an inherent sensitivity disadvantage. In a typical assay of the invention, the magnetic particles are selected such that T_2 increases with an increase in the fraction of aggregated particles.

The assay of the invention can be designed to change the direction of T_2 in the presence of analyte (see FIGS. 5A-5C). For example, the assay can be an agglomerative sandwich immunoassay in which two populations of magnetic particles bind to different epitopes of an analyte (see FIG. 5A); a competitive assay in which analyte competes with a multivalent binding agents to inhibit the aggregation of magnetic particles (see FIG. 5B); or a hybridization-mediated agglomeration in which two populations of magnetic particles bind to a first and second portion of an oligonucleotide (see FIG. 5C).

Other formats for carrying out the assays of the invention can be used, such as: (i) a target sample can be incubated in the presence of a magnetic particle that has been decorated with binding moieties specific to a target analyte and a multivalent binding agent, in an inhibition assay the binding of the analyte to the magnetic particles blocks agglomeration of the magnetic particles with the multivalent binding agent; (ii) a target sample can be incubated in the presence of a magnetic particle that has been decorated with binding moieties specific to a target analyte and a multivalent binding agent, in a disaggregation assay the analyte is exposed to a pre-formed aggregate of the multivalent binding agent and the magnetic particle and the analyte displaces the multivalent binding agent to reduce aggregation in the liquid sample; or (iii) a target sample can be incubated in the presence of a magnetic particle that has been decorated with binding moieties specific to a target analyte and the target analyte itself to form a self-assembling single population of magnetic particles, in an inhibition assay or disaggregation assay the presence the binding of the analyte to the magnetic particles blocks the self agglomeration of the magnetic particles; or (iv) a target sample can be incubated in the presence of a soluble agglomerating agent and a magnetic particle decorated with the analyte or analog of the analyte, in an inhibition assay the presence of the analyte binds the soluble agglomerating agent blocking the agglomeration of the particles.

Where a multivalent binding agent (agglomerant) is employed, multiple analytes are linked to a carrier (e.g., a simple synthetic scaffold, or a larger carrier protein or polysaccharide, such as BSA, transferrin, or dextran).

Magnetic Particles

The magnetic particles described herein include those described, e.g., in U.S. Pat. No. 7,564,245 and U.S. Patent Application Publication No. 2003-0092029, each of which is incorporated herein by reference. The magnetic particles are generally in the form of conjugates, that is, a magnetic particle with one or more binding moieties (e.g., an oligonucleotide, nucleic acid, polypeptide, or polysaccharide) linked thereto. The binding moiety causes a specific interaction with a target analyte. The binding moiety specifically binds to a selected target analyte, for example, a nucleic acid, polypeptide, or polysaccharide. In some instances, binding causes aggregation of the conjugates, resulting in a change, e.g., a decrease (e.g., in the case of smaller magnetic particles) or an

increase (e.g., in the case of larger magnetic particles) in the spin-spin relaxation time (T_2) of adjacent water protons in an aqueous solution (or protons in a non-aqueous solvent). Alternatively, the analyte binds to a preformed aggregate in a competitive disaggregation assay (e.g., an aggregate formed from a multivalent binding agent and magnetic particles), or competes with a multivalent binding agent for binding moieties on the magnetic particles in an inhibition assay (i.e., the formation of aggregates is inhibited in the presence of the analyte).

The conjugates have high relaxivity owing to the superparamagnetism of their iron, metal oxide, or other ferro or ferrimagnetic nanomaterials. Iron, cobalt, and nickel compounds and their alloys, rare earth elements such as gadolinium, and certain intermetallics such as gold and vanadium are ferromagnets can be used to produce superparamagnetic particles. The magnetic particles can be monodisperse (a single crystal of a magnetic material, e.g., metal oxide, such as superparamagnetic iron oxide, per magnetic particle) or polydisperse (e.g., a plurality of crystals per magnetic particle). The magnetic metal oxide can also comprise cobalt, magnesium, zinc, or mixtures of these metals with iron. Important features and elements of magnetic particles that are useful to produce conjugates include: (i) a high relaxivity, i.e., strong effect on water (or other solvent) relaxation, (ii) a functional group to which the binding moiety can be covalently attached, (iii) a low non-specific binding of interactive moieties to the magnetic particle, and/or (iv) stability in solution, i.e., the magnetic particles remain suspended in solution, not precipitated.

The magnetic particles may be linked to the binding moieties via functional groups. In some embodiments, the magnetic particles can be associated with a polymer that includes functional groups selected, in part, to enhance the magnetic particles nonspecific reversibility. The polymer can be a synthetic polymer, such as, but not limited to, polyethylene glycol or silane, natural polymers, or derivatives of either synthetic or natural polymers or a combination of these. The polymer may be hydrophilic. In some embodiments, the polymer "coating" is not a continuous film around the magnetic metal oxide, but is a "mesh" or "cloud" of extended polymer chains attached to and surrounding the metal oxide. The polymer can comprise polysaccharides and derivatives, including dextran, pullan, carboxydextran, carboxymethyl dextran, and/or reduced carboxymethyl dextran. The metal oxide can be a collection of one or more crystals that contact each other, or that are individually entrapped or surrounded by the polymer.

Alternatively, the magnetic particles can be associated with non-polymeric functional group compositions. Methods of synthesizing stabilized, functionalized magnetic particles without associated polymers are described, for example, in Halbreich et al., *Biochimie*, 80:379 (1998).

The magnetic particles typically include metal oxide crystals of about 1-25 nm, e.g., about 3-10 nm, or about 5 nm in diameter per crystal. The magnetic particles can also include a polymer component in the form of a core and/or coating, e.g., about 5 to 20 nm thick or more. The overall size of the magnetic particles can be, e.g., from 20 to 50 nm, from 50 to 200 nm, from 100 to 300 nm, from 250 to 500 nm, from 400 to 600 nm, from 500 to 750 nm, from 700 to 1,200 nm, from 1,000 to 1,500 nm, or from 1,500 to 2,000 nm.

The magnetic particles may be prepared in a variety of ways. It is preferred that the magnetic particle have functional groups that link the magnetic particle to the binding moiety. Carboxy functionalized magnetic particles can be made, for example, according to the method of Gorman (see PCT Pub-

lication No. WO00/61191). In this method, reduced carboxymethyl (CM) dextran is synthesized from commercial dextran. The CM-dextran and iron salts are mixed together and are then neutralized with ammonium hydroxide. The resulting carboxy functionalized magnetic particles can be used for coupling amino functionalized oligonucleotides. Carboxy-functionalized magnetic particles can also be made from polysaccharide coated magnetic particles by reaction with bromo or chloroacetic acid in strong base to attach carboxyl groups. In addition, carboxy-functionalized particles can be made from amino-functionalized magnetic particles by converting amino to carboxy groups by the use of reagents such as succinic anhydride or maleic anhydride.

Magnetic particle size can be controlled by adjusting reaction conditions, for example, by using low temperature during the neutralization of iron salts with a base as described in U.S. Pat. No. 5,262,176. Uniform particle size materials can also be made by fractionating the particles using centrifugation, ultrafiltration, or gel filtration, as described, for example in U.S. Pat. No. 5,492,814.

Magnetic particles can also be synthesized according to the method of Molday (Molday, R. S, and D. MacKenzie, "Immunospecific ferromagnetic iron-dextran reagents for the labeling and magnetic separation of cells," *J. Immunol. Methods*, 52:353 (1982)), and treated with periodate to form aldehyde groups. The aldehyde-containing magnetic particles can then be reacted with a diamine (e.g., ethylene diamine or hexanediamine), which will form a Schiff base, followed by reduction with sodium borohydride or sodium cyanoborohydride.

Dextran-coated magnetic particles can be made and cross-linked with epichlorohydrin. The addition of ammonia reacts with epoxy groups to generate amine groups, see Hogemann, D., et al., Improvement of MRI probes to allow efficient detection of gene expression *Bioconjug. Chem.*, 11:941 (2000), and Josephson et al., "High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates," *Bioconjug. Chem.*, 10:186 (1999)). This material is known as cross-linked iron oxide or "CLIO" and when functionalized with amine is referred to as amine-CLIO or $\text{NH}_2\text{-CLIO}$. Carboxy-functionalized magnetic particles can be converted to amino-functionalized magnetic particles by the use of water-soluble carbodiimides and diamines such as ethylene diamine or hexane diamine.

The magnetic particles can be formed from a ferrofluid (i.e., a stable colloidal suspension of magnetic particles). For example, the magnetic particle can be a composite of including multiple metal oxide crystals of the order of a few tens of nanometers in size and dispersed in a fluid containing a surfactant, which adsorbs onto the particles and stabilizes them, or by precipitation, in a basic medium, of a solution of metal ions. Suitable ferrofluids are sold by the company Liquids Research Ltd under the references: WHKS1S9 (A, B or C), which is a water-based ferrofluid comprising magnetite (Fe_3O_4), having particles 10 nm in diameter; WHJS1 (A, B or C), which is an isoparaffin-based ferrofluid comprising particles of magnetite (Fe_3O_4) 10 nm in diameter; and BKS25 dextran, which is a water-based ferrofluid stabilized with dextran, comprising particles of magnetite (Fe_3O_4) 9 nm in diameter. Other suitable ferrofluids for use in the systems and methods of the invention are oleic acid-stabilized ferrofluids available from Ademtech, which include ca. 70% weight $\alpha\text{-Fe}_2\text{O}_3$ particles (ca. 10 nm in diameter), 15% weight octane, and 15% weight oleic acid.

The magnetic particles are typically a composite including multiple metal oxide crystals and an organic matrix, and having a surface decorated with functional groups (i.e., amine groups or carboxy groups) for the linking binding moieties to the surface of the magnetic particle. For example, the magnetic particles useful in the methods of the invention include those commercially available from Dynal, Seradyn, Kisker, Miltenyi Biotec, Chemicell, Anvil, Biopal, Estapor, Genovis, Thermo Fisher Scientific, JSR micro, Invitrogen, and Ademtech, as well as those described in U.S. Pat. Nos. 4,101,435; 4,452,773; 5,204,457; 5,262,176; 5,424,419; 6,165,378; 6,866,838; 7,001,589; and 7,217,457, each of which is incorporated herein by reference.

Avidin or streptavidin can be attached to magnetic particles for use with a biotinylated binding moiety, such as an oligonucleotide or polypeptide (see, e.g., Shen et al., "Magnetically labeled secretin retains receptor affinity to pancreas acinar cells," *Bioconjug. Chem.*, 7:311 (1996)). Similarly, biotin can be attached to a magnetic particle for use with an avidin-labeled binding moiety. Alternatively, the binding moiety is covalently linked to the surface of the magnetic particle; the particles may be decorated with IgG molecules; the particles may be decorated with anti his antibodies; or the particles may be decorated with his-tagged FAbs.

Low molecular weight materials can be separated from the magnetic particles by ultra-filtration, dialysis, magnetic separation, or other means prior to use. For example, unreacted binding moieties and linking agents can be separated from the magnetic particle conjugates by magnetic separation or size exclusion chromatography. In certain instances the magnetic particles can be fractionated by size to produce mixtures of particles of a particular size range and average diameter.

For certain assays requiring high sensitivity, analyte detection using T_2 relaxation assays can require selecting a proper particle to enable sufficiently sensitive analyte-induced agglomeration. Higher sensitivities can be achieved using particles that contain multiple superparamagnetic iron oxide cores (5-15 nm diameter) within a single larger polymer matrix or ferrofluid assembly (100 nm-1200 nm total diameter, such as particles having an average diameter of 100 nm, 200 nm, 250 nm, 300 nm, 500 nm, 800 nm, or 1000 nm), or by using a higher magnetic moment materials or particles with higher density, and/or particles with higher iron content. Without being limited by theory, it is postulated these types of particles provided a sensitivity gain of over 100x due to a much higher number of iron atoms per particle, which is believed to lead to an increase in sensitivity due to the decreased number of particles present in the assay solution and possibly a higher amount of superparamagnetic iron affected by each clustering event.

Relaxivity per particle and particle size is one useful term for selecting an optimal particle for high sensitivity assays. Ideally, this term will be as large as possible. Relaxivity per particle is a measure of the affect of each particle on the measured T_2 value. The larger this number, the fewer the number of particles needed to elicit a given T_2 response. Furthermore, the lower the concentration of particles in the reactive solution, the higher the analytical sensitivity of the assay. Relaxivity per particle can be a more useful parameter in that the iron density and relaxivity can vary from magnetic particle to magnetic particle, depending upon the components used to make the particles (see Table 1). Relaxivity per particle is proportional to the saturation magnetization of a superparamagnetic material.

TABLE 1

Hydrodynamic Diameter (nm)	# Metal Atoms per Particle	Relativity per Particle ($\text{mM}^{-1}\text{s}^{-1}$)
10-30	1.0E+03-1.0E+06	1.0E+6-1.0E+11
10-50	8.0E+02-4.0E+04	1.0E+04-4.0E+06
10-50	1.0E+04-5.0E+05	1.0E+06-1.0E+08
50-100	1.0E+04-1.0E+07	1.0E+06-1.0E+09
100-200	5.0E+06-5.0E+07	5.0E+08-8.0E+09
200-300	1.0E+07-1.0E+08	3.0E+09-1.0E+10
300-500	5.0E+07-1.0E+09	7.0E+09-5.0E+10
500-800	1.0E+08-4.1E+09	1.0E+10-5.0E+11
800-1000	5.0E+08-5.0E+09	5.0E+10-5.0E+11
1000-1200	1.0E+09-7.0E+09	1.0E+11-1.0E+12

The base particle for use in the systems and methods of the invention can be any of the commercially available particles identified in Table 2.

TABLE 2

Catalogue No.	Source/Description	Diameter (μm)
Kisker		
MAV-1	Polystyrene, Magnet Particles Avidin coated	1.0-1.9
PMSi-0.6	Polystyrene, Magnet Particles Streptavidin coated	0.5-0.69
PMSi-0.7	Polystyrene, Magnet Particles Streptavidin coated	0.7-0.9
PMSi-1.0	Polystyrene, Magnet Particles Streptavidin coated	1.0-1.4
PMB-1	Polystyrene, Magnet Particles Biotin covalently coupled to BSA coating	1.0-1.9
PMP-200	Dextran based, No coating, plain	0.2
PMP-1000	Dextran based, No coating, plain	0.10
PMP-1300	Dextran based, No coating, plain	0.13
PMP-2500	Dextran based, No coating, plain	0.25
PMN-1300	Dextran based, NH ₂ — coated	0.13
PMN-2500	Dextran based, NH ₂ — coated	0.25
PMC-1000	Dextran based, COOH— coated	0.10
PMC-1300	Dextran based, COOH— coated	0.13
PMC-2500	Dextran based, COOH— coated	0.25
PMAV-1300	Dextran based, Avidin coated	0.13
PMAV-2500	Dextran based, Avidin coated	0.25
PMSA-1000	Dextran based, Streptavidin coated	0.1
PMSA-1300	Dextran based, Streptavidin coated	0.13
PMSA-2500	Dextran based, Streptavidin coated	0.25
PMB-1000	Dextran based, Biotin coated	0.1
PMB-1300	Dextran based, Biotin coated	0.13
PMB-2500	Dextran based, Biotin coated	0.25
PMPA-1000	Dextran based, Protein A coated	0.1
PMPA-1300	Dextran based, Protein A coated	0.13
PMPA-2500	Dextran based, Protein A coated	0.25
PMC-0.1	Dextran based, COOH functionalized	0.1-0.4
PMC-0.4	Dextran based, COOH functionalized	0.4-0.7
PMC-0.7	Dextran based, COOH functionalized	0.7-0.9
PMC-1.0	Dextran based, COOH functionalized	1.0-1.4
PMN-1.0	Dextran based, NH ₂ functionalized	1.0-1.4
PMC-0.1	Dextran based, COOH functionalized	0.1-0.4
Accurate Chemical		
ADM01020	Carboxyl-functionality	0.2
ADM01030	Carboxyl-functionality	0.3
ADM02020	Carboxyl-functionality	0.2
ADM02133	high Carboxyl-functionality	0.3
ADM02150	Carboxyl-functionality	0.5
ADM02220	very high Amino-functionality	0.2
ADM02230	very high Amino-functionality	0.3
ADM02250	Carboxyl-functionality	0.5
ADM02030	high Carboxyl-functionality	0.3
ADM02110	high Carboxyl-functionality	0.1
ADM02120	very high Carboxyl-functionality	0.2
ADM02130	very high Carboxyl-functionality	0.3
ADM02252	Carboxyl-functionality	0.5
ADM03120	Streptavidin-functionality	0.2
ADM03121	Streptavidin-functionality	0.2

TABLE 2-continued

Catalogue No.	Source/Description	Diameter (μm)
chemicell		
5		
1201-5 1	Si—(CH ₂) ₃ —COOH	0.5
1201-5 1	Si—(CH ₂) ₃ —COOH	0.75
1201-5 1	Si—(CH ₂) ₃ —COOH	1.0
1202-5 1	Si—(CH ₂) ₃ —SO ₃ H	0.5
1202-5 1	Si—(CH ₂) ₃ —SO ₃ H	0.75
1202-5 1	Si—(CH ₂) ₃ —SO ₃ H	1.0
1205-1	Si—(CH ₂) ₃ —PO ₃ H ₂	0.5
1205-1	Si—(CH ₂) ₃ —PO ₃ H ₂	0.75
1205-1	Si—(CH ₂) ₃ —PO ₃ H ₂	1.0
Estapor		
15		
M1-130/12	Carboxylated polystyrene	0.7-1.3
M1-180/12	Carboxylated Polystyrene	0.9-1.3
M1-180/20	Carboxylated Divinylbenzene	0.8-1.2
M1-050/20	Carboxylated Polystyrene	0.5-0.7
M1-070/40	Carboxylated Polystyrene	0.7-1.3
M1-070/60	Carboxylated Polystyrene	0.7-1.3
20		
M1-020/50	Carboxylated Polystyrene	0.16-0.24
M1-030/40	Carboxylated Polystyrene	0.3-0.5
Genovis		
AMI-25	Dextran	80-150
Thermo Fisher		
25		
4515-2105	Carboxylate-Modified (MG-CM)	1.0
7815-2104	NeutrAvidin (MG-NA)	1.0
5915-2104	Streptavidin (MG-SA)	1.0
2415-2105	Carboxylate-Modified (MG-CM)	1.0
4415-2105	Carboxylate-Modified (MG-CM)	1.0
30		
MB100	Carboxylated	1.1
Invitrogen		
35		
354-01	Carboxylated	1
355-00	Tosylactivated	1
650-11	Carboxylated	1
655-11	Tosylactivated	1
Biopal		
40		
M02Q05	Amino activated	1.5
M02Q05	Biotin activated	1.5
M02Q05	Streptavidin activated	1.5

The magnetic particles for use in the systems and methods of the invention can have a hydrodynamic diameter from 10 nm to 1200 nm, and containing on average from 8×10^2 - 1×10^{10} metal atoms per particle, and having a relativity per particle of from 1×10^4 - 1×10^{13} $\text{mM}^{-1}\text{s}^{-1}$. The magnetic particles used in the systems and methods of the invention can be any of the designs, composites, or sources described above, and can be further modified as described herein for use as a magnetic resonance switch.

In addition to relativity per particle, several other practical issues must be address in the selection and design of magnetic particles for high analytical sensitivity assays.

For example, the use of large particles (i.e., 1000 nm or greater) may be desired to maximize iron content and the relativity per particle. However, we have observed that particles of this size tend to settle rapidly out of solution. We have observed that particle settling does not typically interfere with the assay if magnetic particle sizes are kept below 500 nm. When use of a particle above 500 nm in the described assays or smaller particles with high density are employed, settling is monitored and effect on T₂ measurement is determined. We have found a magnetic particle size of about 100-300 nm particle to be ideal for stability in terms of settling, even after functionalization (increasing the hydrodynamic diameter to 300 nm by approximately 50 nm), and to afford

the high sensitivity enabled by a high relaxivity per particle. Particle density certainly plays a role in buoyancy. As such, the relative density of the solution and particles plays an important role in settling of the particle. Accordingly, a possible solution to this problem is the use of buoyant magnetic particles (i.e., a hollow particle, or particle containing both a low density matrix and high density metal oxide). Settling may affect the T_2 detection, thus, solution additives may be employed to change the ratio of the particle to solution density. T_2 detection can be impacted by settling if there is a significant portioning of the superparamagnetic material from the measured volume of liquid. Settling can be assessed by diluting the particles to a concentration such that UV-Vis absorbance at 410 nm is between 0.6-0.8 absorbance units and then monitoring the absorbance for 90 minutes. If settling occurs, the difference between the initial and final absorbances divided by the initial absorbance will be greater than 5%. If % settling is above 5% then the particle is typically not suitable for use in assays requiring high analytical sensitivity. The magnetic particles used in the assays of the invention can be, but are not limited to, nonsettling magnetic particles. High settling represents handling difficulties and may lead to reproducibility issues.

For magnetic particles on the order of 100 nm or larger, the multiple superparamagnetic iron oxide crystals that typically comprise the particle core results in a net dipole moment when in the presence of external magnetic fields. Nonspecific reversibility is a measure of the colloidal stability and robustness against non-specific aggregation. Nonspecific reversibility is assessed by measuring the T_2 values of a solution of particles before and after incubation in a uniform magnetic field (defined as <5000 ppm). Starting T_2 values are typically 200 ms for a particle with an iron concentration of 0.01 mM Fe. If the difference in T_2 values before and after incubation in the uniform magnetic field is less than 20 ms, the samples are deemed reversible. Further, 10% is a threshold allowing starting T_2 measurements to reflect assay particle concentration. If the difference is greater than 10%, then the particles exhibit irreversibility in the buffer, diluents, and matrix tested. The MAA reversibility of the magnetic particles can be altered as described herein. For example, colloidal stability and robustness against non-specific aggregation can be influenced by the surface characteristics of the particles, the binding moieties, the assay buffer, the matrix and the assay processing conditions. Maintenance of colloidal stability and resistance to non-specific binding can be altered by conjugation chemistry, blocking methods, buffer modifications, and/or changes in assay processing conditions.

We have observed that a very important attribute for robust and reproducible assays is the monodispersity in the size distribution of the magnetic particles used, a distinction observed in polydisperse particles post-coating versus monodisperse particle pre-coating. Polydisperse batches of magnetic particles can lack reproducibility and compromise sensitivity. Polydisperse samples can also present problems in terms of achieving uniform coatings. For certain highly sensitive assays it is desirable that the magnetic particles be substantially monodisperse in size distribution (i.e., having a polydispersity index of less than about 0.8-0.9).

Given that the assays of the invention require monitoring a shift in the clustering states of the agglomeration assays and that measuring a change in clustering likely requires a significant fraction of clustered particles (e.g., thought to be >1-10%), the total number of particles in an assay should be minimized to enable the highest sensitivity. However, sufficient number of particles must be present to allow utilization of the T_2 detection dynamic range. We have found that the

highest sensitivity is observed when the number of magnetic particles (or molar equivalent) is approximately on the same order of magnitude of the number (or molar equivalent) of the analyte being detected, and the magnitude of the number (or molar equivalent) multivalent binding agents employed (i.e., in an inhibition assay).

For proteinaceous samples it may also be required to modify the magnetic particle surface to reduce non-specific binding of background proteins to the magnetic particles. Non-specific binding of background proteins to particles can induce or impede particle clustering, resulting in false signals and/or false lack of signals. For example, in some instances the surface of the magnetic particle can include blocking agents covalently linked to the surface of the magnetic particle which reduce non-specific binding of background proteins. There are a variety of agents that one could use to achieve the desired effect, and in some cases, it is a combination of agents that is optimal (see Table 3; exemplary particles, coatings, and binding moieties).

TABLE 3

Base Particle	Coating	Binding Moiety
NP-COOH:	amino Dextran Transferrin Lysozyme BSA FSG BGG Ovalbumin amino PEG Human albumin	Small molecule
	none amino PEG BSA amino Dextran	Antibody
NP-amino:	none PEG	Small molecule
NP-SA:	none biotinylated amino PEG	biotinylated Ab Antibody
NP-SA:	biotinylated amino PEG	small molecule
NP-anti-species:	none	Antibody
NP-Ni:	none	his-tagged antibody

Thus, we have found a protein block may be required to achieve assay activity and sensitivity, particularly in proteinaceous samples (e.g., plasma samples or whole blood samples), that is comparable to results in nonproteinaceous buffer samples. Some commonly used protein blockers which may be used in provided preparations include, e.g., bovine serum albumin (BSA), fish skin gelatin (FSG), bovine gamma globulin (BGG), lysozyme, casein, peptidase, or non-fat dry milk. In certain embodiments a magnetic particle coating includes BSA or FSG. In other embodiments, a combination of coatings are combinations of those exemplary coatings listed in Table 3.

Furthermore, nonspecific binding can be due to lipids or other non-proteinaceous molecules in the biological sample. For non-proteinaceous mediated non-specific binding, changes in pH and buffer ionic strength maybe selected to enhance the particle repulsive forces, but not enough to limit the results of the intended interactions.

Assay Reagents

The assays of the invention can include reagents for reducing the non-specific binding to the magnetic particles. For example, the assay can include one or more proteins (e.g., albumin, fish skin gelatin, lysozyme, or transferrin); low molecular weight (<500 Daltons) amines (e.g., amino acids, glycine, ethylamine, or mercaptoethanol amine); and/or

water soluble non-ionic surface active agents (e.g., polyethyleneglycol, Tween® 20, Tween® 80, Pluronic®, or Igepal®) (see Table 4).

TABLE 4

Blocking Agents
PEG
BSA—Bovine serum albumin
HSA—Human serum albumin
FSG—Fish skin gelatin
Lysozyme
Transferrin
Glycine or other small amine containing molecules
Ethylamine
Mercaptoethanol amine
Tween 20
Tween 80
Pluronic
Igepal
Triton X-100
Other surfactants/detergents

The surfactant may be selected from a wide variety of soluble non-ionic surface active agents including surfactants that are generally commercially available under the IGEPAL trade name from GAF Company. The IGEPAL liquid non-ionic surfactants are polyethylene glycol p-isooctylphenyl ether compounds and are available in various molecular weight designations, for example, IGEPAL CA720, IGEPAL CA630, and IGEPAL CA890. Other suitable non-ionic surfactants include those available under the trade name TETRONIC 909 from BASF Wyandotte Corporation. This material is a tetra-functional block copolymer surfactant terminating in primary hydroxyl groups. Suitable non-ionic surfactants are also available under the VISTA ALPHONIC trade name from Vista Chemical Company and such materials are ethoxylates that are non-ionic biodegradables derived from linear primary alcohol blends of various molecular weights. The surfactant may also be selected from poloxamers, such as polyoxyethylene-polyoxypropylene block copolymers, such as those available under the trade names Synperonic PE series (ICI), Pluronic® series (BASF), Supronic, Monolan, Pluracare, and Plurodac, polysorbate surfactants, such as Tween® 20 (PEG-20 sorbitan monolaurate), and glycols such as ethylene glycol and propylene glycol.

Such non-ionic surfactants may be selected to provide an appropriate amount of detergency for an assay without having a deleterious effect on assay reactions. In particular, surfactants may be included in a reaction mixture for the purpose of suppressing non-specific interactions among various ingredients of the aggregation assays of the invention. The non-ionic surfactants are typically added to the liquid sample prior in an amount from 0.01% (w/w) to 5% (w/w).

The non-ionic surfactants may be used in combination with one or more proteins (e.g., albumin, fish skin gelatin, lysozyme, or transferring) also added to the liquid sample prior in an amount from 0.01% (w/w) to 5% (w/w).

Furthermore, the assays, methods, and cartridge units of the invention can include additional suitable buffer components (e.g., Tris base, selected to provide a pH of about 7.8 to 8.2 in the reaction milieu); and chelating agents to scavenge cations (e.g., EDTA disodium, ethylene diamine tetraacetic acid (EDTA), citric acid, tartaric acid, glucuronic acid, saccharic acid or suitable salts thereof).

Binding Moieties

In general, a binding moiety is a molecule, synthetic or natural, that specifically binds or otherwise links to, e.g., covalently or non-covalently binds to or hybridizes with, a

target molecule, or with another binding moiety (or, in certain embodiments, with an aggregation inducing molecule). For example, the binding moiety can be an antibody directed toward an antigen or any protein-protein interaction. Alternatively, the binding moiety can be a polysaccharide that binds to a corresponding target or a synthetic oligonucleotide that hybridizes to a specific complementary nucleic acid target. In certain embodiments, the binding moieties can be designed or selected to serve, when bound to another binding moiety, as substrates for a target molecule such as enzyme in solution.

Binding moieties include, for example, oligonucleotide binding moieties (DNA, RNA, or substituted or derivatized nucleotide substitutes), polypeptide binding moieties, antibody binding moieties, aptamers, and polysaccharide binding moieties.

Oligonucleotide Binding Moieties

In certain embodiments, the binding moieties are oligonucleotides, attached/linked to the magnetic particles using any of a variety of chemistries, by a single, e.g., covalent, bond, e.g., at the 3' or 5' end to a functional group on the magnetic particle. Such binding moieties can be used in the systems, devices, and methods of the invention to detect mutations (e.g., SNPs, translocations, large deletions, small deletions, insertions, substitutions) or to monitor gene expression (e.g., the presence of expression, or changes in the level of gene expression, monitoring RNA transcription), or CHP analysis characteristic of the presence of a pathogen, disease state, or the progression of disease.

An oligonucleotide binding moiety can be constructed using chemical synthesis. A double-stranded DNA binding moiety can be constructed by enzymatic ligation reactions using procedures known in the art. For example, a nucleic acid (e.g., an oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the complementary strands, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned.

One method uses at least two populations of oligonucleotide magnetic particles, each with strong effects on water (or other solvent) relaxation. As the oligonucleotide-magnetic particle conjugates react with a target oligonucleotide, they form aggregates (e.g., clusters of magnetic particles). Upon prolonged standing, e.g., overnight at room temperature, the aggregates form large clusters (micron-sized clusters). Using the methods of the invention, the formation of large clusters can be accomplished more quickly by employing multiple cycles of magnetic assisted agglomeration. Magnetic resonance is used to determine the relaxation properties of the solvent, which are altered when the mixture of magnetic oligonucleotide magnetic particles reacts with a target nucleic acid to form aggregates.

Certain embodiments employ a mixture of at least two types of magnetic metal oxide magnetic particles, each with a specific sequence of oligonucleotide, and each with more than one copy of the oligonucleotide attached, e.g., covalently, per magnetic particle. For example, the assay protocol may involve preparing a mixture of populations of oligonucleotide-magnetic particle conjugates and reacting the mixture with a target nucleic acid. Alternatively, oligonucleotide-magnetic particle conjugates can be reacted with the target in a sequential fashion. Certain embodiments feature the use of magnetic resonance to detect the reaction of the oligonucleotide-magnetic particle conjugates with the target

nucleic acid. When a target is present, the dispersed conjugates self-assemble to form small aggregates.

For example, oligonucleotide binding moieties can be linked to the metal oxide through covalent attachment to a functionalized polymer or to non-polymeric surface-functionalized metal oxides. In the latter method, the magnetic particles can be synthesized according to the method of Albrecht et al., *Biochimie*, 80:379 (1998). Dimercapto-succinic acid is coupled to the iron oxide and provides a carboxyl functional group.

In certain embodiments, oligonucleotides are attached to magnetic particles via a functionalized polymer associated with the metal oxide. In some embodiments, the polymer is hydrophilic. In certain embodiments, the conjugates are made using oligonucleotides that have terminal amino, sulfhydryl, or phosphate groups, and superparamagnetic iron oxide magnetic particles bearing amino or carboxy groups on a hydrophilic polymer. There are several methods for synthesizing carboxy and amino derivatized-magnetic particles.

In one embodiment, oligonucleotides are attached to a particle via ligand-protein binding interaction, such as biotin-streptavidin, where the ligand is covalently attached to the oligonucleotide and the protein to the particle, or vice versa. This approach can allow for more rapid reagent preparation.

Polypeptide Binding Moieties

In certain embodiments, the binding moiety is a polypeptide (i.e., a protein, polypeptide, or peptide), attached, using any of a variety of chemistries, by a single covalent bond in such a manner so as to not affect the biological activity of the polypeptide. In one embodiment, attachment is done through the thiol group of single reactive cysteine residue so placed that its modification does not affect the biological activity of the polypeptide. In this regard the use of linear polypeptides, with cysteine at the C-terminal or N-terminal end, provides a single thiol in a manner similar to which alkanethiol supplies a thiol group at the 3' or 5' end of an oligonucleotide. Similar bifunctional conjugation reagents, such as SPDP and reacting with the amino group of the magnetic particle and thiol group of the polypeptide, can be used with any thiol bearing binding moiety. The types of polypeptides used as binding moieties can be antibodies, antibody fragments, and natural and synthetic polypeptide sequences. The peptide binding moieties have a binding partner, that is, a molecule to which they selectively bind.

Use of peptides as binding moieties offers several advantages. For example, polypeptides can be engineered to have uniquely reactive residues, distal from the residues required for biological activity, for attachment to the magnetic particle. The reactive residue can be a cysteine thiol, an N-terminal amino group, a C-terminal carboxyl group or a carboxyl group of aspartate or glutamate, etc. A single reactive residue on the peptide is used to insure a unique site of attachment. These design principles can be followed with chemically synthesized peptides or biologically produced polypeptides.

The binding moieties can also contain amino acid sequences from naturally occurring (wild-type) polypeptides or proteins. For example, the natural polypeptide may be a hormone, (e.g., a cytokine, a growth factor), a serum protein, a viral protein (e.g., hemagglutinin), an extracellular matrix protein, a lectin, or an ectodomain of a cell surface protein. Another example is a ligand binding protein, such as streptavidin or avidin that bind biotin. In general, the resulting binding moiety-magnetic particle is used to measure the presence of analytes in a test media reacting with the binding moiety.

Additionally, a polypeptide binding moiety can be used in a universal reagent configuration, where the target of the

binding moiety (e.g., small molecule, ligand, or binding partner) is pre-attached to the target analyte to create a labeled analyte that, in the presence of the polypeptide decorated particles, induces clustering.

Examples of protein hormones which can be utilized as binding moieties include, without limitation, platelet-derived growth factor (PDGF), which binds the PDGF receptor; insulin-like growth factor-I and -II (Igf), which binds the Igf receptor; nerve growth factor (NGF), which binds the NGF receptor; fibroblast growth factor (FGF), which binds the FGF receptor (e.g., aFGF and bFGF); epidermal growth factor (EGF), which binds the EGF receptor; transforming growth factor (TGF, e.g., TGF α and TGF- β), which bind the TGF receptor; erythropoietin, which binds the erythropoietin receptor; growth hormone (e.g., human growth hormone), which binds the growth hormone receptor; and proinsulin, insulin, A-chain insulin, and B-chain insulin, which all bind to the insulin receptor.

Receptor binding moieties are useful for detecting and imaging receptor clustering on the surface of a cell. Useful ectodomains include those of the Notch protein, Delta protein, integrins, cadherins, and other cell adhesion molecules.

Antibody Binding Moieties

Other polypeptide binding moieties include immunoglobulin binding moieties that include at least one immunoglobulin domain, and typically at least two such domains. An "immunoglobulin domain" refers to a domain of an antibody molecule, e.g., a variable or constant domain. An "immunoglobulin superfamily domain" refers to a domain that has a three-dimensional structure related to an immunoglobulin domain, but is from a non-immunoglobulin molecule. Immunoglobulin domains and immunoglobulin superfamily domains typically include two β -sheets formed of about seven β -strands, and a conserved disulphide bond (see, e.g., Williams and Barclay *Ann. Rev. Immunol.*, 6:381 (1988)). Proteins that include domains of the Ig superfamily domains include T cell receptors, CD4, platelet derived growth factor receptor (PDGFR), and intercellular adhesion molecule (ICAM).

One type of immunoglobulin binding moiety is an antibody. The term "antibody," as used herein, refers to a full-length, two-chain immunoglobulin molecule and an antigen-binding portion and fragments thereof, including synthetic variants. A typical antibody includes two heavy (H) chain variable regions (abbreviated herein as VH), and two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDR's has been precisely defined (see, Kabat, E. A., et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Each VH and VL is composed of three CDR's and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

An antibody can also include a constant region as part of a light or heavy chain. Light chains can include a kappa or lambda constant region gene at the COOH-terminus (termed CL). Heavy chains can include, for example, a gamma constant region (IgG1, IgG2, IgG3, IgG4; encoding about 330 amino acids). A gamma constant region can include, e.g., CH1, CH2, and CH3. The term "full-length antibody" refers

to a protein that includes one polypeptide that includes VL and CL, and a second polypeptide that includes VH, CH1, CH2, and CH3.

The term "antigen-binding fragment" of an antibody, as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target. Examples of antigen-binding fragments include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., *Nature* 341:544 (1989)), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al., *Science* 242:423 (1988); and Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879 (1988)). Such single chain antibodies are also encompassed within the term "antigen-binding fragment."

A single domain antibody (sdAb, nanobody) is an antibody fragment consisting of a single monomeric variable antibody domain, and may also be used in the systems and methods of the invention. Like a whole antibody, sdAbs are able to bind selectively to a specific antigen. With a molecular weight of only 12-15 kDa, single domain antibodies are much smaller than common antibodies (150-160 kDa) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa, two variable domains, one from a light and one from a heavy chain).

Polysaccharide Binding Moieties

In certain embodiments, the binding moiety is a polysaccharide, linked, for example, using any of a variety of chemistries, by a single bond, e.g., a covalent bond, at one of the two ends, to a functional group on the magnetic particle. The polysaccharides can be synthetic or natural. Mono-, di-, tri- and polysaccharides can be used as the binding moiety. These include, e.g., glycosides, N-glycosylamines, O-acyl derivatives, O-methyl derivatives, osazones, sugar alcohols, sugar acids, sugar phosphates when used with appropriate attachment chemistry to the magnetic particle.

A method of accomplishing linking is to couple avidin to a magnetic particle and react the avidin-magnetic particle with commercially available biotinylated polysaccharides, to yield polysaccharide-magnetic particle conjugates. For example, sialyl Lewis based polysaccharides are commercially available as biotinylated reagents and will react with avidin-CLIO (see Syntosome, Gesellschaft für medizinische Biochemie mbH.). The sialyl Lewis x tetrasaccharide (Sle^x) is recognized by proteins known as selecting, which are present on the surfaces of leukocytes and function as part of the inflammatory cascade for the recruitment of leukocytes.

Still other targeting moieties include a non-proteinaceous element, e.g., a glycosyl modification (such as a Lewis antigen) or another non-proteinaceous organic molecule. Another method is covalent coupling of the protein to the magnetic particle.

Another feature of the methods includes identification of specific cell types, for hematological or histopathological

investigations for example CD4/CD3 cell counts and circulating tumor cells using any of the binding moieties described above.

Multivalent Binding Agents

The assays of the invention can include a multivalent binding agent bearing multiple analytes are linked to a carrier (e.g., a simple synthetic scaffold, or a larger carrier protein or polysaccharide, such as BSA, transferrin, or dextran).

Where a multivalent binding agent is employed, multiple analytes are linked to a carrier (e.g., a simple synthetic scaffold, or a larger carrier protein or polysaccharide, such as BSA, transferrin, or dextran). Such a carrier is an agglomerant and the assay architecture is characterized by a competition between the analyte being detected and the multivalent binding agent (e.g., in an inhibition assay, competition assay, or disaggregation assay).

The functional group present in the analyte can be used to form a covalent bond with the carrier. Alternatively, the analyte can be derivatized to provide a linker (i.e., a spacer separating the analyte from the carrier in the conjugate) terminating in a functional group (i.e., an alcohol, an amine, a carboxyl group, a sulfhydryl group, or a phosphate group), which is used to form the covalent linkage with the carrier.

The covalent linking of an analyte and a carrier may be effected using a linker which contains reactive moieties capable of reaction with such functional groups present in the analyte and the carrier. For example, a hydroxyl group of the analyte may react with a carboxyl group of the linker, or an activated derivative thereof, resulting in the formation of an ester linking the two.

Examples of moieties capable of reaction with sulfhydryl groups include α -haloacetyl compounds of the type XCH_2CO- (where $X=Br, Cl$ or I), which show particular reactivity for sulfhydryl groups, but which can also be used to modify imidazolyl, thioether, phenol, and amino groups as described by Gurd, *Methods Enzymol.* 11:532 (1967). N-Maleimide derivatives are also considered selective towards sulfhydryl groups, but may additionally be useful in coupling to amino groups under certain conditions. Reagents such as 2-iminothiolane (Traut et al., *Biochemistry* 12:3266 (1973)), which introduce a thiol group through conversion of an amino group, may be considered as sulfhydryl reagents if linking occurs through the formation of disulphide bridges.

Examples of reactive moieties capable of reaction with amino groups include, for example, alkylating and acylating agents. Representative alkylating agents include:

(i) α -haloacetyl compounds, which show specificity towards amino groups in the absence of reactive thiol groups and are of the type XCH_2CO- (where $X=Cl, Br$ or I), for example, as described by Wong *Biochemistry* 24:5337 (1979); (ii) N-maleimide derivatives, which may react with amino groups either through a Michael type reaction or through acylation by addition to the ring carbonyl group, for example, as described by Smyth et al., *J. Am. Chem. Soc.* 82:4600 (1960) and *Biochem. J.* 91:589 (1964); (iii) aryl halides such as reactive nitrohaloaromatic compounds; (iv) alkyl halides, as described, for example, by McKenzie et al., *J. Protein Chem.* 7:581 (1988); (v) aldehydes and ketones capable of Schiff's base formation with amino groups, the adducts formed usually being stabilized through reduction to give a stable amine; (vi) epoxide derivatives such as epichlorohydrin and bisoxiranes, which may react with amino, sulfhydryl, or phenolic hydroxyl groups; (vii) chlorine-containing derivatives of s-triazines, which are very reactive towards nucleophiles such as amino, sulfhydryl, and hydroxyl groups; (viii) aziridines based on s-triazine compounds detailed above, e.g., as described by Ross, *J. Adv. Cancer Res.* 2:1 (1954), which

react with nucleophiles such as amino groups by ring opening; (ix) squaric acid diethyl esters as described by Tietze, Chem. Ber. 124:1215 (1991); and (x) α -haloalkyl ethers, which are more reactive alkylating agents than normal alkyl halides because of the activation caused by the ether oxygen atom, as described by Benneche et al., Eur. J. Med. Chem. 28:463 (1993).

Representative amino-reactive acylating agents include: (i) isocyanates and isothiocyanates, particularly aromatic derivatives, which form stable urea and thiourea derivatives respectively; (ii) sulfonyl chlorides, which have been described by Herzig et al., Biopolymers 2:349 (1964); (iii) acid halides; (iv) active esters such as nitrophenylesters or N-hydroxysuccinimidyl esters; (v) acid anhydrides such as mixed, symmetrical, or N-carboxyanhydrides; (vi) other useful reagents for amide bond formation, for example, as described by M. Bodansky, Principles of Peptide Synthesis, Springer-Verlag, 1984; (vii) acylazides, e.g. wherein the azide group is generated from a preformed hydrazide derivative using sodium nitrite, as described by Wetz et al., Anal. Biochem. 58:347 (1974); and (viii) imidoesters, which form stable amidines on reaction with amino groups, for example, as described by Hunter and Ludwig, J. Am. Chem. Soc. 84:3491 (1962). Aldehydes and ketones may be reacted with amines to form Schiff's bases, which may advantageously be stabilized through reductive amination. Alkoxyamino moieties readily react with ketones and aldehydes to produce stable alkoxamines, for example, as described by Webb et al., in Bioconjugate Chem. 1:96 (1990).

Examples of reactive moieties capable of reaction with carboxyl groups include diazo compounds such as diazoacetate esters and diazoacetamides, which react with high specificity to generate ester groups, for example, as described by Herriot, Adv. Protein Chem. 3:169 (1947). Carboxyl modifying reagents such as carbodiimides, which react through O-acylurea formation followed by amide bond formation, may also be employed.

It will be appreciated that functional groups in the analyte and/or the carrier may, if desired, be converted to other functional groups prior to reaction, for example, to confer additional reactivity or selectivity. Examples of methods useful for this purpose include conversion of amines to carboxyls using reagents such as dicarboxylic anhydrides; conversion of amines to thiols using reagents such as N-acetylhomocysteine thiolactone, S-acetylmercaptosuccinic anhydride, 2-iminothiolane, or thiol-containing succinimidyl derivatives; conversion of thiols to carboxyls using reagents such as α -haloacetates; conversion of thiols to amines using reagents such as ethylenimine or 2-bromoethylamine; conversion of carboxyls to amines using reagents such as carbodiimides followed by diamines; and conversion of alcohols to thiols using reagents such as tosyl chloride followed by transesterification with thioacetate and hydrolysis to the thiol with sodium acetate. When the C16 and C17 positions of the analyte both have hydroxy substituents, these hydroxy groups, together a vicinal diol, can be converted into a cyclic acetal as described by, for example, J. March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure, John Wiley & Sons, Inc. pp. 889-890, 1992. The acetal can include a reactive group (e.g., an amino or carboxyl group) capable of forming a bond with a carrier.

So-called zero-length linkers, involving direct covalent joining of a reactive chemical group of the analyte with a reactive chemical group of the carrier without introducing additional linking material may, if desired, be used in accordance with the invention. Most commonly, however, the linker will include two or more reactive moieties, as described

above, connected by a spacer element. The presence of such a spacer permits bifunctional linkers to react with specific functional groups within the analyte and the carrier, resulting in a covalent linkage between the two. The reactive moieties in a linker may be the same (homobifunctional linker) or different (heterobifunctional linker, or, where several dissimilar reactive moieties are present, heteromultifunctional linker), providing a diversity of potential reagents that may bring about covalent attachment between the analyte and the carrier.

Spacer elements in the linker typically consist of linear or branched chains and may include a C_{1-10} alkyl, a heteroalkyl of 1 to 10 atoms, a C_{2-10} alkene, a C_{2-10} alkyne, C_{5-10} aryl, a cyclic system of 3 to 10 atoms, or $-(CH_2CH_2O)_n$ CH_2CH_2- , in which n is 1 to 4.

Typically, a multivalent binding agent will include 2, 3, 4, 5, 6, 7, 8, 15, 50, or 100 (e.g., from 3 to 100, from 3 to 30, from 4 to 25, or from 6 to 20) conjugated analytes. The multivalent binding agents are typically from 10 kDa to 200 kDa in size and can be prepared as described in the Examples.

Analytes

Embodiments of the invention include devices, systems, and/or methods for detecting and/or measuring the concentration of one or more analytes in a sample (e.g., a protein, a peptide, an enzyme, a polypeptide, an amino acid, a nucleic acid, an oligonucleotide, a therapeutic agent, a metabolite of a therapeutic agent, RNA, DNA, an antibody, an organism, a virus, bacteria, a carbohydrate, a polysaccharide, glucose, a lipid, a gas (e.g., oxygen, carbon dioxide), an electrolyte (e.g., sodium, potassium, chloride, bicarbonate, BUN, magnesium, phosphate, calcium, ammonia, lactate), general chemistry molecules (creatinine, glucose), a lipoprotein, cholesterol, a fatty acid, a glycoprotein, a proteoglycan, and/or a lipopolysaccharide). The analytes may include identification of cells or specific cell types. The analyte(s) may include one or more biologically active substances and/or metabolite(s), marker(s), and/or other indicator(s) of biologically active substances. A biologically active substance may be described as a single entity or a combination of entities. The term "biologically active substance" includes without limitation, medications; vitamins; mineral supplements; substances used for the treatment, prevention, diagnosis, cure or mitigation of disease or illness; or substances which affect the structure or function of the body; or pro-drugs, which become biologically active or more active after they have been placed in a predetermined physiological environment; or biologically toxic agents such as those used in biowarfare including organisms such as anthrax, ebola, Marburg virus, plague, cholera, tularemia, brucellosis, Q fever, Bolivian hemorrhagic fever, *Coccidioides mycosis*, glanders, *Melioidosis*, *Shigella*, Rocky Mountain spotted fever, typhus, *Psittacosis*, yellow fever, Japanese B encephalitis, Rift Valley fever, and smallpox; naturally-occurring toxins that can be used as weapons include ricin, SEB, botulism toxin, saxitoxin, and many mycotoxins. Analytes may also include organisms such as *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Coagulase negative Staphylococcus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Aspergillus fumigatus*, *Bacteroides fragilis*, *Bacteroides fragilis*, blaSHV, *Burkholderia cepacia*, *Campylobacter jejuni/coli*, *Candida guilliermondii*, *Candida lusitanae*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacteriaceae* spp., *Haemophilus influenza*, *Kingella kingae*, *Klebsiella oxytoca*, *Listeria monocytogenes*, *Mec A* gene-bearing bacteria (MRSA), *Morganella*

morgana, *Neisseria meningitidis*, *Neisseria* spp., Non-meningitidis, *Prevotella buccae*, *Prevotella intermedia*, *Prevotella melaminogenica*, *Propionibacterium acnes*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella enteric*, *Serratia marcescens*, *Staphylococcus haemolyticus*, *Staphylococcus maltophilia*, *Staphylococcus saprophyticus*, *Stenotrophomonas maltophilia*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus bovis*, *Streptococcus dysgalactiae*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus sanguinis*, Van A gene, Van B gene. Analytes may also include viral organisms such as dsDNA viruses (e.g., adenoviruses, herpes viruses, poxviruses); ssDNA viruses (+)sense DNA (e.g., parvoviruses); dsRNA viruses (e.g., reoviruses); (+)ssRNA viruses (+)sense RNA (e.g., picornaviruses, togaviruses); (–)ssRNA viruses (–)sense RNA (e.g., orthomyxoviruses, rhabdoviruses); ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g., retroviruses); and dsDNA-RT viruses (e.g., hepadnaviruses).

Opportunistic infections which can be detected using the systems and methods of the invention include, without limitation, fungal, viral, bacterial, protozoan infections, such as: 1) fungal infections, such as those by *Candida* spp. (drug resistant and non-resistant strains), *C. albicans*, *C. krusei*, *C. glabrata*, and *Aspergillus fumigatus*; 2) gram negative infections, such as those by *E. coli*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae/oxytoca*, and *Pseudomonas aeruginosa*; and 3) gram positive infections, such as those by *Staphylococcus* spp., *S. aureus*, *S. pneumoniae*, *Enterococcus faecalis*, and *E. faecium*. The infection can be by coagulase negative *staphylococcus*, *comybacterium* spp., *Fusobacterium* spp., *Morganella morganii*, *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii*), *F. hominis*, *S. pyogenes*, *Pseudomonas aeruginosa*, polyomavirus JC polyomavirus (the virus that causes Progressive multifocal leukoencephalopathy), *Acinetobacter baumannii*, *Toxoplasma gondii*, cytomegalovirus, *Aspergillus* spp., Kaposi's Sarcoma, *cryptosporidium* spp., *Cryptococcus neoformans*, and *Histoplasma capsulatum*.

Non-limiting examples of broad categories of analytes which can be detected using the devices, systems, and methods of the invention include, without limitation, the following therapeutic categories: anabolic agents, antacids, anti-asthmatic agents, anti-cholesterolemic and anti-lipid agents, anticoagulants, anti-convulsants, anti-diarrheals, anti-emetics, anti-infective agents, anti-inflammatory agents, anti-manic agents, anti-nauseants, anti-neoplastic agents, anti-obesity agents, anti-pyretic and analgesic agents, anti-spasmodic agents, anti-thrombotic agents, anti-uricemic agents, anti-anginal agents, antihistamines, anti-tussives, appetite suppressants, biologicals, cerebral dilators, coronary dilators, decongestants, diuretics, diagnostic agents, erythropoietic agents, expectorants, gastrointestinal sedatives, hyperglycemic agents, hypnotics, hypoglycemic agents, ion exchange resins, laxatives, mineral supplements, mucolytic agents, neuromuscular drugs, peripheral vasodilators, psychotropics, sedatives, stimulants, thyroid and anti-thyroid agents, uterine relaxants, vitamins, and prodrugs.

More specifically, non-limiting examples of analytes which can be detected using the devices, systems, and methods of the invention include, without limitation, the following therapeutic categories: analgesics, such as nonsteroidal anti-inflammatory drugs, opiate agonists and salicylates; antihistamines, such as H₁-blockers and H₂-blockers; anti-infective agents, such as anthelmintics, antianaerobics, antibiotics, aminoglycoside antibiotics, antifungal antibiotics, cephalosporin antibiotics, macrolide antibiotics, miscellaneous

β-lactam antibiotics, penicillin antibiotics, quinolone antibiotics, sulfonamide antibiotics, tetracycline antibiotics, antimycobacterials, antituberculosis antimycobacterials, antiprotozoals, antimalarial antiprotozoals, antiviral agents, antiretroviral agents, scabicides, and urinary anti-infectives; antineoplastic agents, such as alkylating agents, nitrogen mustard alkylating agents, nitrosourea alkylating agents, antimetabolites, purine analog antimetabolites, pyrimidine analog antimetabolites, hormonal antineoplastics, natural antineoplastics, antibiotic natural antineoplastics, and vinca alkaloid natural antineoplastics; autonomic agents, such as anticholinergics, antimuscarinic anticholinergics, ergot alkaloids, parasympathomimetics, cholinergic agonist parasympathomimetics, cholinesterase inhibitor parasympathomimetics, sympatholytics, alpha-blocker sympatholytics, beta-blocker sympatholytics, sympathomimetics, and adrenergic agonist sympathomimetics; cardiovascular agents, such as antianginals, beta-blocker antianginals, calcium-channel blocker antianginals, nitrate antianginals, antiarrhythmics, cardiac glycoside antiarrhythmics, class I antiarrhythmics, class II antiarrhythmics, class III antiarrhythmics, class IV antiarrhythmics, antihypertensive agents, alpha-blocker antihypertensives, angiotensin-converting enzyme inhibitor (ACE inhibitor) antihypertensives, beta-blocker antihypertensives, calcium-channel blocker antihypertensives, central-acting adrenergic antihypertensives, diuretic antihypertensive agents, peripheral vasodilator antihypertensives, antilipemics, bile acid sequestrant antilipemics, HMG-CoA reductase inhibitor antilipemics, inotropes, cardiac glycoside inotropes, and thrombolytic agents; dermatological agents, such as antihistamines, anti-inflammatory agents, corticosteroid anti-inflammatory agents, antipruritics/local anesthetics, topical anti-infectives, antifungal topical anti-infectives, antiviral topical anti-infectives, and topical antineoplastics; electrolytic and renal agents, such as acidifying agents, alkalizing agents, diuretics, carbonic anhydrase inhibitor diuretics, loop diuretics, osmotic diuretics, potassium-sparing diuretics, thiazide diuretics, electrolyte replacements, and uricosuric agents; enzymes, such as pancreatic enzymes and thrombolytic enzymes; gastrointestinal agents, such as antidiarrheals, antiemetics, gastrointestinal anti-inflammatory agents, salicylate gastrointestinal anti-inflammatory agents, antacid anti-ulcer agents, gastric acid-pump inhibitor anti-ulcer agents, gastric mucosal anti-ulcer agents, H₂-blocker anti-ulcer agents, cholelitholytic agents, digestants, emetics, laxatives and stool softeners, and prokinetic agents; general anesthetics, such as inhalation anesthetics, halogenated inhalation anesthetics, intravenous anesthetics, barbiturate intravenous anesthetics, benzodiazepine intravenous anesthetics, and opiate agonist intravenous anesthetics; hematological agents, such as antianemia agents, hematopoietic antianemia agents, coagulation agents, anticoagulants, hemostatic coagulation agents, platelet inhibitor coagulation agents, thrombolytic enzyme coagulation agents, and plasma volume expanders; hormones and hormone modifiers, such as abortifacients, adrenal agents, corticosteroid adrenal agents, androgens, anti-androgens, antidiabetic agents, sulfonylurea antidiabetic agents, antihypoglycemic agents, oral contraceptives, progestin contraceptives, estrogens, fertility agents, oxytocics, parathyroid agents, pituitary hormones, progestins, antithyroid agents, thyroid hormones, and tocotryls; immunobiologic agents, such as immunoglobulins, immunosuppressives, toxoids, and vaccines; local anesthetics, such as amide local anesthetics and ester local anesthetics; musculoskeletal agents, such as anti-gout anti-inflammatory agents, corticosteroid anti-inflammatory agents, gold compound anti-inflammatory agents, immuno-suppressive

anti-inflammatory agents, nonsteroidal anti-inflammatory drugs (NSAIDs), salicylate anti-inflammatory agents, skeletal muscle relaxants, neuromuscular blocker skeletal muscle relaxants, and reverse neuromuscular blocker skeletal muscle relaxants; neurological agents, such as anticonvulsants, barbiturate anticonvulsants, benzodiazepine anticonvulsants, anti-migraine agents, anti-parkinsonian agents, anti-vertigo agents, opiate agonists, and opiate antagonists; ophthalmic agents, such as anti-glaucoma agents, beta-blocker anti-glaucoma agents, miotic anti-glaucoma agents, mydriatics, adrenergic agonist mydriatics, antimuscarinic mydriatics, ophthalmic anesthetics, ophthalmic anti-infectives, ophthalmic aminoglycoside anti-infectives, ophthalmic macrolide anti-infectives, ophthalmic quinolone anti-infectives, ophthalmic sulfonamide anti-infectives, ophthalmic tetracycline anti-infectives, ophthalmic anti-inflammatory agents, ophthalmic corticosteroid anti-inflammatory agents, and ophthalmic nonsteroidal anti-inflammatory drugs (NSAIDs); psychotropic agents, such as antidepressants, heterocyclic antidepressants, monoamine oxidase inhibitors (MAOIs), selective serotonin re-uptake inhibitors (SSRIs), tricyclic antidepressants, antimanics, antipsychotics, phenothiazine antipsychotics, anxiolytics, sedatives, and hypnotics, barbiturate sedatives and hypnotics, benzodiazepine anxiolytics, sedatives, and hypnotics, and psychostimulants; respiratory agents, such as antitussives, bronchodilators, adrenergic agonist bronchodilators, antimuscarinic bronchodilators, expectorants, mucolytic agents, respiratory anti-inflammatory agents, and respiratory corticosteroid anti-inflammatory agents; toxicology agents, such as antidotes, heavy metal antagonists/chelating agents, substance abuse agents, detergent substance abuse agents, and withdrawal substance abuse agents; minerals; and vitamins, such as vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, and vitamin K.

Examples of classes of biologically active substances from the above categories which can be detected using the devices, systems, and methods of the invention include, without limitation, nonsteroidal anti-inflammatory drugs (NSAIDs) analgesics, such as diclofenac, ibuprofen, ketoprofen, and naproxen; opiate agonist analgesics, such as codeine, fentanyl, hydromorphone, and morphine; salicylate analgesics, such as aspirin (ASA) (enteric coated ASA); H₁-blocker antihistamines, such as clemastine and terfenadine; H₂-blocker antihistamines, such as cimetidine, famotidine, nizatidine, and ranitidine; anti-infective agents, such as mupirocin; anti-anaerobic anti-infectives, such as chloramphenicol and clindamycin; antifungal antibiotic anti-infectives, such as amphotericin b, clotrimazole, fluconazole, and ketoconazole; macrolide antibiotic anti-infectives, such as azithromycin and erythromycin; miscellaneous beta-lactam antibiotic anti-infectives, such as aztreonam and imipenem; penicillin antibiotic anti-infectives, such as nafcillin, oxacillin, penicillin G, and penicillin V; quinolone antibiotic anti-infectives, such as ciprofloxacin and norfloxacin; tetracycline antibiotic anti-infectives, such as doxycycline, minocycline, and tetracycline; antituberculosis antimycobacterial anti-infectives such as isoniazid (INH), and rifampin; antiprotozoal anti-infectives, such as atovaquone and dapsone; antimalarial antiprotozoal anti-infectives, such as chloroquine and pyrimethamine; anti-retroviral anti-infectives, such as ritonavir and zidovudine; antiviral anti-infective agents, such as acyclovir, ganciclovir, interferon alfa, and rimantadine; alkylating antineoplastic agents, such as carboplatin and cisplatin; nitrosourea alkylating antineoplastic agents, such as carmustine (BCNU); antimetabolite antineoplastic agents, such as methotrexate; pyrimidine analog antimetabolite antineoplastic agents, such as fluorouracil (5-FU) and gemcitab-

ine; hormonal antineoplastics, such as goserelin, leuprolide, and tamoxifen; natural antineoplastics, such as aldesleukin, interleukin-2, docetaxel, etoposide (VP-16), interferon alfa, paclitaxel, and tretinoin (ATRA); antibiotic natural antineoplastics, such as bleomycin, dactinomycin, daunorubicin, doxorubicin, and mitomycin; vinca alkaloid natural antineoplastics, such as vinblastine and vincristine; autonomic agents, such as nicotine; anticholinergic autonomic agents, such as benztropine and trihexyphenidyl; antimuscarinic anticholinergic autonomic agents, such as atropine and oxybutynin; ergot alkaloid autonomic agents, such as bromocriptine; cholinergic agonist parasympathomimetics, such as pilocarpine; cholinesterase inhibitor parasympathomimetics, such as pyridostigmine; alpha-blocker sympatholytics, such as prazosin; 9-blocker sympatholytics, such as atenolol; adrenergic agonist sympathomimetics, such as albuterol and dobutamine; cardiovascular agents, such as aspirin (ASA) (enteric coated ASA); i-blocker antianginals, such as atenolol and propranolol; calcium-channel blocker antianginals, such as nifedipine and verapamil; nitrate antianginals, such as isosorbide dinitrate (ISDN); cardiac glycoside antiarrhythmics, such as digoxin; class I antiarrhythmics, such as lidocaine, mexiletine, phenytoin, procainamide, and quinidine; class II antiarrhythmics, such as atenolol, metoprolol, propranolol, and timolol; class III antiarrhythmics, such as amiodarone; class IV antiarrhythmics, such as diltiazem and verapamil; alpha-blocker antihypertensives, such as prazosin; angiotensin-converting enzyme inhibitor (ACE inhibitor) antihypertensives, such as captopril and enalapril; beta-blocker antihypertensives, such as atenolol, metoprolol, nadolol, and propranolol; calcium-channel blocker antihypertensive agents, such as diltiazem and nifedipine; central-acting adrenergic antihypertensives, such as clonidine and methyldopa; diuretic antihypertensive agents, such as amiloride, furosemide, hydrochlorothiazide (HCTZ), and spironolactone; peripheral vasodilator antihypertensives, such as hydralazine and minoxidil; antilipemics, such as gemfibrozil and probucol; bile acid sequestrant antilipemics, such as cholestyramine; HMG-CoA reductase inhibitor antilipemics, such as lovastatin and pravastatin; inotropes, such as amilone, dobutamine, and dopamine; cardiac glycoside inotropes, such as digoxin; thrombolytic agents, such as alteplase (TPA), anistreplase, streptokinase, and urokinase; dermatological agents, such as colchicine, isotretinoin, methotrexate, minoxidil, tretinoin (ATRA); dermatological corticosteroid anti-inflammatory agents, such as betamethasone and dexamethasone; antifungal topical anti-infectives, such as amphotericin B, clotrimazole, miconazole, and nystatin; antiviral topical anti-infectives, such as acyclovir; topical antineoplastics, such as fluorouracil (5-FU); electrolytic and renal agents, such as lactulose; loop diuretics, such as furosemide; potassium-sparing diuretics, such as triamterene; thiazide diuretics, such as hydrochlorothiazide (HCTZ); uricosuric agents, such as probenecid; enzymes such as RNase and DNase; thrombolytic enzymes, such as alteplase, anistreplase, streptokinase and urokinase; antiemetics, such as prochlorperazine; salicylate gastrointestinal anti-inflammatory agents, such as sulfasalazine; gastric acid-pump inhibitor anti-ulcer agents, such as omeprazole; H₂-blocker anti-ulcer agents, such as cimetidine, famotidine, nizatidine, and ranitidine; digestants, such as pancrelipase; prokinetic agents, such as erythromycin; opiate agonist intravenous anesthetics such as fentanyl; hematopoietic antianemia agents, such as erythropoietin, filgrastim (G-CSF), and sargramostim (GM-CSF); coagulation agents, such as antihemophilic factors 1-10 (AHF 1-10); anticoagulants, such as warfarin; thrombolytic enzyme coagulation agents, such as alteplase, anistreplase,

streptokinase and urokinase; hormones and hormone modifiers, such as bromocriptine; abortifacients, such as methotrexate; antidiabetic agents, such as insulin; oral contraceptives, such as estrogen and progestin; progestin contraceptives, such as levonorgestrel and norgestrel; estrogens such as conjugated estrogens, diethylstilbestrol (DES), estrogen (estradiol, estrone, and estropipate); fertility agents, such as clomiphene, human chorionic gonadotropin (HCG), and menotropins; parathyroid agents such as calcitonin; pituitary hormones, such as desmopressin, goserelin, oxytocin, and vasopressin (ADH); progestins, such as medroxyprogesterone, norethindrone, and progesterone; thyroid hormones, such as levothyroxine; immunobiologic agents, such as interferon beta-1b and interferon gamma-1b; immunoglobulins, such as immune globulin IM, IMiG, IGIM and immune globulin IV, IViG, IGIV; amide local anesthetics, such as lidocaine; ester local anesthetics, such as benzocaine and procaine; musculoskeletal corticosteroid anti-inflammatory agents, such as beclomethasone, betamethasone, cortisone, dexamethasone, hydrocortisone, and prednisone; musculoskeletal anti-inflammatory immunosuppressives, such as azathioprine, cyclophosphamide, and methotrexate; musculoskeletal nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, ibuprofen, ketoprofen, ketorlac, and naproxen; skeletal muscle relaxants, such as baclofen, cyclobenzaprine, and diazepam; reverse neuromuscular blocker skeletal muscle relaxants, such as pyridostigmine; neurological agents, such as nimodipine, riluzole, tacrine and ticlopidine; anticonvulsants, such as carbamazepine, gabapentin, lamotrigine, phenytoin, and valproic acid; barbiturate anticonvulsants, such as phenobarbital and primidone; benzodiazepine anticonvulsants, such as clonazepam, diazepam, and lorazepam; anti-parkinsonian agents, such as bromocriptine, levodopa, carbidopa, and pergolide; anti-vertigo agents, such as meclizine; opiate agonists, such as codeine, fentanyl, hydromorphone, methadone, and morphine; opiate antagonists, such as naloxone; beta-blocker anti-glaucoma agents, such as timolol; miotic anti-glaucoma agents, such as pilocarpine; ophthalmic aminoglycoside anti-infectives, such as gentamicin, neomycin, and tobramycin; ophthalmic quinolone anti-infectives, such as ciprofloxacin, norfloxacin, and ofloxacin; ophthalmic corticosteroid anti-inflammatory agents, such as dexamethasone and prednisolone; ophthalmic nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac; antipsychotics, such as clozapine, haloperidol, and risperidone; benzodiazepine anxiolytics, sedatives and hypnotics, such as clonazepam, diazepam, lorazepam, oxazepam, and prazepam; psychostimulants, such as methylphenidate and pemoline; antitussives, such as codeine; bronchodilators, such as theophylline; adrenergic agonist bronchodilators, such as albuterol; respiratory corticosteroid anti-inflammatory agents, such as dexamethasone; antidotes, such as flumazenil and naloxone; heavy metal antagonists/chelating agents, such as penicillamine; deterrent substance abuse agents, such as disulfiram, naltrexone, and nicotine; withdrawal substance abuse agents, such as bromocriptine; minerals, such as iron, calcium, and magnesium; vitamin B compounds, such as cyanocobalamin (vitamin B₁₂) and niacin (vitamin B₃); vitamin C compounds, such as ascorbic acid; and vitamin D compounds, such as calcitriol; recombinant beta-glucan; bovine immunoglobulin concentrate; bovine superoxide dismutase; the formulation comprising fluorouracil, epinephrine, and bovine collagen; recombinant hirudin (r-Hir), HIV-1 immunogen; human anti-TAC antibody; recombinant human growth hormone (r-hGH); recombinant human hemoglobin (r-Hb); recombinant human mectasermin (r-IGF-1); recombinant interferon beta-1a;

lenograstim (G-CSF); olanzapine; recombinant thyroid stimulating hormone (r-TSH); topotecan; acyclovir sodium; aldesleukin; atenolol; bleomycin sulfate, human calcitonin; salmon calcitonin; carboplatin; carmustine; dactinomycin, daunorubicin HCl; docetaxel; doxorubicin HCl; epoetin alfa; etoposide (VP-16); fluorouracil (5-FU); ganciclovir sodium; gentamicin sulfate; interferon alfa; leuprolide acetate; mepiridine HCl; methadone HCl; methotrexate sodium; paclitaxel; ranitidine HCl; vinblastin sulfate; and zidovudine (AZT).

Further specific examples of biologically active substances from the above categories which can be detected using the devices, systems, and methods of the invention include, without limitation, antineoplastics such as androgen inhibitors, antimetabolites, cytotoxic agents, and immunomodulators; anti-tussives such as dextromethorphan, dextromethorphan hydrobromide, noscapine, carbetapentane citrate, and chlorphedianol hydrochloride; antihistamines such as chlorpheniramine maleate, phenindamine tartrate, pyrilamine maleate, doxylamine succinate, and phenyltoloxamine citrate; decongestants such as phenylephrine hydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine hydrochloride, and ephedrine; various alkaloids such as codeine phosphate, codeine sulfate and morphine; mineral supplements such as potassium chloride, zinc chloride, calcium carbonates, magnesium oxide, and other alkali metal and alkaline earth metal salts; ion exchange resins such as cholestyramine; anti-arrhythmics such as N-acetylprocainamide; antipyretics and analgesics such as acetaminophen, aspirin and ibuprofen; appetite suppressants such as phenylpropanolamine hydrochloride or caffeine; expectorants such as guaifenesin; antacids such as aluminum hydroxide and magnesium hydroxide; biologicals such as peptides, polypeptides, proteins and amino acids, hormones, interferons or cytokines, and other bioactive peptidic compounds, such as interleukins 1-18 including mutants and analogues, RNase, DNase, luteinizing hormone releasing hormone (LHRH) and analogues, gonadotropin releasing hormone (GnRH), transforming growth factor-beta (TGF-beta), fibroblast growth factor (FGF), tumor necrosis factor-alpha & beta (TNF-alpha & beta), nerve growth factor (NGF), growth hormone releasing factor (GHRF), epidermal growth factor (EGF), fibroblast growth factor homologous factor (FGFHF), hepatocyte growth factor (HGF), insulin growth factor (IGF), invasion inhibiting factor-2 (IIF-2), bone morphogenetic proteins 1-7 (BMP 1-7), somatostatin, thymosin- α -1, T-globulin, superoxide dismutase (SOD), complement factors, hGH, tPA, calcitonin, ANF, EPO and insulin; and anti-infective agents such as antifungals, anti-virals, antiseptics and antibiotics.

Biologically active substances which can be detected using the devices, systems, and methods of the invention also include radiosensitizers, such as metoclopramide, sensamide or neusensamide (manufactured by Oxigene); proflomycin (made by Vion); RSR13 (made by Allos); Thymitaq (made by Agouron), etanidazole or lobenguane (manufactured by Nycomed); gadolinium texaphrin (made by Pharmacyclics); BuDR/Broxine (made by NeoPharm); IPdR (made by Sparta); CR2412 (made by Cell Therapeutic); L1X (made by Terrapin); or the like.

Biologically active substances which can be detected using the devices, systems, and methods of the invention include, without limitation, medications for the gastrointestinal tract or digestive system, for example, antacids, reflux suppressants, antiflatulents, antidopaminergics, proton pump inhibitors, H₂-receptor antagonists, cytoprotectants, prostaglandin analogues, laxatives, antispasmodics, antidiarrheals,

bile acid sequestrants, and opioids; medications for the cardiovascular system, for example, beta-receptor blockers, calcium channel blockers, diuretics, cardiac glycosides, antiarrhythmics, nitrate, antianginales, vasoconstrictors, vasodilators, peripheral activators, ACE inhibitors, angiotensin receptor blockers, alpha blockers, anticoagulants, heparin, HSGAGs, antiplatelet drugs, fibrinolytics, anti-hemophilic factors, haemostatic drugs, hypolipemic agents, and statins; medications for the central nervous system, for example, hypnotics, anaesthetics, antipsychotics, antidepressants, anti-emetics, anticonvulsants, antiepileptics, anxiolytics, barbiturates, movement disorder drugs, stimulants, benzodiazepine, cyclopyrrolone, dopamine antagonists, antihistamine, cholinergics, anticholinergics, emetics, cannabinoids, 5-HT antagonists; medications for pain and/or consciousness, for example, NSAIDs, opioids and orphans such as paracetamol, tricyclic antidepressants, and anticonvulsants; for musculo-skeletal disorders, for example, NSAIDs, muscle relaxants, and neuromuscular drug anticholinesterase; medications for the eye, for example, adrenergic neurone blockers, astringents, ocular lubricants, topical anesthetics, sympathomimetics, parasympatholytics, mydriatics, cycloplegics, antibiotics, topical antibiotics, sulfa drugs, aminoglycosides, fluoroquinolones, anti-virals, anti-fungals, imidazoles, polyenes, NSAIDs, corticosteroids, mast cell inhibitors, adrenergic agonists, beta-blockers, carbonic anhydrase inhibitors/hyperosmotics, cholinergics, miotics, parasympathomimetics, prostaglandin, agonists/prostaglandin inhibitors, nitroglycerin; medications for the ear, nose and oropharynx, for example, sympathomimetics, antihistamines, anticholinergics, NSAIDs, steroids, antiseptics, local anesthetics, antifungals, cerumenolytics; medications for the respiratory system, for example, bronchodilators, NSAIDs, anti-allergics, antitussives, mucolytics, decongestants, corticosteroids, beta-receptor antagonists, anticholinergics, steroids; medications for endocrine problems, for example, androgen, antiandrogen, gonadotropin, corticosteroids, growth hormone, insulin, antidiabetics, thyroid hormones, antithyroid drugs, calcitonin, diphosphonate, and vasopressin analogues; medications for the reproductive system or urinary system, for example, antifungals, alkalinising agents, quinolones, antibiotics, cholinergics, anticholinergics, anticholinesterase, antispasmodics, 5-alpha reductase inhibitor, selective alpha-1 blockers, and sildenafil; medications for contraception, for example, oral contraceptives, spermicides, and depot contraceptives; medications for obstetrics and gynecology, for example, NSAIDs, anticholinergics, haemostatic drugs, antifibrinolytics, hormone replacement therapy, bone regulator, beta-receptor agonists, follicle stimulating hormone, luteinising hormone, LHRH gamolenic acid, gonadotropin release inhibitor, progesterone, dopamine agonist, oestrogen, prostaglandin, gonadorelin, clomiphene, tamoxifen, and diethylstilbestrol; medications for the skin, for example, emollients, anti-pruritics, antifungals, disinfectants, scabicide, pediculicide, tar products, vitamin A derivatives, vitamin D analogue, keratolytics, abrasives, systemic antibiotics, topical antibiotics, hormones, desloughing agents, exudate absorbents, fibrinolytics, proteolytics, sunscreens, antiperspirants, and corticosteroids; medications for infections and infestations, for example, antibiotics, antifungals, antileprotics, antituberculous drugs, antimalarials, anthelmintics, amoebicide, antivirals, antiprotozoals, and antiserum; medications for the immune system, for example, vaccines, immunoglobulin, immunosuppressants, interferon, monoclonal antibodies; medications for allergic disorders, for example, anti-allergics, antihistamines, and NSAIDs; medications for nutrition, for example, tonics, iron prepara-

tions, electrolytes, vitamins, anti-obesity drugs, anabolic drugs, haematopoietic drugs, and food product drugs; medications for neoplastic disorders, for example, cytotoxic drugs, sex hormones, aromatase inhibitors, somatostatin inhibitors, recombinant interleukins, G-CSF, and erythropoietin; medications for diagnostics, for example, contrast agents; and medications for cancer (anti-cancer agents).

Examples of pain medications (e.g., analgesics) which can be detected using the devices, systems, and methods of the invention include opioids such as buprenorphine, butorphanol, dextropropoxyphene, dihydrocodeine, fentanyl, diamorphine (heroin), hydromorphone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, pethidine (meperidine), and tramadol; salicylic acid and derivatives such as acetylsalicylic acid (aspirin), diflunisal, and ethenzamide; pyrazolones such as aminophenazone, metamizole, and phenazone; anilides such as paracetamol (acetaminophen), phenacetin; and others such as ziconotide and tetradycannabinol.

Examples of blood pressure medications (e.g., antihypertensives and diuretics) which can be detected using the devices, systems, and methods of the invention include antidiurenergic agents such as clonidine, doxazosin, guanethidine, guanfacine, mecamlamine, methyl dopa, moxonidine, prazosin, rescinamine, and reserpine; vasodilators such as diazoxide, hydralazine, minoxidil, and nitroprusside; low ceiling diuretics such as bendroflumethiazide, chlorothiazide, chlortalidone, hydrochlorothiazide, indapamide, quinethazone, mersalyl, metolazone, and theobromine; high ceiling diuretics such as bumetanide, furosemide, and torasemide; potassium-sparing diuretics such as amiloride, eplerenone, spironolactone, and triamterene; and other antihypertensives such as bosentan and ketanserin.

Examples of anti-thrombotics (e.g., thrombolytics, anticoagulants, and antiplatelet drugs) which can be detected using the devices, systems, and methods of the invention include vitamin K antagonists such as acenocoumarol, clorindione, dicumarol, diphenadione, ethyl biscoumacetate, phenprocoumon, phenindione, tiocloamarol, and warfarin; heparin group (platelet aggregation inhibitors) such as antithrombin III, bemparin, dalteparin, danaparoid, enoxaparin, heparin, nadroparin, parnaparin, reviparin, sulodexide, and tinzaparin; other platelet aggregation inhibitors such as abciximab, acetylsalicylic acid (aspirin), aloxiprin, beraprost, diltazole, carbazate calcium, cloricromen, clopidogrel, dipyridamole, epoprostenol, eptifibatide, indobufen, iloprost, picotamide, prasugrel, ticlopidine, tirofiban, treprostinal, and triflusal; enzymes such as alteplase, aniclod, anistreplase, brinase, drotrecogin alfa, fibrinolysin, procein C, reteplase, saruplase, streptokinase, tenecteplase, and urokinase; direct thrombin inhibitors such as argatroban, bivalirudin, desirudin, lepirudin, melagatran, and ximelagatran; other antithrombotics such as dabigatran, defibrotide, dermatan sulfate, fondaparinux, and rivaroxaban; and others such as citrate, EDTA, and oxalate.

Examples of anticonvulsants which can be detected using the devices, systems, and methods of the invention include barbiturates such as barbexalone, metharbital, methylphenobarbital, phenobarbital, and primidone; hydantoins such as ethosuximide, fosphenytoin, mephenterol, and phenytoin; oxazolinediones such as ethadione, paramethadione, and trimethadione; succinimides such as ethosuximide, mesuximide, and phensuximide; benzodiazepines such as clobazam, clonazepam, clorazepate, diazepam, lorazepam, midazolam, and nitrazepam; carboxamides such as carbamazepine, oxcarbazepine, rufinamide; fatty acid derivatives such as valpromide and valnoctamide; carboxylic acids such as valproic acid, tiagabine; GABA analogs such as gabapentin, pregaba-

lin, progabide, and givabatin; monosaccharides such as topiramate; aromatic allylic alcohols such as stiripentol; ureas such as phenacemide and pheneturide; carbamates such as emylcamate, felbamate, and meprobamate; pyrrolidines such as brivaracetam, levetiracetam, nefiracetam, and seletracetam; sulfa drugs such as acetazolamide, ethoxzolamide, sulfatam, and zonisamide; propionates such as beclamide; aldehydes such as paraldehyde; and bromides such as potassium bromide.

Examples of anti-cancer agents which can be detected using the devices, systems, and methods of the invention include acivicin; aclarubicin; acodazole hydrochloride; acronine; adriamycin; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1 a; interferon gamma-1 b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; topotecan hydrochloride; toremifene citrate; trestolone acetate; tricitribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; Uracil mustard; rredopa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycin sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinstatin; and zorubicin hydrochloride.

Other biologically active substances which can be detected using the devices, systems, and methods of the invention include those mentioned in Basic and Clinical Pharmacology (LANGE Basic Science), Katzung and Katzung, ISBN 0071410929, McGraw-Hill Medical, 9th edition (2003).

Medical Conditions

Embodiments of the invention may be used in the monitoring of one or more analytes in the diagnosis, management, and/or treatment of any of a wide range of medical conditions. Various categories of medical conditions include, for example, disorders of pain; of alterations in body temperature (e.g., fever); of nervous system dysfunction (e.g., syncope, myalgias, movement disorders, numbness, sensory loss, delirium, dementia, memory loss, sleep disorders); of the eyes, ears, nose, and throat; of circulatory and/or respiratory functions (e.g., dyspnea, pulmonary edema, cough, hemoptysis, hypertension, myocardial infarctions, hypoxia, cyanosis, cardiovascular collapse, congestive heart failure, edema, shock); of gastrointestinal function (e.g., dysphagia, diarrhea, constipation, GI bleeding, jaundice, ascites, indigestion, nausea, vomiting); of renal and urinary tract function (e.g., acidosis, alkalosis, fluid and electrolyte imbalances, azotemia, urinary abnormalities); of sexual function and reproduction (e.g., erectile dysfunction, menstrual disturbances, hirsutism, virilization, infertility, pregnancy associated disorders and standard measurements); of the skin (e.g., eczema, psoriasis, acne, rosacea, cutaneous infection, immunological skin diseases, photosensitivity); of the blood (e.g., hematology); of genes (e.g., genetic disorders); of drug response (e.g., adverse drug responses); and of nutrition (e.g., obesity, eating disorders, nutritional assessment). Other medical fields with which embodiments of the invention find utility include oncology (e.g., neoplasms, malignancies, angiogenesis, paraneoplastic syndromes, oncologic emergencies); hematology (e.g., anemia, hemoglobinopathies, megaloblastic anemias, hemolytic anemias, aplastic anemia, myelodysplasia, bone marrow failure, polycythemia vera, myeloproliferative diseases, acute myeloid leukemia, chronic myeloid leukemia, lymphoid malignancies, plasma cell disorders, transfusion biology, transplants); hemostasis (e.g., disorders of coagulation and thrombosis, disorders of the platelet and vessel wall); and infectious diseases (e.g., sepsis, septic shock, fever of unknown origin, endocarditis, bites, burns, osteomyelitis, abscesses, food poisoning, pelvic inflammatory disease, bacterial (e.g., gram positive, gram negative, miscellaneous (nocardia, actinomyces, mixed), mycobacterial, spirochetal, *rickettsia*, mycoplasma); chlamydia; viral (DNA, RNA), fungal and algal infections; protozoal and helminthic infections; endocrine diseases; nutritional diseases; and metabolic diseases.

Other medical conditions and/or fields with which embodiments of the invention find utility include those mentioned in Harrison's Principles of Internal Medicine, Kasper et al., ISBN 0071402357, McGraw-Hill Professional, 16th edition (2004), as well as those mentioned in Robbins Basic Pathology, Kumar, Cotran, and Robbins, eds., ISBN 1416025340, Elsevier, 7th edition (2005).

Medical tests (e.g., blood tests, urine tests, and/or other human or animal tissue tests) that may be performed using various embodiments of the invention described herein include, for example, general chemistry tests (e.g., analytes include albumin, blood urea nitrogen, calcium, creatinine, magnesium, phosphorus, total protein, and/or uric acid); electrolyte tests (e.g., analytes include sodium, potassium, chloride, and/or carbon dioxide); diabetes tests (e.g., analytes include glucose, hemoglobin A1C, and/or microalbumin); lipids tests (e.g., analytes include apolipoprotein A1, apoli-

poprotein B, cholesterol, triglyceride, low density lipoprotein cholesterol, and/or high density lipoprotein cholesterol); nutritional assessment (e.g., analytes include albumin, prealbumin, transferrin, retinol binding protein, alpha1-acid glycoprotein, and/or ferritin); hepatic tests (e.g., analytes include alanine transaminase, albumin, alkaline phosphatase, aspartate transaminase, direct bilirubin, gamma glutamyl transaminase, lactate dehydrogenase, immunoglobulin A, immunoglobulin G, immunoglobulin M, prealbumin, total bilirubin, and/or total protein); cardiac tests (e.g., analytes include apolipoprotein A1, apolipoprotein B, cardiac troponin-1, creatine kinase, creatine kinase MB isoenzyme, high sensitivity CRP, mass creatine kinase MB isoenzyme myoglobin, and/or N-terminal pro-brain natriuretic peptide); tests for anemia (e.g., analytes include ferritin, folate, homocysteine, haptoglobin, iron, soluble transferrin receptor, total iron binding capacity, transferrin, and/or vitamin B12); pancreatic tests (e.g., analytes include amylase and/or lipase); nephropathies (e.g., analytes include albumin, alpha1-microglobulin, alpha2-macroglobulin, beta2-microglobulin, cystatin C, retinol binding protein, and/or transferrin); bone tests (e.g., analytes include alkaline phosphatase, calcium, and/or phosphorous); cancer marker monitoring (e.g., analytes include total PSA); thyroid tests (e.g., analytes include free thyroxine, free triiodothyronine, thyroxine, thyroid stimulating hormone, and/or triiodothyronine); fertility tests (e.g., analytes include beta-human chorionic gonadotropin); therapeutic drug monitoring (e.g., analytes include carbamazepine, digoxin, digitoxin, gentamicin, lidocaine, lithium, N-acetyl procainamide, phenobarbital, phenytoin, procainamide, theophylline, tobramycin, valproic acid, and/or vancomycin); immunosuppressive drugs (e.g., analytes include cyclosporine A, sirolimus, and/or tacrolimus); tests for complement activity and/or autoimmune disease (e.g., analytes include C3 complement, C4 complement, C1 inhibitor, C-reactive protein, and/or rheumatoid factor); polyclonal/monoclonal gammopathies (e.g., analytes include immunoglobulin A, immunoglobulin G, immunoglobulin M, 1 g light chains types kappa and/or lambda, immunoglobulin G subclasses 1, 2, 3, and/or 4); tests for infectious disease (e.g., analytes include antistreptolysin O); tests for inflammatory disorders (e.g., analytes include alpha1-acid glycoprotein, alpha1-antitrypsin, ceruloplasmin, C-reactive protein, and/or haptoglobin); allergy testing (e.g., analytes include immunoglobulin E); urine protein tests (e.g., analytes include alpha1-microglobulin, immunoglobulin G, 1 g light chains type kappa and/or lambda, microalbumin, and/or urinary/cerebrospinal fluid protein); tests for protein—CSF (e.g., analytes include immunoglobulin G and/or urinary/cerebrospinal fluid protein); toxicology tests (e.g., analytes include serum acetaminophen, serum barbiturates, serum benzodiazepines, serum salicylate, serum tricyclic antidepressants, and/or urine ethyl alcohol); and/or tests for drugs of abuse (e.g., analytes include amphetamine, cocaine, barbiturates, benzodiazepines, ecstasy, methadone, opiate, phencyclidine, tetrahydrocannabinoids, propoxyphene, and/or methaqualone).

Other analytes which can be detected using the devices, systems, and methods of the invention include those mentioned in the Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Burtis, Ashwood, and Bruns, ISBN 0721601898, Elsevier, 4th edition (2006).

For example, the systems and methods of the invention can be used to monitor immuno-compromised subjects following allogeneic/allogeneic transplantation. In transplant subjects that receive solid organ, bone marrow, hematopoietic stem cell, or other allogeneic donations, there is a need to monitor the immune status, organ function, and if necessary, rapidly

and accurately identify opportunistic infections. Tacrolimus (also FK-506, Prograf, or Fujimycin) is an immunosuppressive drug whose main use is after allogeneic organ transplant to reduce the activity of the subject's immune system and so lower the risk of organ rejection. It reduces interleukin-2 (IL-2) production by T-cells. It is also used in a topical preparation in the treatment of severe atopic dermatitis (eczema), severe refractory uveitis after bone marrow transplants, and the skin condition vitiligo. It is a 23-membered macrolide lactone discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria *Streptomyces tsukubaensis*. It has similar immunosuppressive properties to cyclosporin, but is much more potent in equal volumes. Immunosuppression with tacrolimus was associated with a significantly lower rate of acute rejection compared with cyclosporin-based immunosuppression (30.7% vs. 46.4%) in one study. Long term outcome has not been improved to the same extent. Tacrolimus is normally prescribed as part of a post-transplant cocktail including steroids, mycophenolate and IL-2 receptor inhibitors. Dosages are titrated to target blood levels. Side effects can be severe and include infection, cardiac damage, hypertension, blurred vision, liver and kidney problems, seizures, tremors, hyperkalemia, hypomagnesemia, hyperglycemia, diabetes mellitus, itching, insomnia, and neurological problems such as confusion, loss of appetite, weakness, depression, cramps, and neuropathy. In addition tacrolimus may potentially increase the severity of existing fungal or infectious conditions such as herpes zoster or polyoma viral infections, and certain antibiotics cross-react with tacrolimus. Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function. A rise in blood creatinine levels is observed only with marked damage to functioning nephrons. Therefore, this test is not suitable for detecting early stage kidney disease. A better estimation of kidney function is given by the creatinine clearance test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race as suggested by the American Diabetes Association without a 24 hour urine collection. Some laboratories will calculate the creatinine clearance if written on the pathology request form; and, the necessary age, sex, and weight are included in the subject information. There is a need to monitor creatinine and tacrolimus levels from the same blood sample from a subject as the monitoring of the drug concentration and the renal function can assist and guide the physician to optimal therapy post-transplantation. Optimizing therapy is a tight balance of preventing rejection but also to ensure immune function to fight opportunistic infections and overall results in enhanced subject compliance to the immunosuppressive therapy. In large part, transplant recipients succumb to transplant rejection, graft versus host disease, or opportunistic infections. In the first two, immunosuppressive agents can ablate or inhibit the reactions. However, if the subject has an underlying infection, then clinical management is challenging. For a specific example, a heart, lung transplant subject presenting with fever of unknown origin enters a health care facility. The subject is started on broad spectrum antibiotics until the culture results are known. If the condition worsens, and the culture reveals a specific infection, for example *candida*, a specific antifungal, fluconazole, can be administered to the known subject. However, this antifungal may alter the levels of the immunosuppressive agent given to almost all allogeneic transplant recipients, tacrolimus. Upon testing for both tacrolimus and creatinine levels, the physician halts the tacrolimus, believing that the fluconazole will defeat the infection, and in a rapid manner. Under this regimen, the subject may

worsen if the *candida* species is resistant to fluconazole, and the subject is then started on an appropriate anti-fungal agent. However, since the tacrolimus may be halted, the immunosuppressive therapy is unmanaged and the subject may become unresponsive to any additional therapy and death may ensue. Thus, if there was a test to simultaneously monitor creatinine (kidney function), tacrolimus blood levels, and accurate identification of opportunistic infections, the above subject may have been saved. The systems and methods of the invention can include a multiplexed, no sample preparation, single detection method, automated system to determine the drug level, the toxicity or adverse effect determinant, and the pathogen identification having a critical role in the immunocompromised subject setting. For example, a cartridge having portals or wells containing 1) magnetic particles having creatinine specific antibodies decorated on their surface, 2) magnetic particles having tacrolimus specific antibodies on their surface, and 3) magnetic particles having specific nucleic acid probes to identify pathogen species could be employed to rapidly determine and provide clinical management values for a given transplant subject. Opportunistic infections that can be monitored in such subjects, and any other patient populations at risk of infection, include, without limitation, fungal; *candida* (resistant and non-resistant strains); gram negative bacterial infections (e.g., *E. coli*, *stenotrophomonas maltophilia*, *Klebsiella pneumoniae oxytoca*, *Pseudomonas aeruginosa*); and gram positive bacterial infections (e.g., *staphylococcus* species: *S. aureus*, *S. pneumoniae*, *E. faecalis*, and *E. faecium*). Other opportunistic infections that can be monitored include coagulase negative *staphylococcus*, *Corynebacterium* spp., *Fusobacterium* spp., and *Morganella morganii*, and viral organisms, such as CMV, BKV, EBC, HHV-6, HIV, HCV, HBV, and HAV.

The systems and methods of the invention can also be used to monitor and diagnose cancer patients as part of a multiplexed diagnostic test. One specific form of cancer, colorectal cancer, has demonstrated positive promise for personalized medical treatment for a specific solid tumor. Pharmacogenetic markers can be used to optimize treatment of colorectal and other cancers. Significant individual genetic variation exists in drug metabolism of 5FU, capecitabine, irinotecan, and oxaliplatin that influences both the toxicity and efficacy of these agents. Examples of genetic markers include UGT1A1*28 leads to reduced conjugation of SN-38, the active metabolite of irinotecan, resulting in an increased rate of adverse effects, especially neutropenia. To a lesser extent, increased 5-FU toxicity is predicted by DPYD*2A. A variable number of tandem repeats polymorphism in the thymidylate synthase enhancer region, in combination with a single nucleotide polymorphism C>G, may predict poorer response to 5-FU. Efficacy of oxaliplatin is influenced by polymorphisms in components of DNA repair systems, such as ERCC1 and XRCC1. Polymorphic changes in the endothelial growth factor receptor probably predict cetuximab efficacy. Furthermore, the antibody-dependent cell-mediated cytotoxic effect of cetuximab may be reduced by polymorphisms in the immunoglobulin G fragment C receptors. Polymorphic changes in the VEGF gene and the hypoxia inducible factor 1alpha gene are also believed to play a role in the variability of therapy outcome. Thus, identification of such polymorphisms in subjects can be used to assist physicians with treatment decisions. For example, PCR-based genetics tests have been developed to assist physicians with therapeutic treatment decisions for subjects with non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and gastric cancer. Expression of ERCC1, TS, EGFR, RRM1, VEGFR2, HER2, and detection of mutations in KRAS, EGFR, and BRAF are

available for physicians to order to identify the optimal therapeutic option. However, these PCR tests are not available on site, and thus the sample must be delivered to the off-site laboratory. These solid tumors are often biopsied and FFPE (Formalin-Fixed, Paraffin-Embedded (tissue)) samples are prepared. The systems and methods of the invention can be used without the 5-7 day turnaround to get the data and information and use of fixed samples required for existing methods. The systems and methods of the invention can provide a single platform to analyze samples, without sample prep, for multiple analyte types, as in cancer for chemotherapeutic drugs, genotyping, toxicity and efficacy markers can revolutionize the practice of personalized medicine and provide rapid, accurate diagnostic testing.

The systems and methods of the invention can also be used to monitor and diagnose neurological disease, such as dementia (a loss of cognitive ability in a previously-unimpaired person) and other forms of cognitive impairment. Without careful assessment of history, the short-term syndrome of delirium (often lasting days to weeks) can easily be confused with dementia, because they have all symptoms in common, save duration, and the fact that delirium is often associated with over-activity of the sympathetic nervous system. Some mental illnesses, including depression and psychosis, may also produce symptoms that must be differentiated from both delirium and dementia. Routine blood tests are also usually performed to rule out treatable causes. These tests include vitamin B12, folic acid, thyroid-stimulating hormone (TSH), C-reactive protein, full blood count, electrolytes, calcium, renal function, and liver enzymes. Abnormalities may suggest vitamin deficiency, infection or other problems that commonly cause confusion or disorientation in the elderly. The problem is complicated by the fact that these cause confusion more often in persons who have early dementia, so that "reversal" of such problems may ultimately only be temporary. Testing for alcohol and other known dementia-inducing drugs may be indicated. Acetylcholinesterase inhibitors-Tacrine (Cognex), donepezil (Aricept), galantamine (Razadyne), and rivastigmine (Exelon) are approved by the United States Food and Drug Administration (FDA) for treatment of dementia induced by Alzheimer disease. They may be useful for other similar diseases causing dementia such as Parkinsons or vascular dementia. N-methyl-D-aspartate Blockers-Memantine (Namenda) is a drug representative of this class. It can be used in combination with acetylcholinesterase inhibitors. Amyloid deposit inhibitors-Minocycline and Clioquinoline, antibiotics, may help reduce amyloid deposits in the brains of persons with Alzheimer disease. Depression is frequently associated with dementia and generally worsens the degree of cognitive and behavioral impairment. Antidepressants effectively treat the cognitive and behavioral symptoms of depression in subjects with Alzheimer's disease, but evidence for their use in other forms of dementia is weak. Many subjects with dementia experience anxiety symptoms. Although benzodiazepines like diazepam (Valium) have been used for treating anxiety in other situations, they are often avoided because they may increase agitation in persons with dementia and are likely to worsen cognitive problems or are too sedating. Buspirone (Buspar) is often initially tried for mild-to-moderate anxiety. There is little evidence for the effectiveness of benzodiazepines in dementia, whereas there is evidence for the effectiveness of antipsychotics (at low doses). Selegiline, a drug used primarily in the treatment of Parkinson's disease, appears to slow the development of dementia. Selegiline is thought to act as an antioxidant, preventing free radical damage. However, it also acts as a stimulant, making it difficult to determine whether the delay in

onset of dementia symptoms is due to protection from free radicals or to the general elevation of brain activity from the stimulant effect. Both typical antipsychotics (such as Haloperidol) and atypical antipsychotics such as (risperidone) increases the risk of death in dementia-associated psychosis. This means that any use of antipsychotic medication for dementia-associated psychosis is off-label and should only be considered after discussing the risks and benefits of treatment with these drugs, and after other treatment modalities have failed. In the UK around 144,000 dementia sufferers are unnecessarily prescribed antipsychotic drugs, around 2000 subjects die as a result of taking the drugs each year. Dementia can be broadly categorized into two groups: cortical dementias and subcortical dementias. Cortical dementias include: Alzheimer's disease, Vascular dementia (also known as multi-infarct dementia), including Binswanger's disease, Dementia with Lewy bodies (DLB), Alcohol-Induced Persisting Dementia, Korsakoff's syndrome, Wernicke's encephalopathy, Frontotemporal lobar degenerations (FTLD), including Pick's disease, Frontotemporal dementia (or frontal variant FTLD), Semantic dementia (or temporal variant FTLD), Progressive non-fluent aphasia, Creutzfeldt-Jakob disease, Dementia pugilistica, Moyamoya disease, Thebestia (Often mistaken for a cancer), Posterior cortical atrophy or Benson's syndrome. Subcortical dementias include Dementia due to Huntington's disease, Dementia due to Hypothyroidism, Dementia due to Parkinson's disease, Dementia due to Vitamin B1 deficiency, Dementia due to Vitamin B12 deficiency, Dementia due to Folate deficiency, Dementia due to Syphilis, Dementia due to Subdural hematoma, Dementia due to Hypercalcaemia, Dementia due to Hypoglycemia, AIDS dementia complex, Pseudodementia (a major depressive episode with prominent cognitive symptoms), Substance-induced persisting dementia (related to psychoactive use and formerly Absinthism), Dementia due to multiple etiologies, Dementia due to other general medical conditions (i.e., end stage renal failure, cardiovascular disease etc.), Dementia not otherwise specified (used in cases where no specific criteria is met). Alzheimer's disease is a common form of dementia. There are three companies that currently offer for research only diagnostic testing of proteins (Satoris), splice variants (exonhit), or protein expression levels (Diagenic) in subjects suffering from dementia, Lewy Body disease, or mild cognitive impairment. Since dementia is fundamentally associated with many neurodegenerative diseases, the ability to test for these proteins, as biomarkers of the disease, along with drug or drug metabolite levels in a single platform will assist a physician to adjust the dosage, alter a regimen, or generally monitor the progression of the disease. These tests are currently run off-site at locations far from the subject and care giver. Thus, to have the ability to monitor the drug levels and the biomarker in the same detection system, on-site will provide a huge advantage to this debilitating and devastating disease. The method of the invention can be a multiplexed, no sample preparation, single detection method, automated system to determine the drug level, the toxicity or adverse effect determinant, and the potential biomarker of the progression of the disease. For example, a cartridge having portals or wells containing 1) magnetic particles having protein biomarker specific antibodies decorated on their surface, 2) magnetic particles having specific antibodies on their surface, and 3) magnetic particles having nucleic acid specific probes to identify protein expression levels could be employed to rapidly determine and provide clinical management values for a given dementia subject.

The systems and methods of the invention can also be used to monitor and diagnose infectious disease in a multiplexed, automated, no sample preparation system. Examples of pathogens that may be detected using the devices, systems, and methods of the invention include, e.g., *Candida* (resistant and non-resistant strains), e.g., *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*; *A. fumigatus*; *E. coli*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae/oxytoca*, *P. aeruginosa*; *Staphylococcus* spp. (e.g., *S. aureus* or *S. pneumoniae*); *E. faecalis*, *E. faecium*, Coagulase negative *staphylococcus* spp., *Corynebacterium* spp., *Fusobacterium* spp., *Morganella morganii*, *Pneumocystis jirovecii*, previously known as *pneumocystis carinii*, *F. hominis*, *streptococcus pyogenes*, *Pseudomonas aeruginosa*, Polyomavirus JC polyomavirus (the virus that causes Progressive multifocal leukoencephalopathy), *Acinetobacter baumannii*, *Toxoplasma gondii*, Cytomegalovirus, *Aspergillus* spp., Kaposi's Sarcoma, cryptosporidium, *cryptococcus neoformans*, and *Histoplasma capsulatum*, among other bacteria, yeast, fungal, virus, prion, mold, actinomycetes, protozoal, parasitic, protist and helminthic infectious organisms. The systems and methods of the invention can also be used to monitor HIV/AIDS patients. When clinicians suspect acute infection (e.g., in a subject with a report of recent risk behavior in association with symptoms and signs of the acute retroviral syndrome), a test for HIV RNA is usually performed. High levels of HIV RNA detected in plasma through use of sensitive amplification assays (PCR, bDNA, or NASBA), in combination with a negative or indeterminate HIV antibody test, support the diagnosis of acute HIV infection. Low-level positive PCR results (<5000 copies/mL) are often not diagnostic of acute HIV infection and should be repeated to exclude a false-positive result. HIV RNA levels tend to be very high in acute infection; however, a low value may represent any point on the upward or downward slope of the viremia associated with acute infection. Plasma HIV RNA levels during seroconversion do not appear significantly different in subjects who have acute symptoms versus those who are asymptomatic. Viremia occurs approximately 2 weeks prior to the detection of a specific immune response. Subjects diagnosed with acute HIV infection by HIV RNA. Fever and flu- or mono-like symptoms are common in acute HIV infection but are nonspecific rash, mucocutaneous ulcers, or pharyngeal candidiasis and meningismus are more specific and should raise the index of suspicion testing still require antibody testing with confirmatory Western blot 3 to 6 weeks later. Subjects undergoing HIV testing who are not suspected to be in the acute stages of infection should receive HIV antibody testing according to standard protocol. Antibody test results that are initially negative should be followed up with HIV antibody testing at 3 months to identify HIV infection in individuals who may not yet have seroconverted at the time of initial presentation. Plasma HIV RNA levels indicate the magnitude of HIV replication and its associated rate of CD4+ T cell destruction, while CD4+ T-cell counts indicate the extent of HIV-induced immune damage already suffered. Regular, periodic measurement of plasma HIV RNA levels and CD4+ T-cell counts is necessary to determine the risk of disease progression in an HIV-infected individual and to determine when to initiate or modify antiretroviral treatment regimens. As rates of disease progression differ among individuals, treatment decisions should be individualized by level of risk indicated by plasma HIV RNA levels and CD4+ T-cell counts. Current WHO guidelines and recommendations for HIV therapy includes a combination of the following drugs, AZT (zidovudine), 3TC (lamivudine), ABC (abacavir), ATV (atazanavir), d4T (stavudine), ddI (didanosine), NVP (nevi-

rapine), EFV (efavirenz), FTC (emtricitabine), LPV (lopinavir), RTV (ritonavir), TDF (tenofovir disoproxil fumarate) in established regimens. Drug therapy for HIV is to commence in subjects who have a CD4 count <350 cell/mm³ irrespective of clinical symptoms. At least one of the four following regimens for antiretroviral naïve subjects is begun: 1) AZT+3TC+EFV, 2) AZT+3TC+NVP, 3) TDF+3TC or FTC+EFV, or 4) TDF+3TC or FTC+NVP. These regimens avoid d4T (stavudine) to limit the disfiguring, unpleasant, and potentially life-threatening toxicities of this drug. Treatment failure is usually determined by viral load, a persistent value of 5,000 copies/ml confirms treatment failure. In cases whereby viral load measurement is not available, immunological criteria (CD4 cell count) can be used to determine therapeutic progress. In cases of treatment failure, a boosted protease inhibitor plus two nucleoside analogs are added to the regimen and is considered second line antiretroviral therapy. ATV plus low dose RTV, or LPV with low dose RTV is also considered second line therapy. Often the goal in treatment failure cases is simpler timed regimens and fixed doses. For subjects failing the second line treatment regimens should be maintained on a tolerated regimen for the duration. The use of potent combination antiretroviral therapy to suppress HIV replication to below the levels of detection of sensitive plasma HIV RNA assays limits the potential for selection of antiretroviral-resistant HIV variants, the major factor limiting the ability of antiretroviral drugs to inhibit virus replication and delay disease progression. Therefore, maximum achievable suppression of HIV replication should be the goal of therapy. The most effective means to accomplish durable suppression of HIV replication is the simultaneous initiation of combinations of effective anti-HIV drugs with which the subject has not been previously treated and that are not cross-resistant with antiretroviral agents with which the subject has been treated previously. Each of the antiretroviral drugs used in combination therapy regimens should always be used according to optimum schedules and dosages. The available effective antiretroviral drugs are limited in number and mechanism of action, and cross-resistance between specific drugs has been documented. Therefore, any change in antiretroviral therapy increases future therapeutic constraints. Monitoring HIV/AIDS subjects for viral load, drug levels, CD4 cell counts, and toxicity patterns in a single platform diagnostic method would provide distinct advantages to a subject. The systems and methods of the invention can be used in a multiplexed, no sample preparation, single detection method, automated system to determine the drug level, the toxicity or adverse effect determinants, and the potential biomarker of the progression of the disease. For example, a cartridge having portals or wells containing 1) magnetic particles having CD4 cell specific antibodies decorated on their surface, 2) magnetic particles having toxicity biomarker specific antibodies on their surface, and 3) magnetic particles having nucleic acid specific probes to identify viral load levels could be employed to rapidly determine and provide clinical management values for a given HIV/AIDS subject.

The systems and methods of the invention can also be used to monitor and diagnose immune disease in a subject (e.g., Crohn's disease, ileitis, enteritis, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis, as well as non-gastrointestinal immune disease). The relatively recent development of genetically engineered agents has the potential to alter the treatment of immune disease radically, and Remicade (also known as Infliximab, an anti-TNF antibody) was introduced as a new therapeutic class with high efficacy, rapid onset of action, prolonged effect, and improved toler-

ance. However these agents are expensive and at least one-third of the eligible patients fail to show any useful response. Finding a means to predict those who will respond, and to anticipate relapse is, therefore, of obvious importance. T helper-type 1 (Th1) lymphocytes orchestrate much of the inflammation in Crohn's disease mainly via production of TNF-alpha, which appears to play a pivotal role as a pro-inflammatory cytokine. It exerts its effects through its own family of receptors (TNFR1 and TNFR2), the end results of which include apoptosis, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) activation and NF-kappaB activation. Activated NF-kappaB enters the nucleus and induces transcription of genes associated with inflammation, host defense and cell survival. The promoter region of the TNF gene lies between nucleotides -1 and -1300, and encompasses numerous polymorphic sites associated with potential binding sites for various transcription factors. Carriers of the TNF allele 2 (TNF2) (which contains a single base-pair polymorphism at the -308 promoter position) produce slightly more TNF-alpha in their intestinal mucosa than non-TNF2 carriers. TNF polymorphisms also appear to influence the nature and frequency of extra-intestinal manifestations of inflammatory bowel disease (IBD). A number of routes of inhibition of TNF are being investigated. Most extensively evaluated is the use of remicade. Several large controlled trials indicate that remicade has a role in treating patients with moderate to severely active Crohn's disease and in fistulating Crohn's disease. Small studies have shown possible associations between poor response to remicade and increasing mucosal levels of activated NF-kappaB, homozygosity for the polymorphism in exon 6 of TNFR2 (genotype Arg196Arg), positivity for perinuclear antineutrophil cytoplasmic antibodies (ANCA), and with the presence of increased numbers of activated lamina propria mononuclear cells producing interferon-gamma and TNF-alpha. Thus, monitoring Crohn's disease patients for TNF-alpha and toxicity patterns in a single platform diagnostic method would have distinct advantages. The method of the invention can be a multiplexed, no sample preparation, single detection method, automated system to determine the drug level, the toxicity or adverse effect determinants, and the potential biomarker of the progression of the disease. For example, a cartridge having portals or wells containing 1) magnetic particles having anti-TNF-alpha specific antibodies decorated on their surface, 2) magnetic particles having toxicity biomarker specific antibodies on their surface, and 3) magnetic particles having specific probes to identify disease markers of progression could be employed to rapidly determine and provide clinical management values for a given Crohn's disease patient.

The systems and methods of the invention can also be used to monitor and diagnose heart disease in a subject, such as a myocardial infarction. Cardiac markers or cardiac enzymes are proteins that leak out of injured myocardial cells and are used to assess cardiac injury. Cardiac markers include, without limitation, the enzymes SGOT, LDH, the MB subtype of the enzyme creatine kinase, and cardiac troponins (T and I). The cardiac troponins T and I which are released within 4-6 hours of an attack of myocardial infarction (and remain elevated for up to 2 weeks) have nearly complete tissue specificity and are now the preferred markers for assessing myocardial damage. Elevated troponins in the setting of chest pain may accurately predict a high likelihood of a myocardial infarction in the near future. The diagnosis of myocardial infarction is typically based upon subject history, ECG, and cardiac markers. When damage to the heart occurs, levels of cardiac markers rise over time, which is why blood tests for

them are taken over a 24-hour period. Because these enzyme levels are not elevated immediately following a heart attack, patients presenting with chest pain are generally treated with the assumption that a myocardial infarction has occurred and then evaluated for a more precise diagnosis. A MI is a medical emergency which requires immediate medical attention. Treatment attempts to salvage as much myocardium as possible and to prevent further complications, thus the phrase “time is muscle”. Oxygen, aspirin, and nitroglycerin are usually administered as soon as possible. Thus, in the acute setting, monitoring Troponin I and T, as well as potential other biomarkers of cardiac ischemia, in addition to drug therapy and toxicity patterns in a single platform diagnostic method would have distinct advantages. The systems and methods of the invention can be used to provide a multiplexed, no sample preparation, single detection method, automated system to determine the drug level, the toxicity or adverse effect determinants, and the potential biomarker of the progression of the disease. For example, a cartridge having portals or wells containing 1) magnetic particles having anti-troponin I or troponin T specific antibodies decorated on their surface, 2) magnetic particles having toxicity biomarker specific antibodies on their surface, and 3) magnetic particles having specific probes to identify disease markers of progression could be employed to rapidly determine and provide clinical management values for a given myocardial infarction patient.

One or more multi-well cartridges can be configured for use in the systems and methods of the invention and prepared with at least one whole blood sample from the patient; magnetic particles for detecting each of the analytes to be detected (one or more small molecules; one or more metabolites of the one or more small molecules; metabolic biomarker such as described for the hepatic function panel); and dilution and wash buffers. Liver function tests are done on a patient's serum or plasma sample and clinical biochemistry laboratory blood analysis furnishes crucial data regarding the condition of the patient's liver. A “hepatic function panel” is a blood test wherein low or high levels of one or more enzymes may point to liver diseases or damage. For example, the hepatic function panel can include one or more of the following analyte detection assays: one or more small molecules; one or more metabolites of the one or more small molecules; a biologic, metabolic biomarkers; genotyping, gene expression profiling; and proteomic analysis.

A “small molecule” means a drug, medication, medication, or other chemically synthesized compound that is contemplated for human therapeutic use. A “biologic” is defined as a substance derived from a biological source, not synthesized and that is contemplated for human therapeutic use. A “metabolic biomarker” is a substance, molecule, or compound that is synthesized or biologically derived that is used to determine the status of a patient or subject's liver function. “Genotyping” is the ability to determine genetic differences in specific genes that may or may not affect the phenotype of the specific gene. “Phenotype” is the resultant biological expression, (metabolic or physiological) of the protein set by the genotype. “Gene expression profiling” is the ability to determine the rate or amount of the production of a gene or gene product in a specific tissue, in a temporal or spatial manner. “Proteomic analysis” is a protein pattern or array to identify key differences between normal and diseased tissues.

A hepatic function panel can include analysis of one or more of the following proteins in a patient or subject biological sample: 1) albumin (the major constituent of the total protein in the liver; while the remnant is called globulin; albumin must be present as 3.9 to 5.0 g/dL, hypoalbumin-

aemia indicates poor nutrition, lower protein catabolism, cirrhosis or nephrotic syndrome); 2) aspartate transaminase (AST) (also known as serum glutamic oxaloacetic transaminase or aspartate aminotransferase, is an enzyme in liver parenchymal cells and is normally 10 to 34 IU/L; elevated levels are indicative of acute liver damage); 3) alanine transaminase (ALT) (also known as serum glutamic pyruvic transaminase or alanine aminotransferase, is an enzyme is present in hepatocytes at levels between 8 to 37 IU/L; elevated levels are indicative of acute liver damage in viral hepatitis or paracetamol overdose; the ratio of AST to ALT is used to differentiate between the reasons of liver damage); 4) alkaline phosphatase (ALP) (an enzyme that is present in the cells lining the biliary ducts of the liver; the normal range is 44 to 147 IU/L and the level rises in case of infiltrative diseases of the liver, intrahepatic cholestasis or large bile duct obstruction); 5) Gamma glutamyl transpeptidase (GGT) (a more sensitive marker for cholestatic damage than ALP, is very specific to the liver; the standard range is 0 to 51 IU/L; both acute and chronic alcohol toxicity raise GGT; the reason of an isolated elevation in ALP can be detected by GGT); 6) total bilirubin (TBIL) (an increase in the total bilirubin can lead to jaundice and can be attributed to cirrhosis, viral hepatitis, hemolytic anemias, or internal hemorrhage); 7) direct bilirubin; 8) prothrombin time (PTT) (hepatic cell damage and bile flow obstruction can cause changes to blood clotting time); 9) alpha-fetoprotein test (elevated levels indicate hepatitis or cancer); 10) lactate dehydrogenase; and 11) mitochondrial antibodies (if present may indicate chronic active hepatitis, primary biliary cirrhosis, or other autoimmune disorders). The proteins described above would be analyzed in the hepatic function panel using the systems and methods of the invention.

An additional hepatic function panel may include genotyping of cytochrome P450 enzymes. The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes. The function of most CYP enzymes is to catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for ca. 75% of the total metabolism. Most drugs undergo biotransformation and are eventually excreted from the body; and many require bioactivation to form the active compound. The CYP enzymes that metabolize many medications include CYP3A4/5 (36%), CYP2D6 (19%), CYP2C8/9 (16%), and CYP1A2 (11%).

Cytochrome P450 genotyping tests are used to determine how well a patient or subject metabolizes a drug. The results of cytochrome P450 tests can be used to divide individuals into four main types:

(i) Poor metabolizers. Certain drugs are metabolized more slowly than normal and the medication will have a longer half life and possibly increase the likelihood that it will cause side effects.

(ii) Normal metabolizers. Drugs will be metabolized at an average rate and thus is indicative that there is a benefit from treatment and points to fewer side effects than are other individuals who don't metabolize those particular medications as well.

(iii) Intermediate metabolizers. Drugs may or may not be metabolized at an average rate. At least one gene involved in drug metabolism is suspected to function abnormally. There then is a predisposition to metabolize certain drugs differently.

(iv) Ultra rapid metabolizers. Drugs are metabolized faster and more efficiently than the average. Since the metabolic rate is higher than average, some medications are inactivated sooner or are excreted sooner than normal and the medication may not have the desired efficacy.

Currently, genotyping the genes responsible for these enzymes across a population has been shown that polymorphic differences in these enzymes can lead to variation in efficacy and toxicity of some drugs. Assessing cytochrome P450 status in a patient sample can be accomplished by measuring the enzyme activity of the sample, or determining if a genetic difference occurs in one of the genes of this metabolic system in the genome. Genotyping requires a cell sample representative of the patient or subject's genome and the analysis is aimed at determining genetic differences in these clinically important genes. Alternatively, CYP450 enzyme phenotyping (identifying enzymatic metabolizer status) can be accomplished by administering a test enzyme substrate to a patient and monitoring parent substrate and metabolite concentrations over time (e.g., in urine). However, testing and interpretation are time-consuming and inconvenient; as a result, phenotyping is seldom performed.

Below is a listing of the possible hepatic metabolic enzymes that may be part of a hepatic function panel.

CYP2C19 metabolizes several important types of drugs, including proton-pump inhibitors, diazepam, propranolol, imipramine, and amitriptyline. FDA cleared the test "based on results of a study conducted by the manufacturers of hundreds of DNA samples as well as on a broad range of supporting peer-reviewed literature." According to FDA labeling, "Information about CYP2D6 genotype may be used as an aid to clinicians in determining therapeutic strategy and treatment doses for therapeutics that are metabolized by the CYP2D6 product." Thus, a hepatic function panel employing the methods of the invention, may be used to genotype patient or subject samples to assess the status of the cytochrome P450 enzyme system to then optimize therapeutic efficacy and safety.

CYP2D6 (cytochrome P450 2D6) is the best studied of the DMEs and acts on one-fourth of all prescription drugs, including the selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA), beta-blockers such as Inderal and the Type 1A antiarrhythmics. Approximately 10% of the population has a slow acting form of this enzyme and 7% a super-fast acting form. Thirty-five percent are carriers of a non-functional 2D6 allele, especially elevating the risk of ADRs when these individuals are taking multiple drugs. Drugs that CYP2D6 metabolizes include Prozac, Zoloft, Paxil, Effexor, hydrocodone, amitriptyline, Claritin, cyclobenzaprine, Haldol, metoprolol, Rythmol, Tagamet, tamoxifen, dextromethorphan, beta-blockers, antiarrhythmics, antidepressants, and morphine derivatives, including many of the most prescribed drugs and the over-the-counter diphenylhydramine drugs (e.g., Allegra, Dytuss, and Tusstat). CYP2D6 is responsible for activating the pro-drug codeine into its active form and the drug is therefore inactive in CYP2D6 slow metabolizers.

CYP2C9 (cytochrome P450 2C9) is the primary route of metabolism for Coumadin (warfarin). Approximately 10% of the population are carriers of at least one allele for the slow-metabolizing form of CYP2C9 and may be treatable with 50% of the dose at which normal metabolizers are treated. Other drugs metabolized by CYP2C9 include Amaryl, isoniazid, ibuprofen, amitriptyline, Dilantin, Hyzaar, THC (tetrahydrocannabinol), naproxen, and Viagra.

CYP2C19 (cytochrome P450 2C19) is associated with the metabolism of carisoprodol, diazepam, Dilantin, and Prevacid.

CYP1A2 (cytochrome P450 1A2) is associated with the metabolism of amitriptyline, olanzapine, haloperidol, duloxetine, propranolol, theophylline, caffeine, diazepam, chlor-diazepoxide, estrogens, tamoxifen, and cyclobenzaprine.

NAT2 (N-acetyltransferase 2) is a secondary drug metabolizing enzyme that acts on isoniazid, procainamide, and Azulfidine. The frequency of the NAT2 "slow acetylator" in various worldwide populations ranges from 10% to more than 90%.

DPD (Dihydropyrimidine dehydrogenase) is responsible for the metabolism of Fluorouracil (5-FU), one of the most successful and widely used chemotherapy drugs.

UGT1A1 (UDP-glucuronosyltransferase) variations can lead to severe even fatal reactions to the first dose of Camp-tosar (irinotecan).

5HTT (Serotonin Transporter) helps determine whether people are likely to respond to SSRIs, a class of medications that includes citalopram, fluoxetine, paroxetine, and sertraline, among others, and often is prescribed for depression or anxiety.

Diagnostic genotyping tests for certain CYP450 enzymes are now available. Some tests are offered as in house laboratory-developed test services, which do not require U.S. Food and Drug Administration (FDA) approval but which must meet CLIA quality standards for high complexity testing. The AmpliChip® (Roche Molecular Systems, Inc.) is the only FDA-cleared test for CYP450 genotyping. The AmpliChip® is a microarray consisting of many DNA sequences complementary to 2 CYP450 genes and applied in microscopic quantities at ordered locations on a solid surface (chip). The AmpliChip® tests the DNA from a patient's white blood cells collected in a standard anticoagulated blood sample for 29 polymorphisms and mutations for the CYP2D6 gene and 2 polymorphisms for the CYP2C19 gene.

Therefore, the invention features a multiplexed analysis of a single blood sample (e.g., a single blood draw, or any other type of patient sample described herein) from a patient to determine a) liver enzymatic status, as well as b) the genotype of key metabolic enzymes to then be able to design pharmacotherapy regimes for optimal therapeutic care using the systems and methods of the invention.

The systems and methods of the invention can include one or more multi-well cartridges prepared with at least one whole blood sample from the patient; magnetic particles for detecting each of the analytes to be detected; analyte antibodies; multivalent binding agents; and/or dilution and wash buffers for use in a multiplexed assay as described above.

Non-Agglomeration-Based Assays and Methods

In some embodiments, the magnetic particles described herein may be utilized in an assay that does not feature particle agglomeration. For example, the magnetic particles may be used to capture or concentrate an analyte, e.g., by passing a liquid sample containing the analyte over magnetic particles that include binding moieties specific for the analyte. Some advantages of this approach include a) no clusters need be formed (the clusters may be inherently unstable over a certain size, leading to increased CV's); b) no clustering may not require vortexing as flow shear forces may dislodge non-specific binding of magnetic particles, c) fluidic handling steps may be reduced, and d) miniaturization of the assay may favor these non-agglomerative methods.

The magnetic particles derivatized with a binding moiety can be held in position by an external magnetic field while sample containing the corresponding analyte is circulated

past the “trapped” magnetic particles allowing for capture and/or concentrate the analyte of interest. The particles may be pulled to the side or bottom of the assay vessel, or a magnetizable mesh or magnetizable metal foam with appropriate pore size can be present in the reaction vessel, creating very high local magnetic gradients. An advantage of having the mesh/metal foam in the reaction vessel is that the distance each magnetic particle needs to travel to be “trapped” or “captured” can be very short, improving assay kinetics.

Another non-agglomerative assay is to have surfaces derivatized with ligands complementary to the binding moiety present on the magnetic particle and using a capture/depletion/flow through format. Specific binding of magnetic particles to a surface depletes magnetic particles from the bulk particle suspension used in the assay, thus leading to a change in the T_2 value in the reaction volume interrogated by the MR reader. Pre-incubation of the particles with the sample containing analyte can reduce/inhibit the specific binding/capture/depletion of the magnetic particle by the derivatized surface in proportion to the concentration of analyte in the sample. One example of this type of assay approach has been demonstrated using PhyNexus affinity chromatography micropipette tips. The 200 μ l PhyTips contain a 20 μ l volume of resin bed trapped between 2 frits. The resin bed consists of 200 μ m cross-linked agarose beads derivatized with avidin, protein A, protein G, or an analyte. A programmable electronic pipettor can aspirate and dispense various volumes at various flow rates. The magnetic particles flow through the pores created by the packed agarose bead resin bed. By repeatedly passing the appropriate magnetic particle suspension over the trapped resin bed to allow for productive interactions to occur between, say, an avidin-derivatized agarose bead resin bed and biotin-derivatized magnetic particles, some of the magnetic particles will specifically bind to and be depleted from the particles suspension. By measuring the T_2 of the particle suspension before and after exposure to the agarose resin bed, the amount of particle depletion can be quantified.

Another non-agglomerative assay format is similar to that described above, but uses derivatized magnetizable metal foam to replace the resin bed. The advantage of the metal foam as the solid phase substrate is that when placed in a magnetic field, the metal foam generates very high local magnetic field gradients over very short distances which can attract the derivatized magnetic particles and bring them in contact with the complementary binding partner on the metal foam and improve the chances of a specific productive interaction. By optimizing the pore size and surface area of the

metal foam, the assay kinetics can be vastly improved because the particles need to travel much shorter distances to find a complimentary surface to bind. The particle concentration in the flow-through reaction volume will be reduced inversely proportional to the analyte concentration in the sample and can be quantified using the MR reader. The metal foam can be nickel bearing directly bound his-tagged moieties, or can be nickel treated with aminosilane covalently linked to binding moieties. This process has been demonstrated using aminosilane-treated nickel metal foam with 400 μ m pores decorated with anti-creatinine antibodies and shown to specifically bind creatinine-derivatized magnetic particles.

Further, there are examples of assays that would be aimed at detecting a physical property change in a liquid sample. As described in pending cases, PCT/US/2009/062537 and PCT/US2008/073346, coagulation of blood can be determined by the instant methods described. Further, other physical properties may be detected such as solidification, changes in density and may have uses in determining curing of materials (plastic compositions), changes in food and food products with time, contamination of products found in nature, and monitoring certain biological fluids such as urine as a function of kidney function.

Amplification and Detection of Nucleic Acids from Complex Samples

Systems and methods of the invention can include amplification based nucleic acid detection assays conducted starting with complex samples (e.g., for diagnostic, forensic, and environmental analyses).

Sample preparation must also remove or provide resistance for common PCR inhibitors found in complex samples (e.g., body fluids, soil, or other complex milieu). Common inhibitors are listed in Table 5 (see also, Wilson, I. G., Appl. Environ. Microbiol., 63:3741 (1997)). Inhibitors typically act by either prevention of cell lysis, degradation or sequestering a target nucleic acid, and/or inhibition of a polymerase activity. The most commonly employed polymerase, Taq, is inhibited by the presence of 0.1% blood in a reaction. Very recently, mutant Taq polymerases have been engineered that are resistant to common inhibitors (e.g., hemoglobin, humic acid) found in blood and soil (Kermekchiev et al., Nucl. Acid. Res., 37(5): e40, (2009)). Manufacturer recommendations indicate these mutations enable direct amplification from up to 20% blood. Despite resistance afforded by the mutations, accurate real time PCR detection is complicated due to fluorescence quenching observed in the presence of blood sample (Kermekchiev, M B, et al., Nucl. Acid. Res., 37:e40 (2009)).

TABLE 5

PCR inhibitors and facilitators/methods for overcoming inhibition.			
Substrate	Target	Inhibitor	Facilitator
feces	<i>Escherichia coli</i>	>10 ³ bacterial cells	ion-exchange column
CSF	<i>Treponema pallidum</i>	Cellular debris causing nonspecific amplification	nested primers
whole blood	mammalian tissue	>4 μ l of blood/100-ml reaction mix (hemoglobin)	1-2% blood per reaction
feces	Rotavirus	unknown dilution	cellulose fiber
clinical specimens	Cytomegalovirus	unidentified components	glass bead extraction
human blood and tissue	human genes	DNA binding proteins	thermophilic protease from <i>Thermus</i> strain rt44A
mammalian tissue	Mammalian tissue genetics	thermal cycler variations	formamide

TABLE 5-continued

PCR inhibitors and facilitators/methods for overcoming inhibition.			
Substrate	Target	Inhibitor	Facilitator
mammalian tissue	Mammalian tissue genetics	thermal cycler variations	DMSO, glycerol, PEG, organic solvents
clinical specimens	<i>Treponema pallidum</i>	unknown factors	Various substrate-specific physicochemical methods
forensic semen samples	Sperm	Genotyping errors; selective/total PCR inhibition by vaginal microorganisms	
feces	<i>Salmonella enterica</i>	various body fluids	immunomagnetic separation
feces	Various enteric viruses	unknown	size exclusion chromatography, physicochemical extraction
clinical specimens	Herpes simplex virus	endogenous inhibitors, random effects	repurification, coamplified positive control
feces	<i>Escherichia coli</i>	nonspecific inhibitors, urea, hemoglobin, heparin, phenol, SDS	additional primers and reaction cyclers, booster PCR
tissue culture	Cytomegalovirus HIV	glove powder	
suspensions, skin biopsies	<i>Mycobacterium leprae</i>	mercury-based fixatives, neutral buffered formaline	reduced fixation times, ethanol fixation
clinical specimens	<i>Mycobacterium tuberculosis</i>	unknown inhibitors in pus, tissue biopsies, sputum, pleural fluid	physicochemical extraction
mammalian tissue	genetics	unknown contaminant of reverse transcriptase	additional DNA
formalin-fixed paraffin tissue	Hepatitis C virus	ribonucleotide vanadyl complexes	phenol/chloroform extraction
nasopharyngeal aspirates and swabs	<i>Bordetella pertussis</i>	unknown inhibitors	phenol/chloroform extraction
human mononuclear blood cells	HIV type I	detergents	mineral oil
bloodstain	human mitochondrial DNA	unidentified heme compound, hemin	BSA
blood	various	heparin	alternative polymerases and buffers, chelex, spermine, [Mg ²⁺], glycerol, BSA, heparinase
sputa	<i>Mycoplasma pneumonia</i>	N-acetyl-L-cysteine, dithiothreitol, mucolytic agents	
human tissue	HLA-DRB1 genotyping	pollen, glove powder, impure DNA, heparin, hemoglobin	
clinical specimens	<i>Mycobacterium tuberculosis</i>	unknown	competitive internal control
dental plaque	many	unknown	diatomaceous earth, guanidium isothiocyanate, ethanol, acetone
ancient mammalian tissues	Cytochrome b gene	unknown	ammonium acetate, ethidium bromide

Polymerase chain reaction amplification of DNA or cDNA is a tried and trusted methodology; however, as discussed above, polymerases are inhibited by agents contained in crude samples, including but not limited to commonly used anticoagulants and hemoglobin. Recently mutant Taq polymerases have been engineered to harbor resistance to common inhibitors found in blood and soil. Currently available polymerases, e.g., HemoKlenTaq™ (New England BioLabs, Inc., Ipswich, Mass.) as well as OmniTaq™ and OmniKlenTaq™ (DNA Polymerase Technology, Inc., St. Louis, Mo.) are mutant (e.g., N-terminal truncation and/or point mutations) Taq polymerase that render them capable of amplifying DNA in the presence of up to 10%, 20% or 25% whole blood, depending on the product and reaction conditions (See, e.g., Kermekchiev, et al. Nucl. Acids Res. 31:6139 (2003); and Kermekchiev, et al., Nucl. Acid. Res., 37:e40 (2009); and see U.S. Pat. No. 7,462,475). Additionally, Phusion® Blood Direct PCR Kits (Finnzymes Oy, Espoo, Finland), include a

unique fusion DNA polymerase enzyme engineered to incorporate a double-stranded DNA binding domain, which allows amplification under conditions which are typically inhibitory to conventional polymerases such as Taq or Pfu, and allow for amplification of DNA in the presence of up to about 40% whole blood under certain reaction conditions. See Wang et al., Nuc. Acids Res. 32:1197 (2004); and see U.S. Pat. Nos. 5,352,778 and 5,500,363. Furthermore, Kapa Blood PCR Mixes (Kapa Biosystems, Woburn, Mass.), provide a genetically engineered DNA polymerase enzyme which allows for direct amplification of whole blood at up to about 20% of the reaction volume under certain reaction conditions. Despite these breakthroughs, direct optical detection of generated amplicons is not possible with existing methods since fluorescence is quenched by the presence of blood. See Kermekchiev, et al., Nucl. Acid. Res., 37:e40 (2009).

We have found that complex samples such as whole blood can be directly amplified using about 5%, about 10%, about

20%, about 25%, about 30%, about 25%, about 40%, and about 45% or more whole blood in amplification reactions, and that the resulting amplicons can be directly detected from amplification reaction using magnetic resonance (MR) relaxation measurements upon the addition of conjugated magnetic particles bound to oligonucleotides complementary to the target nucleic acid sequence. Alternatively, the magnetic particles can be added to the sample prior to amplification. Thus, provided are methods for the use of nucleic acid amplification in a complex dirty sample, hybridization of the resulting amplicon to paramagnetic particles, followed by direct detection of hybridized magnetic particle conjugate and target amplicons using magnetic particle based detection systems. In particular embodiments, direct detection of hybridized magnetic particle conjugates and amplicons is via MR relaxation measurements (e.g., T_2 , T_1 , T_2^* , etc). Further provided are methods which are kinetic, in order to quantify the original nucleic acid copy number within the sample (e.g., sampling and nucleic acid detection at pre-defined cycle numbers, comparison of endogenous internal control nucleic acid, use of exogenous spiked homologous competitive control nucleic acid).

The terms "amplification" or "amplify" or derivatives thereof as used herein mean one or more methods known in the art for copying a target or template nucleic acid, thereby increasing the number of copies of a selected nucleic acid sequence. Amplification may be exponential or linear. A target or template nucleic acid may be either DNA or RNA. The sequences amplified in this manner form an "amplified region" or "amplicon." Primer probes can be readily designed by those skilled in the art to target a specific template nucleic acid sequence. In certain preferred embodiments, resulting amplicons are short to allow for rapid cycling and generation of copies. For example, in particular embodiments, amplicons are preferably less than about 500 nucleotides in length, less than about 250 nucleotides in length, less than about 200 nucleotides in length, less than about 150 nucleotides in length, less than about 100 nucleotides in length, greater than about 50 nucleotides in length. In certain embodiments, amplicons are preferably less than 200 nucleotides in length and greater than 50 nucleotides in length. In particular embodiments, amplicons are preferably about 150 nucleotides in length, about 125 nucleotides in length, or about 100 nucleotides in length.

While the exemplary methods described hereinafter relate to amplification using polymerase chain reaction ("PCR"), numerous other methods are known in the art for amplification of nucleic acids (e.g., isothermal methods, rolling circle methods, etc.). Those skilled in the art will understand that these other methods may be used either in place of, or together with, PCR methods. See, e.g., Saiki, "Amplification of Genomic DNA" in PCR Protocols, Innis et al., Eds., Academic Press, San Diego, Calif., pp 13-20 (1990); Wharam et al., *Nucleic Acids Res.* 29:E54 (2001); Hafner et al., *Biotechniques*, 30:852 (2001). Further amplification methods suitable for use with the present methods include, for example, polymerase chain reaction (PCR) method, reverse transcription PCR (RT-PCR), ligase chain reaction (LCR), transcription based amplification system (TAS), transcription mediated amplification (TMA), nucleic acid sequence based amplification (NASBA) method, the strand displacement amplification (SDA) method, the loop mediated isothermal amplification (LAMP) method, the isothermal and chimeric primer-initiated amplification of nucleic acid (ICAN) method, and the smart amplification system (SMAP) method. These methods, as well as others are well known in the art and

can be adapted for use in conjunction with provided methods of detection of amplified nucleic acid.

The PCR method is a technique for making many copies of a specific template DNA sequence. The PCR process is disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202; and 4,965,188, each of which is incorporated herein by reference. One set of primers complementary to a template DNA are designed, and a region flanked by the primers is amplified by DNA polymerase in a reaction including multiple amplification cycles. Each amplification cycle includes an initial denaturation, and up to 50 cycles of annealing, strand elongation (or extension) and strand separation (denaturation). In each cycle of the reaction, the DNA sequence between the primers is copied. Primers can bind to the copied DNA as well as the original template sequence, so the total number of copies increases exponentially with time. PCR can be performed as according to Whelan, et al, *Journal of Clinical Microbiology*, 33:556 (1995). Various modified PCR methods are available and well known in the art. Various modifications such as the "RT-PCR" method, in which DNA is synthesized from RNA using a reverse transcriptase before performing PCR; and the "Taq-Man PCR" method, in which only a specific allele is amplified and detected using a fluorescently labeled TaqMan probe, and Taq DNA polymerase, are known to those skilled in the art. RT-PCR and variations thereof have been described, for example, in U.S. Pat. Nos. 5,804,383; 5,407,800; 5,322,770; and 5,310,652, and references described therein, which are hereby incorporated by reference; and TaqMan PCR and related reagents for use in the method have been described, for example, in U.S. Pat. Nos. 5,210,015; 5,876,930; 5,538,848; 6,030,787; and 6,258,569, which are hereby incorporated by reference.

LCR is a method of DNA amplification similar to PCR, except that it uses four primers instead of two and uses the enzyme ligase to ligate or join two segments of DNA. Amplification can be performed in a thermal cycler (e.g., LCx of Abbott Labs, North Chicago, Ill.). LCR can be performed for example, as according to Moore et al., *Journal of Clinical Microbiology* 36:1028 (1998). LCR methods and variations have been described, for example, in European Patent Application Publication No. EP0320308, and U.S. Pat. No. 5,427,930, each of which is incorporated herein by reference.

The TAS method is a method for specifically amplifying a target RNA in which a transcript is obtained from a template RNA by a cDNA synthesis step and an RNA transcription step. In the cDNA synthesis step, a sequence recognized by a DNA-dependent RNA polymerase (i.e., a polymerase-binding sequence or PBS) is inserted into the cDNA copy downstream of the target or marker sequence to be amplified using a two-domain oligonucleotide primer. In the second step, an RNA polymerase is used to synthesize multiple copies of RNA from the cDNA template. Amplification using TAS requires only a few cycles because DNA-dependent RNA transcription can result in 10-1000 copies for each copy of cDNA template. TAS can be performed according to Kwok et al., *PNAS* 86:1173 (1989). The TAS method has been described, for example, in International Patent Application Publication No. WO/1988/010315, which is incorporated herein by reference.

Transcription mediated amplification (TMA) is a transcription-based isothermal amplification reaction that uses RNA transcription by RNA polymerase and DNA transcription by reverse transcriptase to produce an RNA amplicon from target nucleic acid. TMA methods are advantageous in that they can produce 100 to 1000 copies of amplicon per amplification cycle, as opposed to PCR or LCR methods that produce only 2 copies per cycle. TMA has been described, for

example, in U.S. Pat. No. 5,399,491 which is incorporated herein by reference. NASBA is a transcription-based method which for specifically amplifying a target RNA from either an RNA or DNA template. NASBA is a method used for the continuous amplification of nucleic acids in a single mixture at one temperature. A transcript is obtained from a template RNA by a DNA-dependent RNA polymerase using a forward primer having a sequence identical to a target RNA and a reverse primer having a sequence complementary to the target RNA on the 3' side and a promoter sequence that recognizes T7 RNA polymerase on the 5' side. A transcript is further synthesized using the obtained transcript as template. This method can be performed as according to Heim, et al., *Nucleic Acids Res.*, 26:2250 (1998). The NASBA method has been described in U.S. Pat. No. 5,130,238, which is incorporated herein by reference.

The SDA method is an isothermal nucleic acid amplification method in which target DNA is amplified using a DNA strand substituted with a strand synthesized by a strand substitution type DNA polymerase lacking 5'→3' exonuclease activity by a single stranded nick generated by a restriction enzyme as a template of the next replication. A primer containing a restriction site is annealed to template, and then amplification primers are annealed to 5' adjacent sequences (forming a nick). Amplification is initiated at a fixed temperature. Newly synthesized DNA strands are nicked by a restriction enzyme and the polymerase amplification begins again, displacing the newly synthesized strands. SDA can be performed according to Walker, et al., *PNAS*, 89:392 (1992). SDA methods have been described in U.S. Pat. Nos. 5,455,166 and 5,457,027, each of which are incorporated by reference.

The LAMP method is an isothermal amplification method in which a loop is always formed at the 3' end of a synthesized DNA, primers are annealed within the loop, and specific amplification of the target DNA is performed isothermally. LAMP can be performed according to Nagamine et al., *Clinical Chemistry*, 47:1742 (2001). LAMP methods have been described in U.S. Pat. Nos. 6,410,278; 6,974,670; and 7,175,985, each of which are incorporated by reference.

The ICAN method is an isothermal amplification method in which specific amplification of a target DNA is performed isothermally by a strand substitution reaction, a template exchange reaction, and a nick introduction reaction, using a chimeric primer comprising RNA-DNA and DNA polymerase having a strand substitution activity and RNase H. ICAN can be performed according to Mukai et al., *J. Biochem.* 142: 273 (2007). The ICAN method has been described in U.S. Pat. No. 6,951,722, which is incorporated herein by reference.

The SMAP (MITANI) method is a method in which a target nucleic acid is continuously synthesized under isothermal conditions using a primer set comprising two kinds of primers and DNA or RNA as a template. The first primer included in the primer set includes, in the 3' end region thereof, a sequence (Ac') hybridizable with a sequence (A) in the 3' end region of a target nucleic acid sequence as well as, on the 5' side of the above-mentioned sequence (Ac'), a sequence (B') hybridizable with a sequence (Bc) complementary to a sequence (B) existing on the 5' side of the above-mentioned sequence (A) in the above-mentioned target nucleic acid sequence. The second primer includes, in the 3' end region thereof, a sequence (Cc') hybridizable with a sequence (C) in the 3' end region of a sequence complementary to the above-mentioned target nucleic acid sequence as well as a loopback sequence (D-Dc') comprising two nucleic acid sequences hybridizable with each other on an identical strand on the 5'

side of the above-mentioned sequence (Cc'). SMAP can be performed according to Mitani et al., *Nat. Methods*, 4(3): 257 (2007). SMAP methods have been described in U.S. Patent Application Publication Nos. 2006/0160084, 2007/0190531 and 2009/0042197, each of which is incorporated herein by reference.

The amplification reaction can be designed to produce a specific type of amplified product, such as oligonucleotides that are double stranded; single stranded; double stranded with overhanging 5' and 3' sticky ends; or double stranded with chemical binding moieties on the 5' and 3' ends. The amplified PCR product can be detected by: (i) hybridization mediated detection of the amplified product via oligonucleotide probes coupled to magnetic particles, where two different oligonucleotides are used that hybridize to the amplified product such that hybridization cross-links the particles; (ii) hybridization mediated detection where the DNA of the amplified product must first be melted; (iii) hybridization mediated detection where the particles hybridize to the sticky ends of the amplified product; (iv) binding of the particles to the binding moieties on the termini of the amplified product, such as streptavidin functionalized particles binding to biotin functionalized amplified product.

NMR Units

The systems for carrying out the methods of the invention can include one or more NMR units. FIG. 1 is a schematic diagram 100 of an NMR system for detection of a signal response of a liquid sample to an appropriate RF pulse sequence. A bias magnet 102 establishes a bias magnetic field Bb 104 through a sample 106. The magnetic particles are in a liquid or lyophilized state in the cartridge prior to their introduction to a sample well (the term "well" as used herein includes any indentation, vessel, container, or support) 108 until introduction of the liquid sample 106 into the well 108, or the magnetic particles can be added to the sample 106 prior to introduction of the liquid sample into the well 108. An RF coil 110 and RF oscillator 112 provides an RF excitation at the Larmor frequency which is a linear function of the bias magnetic field Bb. In one embodiment, the RF coil 110 is wrapped around the sample well 108. The excitation RF creates a nonequilibrium distribution in the spin of the water protons (or free protons in a non-aqueous solvent). When the RF excitation is turned off, the protons "relax" to their original state and emit an RF signal that can be used to extract information about the presence and concentration of the analyte. The coil 110 acts as an RF antenna and detects a signal, which based on the applied RF pulse sequence, probes different properties of the material, for example a T₂ relaxation. The signal of interest for some cases of the technology is the spin-spin relaxation (generally 10-2000 milliseconds) and is called the T₂ relaxation. The RF signal from the coil 110 is amplified 114 and processed to determine the T₂ (decay time) response to the excitation in the bias field Bb. The well 108 may be a small capillary or other tube with nanoliters to microliters of the sample, including the analyte and a micro-coil wound around it. Alternatively, the coil may be configured as shown in any of FIGS. 2A-2E about or in proximity to the well. An NMR system may also contain multiple RF coils for the detection of multiplexing purposes. In certain embodiments, the RF coil has a conical shape with the dimensions 6 mm×6 mm×2 mm.

FIGS. 2A-2E illustrate exemplary micro NMR coil (RF coil) designs. FIG. 2A shows a wound solenoid micro coil 200 about 100 μm in length, however one could envision a coil having 200 μm, 500 μm or up to 1000 μm in length. FIG. 2B shows a "planar" coil 202 (the coil is not truly planar, since the coil has finite thickness) about 1000 μm in diameter. FIG.

2C shows a MEMS solenoid coil **204** defining a volume of about 0.02 μL . FIG. 2D shows a schematic of a MEMS Helmholtz coil **206** configuration, and FIG. 2E shows a schematic of a saddle coil **220** configuration.

A wound solenoid micro coil **200** used for traditional NMR detection is described in Seeber et al., "Design and testing of high sensitivity micro-receiver coil apparatus for nuclear magnetic resonance and imaging," Ohio State University, Columbus, Ohio. A planar micro coil **202** used for traditional NMR detection is described in Massin et al., "High Q factor RF planar microcoil for micro-scale NMR spectroscopy," Sensors and Actuators A 97-98, 280-288 (2002). A Helmholtz coil configuration **206** features a well **208** for holding a sample, a top Si layer **210**, a bottom Si layer **212**, and deposited metal coils **214**. An example of a Helmholtz coil configuration **206** used for traditional NMR detection is described in Syms et al., "MEMS Helmholtz Coils for Magnetic Resonance Spectroscopy," Journal of Micromechanics and Micromachining 15 (2005) S1-S9.

The coil configuration may be chosen or adapted for specific implementation of the micro-NMR-MRS technology, since different coil configurations offer different performance characteristics. For example, each of these coil geometries has a different performance and field alignment. The planar coil **202** has an RF field perpendicular to the plane of the coil. The solenoid coil **200** has an RF field down the axis of the coil, and the Helmholtz coil **206** has an RF field transverse to the two rectangular coils **214**. The Helmholtz **206** and saddle coils **220** have transverse fields which would allow the placement of the permanent magnet bias field above and below the well. Helmholtz **206** and saddle coils **220** may be most effective for the chip design, while the solenoid coil **200** may be most effective when the sample and MRS magnetic particles are held in a micro tube.

The micro-NMR devices may be fabricated by winding or printing the coils or by microelectromechanical system (MEMS) semiconductor fabrication techniques. For example, a wound or printed coil/sample well module may be about 100 μm in diameter, or as large as a centimeter or more. A MEMS unit or chip (thusly named since it is fabricated in a semiconductor process as a die on a wafer) may have a coil that is from about 10 μm to about 1000 μm in characteristic dimension, for example. The wound or printed coil/sample well configuration is referenced herein as a module and the MEMS version is referenced herein as a chip. For example, the liquid sample **108** may be held in a tube (for example, a capillary, pipette, or micro tube) with the coil wound around it, or it may be held in wells on the chip with the RF coil surrounding the well. Alternatively, the sample is positioned to flow through a tube, capillary, or cavity in the proximity to the RF coil.

The basic components of an NMR unit include electrical components, such as a tuned RF circuit within a magnetic field, comprising an MR sensor, receiver and transmitter electronics that could be including preamplifiers, amplifiers and protection circuits, data acquisitions components, pulse programmer and pulse generator.

Systems containing NMR units with RF coils and micro wells containing magnetic particle sensors described herein may be designed for detection and/or concentration measurement of specific analyte(s) of interest by development of a model for particle aggregation phenomena and by development of an RF-NMR signal chain model. For example, experiments can be conducted for analyte/magnetic particle systems of interest by characterizing the physics of particle aggregation, including, for example, the effects of affinities, relevant dimensions, and concentrations. Also, experiments

can be conducted to characterize the NMR signal(s) (T_2 , T_1 , T_2^* , $T_{2\rho}$, $T_{1\rho}$ and/or other signal characteristics, such as but not limited to diffusion, susceptibility, frequency) as functions of particle aggregation or depletion and magnetic particle characteristics. Signal characteristics specific to the MRS (magnetic resonance switch) phenomenon in a given system can be used to enhance detection sensitivity and/or otherwise improve performance.

The NMR system may include a chip with RF coil(s) and electronics micromachined thereon. For example, the chip may be surface micromachined, such that structures are built on top of a substrate. Where the structures are built on top of the substrate and not inside it, the properties of the substrate are not as important as in bulk micromachining, and expensive silicon wafers used in bulk micromachining can be replaced by less expensive materials such as glass or plastic. Alternative embodiments, however, may include chips that are bulk micromachined. Surface micromachining generally starts with a wafer or other substrate and grows layers on top. These layers are selectively etched by photolithography and either a wet etch involving an acid or a dry etch involving an ionized gas, or plasma. Dry etching can combine chemical etching with physical etching, or ion bombardment of the material. Surface micromachining may involve as many layers as is needed.

In some cases, an inexpensive RF coil maybe integrated into a disposable cartridge and be a disposable component. The coil could be placed in a manner that allows electrical contact with circuitry on the fixed NMR setup, or the coupling could be made inductively to a circuit.

Where the relaxation measurement is T_2 , accuracy and repeatability (precision) will be a function of temperature stability of the sample as relevant to the calibration, the stability of the assay, the signal-to-noise ratio (S/N), the pulse sequence for refocusing (e.g., CPMG, BIRD, Tango, and the like), as well as signal processing factors, such as signal conditioning (e.g., amplification, rectification, and/or digitization of the echo signals), time/frequency domain transformation, and signal processing algorithms used. Signal-to-noise ratio is a function of the magnetic bias field (B_b), sample volume, filling factor, coil geometry, coil Q-factor, electronics bandwidth, amplifier noise, and temperature.

In order to understand the required precision of the T_2 measurement, one should look at a response curve of the assay at hand and correlate the desired precision of determining the analyte concentration and the precision of the measurable, e.g. T_2 for some cases. Then a proper error budget can be formed.

For example, to obtain a 10-fold improvement in the 0.02 ng/mL detection limit for Troponin (10-fold increase in sensitivity), it would be necessary to discern a ΔT_2 less than about 5.6 milliseconds from a traditional (non-MRS-measured) T_2 of about 100 milliseconds. The minimum signal-to-noise ratio (S/N) would need to be about 20 to detect this difference.

The NMR units for use in the systems and methods of the invention can be those described in U.S. Pat. No. 7,564,245, incorporated herein by reference.

The NMR units of the invention can include a small probehead for use in a portable magnetic resonance relaxometer as described in PCT Publication No. WO09/061,481, incorporated herein by reference.

The systems of the invention can be implantable or partially implantable in a subject. For example, the NMR units of the invention can include implantable radiofrequency coils and optionally implantable magnets as described in PCT Pub-

lication Nos. WO09/085,214 and WO08/057,578, each of which is incorporated herein by reference.

The systems of the invention can include a polymeric sample container for reducing, partly or completely, the contribution of the NMR signal associated with the sample container to the nuclear magnetic resonance parameter of the liquid sample as described in PCT Publication No. WO09/045,354, incorporated herein by reference.

The systems of the invention can include a disposable sample holder for use with the MR reader that is configured to permit a predetermined number of measurements (i.e., is designed for a limited number of uses). The disposable sample holder can include none, part, or all, of the elements of the RF detection coil (i.e., such that the MR reader lacks a detection coil). For example, the disposable sample holder can include a "read" coil for RF detection that is inductively coupled to a "pickup" coil present in the MR reader. When the sample container is inside the MR reader it is in close proximity to the pickup coil and can be used to measure NMR signal. Alternatively, the disposable sample holder includes an RF coil for RF detection that is electrically connected to the MR reader upon insertion of the sample container. Thus, when the sample container is inserted into the MR reader the appropriate electrical connection is established to allow for detection. The number of uses available to each disposable sample holder can be controlled by disabling a fusible link included either in the electrical circuit within the disposable sample holder, or between the disposable sample holder and the MR reader. After the disposable sample holder is used to detect an NMR relaxation in a sample, the instrument can be configured to apply excess current to the fusible link, causing the link to break and rendering the coil inoperable. Optionally, multiple fusible links could be used, working in parallel, each connecting to a pickup on the system, and each broken individually at each use until all are broken and the disposable sample holder rendered inoperable.

Cartridge Units

The systems for carrying out the methods of the invention can include one or more cartridge units to provide a convenient method for placing all of the assay reagents and consumables onto the system. For example, the system may be customized to perform a specific function, or adapted to perform more than one function, e.g., via changeable cartridge units containing arrays of micro wells with customized magnetic particles contained therein. The system can include a replaceable and/or interchangeable cartridge containing an array of wells pre-loaded with magnetic particles, and designed for detection and/or concentration measurement of a particular analyte. Alternatively, the system may be usable with different cartridges, each designed for detection and/or concentration measurements of different analytes. The cartridge may be sized to facilitate insertion into and ejection from a housing for the preparation of a liquid sample which is transferred to other units in the system (i.e., a magnetic assisted agglomeration unit, or an NMR unit). The cartridge unit itself could potentially interface directly with manipulation stations as well as with the MR reader(s).

The cartridge unit can be a modular cartridge having an inlet module that can be sterilized independent of the reagent module.

For handling biological samples, such as blood samples, there are numerous competing requirements for the cartridge design, including the need for sterility for the inlet module to prevent cross contamination and false positive test results, and the need to include reagents in the package which cannot be easily sterilized using standard terminal sterilization techniques like irradiation. A modular cartridge can provide a

simple means for cross contamination control during certain assays, including but not limited to distribution of PCR products into multiple detection aliquots. In addition, a modular cartridge can be compatible with automated fluid dispensing, and provides a way to hold reagents at very small volumes for long periods of time (in excess of a year). Finally, pre-dispensing these reagents allows concentration and volumetric accuracy to be set by the manufacturing process and provides for a point of care use instrument that is more convenient as it can require much less precise pipetting.

The modular cartridge of the invention is a cartridge that is separated into modules that can be packaged and if necessary sterilized separately. They can also be handled and stored separately, if for example the reagent module requires refrigeration but the detection module does not. FIG. 6 shows a representative cartridge with an inlet module, a reagent module and a detection module that are snapped together. In this embodiment, the inlet module would be packaged separately in a sterile package and the reagent and detection modules would be pre-assembled and packaged together.

During storage, the reagent module could be stored in a refrigerator while the inlet module could be stored in dry storage. At time of use, the operator would retrieve a detection module and open the package using sterile technique. The Vacutainer tube is then decapped and the inverted inlet module is placed onto the tube as shown in FIG. 7A. This module has been designed to be easily moldable using single draw tooling as shown in FIGS. 7B and 7C and the top and bottom of the cartridge are sealed with foil to prevent contamination and also to close the channels. Once the tube has been resealed using the inlet module, the assembly is turned right side up and snapped onto the remainder of the cartridge. The inlet section includes a well with an overflow that allows sample tubes with between 2 and 6 ml of blood to be used and still provide a constant depth interface to the system automation. It accomplishes this by means of the overflow shown in FIG. 8, where blood that overflows the sampling well simply falls into the cartridge body, preventing contamination.

FIGS. 9A-9C show the means of storing precisely pipetted small volume reagents. The reagents are kept in pipette tips that are shown in FIG. 9C. These are filled by manufacturing automation and then are placed into the cartridge to seal their tips in tight fitting wells which are shown in a cutaway view FIG. 9B. Finally, foil seals are placed on the back of the tips to provide a complete water vapor proof seal. It is also possible to seal the whole module with a seal that will be removed by the operator, either in place of or in addition to the aforementioned foils. This module also provides storage for empty reaction vessels and pipette tips for use by the instrument while the detection module provides storage for capped 200 μ l PCR vials used by the instrument to make final measurements from.

FIGS. 10-13C show an alternative embodiment of the detection module of the cartridge which is design to provide for contamination control during, for example, pipetting of post-PCR (polymerase chain reaction) products. This is required because the billion fold amplification produced by PCR presents a great risk of cross contamination and false positives. However, it is desirable to be able to aliquot this mixture safely, because low frequency analytes will have been amplified up and can be distributed for separate detection or identification. There are three ways in which this portion of the cartridge aids in contamination control during this aliquoting operation.

First, the cartridge contains a recessed well to perform the transfer operations in as shown in FIG. 10. Second, the machine provides airflow through this well and down into the

cartridge through holes in the bottom of the well, as shown in FIG. 11. The depth of the well is such that a pipette tip will remain in the airflow and prevent any aerosol from escaping. FIG. 12 depicts a bottom view of the detection module, showing the bottom of the detection tubes and the two holes used to ensure airflow. An optional filter can be inserted here to capture any liquid aerosol and prevent it from entering the machine. This filter could also be a sheet of a hydrophobic material like Gore-tex that will allow air but not liquids to escape. Finally, there is a special seal cap on each 200 ul tube to provide a make then break seal for each pipette tip as it enters the vessel, as shown in FIGS. 13A-13C. It is contemplated that the pipette tip used for aliquoting be stored in this well at all, thus making it possible for the tip never to leave the controlled air flow region.

Alternatively, the modular cartridge is designed for a multiplexed assay. The challenge in multiplexing assays is combining multiple assays which have incompatible assay requirements (i.e., different incubation times and/or temperatures) on one cartridge. The cartridge format depicted in FIGS. 14A-14C allows for the combination of different assays with dramatically different assay requirements. The cartridge features two main components: (i) a reagent module (i.e., the reagent strip portion) that contains all of the individual reagents required for the full assay panel, and (ii) the detection module. The detection modules contain only the parts of the cartridge that carry through the incubation, and can carry single assays or several assays, as needed. The detection module depicted in FIG. 14B includes two detection chambers for a single assay, the first detection chamber as the control and the second detection chamber for the sample. This cartridge format is expandable in that additional assays can be added by including reagents and an additional detection module.

The operation of the module begins when the user inserts the entire or a portion of the cartridge into the instrument. The instrument performs the assay actuation, aliquoting the assays into the separate detection chambers. These individual detection chambers are then disconnected from the reagent strip and from each other, and progress through the system separately. Because the reagent module is separated and discarded, the smallest possible sample unit travels through the instrument, conserving internal instrument space. By splitting up each assay into its own unit, different incubation times and temperatures are possible as each multiplexed assay is physically removed from the others and each sample is individually manipulated.

The cartridge units of the invention can include one or more populations of magnetic particles, either as a liquid suspension or dried magnetic particles which are reconstituted prior to use. For example, the cartridge units of the invention can include a compartment including from 1×10^6 to 1×10^{13} magnetic particles (e.g., from 1×10^6 to 1×10^8 , 1×10^7 to 1×10^9 , 1×10^8 to 1×10^{10} , 1×10^9 to 1×10^{11} , 1×10^{10} to 1×10^{12} , 1×10^{11} to 1×10^{13} , or from 1×10^7 to 5×10^8 magnetic particles) for assaying a single liquid sample.

MAA Units

The systems for carrying out the methods of the invention can include one or more magnetic assisted agglomeration (MAA) units to expedite agglomeration of the magnetic particles, allowing the assay reactions to reach completion (i.e., a stable reading) more quickly. The methods of the invention utilize functionalized magnetic particles to interact with analytes or multivalent binding agents (with multiple binding sites). Agglomeration of the magnetic particles alters the

spin-spin relaxation rate of the sample when exposed to a magnetic field with a subsequent change in T_2 relaxation time.

For example, a field gradient can be used to sweep magnetic particles (MPs) through the liquid sample, allowing the magnetic particles to bind to either specific antibody (analyte-coated magnetic particles) or analyte (antibody-coated magnetic particles), and then concentrating the magnetic particles in a portion of the reaction chamber so as to facilitate particle-particle interactions that lead to specific, ligand/analyte induced agglomeration. The magnetic particles can optionally be allowed to diffuse in the absence of a magnetic field, sonicated, vortexed, shaken, or subjected to ultrasonic mixing to break apart non-specific magnetic particle interactions and re-distribute the magnetic particles back into the liquid sample. The process can be repeated to promote further specific agglomeration. This cycling of magnetic particles between being dispersed in the liquid sample and then concentrated at the side or bottom of the reaction vessel can be repeated as many times as necessary to maximize specific agglomeration, and consequently maximize the assay signal. The agglomeration state of the magnetic particles can be determined using an NMR relaxation measurement.

The MAA method of the invention can employ a gradient magnetic field in order to promote rapid magnetic particle-particle interactions. In one example, analyte coated magnetic particles are added to a solution with a multimeric-analyte specific ligand and placed in a gradient magnetic field. The magnetic field causes particles to concentrate on the side or bottom of a reaction vessel (highest magnetic field strength) resulting in enhanced particle-particle interaction and subsequent aggregation. Aggregation is measured by observing a change in, for example, T_2 signal. Improvements of 10 to 1000 percent signal change (e.g., from 10 to 30%, from 20% to 50%, from 40% to 80%, from 50% to 200%, from 100% to 500%, or from 500% to 1000% signal change) can be observed.

Traditional homogenous MAA takes advantage of dipole-dipole forces for assisting particle-particle interactions while particle dipoles are aligned with the magnetic field of the hMAA unit throughout the liquid sample. In contrast, gradient MAA rapidly concentrates magnetic particles to a locus, thereby greatly facilitating particle-particle interactions.

The cycling MAA approach described herein can accelerate the kinetics of magnetic particle-analyte clustering by (i) reducing the spatial entropy of the binding interaction step by maintaining local concentration of the magnetic particles, (ii) introducing localized mixing by magnet mediated transportation of the pellet from position to position, (iii) reducing shearing of the specific-bound clusters by reducing the need for more energetic dispersion methods, such as vortexing, and/or (iv) changing the magnetic field direction, and thereby causing a local dispersion and re-aggregation of magnetically clustered particles as they re-align their dipoles with the new magnetic field direction, and allowing the locally dispersed magnetic particles to form specific binding interactions involving the target analyte.

In one example, magnet assemblies producing a magnetic field gradient are placed in two positions relative to the assay tube, one to the side of the tube and one at the bottom of the tube (side-bottom configuration). Alternatively, the second magnet position can be located on a different side of the tube (side-side configuration). The tube then is moved to ensure exposure to one magnet followed by exposure to the other magnet (see FIG. 15). This has also been observed to produce a similar enhancement in clustering.

An alternate methodology is to rotate the liquid sample within a gradient magnetic field (or to rotate the magnetic field gradient about the sample) to simultaneously effect a re-orientation of particles within the pellet (relative to the remainder of the liquid sample) and to sweep the pellet through the liquid sample. The rate of rotation can be slow to allow the pellet of magnetic particles to largely remain held in proximity to the gradient magnet (rather than moving in concert with the solvent and analytes in liquid sample). For example, the rotation is typically slower than 0.0333 Hz (e.g., from 0.000833 Hz to 0.0333 Hz, from 0.00166 Hz to 0.0333 Hz, or from 0.00333 Hz to 0.0333 Hz), such that the particles are retained adjacent to the magnetic field source, while the remaining contents in the tube are rotated.

A single gradient magnet can be used, while the sample can be moved around the magnet (or use the same location close to the magnet and alternate with a position removed from the field of the single magnet. The magnet could be moved to the proximity or away from the sample.

The sample can be placed between magnets of the same field orientation for a "field averaging" effect in alternating fashion, in order to simplify the fabrication of a gMAA system (i.e., eliminate the need to carefully select magnets that generate same field profiles). For example a plurality of such magnets could be placed in a circular setup, and samples rotated via a carousel setup, from the first magnet to a null (small magnetic field exposure) to the second magnet etc.

In another example, a homogenous field is used to expedite the agglomeration of magnetic particles in an assay of the invention. We have observed that hMAA is not as effective as exposure to field gradients in terms of concentrating particles and sweeping them through the sample, for timescales relevant to applications. However hMAA has advantages over the field gradient assisted agglomeration method. Using hMAA the magnetic particles are not enticed to move towards a specific location in the tube (see FIG. 16), minimizing non-specific trapping of particles within specific cluster fragments. Agitation after hMAA appears to minimize the non-specific binding. The hMAA treatment appears to enhance analyte induced clustering by increasing the collision frequency (a possible result of decreasing the particle's position and rotational entropies due to localization in an ordered state). The magnetic particles can subsequently be sonicated, vortexed, shaken (i.e., energy additions) to break apart any non-specific particle interactions and re-distribute the particles back into the sample. Additional mixing or gentle agitation during this process would potentially further increase the analyte-specific binding events for enhancement of the overall assay signal. The agglomeration/clustering state of the magnetic particles can be determined by monitoring changes in an NMR relaxation rate. It is also possible to rotate the liquid sample within a homogenous magnetic field (or to rotate a homogenous magnetic field about the sample) to expedite the aggregation of magnetic particles in a liquid sample.

We have observed that longer MAA times leads to increased changes in T_2 , presumably from an increased fraction of clustered particles. We have found that cycled magnetic separation and resuspension leads to increased changes in T_2 and increased clustering. All of these observations point towards a system that must be driven to a steady state or completion (e.g., maximally clustered).

The systems of the invention can include one or more MAA units. For example, the MAA unit can be one or more magnets configured to apply a gradient magnetic field in a first direction relative to the liquid sample, and, after repositioning the sample chamber, apply a gradient magnetic field in a second

direction relative to the liquid sample (see FIG. 17). Alternatively, the MAA unit can be an array of magnets configured to apply a gradient magnetic field to, e.g., the side of a liquid sample, and, after repositioning the sample chamber, to, e.g., the bottom of the liquid sample (see FIGS. 18A-18C). The systems of the invention can include an MAA unit configured to apply a homogenous magnetic field to one or more liquid samples (see FIGS. 19A and 19B).

Agitation Units

The systems for carrying out the methods of the invention can include one or more agitation units to break apart non-specific magnetic particle interactions and re-distribute the magnetic particles back into the liquid sample, or to simply agitate the sample tube to completely mix the assay reagents. For example, the agitation units can include a sonication, vortexing, shaking, or ultrasound station for mixing one or more liquid samples. Mixing could be achieved by aspiration dispensing or other fluid motion (e.g., flow within a channel). Also, mixing could be provided by a vibrating pipette or a pipette that moves from side to side within the sample tube.

The agitation unit can be vortexer or a compact vortexer each of which can be designed to provide a stable motion for the desired sample mixing.

The vortexer includes the following components: (i) a sample support, (ii) a main plate, (iii) four linkages, (iv) linear rail and carriage system (x2), (v) a support for driveshaft and rails, (vi) coupling and driveshaft, (vii) a mounting plate, and (viii) a drive motor (see FIG. 20).

The compact vortexer includes the following components: (i) a sample support, (ii) a main plate, (iii) two linkages, (iv) linear rail and carriage system (xi), (v) a support for linear rail, (vi) support for driveshaft, (vii) coupling and driveshaft, (viii) a mounting plate, and (ix) a drive motor (see FIG. 21).

The basic principle of motion for a vortexer is as follows: the driveshaft including one axis coaxial to the motor shaft, and a second that is offset and parallel to the motor shaft. When the motor shaft is attached to the driveshaft (typically through a helical coupling) and rotated, the offset axis of the driveshaft is driven in an orbital path. The typical offset is 1/4" to produce a vortex in a single 0.2 mL sample tube, but this can be easily modified to effectively mix different sample volumes in other tube geometries.

Alternatively, the vortexer can be of the type utilizing a planetary belt drive (see FIGS. 23A-23C). FIG. 23A is an overall view showing the vortexer configured for 1 large tube. FIG. 23B is a section view showing 2 tube holders for small tubes. FIG. 23C is an overall view of vortexer showing 4 tubes and a close-up of planetary belt drive mechanism.

The drive motor is typically a servo or stepper with an encoder. These motors have an "index" mark that allows the motor to find a specific point in its rotation. These index marks are used to home the system, and ensure that the sample can be returned to a known position after mixing. Knowing the exact position of the sample in the vortex station allows these vortexers to be easily accessed by robotic actuators and thus integrated into an automated system. In lieu of index marks, sensing devices external could be employed (see FIG. 22A). These could be mechanical, magnetic, optical or other sensor that is capable of resolving the sample's position at any point along the system's path or at a fixed "home" position. In order to access a vortexers or centrifuge via a robotic sample holder/positioned, the system can include using an index mark or external switch to "home" the system to a set position after running, using a sensor which tracks the sample motion at all times, so that wherever the system stops the robot knows the position, and using a "find" method that includes finding a sample after running that would employ a vision system that

tracks the sample. The guide mechanism is depicted in FIG. 22B. The main plate is connected to the offset axis of the drive shaft and is free to rotate. The plate follows the orbital path around and dictated by the motor shaft. One end of a linkage is connected to the main plate, and is free to rotate. Therefore in this way, the connected linkage is then connected to the orbital rotation of the drive shaft. The other end of the linkage is connected to a carriage of the linear rail system and is free to rotate. Thus this end of the linkage follows the linear path of the rail. Having two linkages connected to both the carriage and main plate in this way prevents the main plate from rotating around its own center. In the vortexer, two linkages are used on two sides of the main plate (4 in total) to balance and stabilize the entire system.

The two vortexers differ because of their use and design requirements. The compact version is designed to occupy less space, and requires less durability than this version because it is run at a lower speed, as limited by its smaller motor. For these reasons only two linkages are used to connect to a single linear rail system in the compact vortexer. This version needs to be capable of higher speeds, and a nearly continuous utilization due to the large throughput capability of this system. For these reasons a second carriage and set of linkages is added to balance the system, and increase its durability.

Systems

The systems for carrying out the methods of the invention can include one or more NMR units, MAA units, cartridge units, and agitation units. Such systems may further include other components for carrying out an automated assay of the invention, such as a PCR unit for the detection of oligonucleotides; a centrifuge, a robotic arm for delivery an liquid sample from unit to unit within the system; one or more incubation units; a fluid transfer unit (i.e., pipetting device) for combining assay reagents and a biological sample to form the liquid sample; a computer with a programmable processor for storing data, processing data, and for controlling the activation and deactivation of the various units according to a one or more preset protocols; and a cartridge insertion system for delivering pre-filled cartridges to the system, optionally with instructions to the computer identifying the reagents and protocol to be used in conjunction with the cartridge. See FIG. 42.

The systems of the invention can provide an effective means for high throughput and real-time detection of analytes present in a bodily fluid from a subject. The detection methods may be used in a wide variety of circumstances including, without limitation, identification and/or quantification of analytes that are associated with specific biological processes, physiological conditions, disorders or stages of disorders. As such, the systems have a broad spectrum of utility in, for example, drug screening, disease diagnosis, phylogenetic classification, parental and forensic identification, disease onset and recurrence, individual response to treatment versus population bases, and monitoring of therapy. The subject devices and systems are also particularly useful for advancing preclinical and clinical stage of development of therapeutics, improving patient compliance, monitoring ADRs associated with a prescribed drug, developing individualized medicine, outsourcing blood testing from the central laboratory to the home or on a prescription basis, and monitoring therapeutic agents following regulatory approval. The devices and systems can provide a flexible system for personalized medicine. The system of the invention can be changed or interchanged along with a protocol or instructions to a programmable processor of the system to perform a wide variety of assays as described herein. The systems of the invention offer many

advantages of a laboratory setting contained in a desk-top or smaller size automated instrument.

The systems of the invention can be used to simultaneously assay analytes that are present in the same liquid sample over a wide concentration range, and can be used to monitor the rate of change of an analyte concentration and/or concentration of PD or PK markers over a period of time in a single subject, or used for performing trend analysis on the concentration, or markers of PD, or PK, whether they are concentrations of drugs or their metabolites. For example, if glucose were the analyte of interest, the concentration of glucose in a sample at a given time as well as the rate of change of the glucose concentration over a given period of time could be highly useful in predicting and avoiding, for example, hypoglycemic events. Thus, the data generated with the use of the subject fluidic devices and systems can be utilized for performing a trend analysis on the concentration of an analyte in a subject.

For example, a patient may be provided with a plurality of cartridge units to be used for detecting a variety of analytes at predetermined times. A subject may, for example, use different cartridge units on different days of the week. In some embodiments the software on the system is designed to recognize an identifier on the cartridge instructing the system computer to run a particular protocol for running the assay and/or processing the data. The protocol can be optimized as needed by prompting the user for various inputs (i.e., for changing the dilution of the sample, the amount of reagent provided to the liquid sample, altering an incubation time or MAA time, or altering the NMR relaxation collection parameters).

The systems of the invention can run a variety of assays, regardless of the analyte being detected from a bodily fluid sample. A protocol dependent on the identity of the cartridge unit being used can be stored on the system computer. In some embodiments, the cartridge unit has an identifier (ID) that is detected or read by the system computer, or a bar code (1D or 2D) on a card that then supplies assay specific or patient or subject specific information needed to be tracked or accessed with the analysis information (e.g., calibration curves, protocols, previous analyte concentrations or levels). Where desired, the cartridge unit identifier is used to select a protocol stored on the system computer, or to identify the location of various assay reagents in the cartridge unit. The protocol to be run on the system may comprise instructions to the controller of the system to perform the protocol, including but not limited to a particular assay to be run and a detection method to be performed. Once the assay is performed by the system, data indicative of an analyte in the biological sample is generated and communicated to a communications assembly, where it can either be transmitted to the external device for processing, including without limitation, calculation of the analyte concentration in the sample, or processed by the system computer and the result presented on a display read-out. For example, the identifier may be a bar code identifier with a series of black and white lines, which can be read by a bar code reader (or another type of detector) upon insertion of the cartridge unit. Other identifiers could be used, such as a series of alphanumeric values, colors, raised bumps, RFID, or any other identifier which can be located on a cartridge unit and be detected or read by the system computer. The detector may also be an LED that emits light which can interact with an identifier which reflects light and is measured by the system computer to determine the identity of a particular cartridge unit. In some embodiments, the system includes a storage or memory device with the cartridge unit or the detector for transmitting information to the system computer.

Thus, the systems of the invention can include an operating program to carry out different assays, and cartridges encoded to: (i) report to the operating program which pre-programmed assay was being employed; (ii) report to the operating program the configuration of the cartridges; (iii) inform the operating system the order of steps for carrying out the assay; (iv) inform the system which pre-programmed routine to employ; (v) prompt input from the user with respect to certain assay variables; (vi) record a patient identification number (the patient identification number can also be included on the Vacutainer holding the blood sample); (vii) record certain cartridge information (i.e., lot #, calibration data, assays on the cartridge, analytic data range, expiration date, storage requirements, acceptable sample specifics); or (viii) report to the operating program assay upgrades or revisions (i.e., so that newer versions of the assay would occur on cartridge upgrades only and not to the larger, more costly system).

The systems of the invention can include one or more fluid transfer units configured to adhere to a robotic arm (see FIGS. 43A-43C). The fluid transfer unit can be a pipette, such as an air-displacement, liquid backed, or syringe pipette. For example, a fluid transfer unit can further include a motor in communication with a programmable processor of the system computer and the motor can move the plurality of heads based on a protocol from the programmable processor. Thus, the programmable processor of a system can include instructions or commands and can operate a fluid transfer unit according to the instructions to transfer liquid samples by either withdrawing (for drawing liquid in) or extending (for expelling liquid) a piston into a closed air space. Both the volume of air moved and the speed of movement can be precisely controlled, for example, by the programmable processor. Mixing of samples (or reagents) with diluents (or other reagents) can be achieved by aspirating components to be mixed into a common tube and then repeatedly aspirating a significant fraction of the combined liquid volume up and down into a tip. Dissolution of reagents dried into a tube can be done in similar fashion.

A system can include one or more incubation units for heating the liquid sample and/or for control of the assay temperature. Heat can be used in the incubation step of an assay reaction to promote the reaction and shorten the duration necessary for the incubation step. A system can include a heating block configured to receive a liquid sample for a predetermined time at a predetermined temperature. The heating block can be configured to receive a plurality of samples.

The system temperature can be carefully regulated. For example, the system includes a casing kept at a predetermined temperature (i.e., 37° C.) using stirred temperature controlled air. Waste heat from each of the units will exceed what can be passively dissipated by simple enclosure by conduction and convection to air. To eliminate waste heat, the system can include two compartments separated by an insulated floor. The upper compartment includes those portions of the components needed for the manipulation and measurement of the liquid samples, while the lower compartment includes the heat generating elements of the individual units (e.g., the motor for the centrifuge, the motors for the agitation units, the electronics for each of the separate units, and the heating blocks for the incubation units). The lower floor is then vented and forced air cooling is used to carry heat away from the system. See FIGS. 44A and 44B.

The MR unit may require more closely controlled temperature (e.g., $\pm 0.1^\circ\text{C}$.), and so may optionally include a separate casing into which air heated at a predetermined temperature is blown. The casing can include an opening through which the

liquid sample is inserted and removed, and out of which the heated air is allowed to escape. See FIGS. 45A and 45B. Other temperature control approaches may also be utilized.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the devices, systems, and methods described herein are performed, made, and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

Example 1

Preparation of Coated Particles

Briefly, 1 mg of substantially monodisperse carboxylated magnetic particles were washed and resuspended in 100 μL of activation buffer, 10 mM MES. 30 μL of 10 mg/ml 10 kDa amino-dextran (Invitrogen) was added to activation buffer and incubated on a rotator for 5 minutes at room temp. For coupling of the carboxyl groups to amines on the dextran, 30 μL of 10 mg/ml 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride (EDC) was added and incubated on rotator for 2 hours at room temperature. Particles were washed away from free dextran 3 \times in 1 ml of PBS using magnetic separation, then resuspended in 1 ml of PBS. 100 μL of a 100 mM solution of Sulfo-NHS-biotin (Invitrogen) was used to decorate the amino groups on the dextran surface with biotin. After 30 minutes of incubation, particles were washed 3 \times in 1 ml activation buffer. Next, a protein block of 100 μL of 0.5 mg/ml of bovine serum albumin (BSA) (Sigma) and 30 μL of 10 mg/ml EDC was introduced and incubated overnight (Sigma). Prepared particles were washed 3 \times in 1 ml PBS and resuspended to the desired concentration.

Prepared particles synthesized with this protocol have been shown to give similar results in T_2 assays for detection of analyte, whether samples comprise buffer or 20% lysed blood (see FIG. 37). Variations of the preparations wherein pre-biotinylated amino dextran was conjugated directly to particles in one step have also resulted in similar performance in T_2 assays in both blood and buffer samples.

Example 2

Assessment of Particles Prepared with and without a Protein Block

Briefly, biotin decorated amino-dextran magnetic particles prepared according to the method described in Example 1 were assayed in PBS and in 20% lysed blood samples in an anti-biotin titration T_2 assay.

The assay was performed with the following procedure. 50 μL of matrix, either PBS or 20% Lysed blood sample, 50 μL of varying concentrations of Anti-biotin antibody, and 50 μL of 1.0 $\mu\text{g}/\text{ml}$ secondary antibody were added to a 5 mm NMR Tube. 150 μL of 0.02 mM Fe particles were then added to each tube (i.e., 2.7×10^8 particles per tube). The samples were then vortexed for 4 seconds and incubated in a 37° C. heat block for 2 minutes. Each sample was then revortexed for 4 seconds, and incubated for an additional minute in the 37° C. heat block. Following incubation, each sample was placed into a Broker Minispec for 10 minutes, under a magnetic field. After 10 minutes, the sample was removed from the magnet, vortexed for 4 seconds, and incubated in 37° C. heat block for 5 minutes. After 5 minutes, each sample was revortexed and

incubated in a 37° C. heat block for an additional 1 minute. T₂ values were taken using the Broker Minispec program with the following parameters:

Scans: 1
Gain: 75
Tau: 0.25
Echo Train: 3500
Total Echo Train: 4500
Dummy Echos: 2

ΔT₂ values were calculated: T₂-(T₂)₀ and results are depicted in FIG. 37.

Particles synthesized with a protein block, AXN4, gave nearly equal performance in blood and buffer (FIG. 37). The graph depicted in FIG. 38 compares particles prepared with (open circle) and without (filled circle) a protein blocking step. We have thus found the protein block may be needed to achieve similar functionality in blood matrices.

Additional protein blocks including but not limited to fish skin gelatin have also been successful. Particles were prepared according to the method described above, with the exception that in lieu of using BSA as the protein block, fish skin gelatin (FSG) was substituted. The graph depicted in FIG. 39 shows results of a T₂ assay (as described above) using antibody titration for particles blocked with BSA and compared to FSG. The data indicates that there is little or no difference between the two protein blocking methods (see FIG. 39). However, BSA has proven to be a more reliable block.

Example 3

Determination of Amount of Dextran Coating

Attempts to increase dextran coating density on particles have been found to reduce functionality of prepared particles in blood. The preparation of particles described in Example 1 above that demonstrated nearly equivalent buffer/blood performance used a 10× excess of dextran base upon a space filling model to determine amount of dextran to include in coating experiments. In an attempt to functionalize particles with a higher fidelity, increasing the dextran coating to a 1000-10000× excess of dextran in coating experiments generated particles having a thicker dextran coating which yielded a reduced response in blood as compared to buffer. We conclude that a moderate density of dextran with a protein block may be desirable to produce a particle coating that functions well in T₂ assays in the presence of blood sample (see FIGS. 40A and 40B).

Example 4

Detection of a Small Molecule Analyte in Whole Blood Samples

Materials and Methods:

Jackson Immuno Research Labs Mouse Anti-Biotin Monoclonal Antibody (200-002-211)
Jackson Immuno Research Labs Sheep Anti-Mouse (515-005-071)
Tween 20
Bovine Serum Albumin (Sigma Product #: B4287-256)
1×PBS Tablets (Sigma P4417)
PEG FITC Biotin Analyte
100 mM Tris HCl in dH2O
0.1% Tween®
EDTA Whole blood lysed 1:5 with 1× Trax buffer
Superparamagnetic, iron oxide, COOH-coated particles

Equipment:

Broker Minispec
Variable Speed Vortexer (VWR)
5 mm NMR Tubes

37° C. Heat block with custom made NMR Tube slots

Buffer/Analyte Preparation:

0.1% BSA, 0.1% Tween® in 1×PBS: A 10% Tween® 20 solution by weight was prepared. Briefly, Tween® in 1×PBS was prepared. 500 mL of 0.2% Tween® solution was prepared by adding 10 mL of 10% Tween® to 490 mL of 1×PBS. A 2% solution of BSA was prepared in 1×PBS solution by weight. A 0.2% solution of BSA solution was prepared by adding 50 mL of 2% BSA in PBS to 450 mL of 1×PBS. Dilutions were combined to make a final volume of 1 L and a final buffer concentration of 0.1% BSA, 0.1% Tween® in 1×PBS.

PEG-FITC-Biotin Analyte: 100 μl of a 0.5 mM solution was prepared from 1 mM Tris HCl. 40 μl of PEG FITC biotin was mixed with 40 μl of 0.5 mM Tris HCl, and incubated for 15 minutes at room temperature. After 15 minutes, 70 μl of PEG-FITC-Biotin in 0.5 mM Tris HCl was added to 630 μl of 0.1% Tween® to make a 100 μM stock solution. Stock solution was vigorously mixed by vortexing. 200 μl of 100 μM solution was added to 900 μl of 0.1% Tween® to make 20,000 nM analyte. 10 fold dilutions were prepared down to 0.02 nM

Procedure:

25 μl of appropriate analyte and 50 μl of 1:5 Lysed blood matrix were pipetted directly into a 5 mm NMR tube. Samples were vortexed for 4 seconds. 25 μl of primary Anti-biotin antibody (0.18 μg/ml) was added, then incubated in a 37° C. heat block for 15 minutes. After 15 minutes, 50 μl of 3.0 μg/ml Secondary Anti-Mouse antibody and 150 μl of 0.02 mM Fe particles (2.7×10⁸ particles per tube) were added to the NMR Tube. The sample was then vortexed for 4 seconds and incubated for 5 minutes at 37° C. The sample was placed in a Broker Minispec for 10 minutes, under magnetic field. After 10 minutes, the sample was removed from the magnet and incubated for an additional 5 minutes. Sample was again vortexed for 4 seconds and incubated for an additional 1 minute. T₂ values were taken using the Broker Minispec program with the following parameters:

Scans: 1
Gain: 75
Tau: 0.25
Echo Train: 3500
Total Echo Train: 4500
Dummy Echos: 2

Example 5

Synthesis of Antibody Decorated Particles

Amino dextran coated magnetic particles prepared as described in Example 1 can be further functionalized with antibodies via an SMCC-SATA linkage (SMCC=succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate; SATA=N-succinimidyl-5-acetylthioacetate). The carboxylated magnetic particles are first conjugated to 10 kDa amino dextran via EDC chemistry as described above. The dextran coated particles are further modified with an excess of sulfo-SMCC to provide a maleimide functional group. Antibodies are modified with a SATA linker, which primarily binds to the amines on the antibody. The SATA linkage is controlled to minimize over-functionalization of the antibody which may lead to cross-linking of the particles or reduced affinity of the antibody. After deacetylation, the SATA linker exposes a thiol functional group which can be

used to directly attach to the maleimide functionalized particles forming a thioether bond. The number of antibodies conjugated to each particle can be measured using a BCA protein assay (Pierce). Linkers that provide similar functionality to SATA have been used successfully, such as SPDP (N-Succinimidyl 3-[2-pyridyldithio]-propionate).

Antibody coated magnetic particles can also be prepared using the chemistries described above, but with direct covalent linkage to the base carboxylated particle. In some instances it may necessary to add additional coating to the particle surface, such as dextran, or a blocking agent. Similar chemistries can be used with alternate coatings to the amino dextran, such as PEG or BSA.

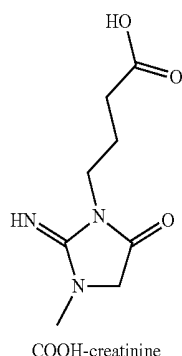
Example 6

Creatinine Assay

Briefly, the assay includes the following: a target sample is incubated in the presence of a magnetic particle that has been decorated with creatinine, which is linked to the surface of the magnetic particles. The creatinine decorated magnetic particles are designed to aggregate in the presence of the creatinine antibody. Each of the creatinine decorated magnetic particles and creatinine antibody is added to the liquid sample containing creatinine, which competes with the magnetic particles for the creatinine antibody. Thus, the binding of the creatinine to the antibody blocks agglomeration of the magnetic particles, and low levels of creatinine are marked by the formation of agglomerates. These agglomerates alter the spin-spin relaxation rates of sample when exposed to a magnetic field and the change in the T_2 relaxation times (measuring a change in the magnetic resonance signal from the surrounding water molecules) can be directly correlated to presence and/or concentration of the analyte in the target sample.

Creatinine Antibody

In establishing an antibody generation program for creatinine, a modified creatine molecule was devised (COOH-creatinine) and conjugated to transferrin for immunization in BALB-C and AJ mice.



Thirty four stable antibody producing clones were generated. These clones arose from either BALB-C (spleen cells) (n=17) or AJ mice (n=17). The two genetically different mouse lines were selected for the known genetic differences in their immune systems. Criteria and a selection process were developed for screening and identification of an optimal monoclonal antibody for use in the assay. The antibody selection process included screening for binding to BSA-creatinine by ELISA, antibody affinity/sensitivity/specificity by ELISA competitive assays using free creatinine and potential

interferents, determination of the ability of the antibody to be conjugated to the magnetic particle and to be functional in a T_2 magnetic relaxation switch assay.

Using the established antibody selection criteria outlined above, seven monoclonal antibodies were identified and selected for these qualities as potential candidates in the assay.

Creatinine-Coated Magnetic Particles

Substantially monodisperse carboxylated magnetic particles were washed and resuspended in 100 μ l of coupling buffer (50 mM MES, pH=4.75). Sulfo-NHS (55 μ mol in 200 μ l MES buffer) was added and the mixture vortexed. To the mixture was added EDC (33.5 μ mol in 200 μ l MES buffer). The solution was briefly vortexed and placed on an end over end mixer for 1 hour at room temperature, allowed to settle, and the supernatant removed. To the resulting solids was added 1 mL of 1% BSA in PBS, and again the mixture was vortexed and placed on an end over end mixer for 15-18 hours at room temperature. The particles were allowed to settle and the supernatant removed.

The BSA-coated particles were suspended in 0.5 mL PBS-0.01% T20 (10 mM phosphate buffer, pH=7.4, 150 mM NaCl, with 0.01% Tween® 20). Unreacted carboxyl groups were subjected to Methyl-PEG4-amine (20 μ l of 10% v/v in DMSO) as a blocking agent. The mixture was vortexed and placed on an end over end mixer for 8 hours at room temperature. The resulting BSA-coated particles were repeatedly washed with 0.5 mL PBS-0.01% T20.

COOH-creatinine (66 μ mol), EDC (140 μ mol), and NHS (260 μ mol) were combined with 300 μ l of dry DMSO to form a slurry, which cleared as the reaction reached completion. BSA-coated particles were suspended in 0.5 mL PBS-0.01% T20 (pH=8), followed by the addition of the activated COOH-creatinine solution. The resulting mixture was vortexed and placed on an end over end mixer for 4 hours at room temperature. The resulting particles were washed 3 \times each with sonication using 1:15 and 1:30 DMSO:PBS-0.01% T20 (vol/vol). The particles were then washed 3 \times each with sonication using PBS-0.01% T20. The particles were resuspended in PBS-0.1% T20 (pH=8) and 2 mg of NHS-PEG 2K in 200 μ l PBS-0.01% T20 was added. The mixture was placed on an end over end mixer for 12-20 hours at room temperature. The particles were then washed 3 \times each with sonication using PBS-0.01% T20 to produce creatinine-conjugated magnetic particles with sequential BSA, creatinine coating, PEG cap and block.

The creatinine coated particles were resuspended in assay buffer (100 mM glycine (pH=9.0), 150 mM NaCl, 1% BSA, 0.05% ProClin®, and 0.05% Tween®).

The creatinine assay protocol using creatinine conjugated particles and soluble creatinine antibody with detection using the T_2 signal. The creatinine competitive assay architecture is depicted in FIG. 24.

Solutions of magnetic particles, antibody, and liquid sample were, where indicated, subject to dilution with an assay buffer that included 100 mM Tris pH 7.0, 800 mM NaCl, 1% BSA, 0.1% Tween®, and 0.05% ProClin®.

The creatinine-coated magnetic particles were diluted to 0.4 mM Fe (5.48×10^9 particles/ml) in assay buffer, vortexed thoroughly, and allowed to equilibrate for 24 hours at 4-8° C.

The anti-creatinine mouse monoclonal antibody (described above) was employed as a multivalent binding agent for the creatinine-conjugated magnetic particles. The antibody was diluted to a concentration of 0.8 μ g/ml in assay buffer and vortexed thoroughly.

Samples and calibrators were diluted 1 part sample to 3 parts assay buffer. The upper assay range is ca. 4 mg/dL.

creatinine. For samples with expected creatinine levels >4 mg/dL an additional sample dilution was performed using 1 part initial diluted sample to 4 parts assay buffer.

The pre-diluted sample, assay buffer, magnetic particle, and antibody solutions were each vortexed. 10 μ L of each solution added to a tube, and the tube was vortexed for 5 minutes.

The tube was then subjected to 12 minutes of gMAA, incubated for 5 minutes at 37° C., placed in the MR Reader (T₂ MR, Reader with 2200 Fluke Temperature Controller, with NDxlient software 0.9.14.1/hardware Version 0.4.13 Build 2, Firmware Version 0.4.13 Build 0) to measure the T₂ relaxation rate of the sample, and the T₂ relaxation rate of the sample was compared to a standard curve (see FIG. 25A) to determine the concentration of creatinine in the liquid sample.

Performance of Modified Creatinine Antibodies

Different creatinine antibodies were tested in the assay to ascertain the effect of the antibody on agglomeration. We observed that the performance of the creatinine antibodies varied in their performance characteristics when combined with creatinine-coated magnetic particles (see FIG. 25B). SDS-PAGE gel analysis of the two preparations revealed significantly enhanced aggregation in preparation 1, believed to arise from an increase in the creatinine binding valency for this antibody, which is aggregated due to its purification process. For comparison, we multimerized another creatinine monoclonal antibody (14HO3) by biotinylating the antibody and multimerizing the antibody in the presence of streptavidin. The monomeric, biotinylated monomeric, and multimerized forms were then tested with creatinine-coated magnetic particles to assess the effect of increased valency on T₂ time. The results are depicted in FIG. 25C, showing the multimerized antibody forms clusters at much lower concentrations than the non-multimerized antibodies. This valency enhancement for particle clustering has also been observed using IgM antibodies.

Example 7

Creatinine Antibody-Coated Magnetic Particle

Using an alternative assay architecture, the assay includes the following: a target sample is incubated in the presence of (i) a magnetic particle that has been decorated with creatinine antibody; and (ii) a multivalent binding agent including multiple creatinine conjugates. The magnetic particles are designed to aggregate in the presence of the multivalent binding agent, but aggregation is inhibited by competition with creatinine in the liquid sample. Thus, the binding of the creatinine to the antibody-coated particle blocks agglomeration of the magnetic particles in the presence of the multivalent binding agent, and low levels of creatinine are marked by the formation of agglomerates. These agglomerates alter the spin-spin relaxation rates of sample when exposed to a magnetic field and the change in the T₂ relaxation times (measuring a change in the magnetic resonance signal from the surrounding water molecules) can be directly correlated to presence and/or concentration of the analyte in the target sample.

Substantially monodisperse carboxylated magnetic particles were washed and resuspended in 300 μ L of coupling buffer (50 mM MES, pH=4.75), and sulfo-NHS (46 μ mol) EDC (25 μ mol) were added to the particles. The solution was briefly vortexed and placed on an end over end mixer for 1 hour at room temperature. The activated particles were washed with mL PBS-0.01% T20, and resuspended in 1 mL

of 10% w/v solution of amine-PEG-amine in PBS-0.01% T20. The mixture was vortexed and placed on an end over end mixer for 2 hours at room temperature, and then washed 3 \times with PBS-0.01% T20.

BSA can be substituted for amine-PEG-amine as an alternate chemistry. The BSA-coated magnetic particles were prepared as described in example 6, in the section describing creatinine coated magnetic particles.

The particles were resuspended in 260 μ L PBS-0.01% T20 and reacted with 198 μ L sulfo SMCC (5 mg/mL in PBS-0.01% T20). The solution was briefly vortexed and placed on an end over end mixer for 1 hour at room temperature, and then washed 3 \times with PBS-0.01% T20 with 10 mM EDTA to produce SMCC-coated particles.

SATA-labeled antibody was prepared by combining SATA (30 nmol in DMSO) with antibody (2 nmol in PBS, pH=7.4). The solution was placed on an end over end mixer for 1 hour at room temperature. Blocked sulfhydryl groups on SATA-labeled antibody were deprotected by treatment with deacetylation buffer (0.5M hydroxylamine hydrochloride in pH 7.4, 10 mM phosphate, 150 mM sodium chloride, 10 mM EDTA) for 1 hour and purified through a desalting column using PBS containing 10 mM EDTA prior to use.

As an alternate to SATA, SPDP-labeled antibody can be used. SPDP-labeled antibody was prepared by adding SPDP (10 nmol in DMSO) with antibody (2 nmol in PBS, pH 7.4). The solution was incubated for 1 hour at room temperature and purified through a desalting column. The disulfide linkage of SPDP on the SPDP-labeled antibody was cleaved in a reaction with 5 mM mercaptoethylamine and incubated for 10 minutes at ambient temperature. The disulfide bond-cleaved SPDP-labeled antibody was purified through a desalting column prior to use.

The SMCC-functionalized particles with PEG- or BSA-coating and deacetylated SATA-modified antibody were combined and placed on an end over end mixer for overnight at room temperature, washed 3 \times with PBS-0.05% Tween® 80, and resuspended in PBS-0.01% T20 with 10 mM EDTA. A blocking agent (m-PEG-SH 2K) was added, the solution was placed on an end over end mixer for 2 hours, washed 2 \times with PBS-0.05% Tween® 80, and resuspended in PBS-0.05% Tween® 80, 1% BSA, and 0.05% ProClin® to produce antibody-coated magnetic particles.

The SMCC-functionalized BSA-coated particles and disulfide-bind cleaved SPDP-labeled antibody were combined and placed on an end over end mixer for 2 hours at room temperature, washed 2 times with PBS-0.01% Tween® 20, 10 mM EDTA, and resuspended in PBS, 0.01% T20, and 10 mM EDTA. A blocking agent, m-PEG-SH 2K (1 mole), was added, and the solution was placed on an end over end mixer for 2 hours. A second blocking agent, n-ethyl maleimide (5 mole), was added. The particles were mixed for 15 minutes, washed twice with PBS-0.01% Tween® 20, and resuspended in pH 9, 100 mM Tris, 0.05% Tween® 80, 1% BSA, and 0.05% ProClin® to produce antibody coated magnetic particles.

The procedure outlined above can be used with creatinine antibodies, or the creatinine antibodies can be coupled directly to the surface of the carboxylated magnetic particles via EDC coupling.

Creatinine Multivalent Binding Agents

COOH-creatinine was conjugated to 3 amino-dextran compounds (Invitrogen; MW 10 k, 40 k, and 70 k with 6.5, 12, and 24 amino groups per molecule of dextran respectively) and BSA via EDC coupling. The resulting BSA-creatinine and amino-dextran-creatinine multivalent binding agents can be used in the competitive inhibition assay described above. Degrees of substitution between 2-30 creatinines per dextran moiety were achieved. An example creatinine inhibition curve is shown in FIG. 33. The binding agent used is a 40 kDa dextran with ~10 creatinines per dextran molecule.

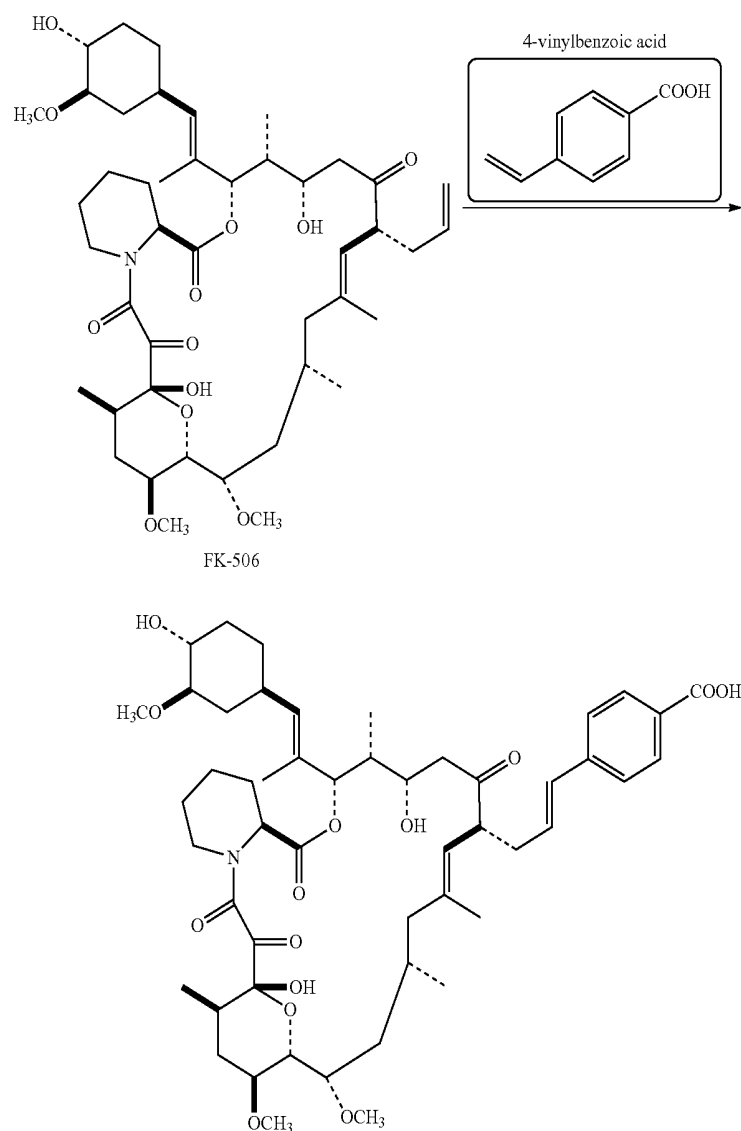
93

Example 8

Preparation of Tacrolimus Multivalent Binding Agents

Tacrolimus conjugates were prepared using dextran and BSA. FK-506 was subjected to the olefin metathesis reaction using Grubbs second generation catalyst in the presence of 4-vinylbenzoic acid as depicted below in Scheme 1. The crude product mixture was purified by normal phase silica gel chromatography.

Scheme 1



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temperature to form the activated tacrolimus-acid derivative (activated Tac solution 4.6 mM). The activated tacrolimus was used immediately.

Various amino-dextran polymers were dissolved in 100 mM sodium phosphate buffer (pH 8.0) to make a 9.5 mg/mL stock solution.

Activated Tac solution was added drop-wise with stirring at room temperature to the stock solution of amino-dextran in the ratios tabulated below. Each reaction was stirred vigorously for at least 2 hours.

Dextran Conjugates

Dextran-tacrolimus conjugates were prepared using three different molecular weight amino-dextrans, each with a different amino group substitution.

2.78 mL of EDC solution (40 mg/mL EDC hydrochloride) and 2.78 mL of sulfo-NHS solution (64 mg/mL sulfo-NHS) were combined with stirring. To this mixture was added 0.96 mL of tacrolimus-acid derivative (C21) solution (28.8 mg/mL in DMSO) and the contents stirred for 30 minutes at room

TABLE 6

Reaction	Amino Dextran m.w.	Ratio of Amine:Tac	Amino Dextran (μL)	Volume Tac (μL)	Estimated Tac:Dextran molar ratio
1	70K	1:0.2	1000	70.8	Not tested
2	70K	1:0.4	1000	141.6	Not tested
3	70K	1:0.8	1000	283.2	4.1
4	70K	1:1.6	1000	566.4	Not tested
5	70K	1:3.2	1000	1132.8	Not tested

TABLE 6-continued

Reaction	Amino Dextran m.w.	Ratio of Amine:Tac	Amino Dextran (μL)	Volume Tac (μL)	Estimated Tac:Dextran molar ratio
6	70K	1:5	1000	1770	15.8
7	10K	1:0.8	1000	283	1.0
8	10K	1:5	1000	1766	2.2
9	40K	1:0.8	1000	287	2.0
10	40K	1:5	1000	1793	8.2

The resulting Tac-dextran conjugates were purified using a 5-step serial dialysis of each reaction product (1st—15% (v/v) aqueous DMSO; 2nd—10% (v/v) aqueous methanol; 3rd to 5th—high purity water; at least 2 hours for each step; using a 3,500 MWCO dialysis membrane for the 10K MW amino-dextran and a 7K MWCO dialysis membrane for the 40K and 70K amino-dextran).

Following purification, each of the samples was lyophilized and the dry weight determined. The multivalent binding agents were reconstituted prior to use.

After reconstitution, the tacrolimus substitution ratios were estimated based upon the absorbance at 254 nm.

Experiments were performed to determine which size dextran provided the most optimal agglomerative performance. Briefly, 10 μL of 10% MeOH, 1% BSA in PBS pH 6.3 buffer, 20 μL of Dextran Tac agglomerator, 10K, 40K, 70K, at varying concentrations, and 10 μL of Anti-Tacrolimus coated magnetic particle at 0.2 mM Fe was added to a 200 μL PCR Tube (2.7×10⁹ particles per tube). The sample was vortexed using a plate mixer at 2000 rpm for 2 minutes, preheated for 15 minutes at 37° C. in an incubation station, exposed to a side and bottom magnet for 1 minute each, repeated for 6 cycles, vortexed again for 2 minutes at 2000 rpm, incubated for 5 minutes in 37° C. incubator containing PCR tube designed heat block, and the T₂ was read on the MR Reader. Data from 3 replicates indicates that increased molecular weight/varied substitution ratios of dextran Tac can result in the improved T₂ signal (see FIG. 34). In addition, higher substitution also resulted in improved response (see FIG. 35).

BSA Conjugates

BSA-tacrolimus conjugates were prepared with varying degrees of tacrolimus substitution.

34.5 μL of NHS solution (66.664 mg/mL in acetonitrile) and 552 μL of EDC (6.481 mg/mL in 50 mM MES pH 4.7) were combined with stirring. 515.2 μL of this EDC NHS mixture was added drop-wise to 220.8 μL of tacrolimus-acid derivative (C21) solution (33.33 mg/mL in acetonitrile) and the contents stirred for 1 hour at room temperature to form the activated tacrolimus-acid derivative. The activated tacrolimus was used immediately.

BSA was dissolved in phosphate buffered saline and acetonitrile to form a solution containing 5 mg/mL BSA in 40% acetonitrile.

Activated Tac solution was added drop-wise with stirring at room temperature to the BSA solution in the ratios tabulated below. Each reaction was stirred vigorously for at least 2 hours.

TABLE 7

Reaction	Ratio of Tac:BSA	BSA (μL)	Volume Tac (μL)
1	5:1	1000	35
2	10:1	1000	70
3	20:1	1000	140

TABLE 7-continued

Reaction	Ratio of Tac:BSA	BSA (μL)	Volume Tac (μL)
4	30:1	1000	210
5	50:1	1000	350

The resulting Tac-BSA conjugates were purified using a PD10 size exclusion column pre-equilibrated with 40% acetonitrile. The eluent was collected in 1 mL fractions and monitored for absorbance at 280 nm to identify fractions containing BSA.

The BSA-containing fractions were combined and the acetonitrile removed under vacuum.

Tac-BSA conjugates were evaluated for clustering ability by performing a titration similar to that used for the dextran-tacrolimus conjugates. As observed, clustering performance differs with Tac substitution ratio (see FIG. 36).

Example 9

Tacrolimus Competitive Assay Protocol (Antibody on Particle Architecture)

A tacrolimus assay was developed using anti-tacrolimus antibody conjugated particles and BSA-tacrolimus multivalent binding agent with detection using an MR Reader (see Example 6). This assay was designed for testing whole blood samples that have been extracted to release tacrolimus from the red blood cells and binding proteins. The tacrolimus competitive assay architecture is depicted in FIG. 28.

Solutions of magnetic particles and multivalent binding agent were, where indicated, subject to dilution with an assay buffer that included 100 mM Glycine pH 9, 0.05% Tween® 80, 1% BSA, 150 mM NaCl, 0.1% CHAPS.

A base particle with COOH functionality was modified by sequential aminated coating (PEG or BSA), antibody covalent attachment, PEG cap and PEG/protein block (as described in the examples above). The antibody-coated magnetic particles were diluted to 0.4 mM Fe (5.48×10⁹ particles/mL) in assay buffer, vortexed thoroughly.

The multivalent binding agent was formed from COOH-modified tacrolimus covalently conjugated to BSA (as described in Example 8). The multivalent binding agent was diluted to 0.02 μg/mL in assay buffer, and vortexed thoroughly.

The extracted sample solution (10 μL) and the magnetic particle solution (10 μL) were combined and vortexed for five seconds and incubated at 37° C. for 15 minutes. To this mixture was added 20 μL of the multivalent binding agent and the resulting mixture vortexed for five seconds and incubated at 37° C. for 5 minutes.

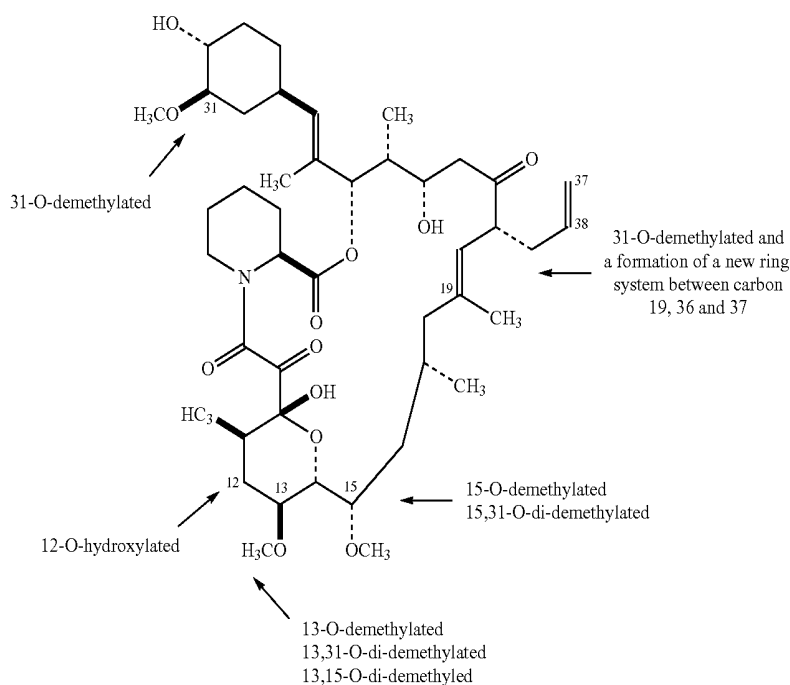
Several samples were prepared as described above. All samples were placed into the gMAA unit for 1 minute. All samples were then placed into a tray removed from the magnetic field. Each sample was vortexed for at least five seconds and returned to the tray. All samples were again placed into the gMAA unit for 1 minute, followed by vortexing. This process was repeated twelve times for each sample.

The sample was incubated for 5 minutes at 37° C., placed in the MR Reader (see Example 6) to measure the T₂ relaxation rate of the sample, and the T₂ relaxation rate of the sample was compared to a standard curve (see FIG. 29) to determine the concentration of tacrolimus in the liquid sample.

Tacrolimus Antibody

Several antibody development programs were pursued to create a high-affinity tacrolimus antibody including traditional mouse monoclonal models, in vitro phage display strategies, and rabbit models. C21 derivatives of tacrolimus were used as the haptens for the immunogen and screening conjugates used in these programs. A set of criteria was developed for screening and identification of an optimal antibody for use in the assay. The criteria include the ability to bind tacrolimus-protein conjugates, the inhibition of that binding in the presence of nanomolar levels of free tacrolimus, all while exhibiting little or no affinity for the metabolites of tacrolimus (depicted below).

Using the established antibody selection criteria outlined above, several monoclonal antibodies, polyclonal antibodies, and Fab fragments have been identified and selected as potential candidates in a tacrolimus assay.



Example 10

Side-Side Gradient Magnetic Assisted Agglomeration (gMAA)

An evaluation of alternative methods of gMAA was performed using the creatinine immunoassay described in Example 6 with sample containing no analyte to compete with the particle-antibody specific agglomeration.

Several identical samples were prepared as described in Example 6. All samples were placed into the gMAA unit for 1 minute. All samples were then placed into a tray removed from the magnetic field. Each sample was vortexed for at least five seconds and returned to the tray. All samples were again placed into the gMAA unit for 1 minute. This process was repeated twelve times for each sample, to obtain replicate measurements.

After the last gMAA cycle, the sample was vortexed for 5 seconds, incubated for 5 minutes at 37° C., and placed in the MR Reader to measure the T₂ relaxation rate of the sample.

The specific aggregation achieved with various methods of gMAA are depicted in FIG. 26, wherein (i) "control" is

gMAA (magnet exposure+vortex, repeat) in which the relative position of the sample and the magnetic field direction are unchanged with each cycle; (ii) "twist is gMAA (magnet exposure+rotation within magnet, repeat) with rotating tube ca. 90° relative to the gradient magnet with each cycle; (iii) "180 turn" is gMAA (magnet exposure+remove tube from magnet, rotate, place back in magnet, repeat) with rotating tube ca. 180° relative to the gradient magnet with each cycle; and (iv) "remove 5 s" is =removal of tube from magnet, 5 sec rest (no rotation), repeat.

In the pulsed (cycled) magnetic assisted agglomeration of the invention, the liquid sample is exposed to magnetic fields from different directions in an alternating fashion. As shown in FIG. 26, the rate at which a steady state degree of agglomeration, and stable T₂ reading, is achieved is expedited by cycling between the two or more positions over a series of gMAA treatments.

Example 11

Side-Bottom Gradient Magnetic Assisted Agglomeration (gMAA)

An evaluation of "side-bottom" gMAA was performed using the creatinine basic immunoassay described in Example 6. For this evaluation, creatinine antibody was diluted to 1 µg/ml and serum calibrators were diluted 1:5 prior to the assay. 10 µl of diluted calibrator, 10 µL of particle reagent and 20 µL of antibody reagent were pipetted into the reaction well. The tube was preheated to 37 C for 5 minutes and then processed through gMAA with a 60 sec exposure in the side magnet, followed by 60 sec in the bottom magnet. This was completed for 6 total cycles or 12 minutes total. A final mix using a vortex for 60 sec was performed prior to the reading operation.

A standard curve for the competitive creatinine creatinine assay with alternating side-bottom gMAA is shown in FIG. 27 demonstrating good response with the side-bottom gMAA configuration.

Effect of Varying the gMAA Dwell Time and Temperature

An evaluation of gMAA dwell time and temperature on assisted agglomeration was performed.

The following conditions were tested to determine the most optimal temperature and dwell time for T_2 performance: Alterations—6, 12, 24, 48; for each number of alterations the following dwell time was evaluated: 30, 60, 120 seconds. A fixed magnet time of 6 minutes with the following dwell times was also evaluated: 30, 60, 120 seconds. Samples were prepared by adding 20 μ L of varied concentrations of Protein A (a target protein) and 20 μ L Anti-Protein A antibody coated magnetic particles at 0.08 mM Fe to a PCR Tube (1.2×10^9 particles per tube). Samples were placed into a 32 position tray, vortexed in a plate shaker for 2 minutes at 2000 rpm and incubated in a 37° C. incubation station for 15 minutes. Samples were then exposed to the aforementioned dwell and alteration conditions between alternating magnetic fields. Following gMAA treatment, samples were vortexed manually for 5 minutes, incubated in a 37° C. heat block compatible with PCR Tubes, and the T_2 was read using the MR Reader (see Example 6). Data in FIGS. 30A and 30B show that T_2 response is directly proportional to temperature and dwell time. Therefore, increased temperature and dwell time/total time results in improved T_2 response.

Example 13

Effect of Varying the Number of gMAA Cycles

An evaluation of varying the number of gMAA cycles was performed using the system and procedure of Example 12.

The following conditions were tested for effect on T_2 performance: cycles—3, 6, 12, 24; for each cycle the following dwell time were evaluated: 30, 60, 120 seconds. A cycle consists of dwell in the side, followed by bottom. 6 cycles=12 total alterations. Samples were prepared as described in Example 12. As shown in FIG. 31, the degree of aggregation is directly proportional to number of gMAA cycles. It was also found that when magnet exposure time reaches or exceeds 24 minutes, there is an increase in non-specific aggregation that cannot be dispersed with vortex (not shown here).

Example 14

Candida Assay

In the assay used for *Candida*, two pools of magnetic particles are used for detection of each *Candida* species. In the first pool, a species specific *Candida* capture oligonucleotide probe is conjugated to the magnetic particles. In the second pool, an additional species-specific capture oligonucleotide probe is conjugated to the magnetic particles. Upon hybridization, the two particles will hybridize to two distinct species-specific sequences within the sense strand of the target nucleic acid, separated by approximately 10 to 100 nucleotides. (Alternatively, the two capture oligonucleotides can be conjugated to a single pool of particles, resulting in individual particles having specificity for both the first and second regions). The oligonucleotide-decorated magnetic particles are designed to aggregate in the presence of nucleic acid molecules from a particular species of *Candida*. Thus, unlike the inhibition assays used for creatinine and tacrolol-

mus, the *Candida* assay features an increase in agglomeration in the presence of the target *Candida* nucleic acid molecules. The hybridization-mediated agglomerative assay architecture is depicted in FIG. 32.

Carboxylated magnetic particles are used in the *Candida* assays. Magnetic particles were conjugated to oligonucleotide capture probes to create oligonucleotide-particle conjugates. For each target amplicon, two populations of oligonucleotide-particle conjugates were prepared. Oligonucleotide-particle conjugates were prepared using standard EDC chemistry between aminated oligonucleotides and carboxylated particles, or, optionally, by coupling biotin-TEG modified oligonucleotides to streptavidin particles. Coupling reactions were typically performed at a particle concentration of 1% solids.

Post-conjugation, functional oligonucleotide densities were measured by hybridizing Cy5-labeled complements to the particles, washing the particles three times to remove non-hybridized oligo; and eluting by heating to 95° C. for 5 minutes. The amount of Cy5 labeled oligonucleotide was quantified via fluorescence spectroscopy.

The coupling reactions were performed at 37° C. overnight with continuous mixing using a rocker or roller. The resulting particle conjugates were washed twice with 1 \times reaction volume of Millipore water; twice with 1 \times reaction volume of 0.1 M Imidazole (pH 6.0) at 37° C. for 5 minutes; three times with 1 \times reaction volume of 0.1 M sodium bicarbonate at 37° C. for 5 minutes; then twice with 1 \times reaction volume of 0.1 M sodium bicarbonate at 65° C. for 30 minutes. The resulting particle conjugates were stored at 1% solids in TE (pH 8), 0.1% Tween®20).

The panel of *Candida* species detected includes *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*. The sequences are amplified using universal primers recognizing highly conserved sequence within the genus *Candida*. The capture oligonucleotides were designed to recognize and hybridize to species-specific regions within the amplicon.

An aliquot of a blood sample was first subjected to lysis as follows:

(i) A whole blood sample was mixed with an excess (1.25 \times , 1.5 \times , or 2 \times) volume of ammonium chloride hypotonic lysis solution. Addition of lysis solution disrupts all RBCs, but does not disrupt WBC, yeast, or bacteria cells. The cellular matter was centrifuged at 9000 rpm for 5 minutes and lysate was removed. Intact cells were reconstituted with 100 μ L TE (tris EDTA, pH=8) to a final volume of about 100 μ L; and

(ii) To the approximately 100 μ L sample, 120 mg of 0.5 mm beads were added. The sample was agitated for 3 minutes at about 3K rpm, thereby forming a lysate.

An aliquot of ca. 50 μ L of lysate was then subjected to PCR amplification by addition of the lysate to a PCR master mix including nucleotides; buffer (5 mM (NH₄)₂SO₄, 3.5 mM MgCl₂, 6% glycerol, 60 mM Tricine, pH=8.7 at 25° C.; primers (forward primer in 4 \times excess (300 mM forward; 0.75 mM reverse) to allow for asymmetric single strand production in the final product); and thermostable polymerase (HemoKlen-Taq (New England Biolabs)). After an initial incubation at 95° C. for 3 minutes, the mixture is subjected to PCR cycles: 62° C. annealing; 68° C. elongation; 95° C.—for 40 cycles. Note: there is a 6° C. difference in the annealing and elongation temperatures; the annealing and elongation may be combined into a single step to reduce the total amplification turn-around time.

The PCR amplicon, now ready for detection, is combined with two populations of particles in a sandwich assay.

The PCR primers and capture probes which can be used in the *Candida* assay are provided below in Table 8.

fixture (side pull 6 position) for one minute and removed touched with a hand demagnetizer, and placed back into the

TABLE 8

PCR Primers	
Pan <i>Candida</i> -PCR Forward Primer	GGC ATG CCT GTT TGA GCG TC (SEQ ID NO. 1)
Pan <i>Candida</i> -PCR Reverse Primer	GCT TAT TGA TAT GCT TAA GTT CAG CGG GT (SEQ ID NO. 2)
Capture Probes	
<i>Candida albicans</i> Probe #1	ACC CAG CGG TTT GAG GGA GAA AC (SEQ ID NO. 3)
<i>Candida albicans</i> Probe #2	AAA GTT TGA AGA TAT ACG TGG TGG ACG TTA (SEQ ID NO. 4)
<i>Candida krusei</i> Probe #1	CGC ACG CGC AAG ATG GAA ACG (SEQ ID NO. 5)
<i>Candida krusei</i> Probe #2	AAG TTC AGC GGG TAT TCC TAC CT (SEQ ID NO. 6)
<i>Candida glabrata</i> Probe #1	CTA CCA AAC ACA ATG TGT TTG AGA AG (SEQ ID NO. 7)
<i>Candida glabrata</i> Probe #2	CCT GAT TTG AGG TCA AAC TTA AAG ACG TCT G (SEQ ID NO. 8)
<i>Candida parapsilosis/tropicalis</i> Probe #1	AGT CCT ACC TGA TTT GAG GTC NitInd ¹ AA (SEQ ID NO. 9)
<i>Candida parapsilosis/tropicalis</i> Probe #2	CCG NitInd ¹ GG GTT TGA GGG AGA AAT (SEQ ID NO. 10)

¹NitInd is 5' 5-Nitroindole, a base that is capable of annealing with any of the four DNA bases.

Optionally, the assay is carried out in the presence of a control sequence, along with magnetic particles decorated with probes for confirming the presence of the control sequence.

Example 15

Non-Agglomerative Methods

This process has been demonstrated using aminosilane-treated nickel metal foam with 400 μm pores decorated with anti-creatinine antibodies and shown to specifically bind creatinine-derivatized magnetic particles. A 1 cm square piece of nickel metal foam (Recemat RCM-Ni-4753.016) was washed by incubating at room temperature for 1 hr in 2M HCL, rinsed thoroughly in deionized water, and dried at 100° C. for 2 hours. The nickel foam was then treated with 2% 3-aminopropyltriethoxysilane in acetone at room temp overnight. The nickel metal foam was then washed extensively with deionized water and dried for 2 hours at 100° C. The aminosilane-treated nickel metal foam was treated with 2% glutaraldehyde in water for 2 hours at room temp and washed extensively with deionized water. Next, the metal foam was exposed to 100 $\mu\text{g}/\text{ml}$ of anti-creatinine antibody (14H03) (see Example 6) in PBS overnight, washed extensively with PBS, and treated with Surmodics Stabilguard to stabilize and block non-specific binding. Two mm square pieces of the derivatized metal foam were cut using a fresh razor blade being careful not to damage the foam structure. A piece of the derivatized metal foam was placed into a PCR tube in 20 μl assay buffer (100 mM glycine (pH=9.0), 150 mM NaCl, 1% BSA, 0.05% ProClin®, and 0.05% Tween®). Twenty microliters of control particles (that should not bind to the metal foam ABX1-11) at 0.2 mMFe were added to the tube to bring the final volume to 40 μl and final particle concentration to 0.1 mM Fe (1×10^6 - 1×10^8 particles/tube). A separate PCR tube with the exact particle and buffer, without the metal foam was also prepared. The PCR tube containing the derivatized metal foam and control particles was placed in a gMAA

gMAA fixture for another minute, removed touched with a hand demagnetizer and placed back into the gMAA fixture for another minute and vortexed (three 1 minute magnetic exposures). Thirty μl of sample was removed from both PCR tubes, heated to 37° C. in a grant block heater for 5 minutes and the T_2 read using the MR Reader (see Example 6). The T_2 from the sample with no foam read 39.2, and the samples from the PCR tube containing the foam read 45.1, demonstrating a low level of particle depletion due to NSB. The derivatized metal foam was de-magnetized, vortexed and rinsed in assay buffer. It was placed in a new PCR tube with 20 μl of assay buffer and 20 μl of AACr2-3-4 particles derivatized with creatinine with a final particle concentration of 0.1 mMFe. A duplicate PCR tube without the derivatized metal foam was also set up as in the control experiment. The PCR tube with the metal foam was cycled twice through the gMAA device exactly as the control experiment (3 one minute exposures with demag after each exposure, and final vortex). Thirty μl samples from both tubes were removed and heated to 37° C. for 5 minutes and then read on the MR reader. The sample from the PCR tube with the derivatized metal foam read 41.5, and the sample from the PCR tube with the metal foam derivatized with the anti-creatinine antibody read 324.2, thus demonstrating specific binding/depletion of the appropriate creatinine-derivatized magnetic particles from the aqueous volume read by the MR reader.

Example 16

Detection of Single Nucleotide Polymorphisms

There are numerous methods by which T_2 measurements could detect single nucleotide polymorphisms.

The simplest application would involve discrimination of mismatches via a thermophilic DNA ligase (Tth ligase). This assay would require lysis of the sample material followed by DNA shearing. Adaptors could be ligated onto the sheared DNA if a universal amplification of the genomic DNA was needed. The SNP would be detected by engineering super-

paramagnetic particle bound capture probes which flank the SNP such that the 5' end of the 3' aminated capture probe would be perfectly complementary to one particular SNP allele and subsequent treatment with Tth ligase would result in the ligation of the two particle-bound capture probes. Ligation would therefore lock the particles into an agglomerated state. Repeated melt, hybridization cycles will result in signal amplification in cases where genomic DNA amplification is not desired because of the amplification bias risk. The same 5' aminated capture probe could be utilized in all case while the 3' aminated probe could be generated to yield 4 distinct pools (an A, G, C, or T) at the extreme 5' end. Detection would require splitting of the sample into the 4 pools to determine which nucleotide(s) were present at the polymorphic site within that particular individual. For example a strong T_2 switch in the G detection tube only would indicate the individual were homozygous for G at that particular sequence location, while a switch at G and A would indicate the individual is a heterozygote for G and A at that particular SNP site. The advantage of this method is Tth polymerase has been demonstrated to have superior discrimination capability even discriminating G-T mismatches (a particular permissive mismatch and also the most common) 1:200 fold against the correct complement. While ligase detection reactions as well as oligonucleotide ligase assays have been employed in the past to define nucleotide sequences at known polymorphic sites, all required amplification either before or after ligation; in this particular example the signal could be amplified via a ligation induced increase in the size of the resulting agglomerated particle complex and thereby increases in the measured relaxation times (T_2).

A modification to this procedure could include hybridization of a particle bound capture probe flanking the hybridization of a biotinylated probe. When a perfectly complementary duplex is formed via hybridization of the particle bound probe, the ligase would covalently bind the biotin probe to the magnetic particle. Again repeated rounds of heat denaturation followed by annealing and ligation should yield a high proportion of long biotinylated oligos on the magnetic particle surface. A wash to remove any free probe would be conducted followed by the addition of a second streptavidin labeled superparamagnetic particle. Agglomeration would ensue only if the biotinylated probes were ligated onto the surface of first particle.

A hybridization discrimination approach could as well be employed. In this example, aminated oligonucleotide complements adjacent to known SNPs would be generated. These aminated oligonucleotides would be used to derivatize the surface of a 96-well plate with 1 SNP detection reaction conducted per well. Genomic DNA would then be sheared, ligated to adaptors, and asymmetrically amplified. This amplified genomic DNA would then be applied to the array as well as a short biotinylated SNP detecting probe. The amplified genomic DNA would hybridize to the well-bound capture probe and the SNP detecting probe would then bind to the tethered genomic DNA. Washing would be conducted to remove free SNP-detecting probe. A streptavidin (SA) magnetic particle would then be added to each well. Washing again would be required to remove free-SA particles. T_2 detection could be conducted directly within the wells by added biotinylated superparamagnetic particles to yield surface tethered agglomerated particles, or the SA magnetic particles could be eluted from each well on the array and incubated in detection reactions with biotinylated magnetic particles.

Lastly a primer extension reaction could be coupled to T_2 detection to discriminate which nucleotide is present at a polymorphic site. In this assay, a pool of dideoxynucleotides would be employed with one nucleotide per pool possessing a biotin (i.e., ddA, ddT, ddbiotin-C, ddG). A superparamagnetic particle bearing a capture probe whose last base upon hybridization lies adjacent to a SNP would be employed.

The sheared genomic DNA would be split and incubated in four separate primer extension reactions. An exo-DNA polymerase would then catalyze the addition of a dideoxy complementary to the nucleotide present in the SNP. Again this reaction could be cycled if a thermophilic polymerase is employed to ensure that most of the capture probes on the particle will be extended. A magnetic separation followed by a wash of the particles would be conducted followed by incubation with streptavidin superparamagnetic particles. Clustering would ensue proportional to the extent of biotinylated capture probe on the surface of the first particle. If two of the dideoxypools generated a gain in T_2 (i.e., facilitate particle agglomeration), the patient would be a heterozygote. If only one pool yielded and increase in T_2 , the patient would be a homozygote.

A final method to detect SNPs employs allele-specific PCR primers, in which the 3' end of the primer encompasses the SNP. Since stringent amplification conditions are employed, if the target sequence is not perfectly complementary to the primer, PCR amplification will be compromised with little or no product generated. In general, multiple forward primers would be designed (one perfectly complementary to each allele) along with a single reverse primer. The amplicon would be detected using two or more capture probe bound superparamagnetic particles to induce hybridization based agglomeration reactions. One advantage of this approach is that it leverages some of the work already conducted at T_2 on PCR within crude samples, and would merely entail primers designed to encompass known SNPs. A disadvantage in this approach is that it cannot determine de novo SNP locations.

An additional method which can be used is simply relying on the discrimination capabilities of particle-particle cross-linking due to hybridization to a short nucleic acid target. Mis-matches in base pairs for oligonucleotides have been shown to dramatically shift the agglomeration state of particles, and the measured T_2 signal, due to reduced hybridization efficiencies from the presence of a single base mismatch.

OTHER EMBODIMENTS

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

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

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What is claimed is:

1. A composition comprising:

(a) a liquid sample containing an analyte; and

(b) within the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^4 to 1×10^{12} and binding moieties specific to the analyte are conjugated to their surface,

wherein the analyte from the sample is bound to at least one of the binding moieties on at least one magnetic particle.

2. A composition comprising:

(a) a liquid sample suspected of containing a *Candida* nucleic acid; and

(b) within the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^9 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$, and having a first nucleic acid probe

and a second nucleic acid probe conjugated to their surface selected so that the first nucleic acid probe is operative to bind to a first segment of the *Candida* nucleic acid and the second nucleic acid probe is operative to bind to a second segment of the *Candida* nucleic acid.

3. The composition of claim 2, wherein the liquid sample is derived from a whole blood sample.

4. A composition, comprising:

(a) a liquid sample containing one or more nucleic acid amplicons generated from an amplification reaction; and

(b) within the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^4 to 1×10^{12} $\text{mM}^{-1}\text{s}^{-1}$, and having a first nucleic acid probe and a second nucleic acid probe conjugated to their surface selected so that the first nucleic acid probe is operative to bind to a first segment of the nucleic acid

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amplicons and the second nucleic acid probe is operative to bind to a second segment of the nucleic acid amplicons.

5. A composition, comprising:

(a) a liquid sample containing *Candida* nucleic acid amplicons generated from an amplification reaction; and

(b) within the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^4 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$, and having a first nucleic acid probe and a second nucleic acid probe conjugated to their surface selected so that the first nucleic acid probe is operative to bind to a first segment of the *Candida* nucleic acid amplicons and the second nucleic acid probe is operative to bind to a second segment of the *Candida* nucleic acid amplicons.

6. The composition of claim 5, wherein said magnetic particles have a T_2 relaxivity per particle of from 1×10^9 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$.

7. The composition of claim 5, the binding of the first probe to a first segment of the *Candida* amplicon and the binding of the second probe to a second segment of the *Candida* amplicon form magnetic particle-amplicon aggregates.

8. The composition of claim 7, wherein said magnetic particles have a T_2 relaxivity per particle of from 1×10^9 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$.

9. The aggregate of claim 7, wherein the *Candida* nucleic acid amplicons are amplified from *Candida albicans*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 3)
5'-ACC CAG CGG TTT GAG GGA GAA AC-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 4)
5'-AAA GTT TGA AGA TAT ACG TGG TGG ACG TTA-3'.

10. The aggregate of claim 7, wherein the *Candida* nucleic acid amplicons are amplified from *Candida krusei*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 5)
5'-CGC ACG CGC AAG ATG GAA ACG-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 6)
5'-AAG TTC AGC GGG TAT TCC TAC CT-3'.

11. The aggregate of claim 7, wherein the *Candida* nucleic acid amplicons are amplified from *Candida glabrata*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 7)
5'-CTA CCA AAC ACA ATG TGT TTG AGA AG-3',

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and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 8)
5'-CCT GAT TTG AGG TCA AAC TTA AAG ACG TCT G-3'.

12. The aggregate of claim 7, wherein the *Candida* nucleic acid amplicons are amplified from *Candida parapsilosis* or *Candida tropicalis*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 9)
5'-AGT CCT ACC TGA TTT GAG GTC NitIndAA-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 10)
5'-CCG NitIndGG GTT TGA GGG AGA AAT-3'.

13. The composition of claim 5, wherein the *Candida* nucleic acid amplicons are amplified from *Candida albicans*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 3)
5'-ACC CAG CGG TTT GAG GGA GAA AC-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 4)
5'-AAA GTT TGA AGA TAT ACG TGG TGG ACG TTA-3'.

14. The composition of claim 5, wherein the *Candida* nucleic acid amplicons are amplified from *Candida krusei*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 5)
5'-CGC ACG CGC AAG ATG GAA ACG-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 6)
5'-AAG TTC AGC GGG TAT TCC TAC CT-3'.

15. The composition of claim 5, wherein the *Candida* nucleic acid amplicons are amplified from *Candida glabrata*, and wherein the first probe comprises the oligonucleotide sequence:

(SEQ ID NO: 7)
5'-CTA CCA AAC ACA ATG TGT TTG AGA AG-3',

and the second probe comprises the oligonucleotide sequence:

(SEQ ID NO: 8)
5'-CCT GAT TTG AGG TCA AAC TTA AAG ACG TCT G-3'.

16. The composition of claim 5, wherein the *Candida* nucleic acid amplicons are amplified from *Candida parapsilosis* or *Candida tropicalis*, and wherein the first probe comprises the oligonucleotide sequence:

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(SEQ ID NO: 9)

5'-AGT CCT ACC TGA TTT GAG GTCNitIndAA-3',

and the second probe comprises the oligonucleotide sequence: 5

(SEQ ID NO: 10)

5'-CCG NitIndGG GTT TGA GGG AGA AAT-3'.

17. A composition comprising:

(a) a liquid sample containing nucleic acid amplicons generated from an amplification reaction;

(b) within the liquid sample from 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^4 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$, and having a first nucleic acid probe and a second nucleic acid probe conjugated to their surface and selected to hybridize to a first segment and a second segment of the nucleic acid amplicons to form aggregates, the aggregates comprising the magnetic particles and the nucleic acid amplicons. 20

18. The composition of claim 17, wherein said magnetic particles have T_2 relaxivity per particle of from 1×10^9 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$. 25

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19. A method of detecting the presence of an analyte, comprising:

(a) combining a liquid sample containing an analyte with a population of 1×10^6 to 1×10^{13} magnetic particles per milliliter of the liquid sample, the magnetic particles having a mean diameter of from 700 nm to 950 nm, a T_2 relaxivity per particle of from 1×10^4 to $1 \times 10^{12} \text{ mM}^{-1} \text{ s}^{-1}$, and binding moieties specific to a first binding site on the analyte are conjugated to their surface;

(b) exposing the sample to a magnetic field to capture or concentrate the magnetic particles and the analyte;

(c) exposing the concentrated magnetic particles to a surface derivatized with ligands complementary to a second binding site on the analyte thereby forming magnetic particle-analyte-ligand complexes;

(d) placing the analyte-bound magnetic particles in a well having an radiofrequency (RF) coil disposed about the well, the RF coil configured to detect a signal produced by exposing the liquid sample to a bias magnetic field created using one or more magnets and an RF pulse sequence;

(e) exposing the sample to a bias magnetic field and an RF pulse sequence;

(f) following step (e), measuring the T_2 relaxation signal; and

(g) wherein an increase in T_2 relaxation measured in step (f) indicates the presence of said analyte in the liquid sample.

* * * * *

专利名称(译)	用于快速检测分析物的NMR系统和方法		
公开(公告)号	US8563298	公开(公告)日	2013-10-22
申请号	US12/910594	申请日	2010-10-22
[标]申请(专利权)人(译)	T2 BIOSYST		
申请(专利权)人(译)	T2 BIOSYSTEMS , INC.		
当前申请(专利权)人(译)	T2 BIOSYSTEMS , INC.		
[标]发明人	LOWERY JR THOMAS JAY AUDEH MARK JOHN CHEPIN JAMES FRANKLIN DHANDA RAHUL NEELY LORI ANNE RITTERSHAUS CHARLES WILLIAM		
发明人	LOWERY, JR., THOMAS JAY AUDEH, MARK JOHN CHEPIN, JAMES FRANKLIN DHANDA, RAHUL NEELY, LORI ANNE RITTERSHAUS, CHARLES WILLIAM		
IPC分类号	C12M1/34 G01N24/00 C07H21/04 C12Q1/68 G01N33/53 C12P19/34 G01N33/553 C12M3/00 A61B5/055 G01N15/06		
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其他公开文献	US20120100546A1		
外部链接	Espacenet USPTO		

摘要(译)

本发明的特征在于检测分析物的系统和方法，以及它们在疾病的治疗和诊断中的用途。

