



US 20160146793A1

(19) **United States**

(12) **Patent Application Publication**
Kasdan et al.

(10) **Pub. No.: US 2016/0146793 A1**

(43) **Pub. Date: May 26, 2016**

(54) **KITS, COMPOSITIONS AND METHODS FOR
DETECTING A BIOLOGICAL CONDITION**

Publication Classification

(71) Applicant: **LEUKODX LTD.**, Jerusalem (IL)

(51) **Int. Cl.**
G01N 33/53 (2006.01)
G01N 33/58 (2006.01)

(72) Inventors: **Harvey Lee Kasdan**, Jerusalem (IL);
Julien Meissonnier, Jerusalem (IL);
Yoav Zuta, Jerusalem (IL); **Bruce
Davis**, Jerusalem (IL); **Micha Rosen**,
Jerusalem (IL); **Yael Himmel**, Jerusalem
(IL); **Yehoshua Broder**, Jerusalem (IL)

(52) **U.S. Cl.**
CPC **G01N 33/5302** (2013.01); **G01N 33/582**
(2013.01); **G01N 2333/70535** (2013.01); **G01N**
2333/70596 (2013.01)

(21) Appl. No.: **14/942,921**

(57) **ABSTRACT**

(22) Filed: **Nov. 16, 2015**

Related U.S. Application Data

(60) Continuation of application No. 14/571,906, filed on
Dec. 16, 2014, now Pat. No. 9,207,239, which is a
continuation of application No. 14/296,317, filed on
Jun. 4, 2014, now Pat. No. 8,945,913, which is a divi-
sion of application No. 13/716,246, filed on Dec. 17,
2012, now abandoned.

The present invention provides kits, apparatus and methods
for determining a biological condition in a mammalian sub-
ject, the method includes incubating a specimen from a
patient with at least one composition in a kit for a predeter-
mined period of time to form at least one reaction product,
when the subject has said biological condition, and receiving
an indication of the at least one reaction product responsive to
at least one reporter element in the kit thereby providing the
indication of the biological condition in the subject.

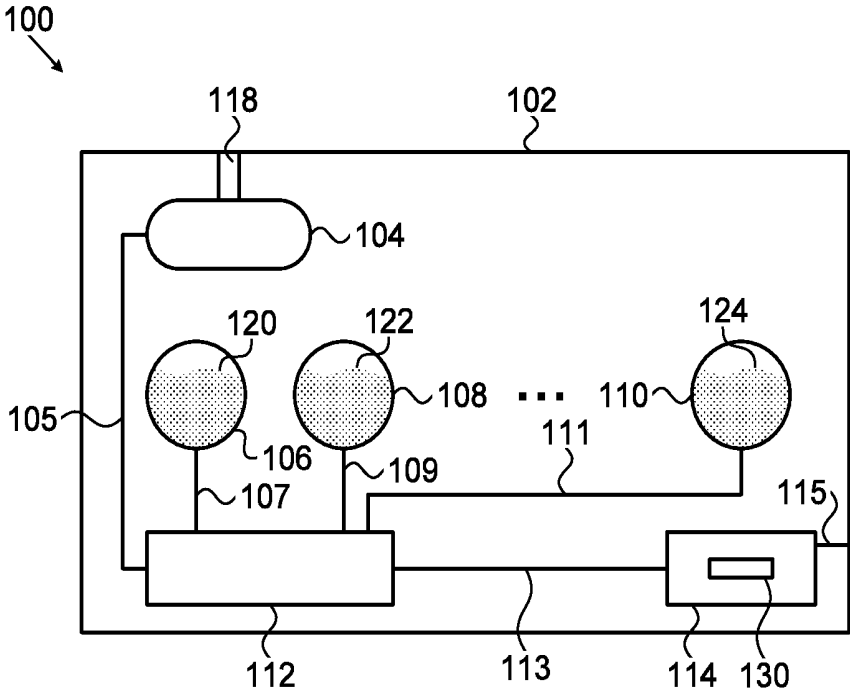


FIG. 1

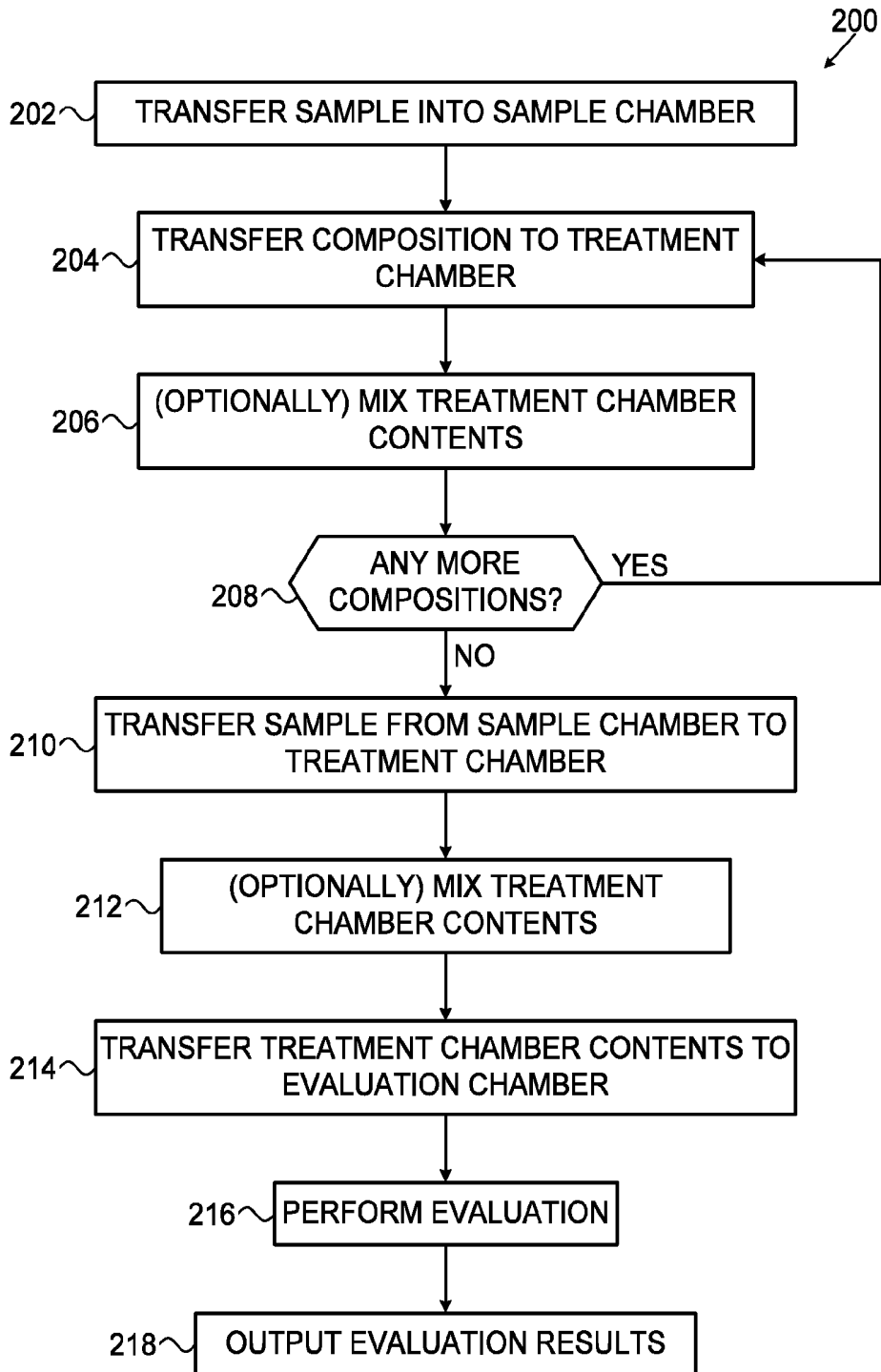


FIG. 2

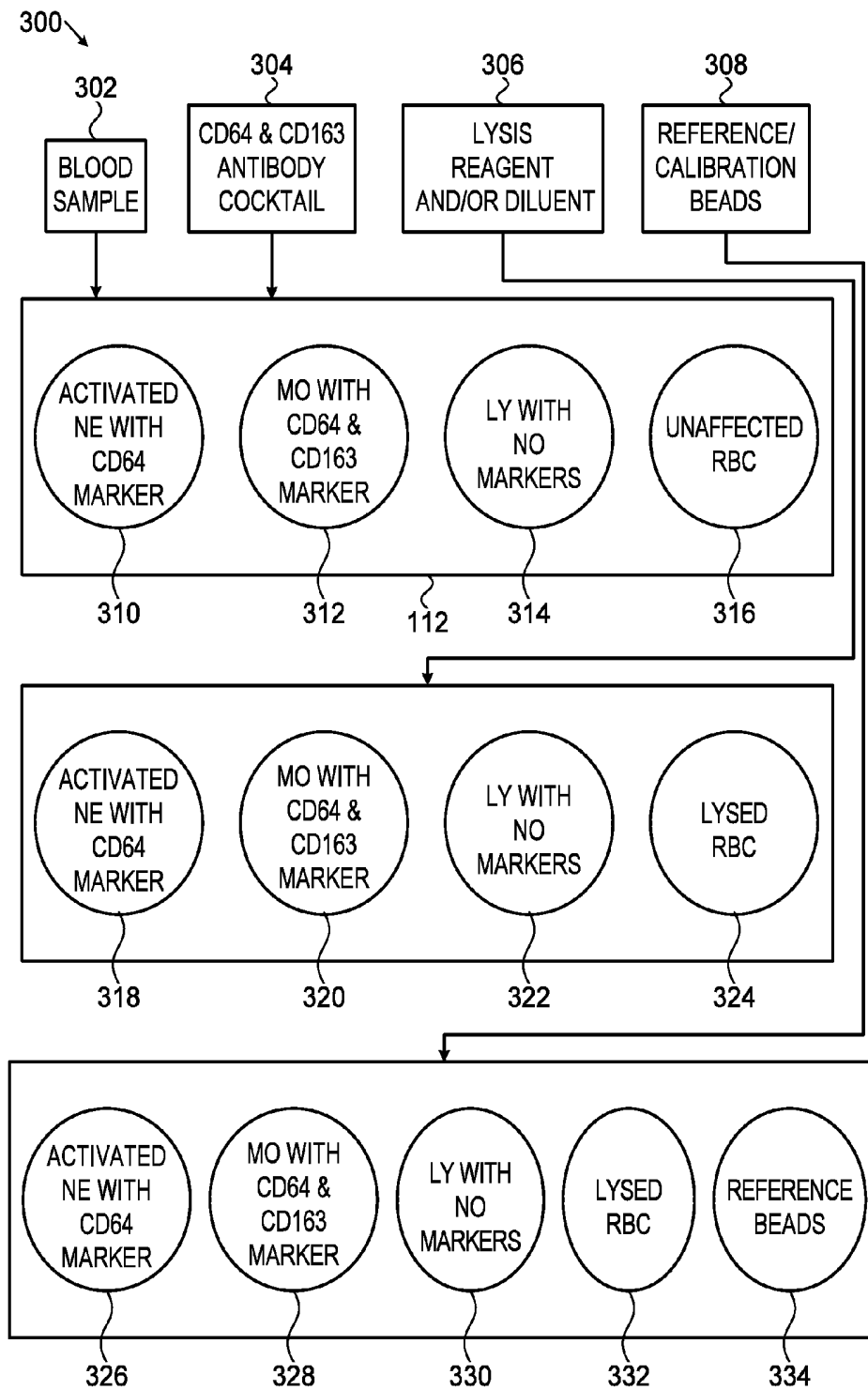


FIG. 3

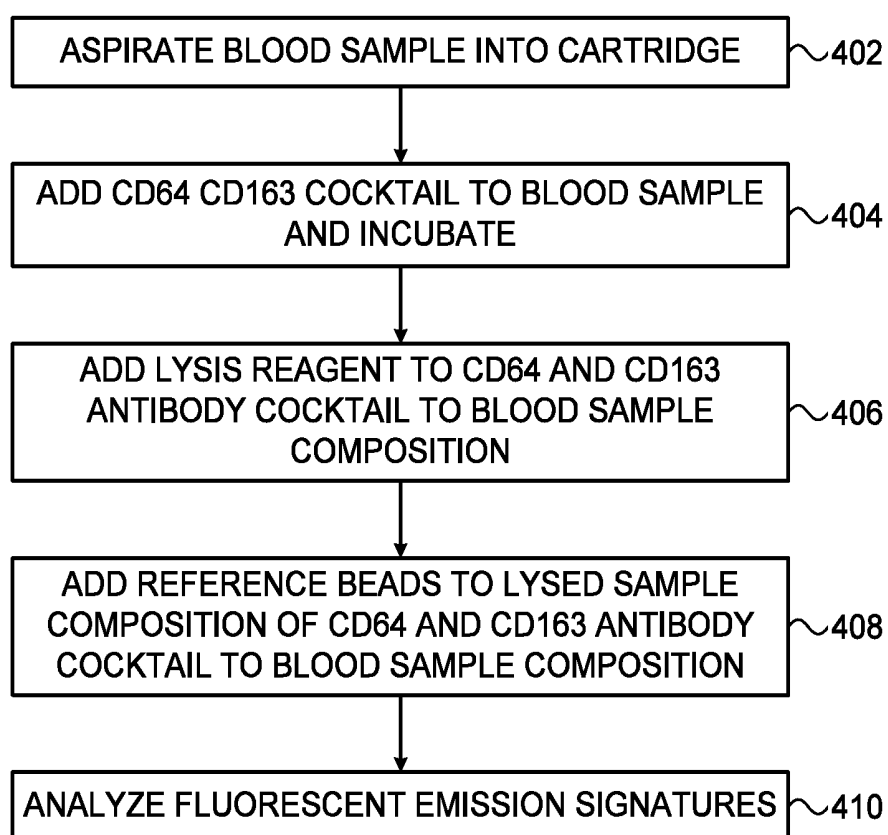


FIG. 4

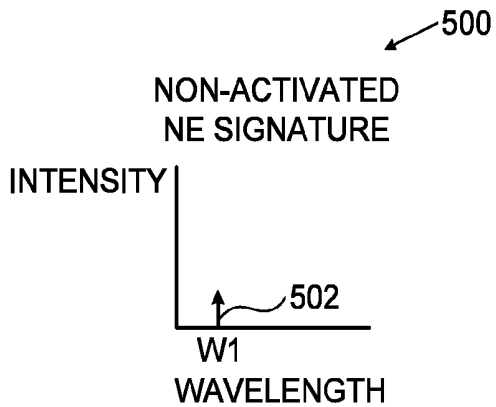


FIG. 5A

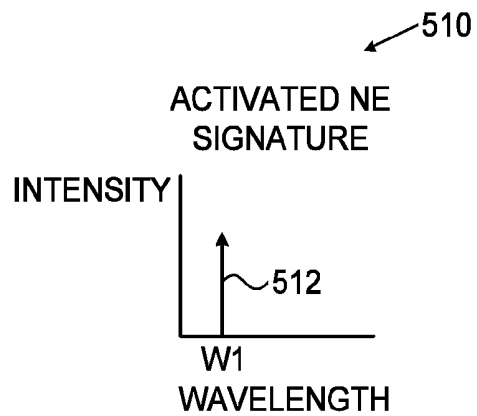


FIG. 5B

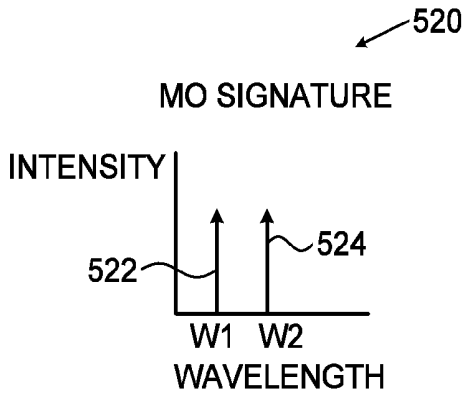


FIG. 5C

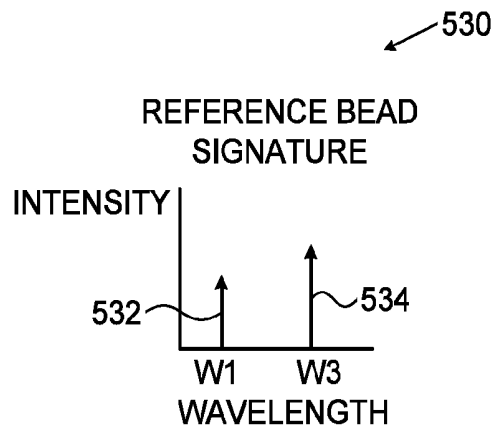


FIG. 5D

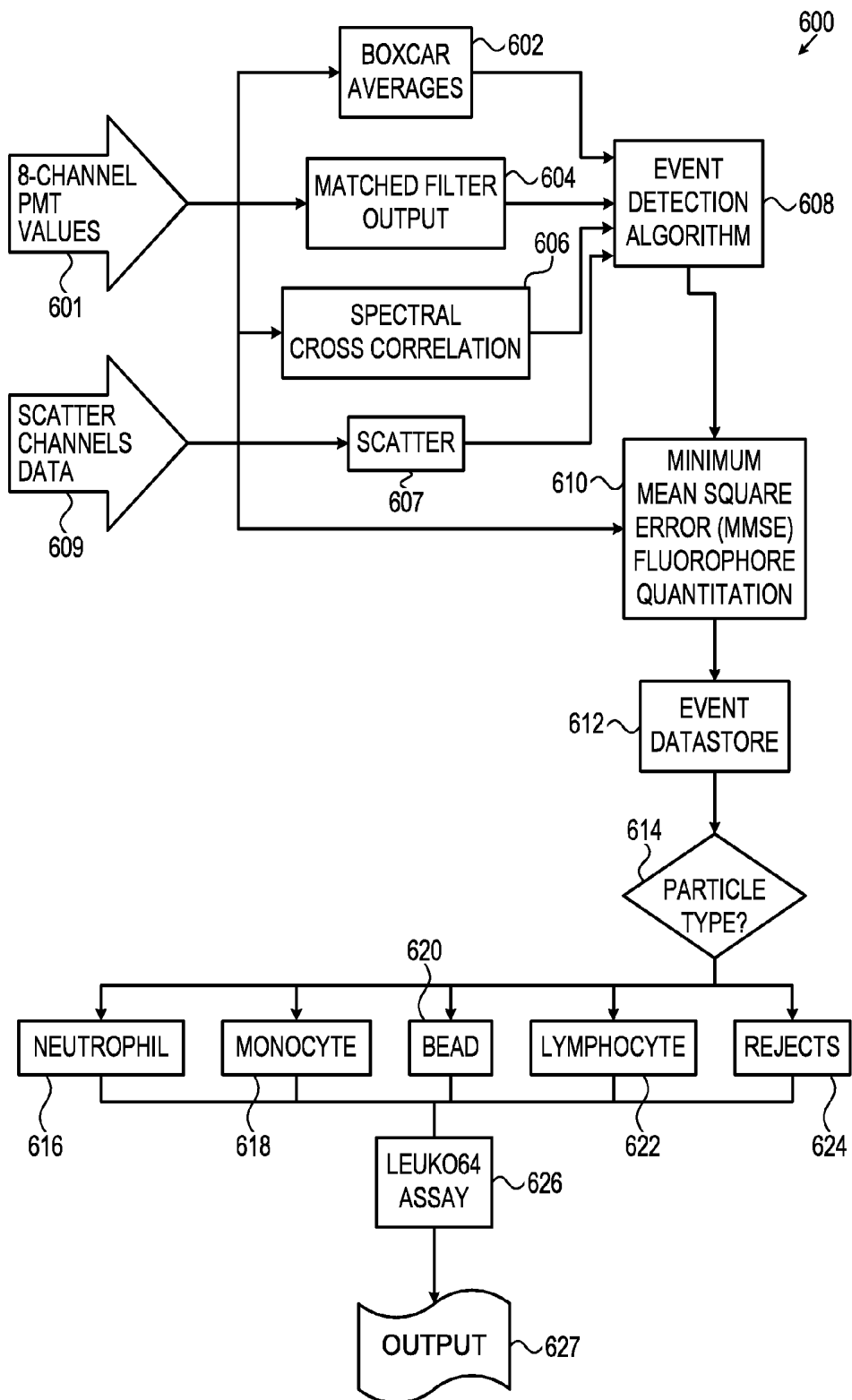


FIG. 6

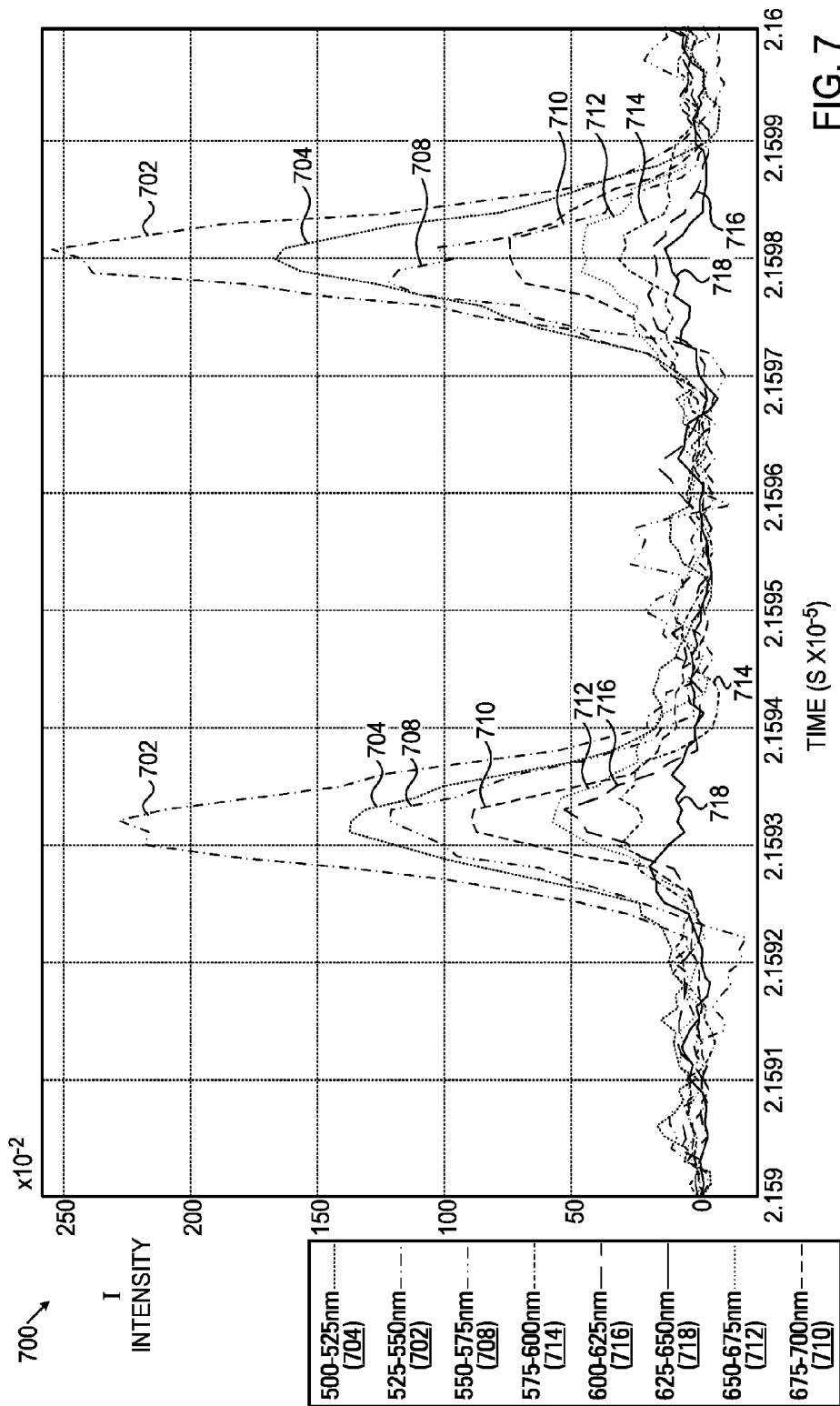


FIG. 7

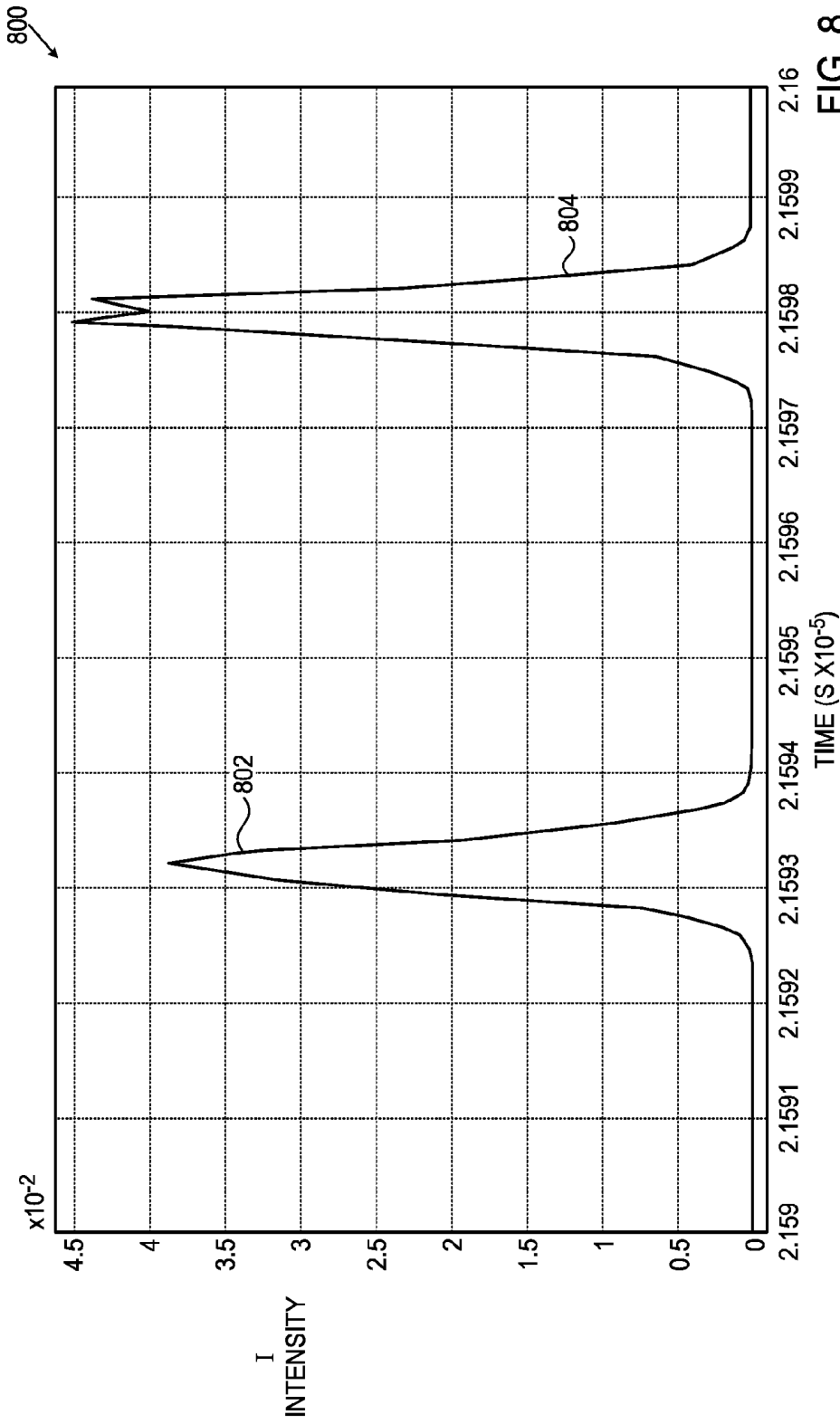


FIG. 8

900

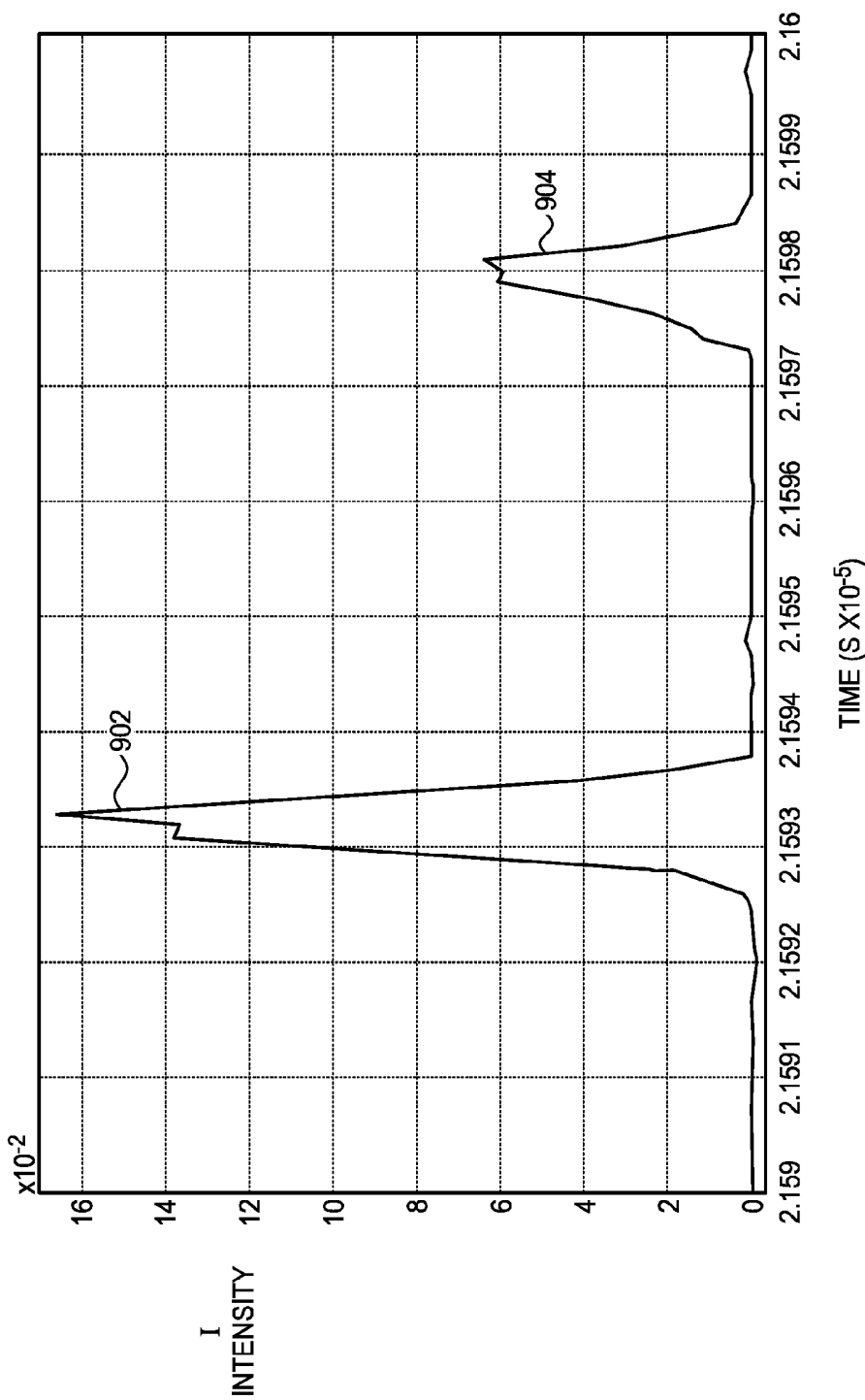


FIG. 9

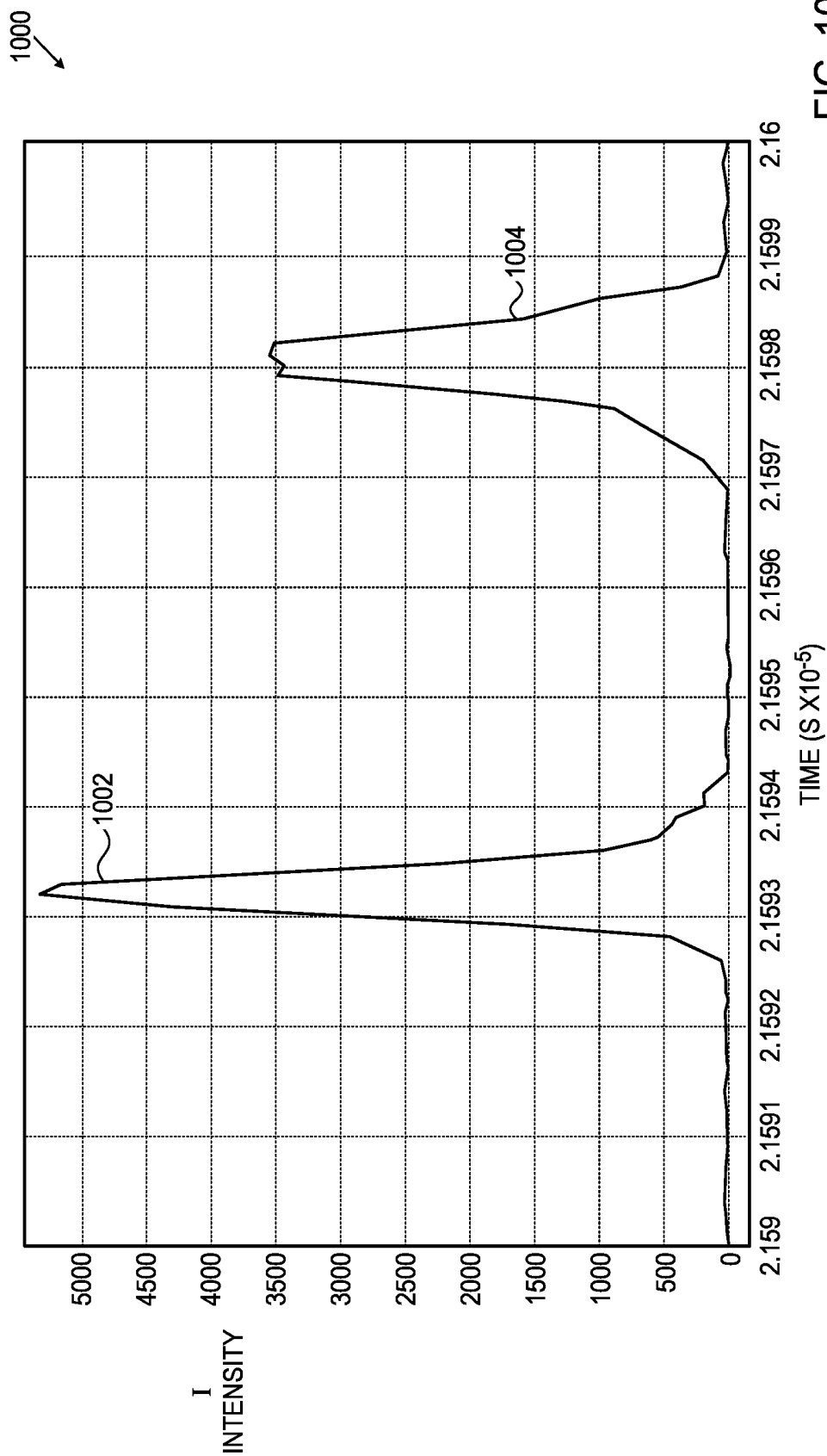


FIG. 10

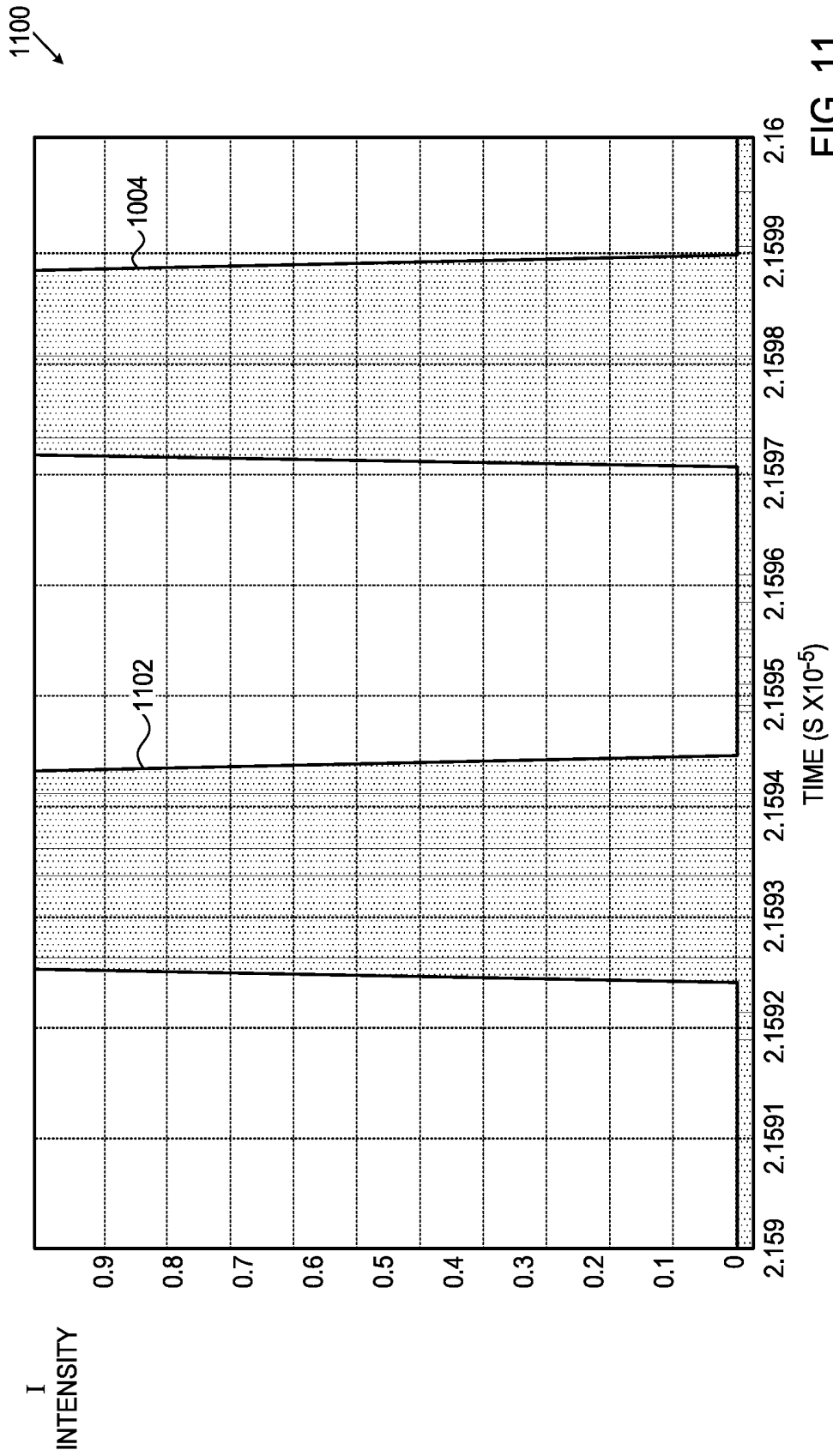


FIG. 11

KITS, COMPOSITIONS AND METHODS FOR DETECTING A BIOLOGICAL CONDITION

CROSS-REFERENCE

[0001] This application is a continuation application of application Ser. No. 14/296,317, filed on Jun. 4, 2014, which is a divisional application of Ser. No. 13/716,246, filed Dec. 17, 2012, which is incorporated herein by reference in its entirety.

[0002] The disclosures of the co-pending US Provisional Patent Application to Kasdan, et al, filed on Nov. 17, 2012, and titled "Kits, Compositions and Methods for Detecting a Biological Condition" and the co-pending US Provisional Patent Application to Kasdan, et al, filed on Nov. 17, 2012, and titled "Kits, Compositions and Methods for Rapid Chemical Detection" are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0003] The present invention relates generally to apparatus and methods for detecting a biological condition, and more specifically to methods and apparatus for detecting a biological condition in small fluid samples.

BACKGROUND OF THE INVENTION

[0004] There are numerous medical conditions which are hard to diagnose. Often diagnosis by a physician is based on the physician's observation of combinations of symptoms in a patient. This sometimes leads to misdiagnosis. Furthermore, the patient's response to a treatment, whether drug or other modality is often followed up by physician's observation.

[0005] Many laboratory tests are performed in the diagnostic arena on a bodily specimen or fluid to determine a biological condition in a patient. However, these tests are performed off-line in diagnostic laboratories. Often, the laboratory services are only provided during a single 8-hour shift during the day and tend to be labor intensive. Some prior art publications in the field include, inter alia, U.S. Pat. No. 8,116,984, US2006215155 and US2012187117.

[0006] Despite the inventions mentioned hereinabove, there still remains an unmet need to provide improved apparatus and methods for detecting and diagnosing biological conditions in a patient.

SUMMARY OF THE INVENTION

[0007] It is an object of some aspects of the present invention to provide improved apparatus and methods for detecting and diagnosing biological conditions in a patient.

[0008] In some embodiments of the present invention, improved methods, apparatus and kits are provided for detecting and diagnosing a biological condition in a patient.

[0009] In other embodiments of the present invention, a method and kit is described for providing rapid detection of biological moieties in a sample from a patient.

[0010] In further embodiments of the present invention, a method and kit is disclosed for providing detection of biological moieties in a small fluid sample from a patient.

[0011] There is thus provided according to an embodiment of the present invention, a kit for evaluating a biological condition in a patient, the kit comprising;

[0012] a) a disposable element for receiving a biological specimen and for combining said specimen with at least one composition;

[0013] b) at least one composition comprising at least one detector moiety adapted to react with said specimen to form a reaction product, when said patient has said biological condition; and

[0014] c) at least one reporter element adapted to provide an indication of reaction product thereby providing the indication of the biological condition. Additionally, according to an embodiment of the present invention, the kit further comprises;

[0015] d) instructions for using the kit.

[0016] Furthermore, according to an embodiment of the present invention, the disposable element is a disposable cartridge.

[0017] Moreover, according to an embodiment of the present invention, the disposable cartridge is a disposable microfluidics cartridge.

[0018] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least one of the following elements:

[0019] a) a reservoir;

[0020] b) a pump;

[0021] c) a valve;

[0022] d) a conduit;

[0023] e) a motor;

[0024] f) a miniaturized flow cell;

[0025] g) a transport channel;

[0026] h) a microfluidic element;

[0027] i) a compressed gas holding element;

[0028] j) a compressed gas releasing element;

[0029] k) a nozzle element;

[0030] l) a mixing element;

[0031] m) a bellows element;

[0032] n) software adapted to activate said elements according to a specific sequence; and

[0033] o) hardware to activate said elements according to a specific sequence.

[0034] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least two of the elements.

[0035] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least three of the elements.

[0036] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least four of the elements.

[0037] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least five of the elements.

[0038] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least ten of the elements.

[0039] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least twenty of the elements.

[0040] Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least thirty of the elements.

[0041] According to an embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one hour.

[0042] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with thirty minutes.

[0043] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with fifteen minutes.

[0044] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with ten minutes.

[0045] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with five minutes.

[0046] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one minute.

[0047] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with thirty seconds.

[0048] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with ten seconds.

[0049] According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one second.

[0050] There is thus provided according to an embodiment of the present invention, a microfluidics assay kit for performing a rapid biological assay, the kit comprising;

[0051] a) a disposable element comprising a reactant, the disposable element being adapted to receive a sample comprising a biological entity and for combining said reactant with said biological entity to form a reaction product; and

[0052] b) at least one reporter element adapted to provide a rapid indication of disappearance of said reactant thereby providing rapid assay of the biological entity.

[0053] There is thus provided according to an embodiment of the present invention, a microfluidics assay kit for performing a rapid assay of a biological entity, the kit comprising;

[0054] a) a disposable element comprising a reactant, the disposable element being adapted to receive a sample comprising the biological entity and for combining said reactant with said biological entity to form a reaction product; and

[0055] b) at least one reporter element adapted to provide a rapid indication of appearance of said reaction product thereby providing rapid assay of the biological entity. There is thus provided according to an embodiment of the present invention, a composition for evaluating a biological condition, the composition comprising;

[0056] a. a sample composition comprising at least one of;

[0057] i. a bodily specimen comprising a target moiety;

[0058] ii. a positive control moiety; and

[0059] iii. a negative control moiety;

[0060] b. a detection composition comprising at least one of;

[0061] i. at least one target antibody;

[0062] ii. at least one positive control identifying antibody; and

[0063] iii. at least one negative control identifying detection moiety or characteristic; and

[0064] c. at least one reference composition comprising at least one of;

[0065] i. a target signal reference composition; and

[0066] ii. a reference identifier composition.

[0067] There is thus provided according to another embodiment of the present invention a composition for evaluating a biological condition, the composition comprising;

[0068] a. a sample composition comprising at least one of;

[0069] i. a bodily specimen comprising a target moiety;

[0070] ii. a positive control moiety; and

[0071] iii. a negative control moiety;

[0072] b. an antibody composition comprising at least one of;

[0073] i. at least one target antibody (CD64 antibody);

[0074] ii. at least one positive control identifying antibody (CD163); and

[0075] iii. at least one negative control identifying antibody or characteristic; and

[0076] c. at least one reference composition comprising at least one of;

[0077] i. a target signal reference composition; and

[0078] ii. a reference identifier composition.

[0079] Additionally, according to an embodiment of the present invention, the composition further comprises at least one conditioning moiety comprising;

[0080] a. at least one lysis reagent; and

[0081] b. at least one diluent.

[0082] Furthermore, according to an embodiment of the present invention, the biological condition is selected from a group consisting of blood diseases such as leukemia, thrombocytopenia immune system disorders, local infections, urinary tract disorders, autoimmune diseases and sepsis.

[0083] Moreover, according to an embodiment of the present invention the bodily specimen is selected from a group consisting of blood, serum, plasma, urine, saliva, cerebrospinal fluid (CSF), serous fluid, peritoneal fluid and synovial fluid.

[0084] According to another embodiment of the present invention, the target moiety includes a CD64 surface antigen on neutrophils.

[0085] Additionally, according to a further embodiment of the present invention, the positive control moiety includes monocytes and the negative control includes lymphocytes. Additionally, according to an embodiment of the present invention, the target moiety is CD64 on neutrophils, the positive control moiety includes CD64 expression on monocytes, and the negative control moiety includes lymphocytes without CD64 expression.

[0086] Further, according to an embodiment of the present invention, the target indicator is bound to a signaling moiety on the at least one target antibody.

[0087] Yet further, according to an embodiment of the present invention, the at least one reference composition includes beads.

[0088] Additionally, according to an embodiment of the present invention, the beads include polystyrene microbeads.

[0089] Moreover, according to an embodiment of the present invention, the target antibody reference composition includes a first fluorescent signal and the reference identifier composition includes a second fluorescent signal.

[0090] Furthermore, according to an embodiment of the present invention, the first fluorescent signal includes FITC and the second fluorescent signal includes Starfire Red fluor.

[0091] There is thus provided according to an embodiment of the present invention, a method of quantifying a biomarker in a sample, comprising;

- [0092] a. contacting the sample with a fluorescently-labeled binding moiety that specifically binds to the biomarker;
- [0093] b. detecting a first fluorescent signal from at least a portion of the labeled sample;
- [0094] c. detecting a second fluorescent signal from a population of fluorescently-labeled particles, wherein the population includes a known fluorescent intensity over a fixed time; and
- [0095] d. normalizing the first fluorescent signal to the second fluorescent signal, thereby quantifying the biomarker, wherein the normalizing includes using a device comprising software capable of comparing the first and second fluorescent signal.
- [0096] Furthermore, according to an embodiment of the present invention, the biomarker is a sepsis biomarker.
- [0097] Moreover, according to an embodiment of the present invention, the biomarker is CD64 or CD163.
- [0098] Additionally, according to an embodiment of the present invention, the sample is a blood sample.
- [0099] According to another embodiment of the present invention, the fluorescent label of the binding moiety and the fluorescent label of the particles is the same fluorescent label.
- [0100] Further, according to an embodiment of the present invention, the binding moiety is an antibody.
- [0101] According to an embodiment of the present invention, the software is capable of recognizing a specific lot of fluorescently-labeled particles.
- [0102] Moreover, according to an embodiment of the present invention, the individual fluorescent signals include at least one first fluorescent signal and at least one second fluorescent signal.
- [0103] Additionally, according to an embodiment of the present invention the fluorescently-labeled binding moiety targets a first cell population and a second cell population in the sample.
- [0104] According to another embodiment of the present invention the detection of binding of the binding moiety to the second cell population provides an internal positive control for the sample.
- [0105] Furthermore, according to an embodiment of the present invention, the binding moiety is anti-CD64 antibody and the first cell population includes polymorphonuclear leukocytes.
- [0106] Yet further, according to an embodiment of the present invention, the second cell population includes monocytes.
- [0107] According to an embodiment of the present invention, the method further comprises the step of determining the presence of at least one cell population in the sample that is not bound by the binding moiety, thus providing an internal negative control for the sample.
- [0108] There is thus provided according to another embodiment of the present invention, a composition for evaluating a biological condition, the composition comprising;
- [0109] a. a sample comprising at least one of;
- [0110] i. a bodily specimen comprising a target moiety;
- [0111] ii. a positive control moiety; and
- [0112] iii. a negative control moiety;
- [0113] b. an antibody composition comprising at least one of;
- [0114] i. at least one target antibody;
- [0115] ii. at least one positive control identifying antibody; and
- [0116] iii. at least one negative control identifying antibody or characteristic; and
- [0117] c. at least one reference composition comprising at least one of;
- [0118] i. a target antibody reference composition; and
- [0119] ii. a reference identifier composition.
- [0120] According to an embodiment of the present invention, the composition further comprises at least one conditioning moiety comprising;
- [0121] a) at least one lysis reagent; and
- [0122] b) at least one diluent.
- [0123] There is thus provided according to another embodiment of the present invention, a method of determining the presence or absence of sepsis in a subject, the method including;
- [0124] a) contacting a blood sample from the subject with a fluorescently-labeled binding moiety specific to a sepsis marker, wherein the volume of the blood sample is 50 μ L or smaller; and
- [0125] b) detecting the presence, absence or level of the binding moiety in the sample, thereby determining the presence or absence of sepsis in the subject.
- [0126] There is thus provided according to another embodiment of the present invention, a method of quantifying a biomarker in a sample, comprising;
- [0127] a) contacting the sample with a fluorescently-labeled binding moiety that specifically binds to the biomarker;
- [0128] b) detecting a first fluorescent signal from at least a portion of the labeled sample;
- [0129] c) detecting a second fluorescent signal from a population of fluorescently-labeled particles, wherein the population includes a known fluorescent intensity over a fixed time; and
- [0130] d) normalizing the first fluorescent signal to the second fluorescent signal, thereby quantifying the biomarker, wherein the normalizing includes using a device comprising software capable of comparing the first and second fluorescent signal.
- [0131] According to some embodiments, the sample may be liquid, according to other embodiments, the sample may be a colloid or suspension. According to further embodiments, the sample may be a solid, such as in a powder or crystal form.
- [0132] Typical turnaround times for diagnostic prior art assays are 30-120 minutes. Often, the time lost in waiting for laboratory results can lead to a further deterioration in a patient, and sometimes death. In some cases, the physician has to act without having the laboratory results. This can lead to providing the patient with the wrong treatment. The present invention provides rapid assays to save lives and provide fast correct treatments to a patient.
- [0133] There is thus provided according to an embodiment of the present invention automated method of determining the presence or absence of sepsis in a subject, including;
- [0134] a) contacting a blood sample from the subject with a fluorescently-labeled binding moiety specific to a sepsis marker, wherein the volume of the blood sample is 50 μ L or smaller; and
- [0135] b) detecting the presence, absence or level of the binding moiety in the sample, thereby determining the presence or absence of sepsis in the subject within twenty minutes.

[0136] Additionally, according to an embodiment of the present invention, the sepsis marker is CD64.

[0137] Furthermore, according to an embodiment of the present invention, a second sepsis marker is CD163.

[0138] Moreover, according to an embodiment of the present invention, the method further includes contacting the blood sample with a second fluorescently-labeled binding moiety specific for a second sepsis marker.

[0139] Further, according to an embodiment of the present invention, the sepsis marker is CD64 and the second sepsis marker is CD163.

[0140] Additionally, according to an embodiment of the present invention, the binding moiety is an antibody.

[0141] Moreover, according to an embodiment of the present invention, the detecting step is performed in a device capable of receiving the sample and capable of detecting the binding moiety.

[0142] Additionally, according to an embodiment of the present invention, the method further includes the step of calibrating the device by detecting a population of the fluorescently-labeled particles.

[0143] According to another embodiment of the present invention, the particles include the same fluorescent label as the fluorescently-labeled binding moiety.

[0144] Additionally, according to an embodiment of the present invention, the method further includes a second population of particles that include the same fluorescent label as the second fluorescently-labeled binding moiety.

[0145] Moreover, according to an embodiment of the present invention, the method further includes performing an internal calibration after the detecting the fluorescently-labeled binding moiety.

[0146] Notably, according to an embodiment of the present invention, the calibration is completed in less than 5 minutes.

[0147] According to some embodiments, the particles are microbeads.

[0148] Additionally, according to an embodiment of the present invention, the method is performed in less than 15 minutes.

[0149] Furthermore, according to an embodiment of the present invention, the method, further includes the step of determining the presence of at least one cell population in the sample that is not bound by the binding moiety, thus providing an internal negative control for the sample.

[0150] The present invention will be more fully understood from the following detailed description of the preferred embodiments thereof, taken together with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0151] The invention will now be described in connection with certain preferred embodiments with reference to the following illustrative figures so that it may be more fully understood.

[0152] With specific reference now to the figures in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings

making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0153] In the drawings:

[0154] FIG. 1 is a simplified schematic illustration showing an apparatus for detecting a biological condition, in accordance with an embodiment of the present invention;

[0155] FIG. 2 is a simplified flow chart of a method for detecting a biological condition, in accordance with an embodiment of the present invention;

[0156] FIG. 3 is a simplified schematic illustration showing a methodology for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention;

[0157] FIG. 4 is a simplified flow chart of a method for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention;

[0158] FIG. 5A is a graphical output of a fluorescent detection assay of a non-activated neutrophil signature associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention;

[0159] FIG. 5B is a graphical output of a fluorescent detection assay of an activated neutrophil signature, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention;

[0160] FIG. 5C is a graphical output of a fluorescent detection assay of a monocyte signature, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention;

[0161] FIG. 5D is a graphical output of a fluorescent detection assay of a reference bead signature, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention;

[0162] FIG. 6 is a simplified flow chart of a method for differentiating between different particles, in accordance with an embodiment of the present invention;

[0163] FIG. 7 is a graphical output of fluorescence from reference beads in eight wavebands, in accordance with an embodiment of the present invention;

[0164] FIG. 8 is a graphical output of data from FIG. 7 after a first mathematical manipulation, in accordance with an embodiment of the present invention;

[0165] FIG. 9 is a graphical output of data from FIG. 7 after a second mathematical manipulation, in accordance with an embodiment of the present invention;

[0166] FIG. 10 is a graphical output of data from FIG. 7 after a third mathematical manipulation, in accordance with an embodiment of the present invention; and

[0167] FIG. 11 is a graphical output of an event locator, based on data from FIG. 8-10, in accordance with an embodiment of the present invention.

[0168] In all the figures similar reference numerals identify similar parts.

DETAILED DESCRIPTION OF THE INVENTION

[0169] In the detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that these are specific embodiments and that the present invention may be practiced also in different ways that embody the characterizing features of the invention as described and claimed herein.

[0170] International patent application publication no. WO2011/128893 to Kasdan et al., describes a device, system

and method for rapid determination of a medical condition and is incorporated herein by reference.

[0171] The microfluidic cartridges of the present invention may be any suitable cartridge as shown in the figures or any of the prior art cartridges described or cited herein, such as, but not limited to, those described in U.S. D669191 S1, US20120266986 A1, EP1846159 A2, US2012275972, W011094577A, US2007292941A and EP1263533 B1.

[0172] Reference is now made to FIG. 1, which is a simplified schematic illustration showing an apparatus 100 for detecting a biological condition, in accordance with an embodiment of the present invention.

[0173] Apparatus 100 is a kit comprising a cartridge 102 and a number of chemical/biochemical reactants termed herein, treatment compositions. The treatment compositions are adapted to react, at least in part, with biological specimen, such as a body specimen, to be introduced to the apparatus. The body specimen may be a bodily fluid such as, but not limited to, blood, serum, plasma, urine, saliva, cerebrospinal fluid (CSF), serous fluid, peritoneal fluid and synovial fluid. Additionally or alternatively, the body specimen may be a solid such as a hair, a tooth part, a bone part or a piece of cartilage.

[0174] Apparatus 100 comprises a specimen receiving element 118, adapted to transfer the specimen to a sample composition chamber 104. The sample composition chamber comprises on or more transfer elements 105, adapted to transfer the specimen from the sample composition chamber to one or more other locations in the cartridge. In the non-limiting example shown in FIG. 1, transfer element 105 is a conduit in fluid connection with a treatment chamber 112.

[0175] Additionally, the cartridge comprises a number of treatment composition chambers 106, 108, 110, adapted to respectively house a corresponding number of treatment compositions 120, 122, 124. These treatment compositions may be liquid, solid or combinations thereof. Apparatus 100 is typically sold commercially as a kit with the treatment compositions disposed therein. In some cases, the apparatus 100 may be adapted for a one-off test and may be disposable. In other cases, the apparatus may be re-used. A re-usable apparatus may be adapted to receive additional external compositions (not shown) or may have a plurality of treatment compositions, wherein only a portion is used for each test.

[0176] The apparatus may be constructed and configured such that the treatment composition comprises proteins attached to a surface, such as to beads. A plurality of beads or

other structural elements with proteins attached to their surfaces can be made by any one or more of the following methodologies:—

[0177] simple attachment such as by adsorption via electrostatic or hydrophobic interactions with the surface, entrapment in immobilized polymers, etc.

[0178] non-covalent or physical attachment;

[0179] covalent bonding of the protein to the bead surface

[0180] biological recognition (e.g., biotin/streptavidin).

[0181] requires two steps: a first layer is formed by silane chemistry such that the surface presents a reactive group (e.g., epoxy, amino, thiol, etc.), and a second layer (e.g., the protein to be immobilized or a linker molecule) is covalently attached via the immobilized reactive groups.

[0182] covalent attachment to functionalized polymer coatings on the interior of the device or linkage to the free end of a self-assembled monolayer (SAM) on a gold surface.

[0183] The reaction type may include any one or more of antigen-antibody binding, sandwich (such as antibody-antigen-antibody), physical entrapment, receptor-ligand, enzyme-substrate, protein-protein, aptamers, covalent bonding or biorecognition.

[0184] Cartridge 102 further comprises at least one transfer element 107, 109, 111 in fluid communication with each respective of treatment composition chamber, each transfer element also being in fluid communication with treatment chamber 112. These elements are typically microfluidic channels and may be designed for mixing, such as being tortuous in shape.

[0185] Various methodologies for transferring the contents of the treatment composition chambers and the sample composition chamber via the transfer elements to the treatment chamber may be employed, some of which are known in microfluidics technologies. These include air blowing, suction, vacuuming, mechanical transfer, pumping and the like.

[0186] Cartridge 102 further comprises at least one transfer element 113 in fluid communication with treatment chamber 112 and with an evaluation chamber 114.

[0187] Optionally, evaluation chamber 114 is further in fluid communication with a transfer element 115, adapted to remove the contents of the evaluation chamber for disposal outside the cartridge. Alternatively, the evaluation chamber may have no external disposal means.

[0188] Table 1 shows some representative applications of apparatus 100 and methods of the present invention.

TABLE 1

Applications of the apparatus and methods of this invention.					
Application	Type of Test	Relevant Figures in this invention	Typical Prior Art Laboratory Turnaround time (TAT)-see references	This invention Turnaround time (TAT)	References
Application #1 - CD64 Infection & Sepsis	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	U.S. Pat. No. 8,116,984, Davis, BH et al., (2006)
1 - Fetal Hemoglobin Test	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Dziegiel et al. (2006)
2 - Low Platelet Count	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Segal, H. C., et al. (2005):
3 - Resolving BLAST Flag for hematology Lab	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Guerti, K., et al.

TABLE 1-continued

Applications of the apparatus and methods of this invention.					
Application	Type of Test	Relevant Figures in this invention	Typical Prior Art Laboratory Turnaround time (TAT)- see references	This invention Turnaround time (TAT)	References
4 - CD34 Stem Cell Enumeration Assay	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Sutherland et al. (1996)
5 - Platelets Activation Assay CD62	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Graff et al. (2002) Divers, S. G., et al. (2003)
6 - D-dimer (Bead based protein)	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Stein et al. (2004) Rylatt, D. B., et al. (1983):
7 - Chorioamniotitis CD64	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Hillier et al. (1988)
8 - CD20 Cell Quantitation (Therapy Monitoring)	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rawstron et al. (2001) Cheson et al. (1996)
9 - CD52 Cell quantitation (Therapy Monitoring)	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rawstron et al. (2001)
10 - Circulating Tumor Cells	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Cristofanilli et al. (2004)
11 - Reticulated Platelet Assay	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Matic et al. (1998) Ault et al (1993) Wang et al. (2002)
12 - Bacteria Detection in platelet packs			4 hours	10 minutes	Blajchman et al (2005) McDonald et al. (2005)
13 - Platelet Associated Antibodies	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Michelson (1996)
14 - Residual Leukocyte Count in blood products	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Bodensteiner, (2003)
15 - CD4 HIV AIDS	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rodriguez (2005). Dieye et al. (2005)
16 - Leukemia Panels - Very complex	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Drexler et al (1986)
17 - Bladder Cancer Screening in Urine - Urine sample	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Ramakumar et al (1999) Lotan et al. (2009)
18 - HLA DR Sepsis and Immunosuppression	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Hershman et al. (2005) Perry et al (2003)
19 - RECAF Protein for Canine and other Cancers	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Moro et al. (2005).
20 - CytoImmun - Cervical Screening			4 hours	10 minutes	Hilfrich et al. (2008)
21 - Procalcitonin (Bead Based Protein) + Feasibility	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Assicot et al. (1993) Christ-Crain et al. (2004)

[0189] Reference is now made to FIG. 2, which is a simplified flow chart 200 of a method for detecting a biological condition, in accordance with an embodiment of the present invention.

[0190] It should be understood that each of the steps of the method may take a predetermined period of time to perform, and in between these steps there may be incubation and/or waiting steps, which are not shown for the sake of simplicity.

[0191] In a sample transferring step 202, a sample, such as a bodily specimen is transferred from outside apparatus 100

via receiving element 118 into sample composition chamber 104 and then to the treatment chamber 112. According to some embodiments, the volume of the specimen or sample is less than 200 μ L, less than 100 μ L, less than 50 μ L, less than 25 μ L or less than 11 μ L.

[0192] Thereafter, treatment composition 120 is transferred via transfer element 107 to the treatment chamber in a composition transfer step 204. In some cases, there may be a treatment composition disposed in the treatment chamber.

[0193] Depending on the nature of the treatment composition and sample/specimen type, there may be a requirement to mix or agitate the treatment chamber contents in an optional mixing step **206**. This may be performed by using a small stirbar (not shown) disposed in the chamber. Additionally or alternatively, this may be effected by the fluid dynamics of kit. Additionally or alternatively, stirbars may be disposed in any of the other chambers in the apparatus.

[0194] Typically, the total sample volumes are in the range of 10 to 1000 μL , 100 to 900 μL , 200 to 800 μL , 300 to 700 μL , 400 to 600 μL , or 420 to 500 μL .

[0195] According to some embodiments, the volume of the treatment composition chambers **106**, **108**, **110** (also called blisters) is from about 1 μL to 1000 μL . According to other embodiments, the volume of the specimen is from about 10 μL to 200 μL . According to other embodiments, the volume of the specimen is about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL .

[0196] According to some embodiments, the volume of the treatment compositions **120**, **122**, **124** is at most about 500 μL . According to other embodiments, the volume of the specimen is at most about 200 μL . According to other embodiments, the volume of the specimen at most about 500, 450, 400, 350, 300, 250, 200, 180, 160, 140, 120, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, or 1 μL .

[0197] According to some embodiments, the volume of a reactant is at least about 1 μL . According to other embodiments, the volume of the specimen is from about 10 μL . According to other embodiments, the volume of the specimen is at least about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL .

[0198] The sequence of transfer of the various treatment compositions may be important to the reaction sequence and is typically predefined. Steps **204-206** may be performed, for example on treatment composition chamber **106**, thereafter on treatment composition chamber **108** and thereafter on treatment composition chamber **110**. In some cases, some of these steps may be performed concurrently.

[0199] In a checking step **208**, it is ascertained whether all the compositions required for the sample treatment have been transferred to the treatment chamber. If any compositions remain, then steps **204-206** are performed on the subsequent treatment composition chamber(s). If no further treatment compositions require transfer, then the sample/specimen is transferred from chamber **104** into the treatment chamber.

[0200] Thereafter, in a second sample transfer step **210**, the sample is transferred from the sample composition chamber into the treatment chamber.

[0201] According to some embodiments, step **210** may be performed before steps **204-208**. If required, an optional mixing step **212** to the contents of the treatment chamber may be performed.

[0202] In a transferring step **214**, the contents of the treatment chamber are transferred to the evaluation chamber.

[0203] The evaluation chamber **114** is configured and constructed for one or more evaluation steps **216**. These may include any of the following, or combinations thereof:

- [0204] a) transfer of radiation there-through,
- [0205] b) impinging radiation thereupon;
- [0206] c) detecting reflected, refracted, and/or transmitted radiation,
- [0207] d) detecting emitted radiation;
- [0208] e) capturing one or more images thereof;

[0209] f) performing image analysis on the captured images;

[0210] g) measuring electrical characteristics of the treated specimen;

[0211] h) impinging sonic energy thereon;

[0212] i) detecting sonic energy therefrom; and

[0213] j) analyzing the outputs of any one or more of the above steps.

[0214] According to some embodiments, the cartridge is introduced into a system as described in International patent application publication no. WO2011/128893 to Kasdan et al., incorporated herein by reference.

[0215] The results of the evaluation step are then outputted in a results outputting step **218**.

[0216] According to some embodiments; the apparatus may have on-board means for showing a result, such as a colorimetric strip (not shown). Additionally or alternatively, the results are displayed in a display unit, separate and remote from apparatus **100**.

[0217] Reference is now made to FIG. 3, which is a simplified schematic illustration showing a methodology **300** for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention.

[0218] According to some embodiments, the method is carried out in the apparatus shown in FIG. 1 and as described herein. A biological specimen, such as a blood sample, is aspirated via specimen receiving element **118** to sample composition chamber **104**, and then to treatment chamber **112**. The sample is typically of a volume in the range of 10-200 μL .

[0219] The blood sample is typically whole blood recently removed from a patient. The whole blood comprises mainly red blood cells (also called RBCs or erythrocytes), platelets and white blood cells (also called leukocytes), including lymphocytes and neutrophils. Increased number of neutrophils, especially activated neutrophils are normally found in the blood stream during the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure and some cancers.

[0220] A cocktail **304** comprising antibodies to CD64 and antibodies to CD163 is introduced to the treatment chamber (see Davis et al. (2006)). Each antibody type is typically tagged by a specific fluorescent tag.

[0221] The contents of the chamber are incubated and/or mixed as is required to bind the activated blood neutrophils with the CD64 tagged antibody (also called a marker) to form activated neutrophils with CD64 marker **310**, and/or monocyte with a CD64 tagged antibody and a CD163 tagged antibody **312**. Lymphocytes with no markers **314** are present in the contents, as well as unaffected RBCs **316**.

[0222] Thereafter, a lysis reagent or diluent **306** is introduced into treatment chamber **112**. In the case of a lysis reagent, it is adapted to lyse red blood cells to form lysed red blood cells **324**. Additionally, reference/calibration beads **308** are added to the treatment chamber. These are used to calibrate the outputs, as is explained with reference to FIGS. 5A-5D hereinbelow.

[0223] CD64 (Cluster of Differentiation 64) is a type of integral membrane glycoprotein known as an Fc receptor that binds monomeric IgG-type antibodies with high affinity. Neutrophil CD64 expression quantification provides improved diagnostic detection of infection/sepsis compared with the standard diagnostic tests used in current medical practice.

[0224] CD163 (Cluster of Differentiation 163) is a human protein encoded by the CD163 gene. It has also been shown to mark cells of monocyte/macrophage lineage.

[0225] Reference is now made to FIG. 4, which is a simplified flow chart 400 of a method for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention.

[0226] According to some embodiments, the method is carried out in the apparatus shown in FIG. 1 and as described herein. In a first transferring step 402, a biological specimen, such as a blood sample is aspirated via specimen receiving element 118 to sample composition chamber 104. The sample is typically of a volume in the range of 10-200 μL .

[0227] Typically, the total sample volumes are in the range of 10 to 1000 μL , 100 to 900 μL , 200 to 800 μL , 300 to 700 μL , 400 to 600 μL , or 420 to 500 μL .

[0228] According to some embodiments, the volume of the treatment composition chambers 106, 108, 110 (also called blisters) is from about 1 μL to 1000 μL . According to other embodiments, the volume of the specimen is from about 10 μL to 200 μL . According to other embodiments, the volume of the specimen is about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL .

[0229] According to some embodiments, the volume of the treatment compositions 120, 122, 124 is at most about 500 μL . According to other embodiments, the volume of the specimen is at most about 200 μL . According to other embodiments, the volume of the specimen at most about 500, 450, 400, 350, 300, 250, 200, 180, 160, 140, 120, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, or 1 μL .

[0230] According to some embodiments, the volume of a reactant is at least about 1 μL . According to other embodiments, the volume of the specimen is from about 10 μL . According to other embodiments, the volume of the specimen is at least about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL .

[0231] In an addition step 404, a cocktail of tagged antibodies to CD64 and to CD163 is added to the treatment chamber 112 and is incubated with the blood sample. In the incubation phase of this step, the antibodies bind activated neutrophils with CD64 marker 310, and/or monocytes activated with a CD64 tagged antibody and a CD163 tagged antibody 312.

[0232] In a lysis reagent addition step 406, the lysis reagent is added to the treatment chamber and thereby lyses at least some of the RBCs in the chamber.

[0233] At any suitable time, typically following lysis step 406, reference beads are added to the contents of the treatment chamber in a reference bead adding step 408.

[0234] After a predefined period of time, an analysis step 410 is performed to analyze the fluorescent emission signatures from the contents. This is described in further detail with reference to FIGS. 5A-5D. According to some examples, the evaluation chamber 114 is constructed and configured to allow cells to pass through a reading zone 130 such that each cell passing therethrough is analyzed individually. The assay sensitivity is around 86% and its specificity is around 87% (Hoffmann, 2011).

[0235] The time required to complete an assay using apparatus 100 of the present invention varies depending on a number of factors, with non-limiting examples that include described herein. In some embodiments, the time required to complete an assay is from about 0.5 to 100 minutes. In other embodiments, the time required to complete an assay is from

about 1 to 20 minutes. In still other embodiments, the time required to complete an assay is from about 1 to 10 minutes. In some examples, the time required to complete an assay is from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, or 100 minutes.

[0236] Reference is now made to FIG. 5A, which is a graphical output of a fluorescent detection assay of a non-activated neutrophil signature 500 associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention. The non-activated tagged neutrophils each emit a signal 502 at wavelength W1 of an intensity I1. The wavelengths shown in FIGS. 5A-5D represent a peak wavelength of waveband outputs detected, as are shown in FIGS. 7-11.

[0237] FIG. 5B shows a graphical output of a fluorescent detection assay of an activated neutrophil signature 510, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention. Each activated tagged neutrophil emits an activated neutrophil signature 512 at wavelength W1 of an intensity I2. Typically I2 is greater than I1. In some cases the difference in signatures 512 and 510 may be detected by an image analysis, a fluorescent emission radiation count or by other qualitative or quantitative methods known in the art. The current example is not meant to be limiting.

[0238] Turning to FIG. 5C, there can be seen a graphical output of a fluorescent detection assay of a monocyte signature 520, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention. The monocyte signature comprises a first signal 522 at a first wavelength W1 of an intensity I3 and a second signal 524 at a second wavelength W2 of an intensity I4.

[0239] FIG. 5D shows a graphical output of a fluorescent detection assay of a reference bead signature 530, associated with the method of FIGS. 3-4, in accordance with an embodiment of the present invention. The reference bead signature comprises a first signal 532 at a first wavelength W1 of an intensity I1 (similar or equal to non-activated tagged neutrophils' signal 502) and a second signal 534 at a second wavelength W3 of an intensity I5.

[0240] This methodology enables the identification and quantification of activated neutrophils by intensity of signature 512 of the CD64 tag. Monocytes are identified by the double signal signature 522, 524, acting as a positive control. Reference beads are identified by the unique signal 534 at wavelength W3. The intensity of signal 532 at wavelength W1 provides a reference level of CD64 for the comparison of intensity of 512 of the neutrophils.

[0241] Lymphocytes with no markers 330 (FIG. 3) act as a negative control and should provide no fluor signature, but may be detected by their scattering or other characteristics. Further details of some embodiment of this assay procedure are described in U.S. Pat. No. 8,116,984 and in Davis, B H et al., (2006).

[0242] Reference is now made to FIG. 6, which is a simplified flow chart of a method 600 for differentiating between different particles, in accordance with an embodiment of the present invention.

[0243] The input to the processing is a time series from each of the channels in the eight channel photomultiplier array 601. In addition, data from multiple scatter channels 609 is introduced. Each fluorescent time series and scatter time series may be processed individually employing respective spectral crosscorrelation algorithm 606 and scatter algorithm

607 to smooth it and minimize noise. Two possible processing methods are boxcar averaging algorithm **602** and matched filtering algorithm **604**. In addition, groups of individual channels may be correlated to yield a multiple spectral cross-correlations **606**. One or more of these derived time series may be used to determine event locations.

[0244] Once an event is located in the eight channel time series the composition of that event in terms of known fluorophore signatures is determined using a minimum mean square error fit **610**. The event is now described in terms of its composition of known fluors. Each event thus described is stored in an event store, i.e. memory, together with the data from the eight time series for that event and its description **612**. Based on the fluor composition for each event in the data store, it is possible to determine the type of particle. For example, a neutrophil **616** is characterized by the single fluor attached to the CD64 antibody shown in FIG. 5 as W1. Thus events that are preponderantly characterized by the single fluor attached to the CD64 antibody are identified as neutrophils.

[0245] Similarly, monocytes **618** are characterized by fluors W1 and W2 so that an event with both of these fluor signatures is identified as a monocyte. Similarly, a bead **620** is characterized by an event that has fluors W1 and W3. Lymphocytes **622** do not express significant fluorescence but are identified by their scatter as events. Events that do not match any of the known combinations of the fluorophores are identified as rejects **626**.

[0246] Given the population of identified events, the median intensity of the neutrophil population and the median intensity of the bead population are determined. The ratio of the neutrophil median to the bead median is the desired Leuko64 index. The positive control value is determined as the median intensity of the CD64 fluorophore bound to monocytes divided by the median intensity of the same fluorophore on the bead population. The negative control value is determined by the median intensity of the CD64 fluorophore bound to lymphocytes. These are the key steps in performing the Leuko64 assay.

[0247] FIG. 7 is a graphical output **700** of fluorescence from reference beads in eight wavebands, in accordance with an embodiment of the present invention. This figure shows the smoothed signals from the eight channel PMT array for two reference beads. The amplitude for each waveband is shown on the same graph. The corresponding wavelength range is shown for each plot **702, 706, 708, 710, 712, 714, 716, 718** in the legend box. The two fluorophores signatures present in this plot are **702, 706** and **708** for FITC, which is the fluorophore attached to the CD64 antibody and **710, 712** for Starfire Red, which is the fluorophore identifying the reference beads.

[0248] Reference is now made to FIG. 8, which is a graphical output **800** of data from FIG. 7 after a first mathematical manipulation, in accordance with an embodiment of the present invention. FIG. 8 shows the cross correlation of wave bands one two and three corresponding to wavelength 500 to 525, 525 to 550, and 552 to 575 nm. This cross correlation is computed by multiplying the boxcar smoothed time series corresponding to these wavelengths. This signal will have a high-value when an event containing the FITC fluorophore is present.

[0249] FIG. 9 is a graphical output **900** of data from FIG. 7 after a second mathematical manipulation, in accordance with an embodiment of the present invention. FIG. 9 shows the cross correlation of wave bands **3, 4** and **5** corresponding

to wavelengths 550 to 575, 575 to 600, and 600 to 625 nm. This signal will have a high-value when an event containing the PE fluorophore is present.

[0250] FIG. 10 is a graphical output **1000** of data from FIG. 7 after a third mathematical manipulation, in accordance with an embodiment of the present invention. FIG. 10 shows the cross correlation of wave bands **7** and **8** corresponding to wavelengths 650 to 675, and 675 to 700 nm. This signal will have a high-value when an event containing the Starfire Red fluorophore is present.

[0251] FIG. 11 is a graphical output **1100** of an event locator, based on data from FIG. 8-10, in accordance with an embodiment of the present invention. FIG. 11 shows the event locations determined from the cross correlations computed in FIGS. 8, 9 and 10. The solid fill area **1102** corresponds to the region where any of the cross correlations **802, 902** and **1002** exceeded a predefined threshold. Similarly, the solid fill area **1104** corresponds to the region where any of the cross correlations **804, 904** and **1004** exceeded a predefined threshold. This then completes the event location process.

Example

Application No. 1-CD64 Infection & Sepsis

[0252] A cartridge **102** (FIG. 1) is prepared for receiving a blood sample. The cartridge comprises a number of treatment composition chambers **106, 108, 110**, adapted to respectively house a corresponding number of treatment compositions **120, 122, 124**. These compositions are described in further detail in U.S. Pat. No. 8,116,984 and in Davis, B H et al., (2006), incorporated herein by reference. In brief, Reagent A comprises a mixture of murine monoclonal antibodies (contains buffered saline), Reagent B—10× Concentrated Trillium Lyse solution (contains ammonium chloride), Reagent C—suspension of 5.2 μm polystyrene beads labeled with Starfire Red and fluorescein isothiocyanate (FITC), (contains <0.1% sodium azide and 0.01% Tween 20).

[0253] In a sample transferring step **202** (FIG. 2), a 10 uL blood sample, is transferred from outside apparatus **100** via receiving element **118** into sample composition chamber **104** and then on to treatment chamber **112** in a transferring step **214**.

[0254] An antibody composition (Reagent A) **120** comprising CD64 antibodies is transferred via transfer element **107** to the treatment chamber **112** in a composition transfer step **204**.

[0255] These two steps combined with mixing step **206** take around four minutes using cartridge **102** of the present invention.

[0256] A lysis buffer (Reagent B) **122** is also added and mixed with the resultant mixed composition. This step and mixing all the compositions takes around three minutes using cartridge **102** of the present invention. Reference beads (Reagent C) **308** are added to the treatment chamber.

[0257] The evaluation chamber **114** is configured and constructed for one or more evaluation steps **216**.

[0258] According to some embodiments, the cartridge is introduced into a system as described in International patent application publication no. WO2011/128893 to Kasdan et al., incorporated herein by reference. This system has software associated therewith for computing the CD64 and CD163 indices on leukocytes.

[0259] The results of the evaluation step are then outputted in a results outputting step **218**. According to this example, the time taken from the introduction of the small blood

sample to obtaining an indication of sepsis is less than 15 minutes, typically around 10 minutes.

[0260] From a user point of view, the following steps are performed:

[0261] 1) The user adds drop of blood to the cartridge 102 and seals it. (10 µL are metered out by micro fluidics).

[0262] 2) Blister A (106) is pressed, releasing 100 µL of Reagent A. Mixing in the cartridge is controlled by the cartridge handling unit (CHU), followed by a 4-minutes incubation.

[0263] 3) Blister B (108) is pressed, releasing 250 µL of Reagent B. Mixing in the cartridge is controlled by the CHU, followed by a 3-5-minutes incubation.

[0264] 4) Magnetic stirbar is activated, stirring the bead suspension (Reagent C).

[0265] 5) Blister C (110) is pressed, releasing 100 µL of Reagent C. Mixing in the cartridge is controlled by the CHU. According to one example, Reagent A is a mixture of murine monoclonal antibodies-diluted 1:5 in buffered saline (PBS+0.5% BSA); Reagent B is a Trillium Lyse solution (at working concentration); Reagent C is a suspension of 5.2 µm polystyrene beads labeled with Star-fire Red and FITC, diluted 1:100 in PBS+0.01% Tween 20.

[0266] 6) The sample is read by the optoelectronics core, and collected to the reading below.

[0267] 7) Data is analyzed automatically and result is presented.

[0268] 8) The cartridge is disposed as biohazard.

TABLE 2

Comparison of Prior art methodology with the methodology of the present invention for detecting sepsis using CD64 and CD163 antibodies. LeukoDx device- present invention				
Step	Description	Volume (uL)	Duration (min)	comments
1	Mixing blood and antibodies	Blood- 10 Abs- 50	4	
2	Adding RBC lysis buffer	250	3	Might require heating the buffer to 37 C.
3	Incubating, Vortexing		3	
4	Adding normalization beads	2	Less than 1	
5	Reading		Less than 1	
Total		312	10	

[0269] In the case of sepsis, by “normalization” is meant taking the ratio of the median of the target population fluorescence emission to the median of the reference bead population fluorescence emission.

[0270] According to some embodiments, the readout may comprise an optoelectronics core, which enables identification and detection of fluorescent signals.

[0271] The CCD in the core, used for focusing, can also be used to read chemiluminescent signals. The readout to user may also indicate where the result falls relative to reference ranges.

[0272] The contents of these publications are incorporated by reference herein where appropriate for teachings of additional or alternative details, features and/or technical background.

[0273] It is to be understood that the invention is not limited in its application to the details set forth in the description contained herein or illustrated in the drawings. The invention is capable of other embodiments and of being practiced and carried out in various ways. Those skilled in the art will readily appreciate that various modifications and changes can be applied to the embodiments of the invention as hereinbefore described without departing from its scope, defined in and by the appended claims.

REFERENCES

[0274] Assicot, Marcel, et al. “High serum procalcitonin concentrations in patients with sepsis and infection.” *The Lancet* 341.8844 (1993): 515-518.

[0275] Aulesa, C., et al. “Validation of the Coulter LH 750 in a hospital reference laboratory.” *Laboratory Hematology* 9.1 (2003): 15-28.

[0276] Ault, Kenneth A. “Flow cytometric measurement of platelet function and reticulated platelets.” *Annals of the New York Academy of Sciences* 677.1 (1993): 293-308.

[0277] Blajchman, Morris A., et al. “Bacterial detection of platelets: current problems and possible resolutions.” *Transfusion medicine reviews* 19.4 (2005): 259-272.

[0278] Bodensteiner, David C. “A flow cytometric technique to accurately measure post-filtration white blood cell counts.” *Transfusion* 29.7 (1989): 651-653.

[0279] Cheson, Bruce D., et al. “National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment.” *Blood* 87.12 (1996): 4990-4997.

[0280] Christ-Crain, Mirjam, et al. “Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial.” *Lancet* 363.9409 (2004): 600-607.

[0281] Cristofanilli, Massimo, et al. “Circulating tumor cells, disease progression, and survival in metastatic breast cancer.” *New England Journal of Medicine* 351.8 (2004): 781-791.

[0282] Davis, Bruce H., et al. “Neutrophil CD64 is an improved indicator of infection or sepsis in emergency department patients.” *Archives of pathology & laboratory medicine* 130.5 (2006): 654-661.

[0283] Dieye, Tandakha Ndiaye, et al. “Absolute CD4 T-cell counting in resource-poor settings: direct volumetric measurements versus bead-based clinical flow cytometry instruments.” *JAIDS Journal of Acquired Immune Deficiency Syndromes* 39.1 (2005): 32-37.

[0284] Divers, S. G., et al. “Quantitation of CD62, soluble CD62, and lysosome-associated membrane proteins 1 and 2 for evaluation of the quality of stored platelet concentrates.” *Transfusion* 35.4 (2003): 292-297.

[0285] Drexler, Hans G., et al. “Diagnostic value of immunological leukemia phenotyping.” *Acta haematologica* 76.1 (1986): 1-8.

[0286] Dziegiel, Morten Hanefeld, Leif Kofoed Nielsen, and Adela Berkowicz. “Detecting fetomaternal hemorrhage by flow cytometry.” *Current opinion in hematology* 13.6 (2006): 490.

[0287] Fischer, Johannes C., et al. “Reducing costs in flow cytometric counting of residual white blood cells in blood

products: utilization of a single platform bead free flow rate calibration method." *Transfusion* 51.7 (2011): 1431-1438.

[0288] Graff, Jochen, et al. "Close relationship between the platelet activation marker CD62 and the granular release of platelet-derived growth factor." *Journal of Pharmacology and Experimental Therapeutics* 300.3 (2002): 952-957.

[0289] Guerti, K., et al. "Performance evaluation of the PENTRA 60C+ automated hematology analyzer and comparison with the ADVIA 2120." *International journal of laboratory hematology* 31.2 (2009): 132-141.

[0290] Hawkins, Robert C. "Laboratory turnaround time." *The Clinical Biochemist Reviews* 28.4 (2007): 179.

[0291] Hershman, M. J., et al. "Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient." *British Journal of Surgery* 77.2 (2005): 204-207.

[0292] Hilfrich, Ralf, and Jalil Hariri. "Prognostic relevance of human papillomavirus L1 capsid protein detection within mild and moderate dysplastic lesions of the cervix uteri in combination with p16 biomarker." *Analytical and Quantitative Cytology and Histology* 30.2 (2008): 78-82.

[0293] Hillier, Sharon L., et al. "A case—control study of chorioamnionic infection and histologic chorioamnionitis in prematurity." *New England Journal of Medicine* 319.15 (1988): 972-978.

[0294] Hoffmann, Johannes J M L. "Neutrophil CD64 as a sepsis biomarker." *Biochimica Medica* 21.3 (2011): 282-290.

[0295] Kibe, Savitri, Kate Adams, and Gavin Barlow. "Diagnostic and prognostic biomarkers of sepsis in critical care." *Journal of Antimicrobial Chemotherapy* 66.suppl 2 (2011): ii33-ii40.

[0296] LaRosa, Steven P., and Steven M. Opal. "Biomarkers: the future." *Critical care clinics* 27.2 (2011): 407.

[0297] Liu, N. I. N. G., A. H. Wu, and Shan S. Wong. "Improved quantitative Apt test for detecting fetal hemoglobin in bloody stools of newborns." *Clinical chemistry* 39.11 (1993): 2326-2329.

[0298] Lotan, Yair, et al. "Bladder cancer screening in a high risk asymptomatic population using a point of care urine based protein tumor marker." *The Journal of urology* 182.1 (2009): 52-58.

[0299] Masse, M., et al. "Validation of a simple method to count very low white cell concentrations in filtered red cells or platelets." *Transfusion* 32.6 (2003): 565-571.

[0300] Matic, Goran B., et al. "Whole blood analysis of reticulated platelets: improvements of detection and assay stability." *Cytometry* 34.5 (1998): 229-234.

[0301] McDonald, C. P., et al. "Use of a solid-phase fluorescent cytometric technique for the detection of bacteria in platelet concentrates." *Transfusion Medicine* 15.3 (2005): 175-183.

[0302] Michelson, Alan D. "Flow cytometry: a clinical test of platelet function." *Open Access Articles* (1996): 290.

[0303] Miller, E. M.; Freire, S. L. S.; Wheeler, A. R. "Proteomics in Microfluidic Devices" In *Encyclopedia of Micro and Nanofluidics*; Li, D. Q., Ed.; Springer: Heidelberg, Germany, 2008; Vol. 3, pp 1749-1758.

[0304] Moro, Ricardo, et al. "A new broad-spectrum cancer marker." *Vitro Diagnostic Technology* (2005).

[0305] Perry, Sara E., et al. "Is low monocyte HLA-DR expression helpful to predict outcome in severe sepsis?." *Intensive care medicine* 29.8 (2003): 1245-1252.

[0306] Ramakumar, Sanjay, et al. "Comparison of screening methods in the detection of bladder cancer." *The Journal of urology* 161.2 (1999): 388-394.

[0307] Rawstron, Andy C., et al. "Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy." *Blood* 98.1 (2001): 29-35.

[0308] Rodriguez, William R., et al. "A microchip CD4 counting method for HIV monitoring in resource-poor settings." *PLoS medicine* 2.7 (2005): e182.

[0309] Rylatt, D. B., et al. "An immunoassay for human D dimer using monoclonal antibodies." *Thrombosis research* 31.6 (1983): 767-778.

[0310] Sacks, David B., et al. "Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus." *Clinical Chemistry* 48.3 (2002): 436-472.

[0311] Segal, H. C., et al. "Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion." *British Journal of Haematology* 128.4 (2005): 520-525.

[0312] Stein, Paul D., et al. "D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: a systematic review." *Annals of internal medicine* 140.8 (2004): 589.

[0313] Sutherland, D. Robert, et al. "The ISHAGE guidelines for CD34+ cell determination by flow cytometry." *Journal of hematology* 5.3 (1996): 213-226.

[0314] Wang, Chao, et al. "Reticulated platelets predict platelet count recovery following chemotherapy." *Transfusion* 42.3 (2002): 368-374.

1.-16. (canceled)

17. A test cartridge for analyzing a sample from a subject, the test cartridge comprising:

- a) a sample composition chamber adapted for receiving the sample from the subject;
- b) a first blister comprising an antibody mixture comprising fluorescently tagged target antibodies and fluorescently tagged control antibodies;
- c) a second blister comprising a cell lysis reagent;
- d) a third blister comprising beads comprising a fluorescent tag, wherein the fluorescent tag is different than those of the target antibodies and control antibodies;
- d) a treatment compartment adapted for fluid mixing, wherein the treatment compartment is coupled to the sample composition chamber, the first blister, the second blister, and the third blister via one or more transfer elements, wherein the one or more transfer elements are microfluidic channels that allow fluid communication between the treatment compartment and (1) the sample composition chamber, (2) the first blister, (3) the second blister, and (4) the third blister; and
- e) an evaluation chamber fluidly connected to the treatment chamber and comprising a reading zone, wherein the reading zone allows the sample to be analyzed as it passes therethrough.

18. The test cartridge of claim 17, wherein the beads comprise a second fluorescent tag different from the fluorescent tag.

19. The test cartridge of claim 17, wherein the target antibodies are CD64 antibodies and wherein the control antibodies are CD163 antibodies.

20. The test cartridge of claim 3017, further comprising a pump or an air blowing element coupled to the treatment compartment.

21. The test cartridge of claim 20, wherein the pump is a bellows pump.

22. The test cartridge of claim 17, wherein a volume of any of the blisters is from about 1 microliter to 1000 microliters.

23. The test cartridge of claim 17, wherein a volume of the sample is about 50 microliters or smaller.

24. The test cartridge of claim 17, wherein the one or more transfer elements are designed to allow mixing of fluid.

25. The test cartridge of claim 24, wherein the one or more transfer elements has a tortuous shaped channel.

26. The microfluidic cartridge of claim 17, wherein the fluorescently tagged beads comprise Starfire Red.

27. The test cartridge of claim 17, wherein the blood sample comprises erythrocytes or leukocytes.

28. The microfluidic cartridge of claim 17, wherein the cell lysis reagent comprises ammonium chloride.

29. The microfluidic cartridge of claim 17, wherein the antibodies are murine monoclonal antibodies.

30. A Cartridge Handling Unit (CHU) for providing an indication of a biological condition in a subject, the CHU adapted to receive a test cartridge and pre-programmed to perform at least the following steps:

- a) pressing a first blister of the test cartridge thereby releasing an antibody mixture and permitting it to contact with the sample, wherein the antibody mixture comprises fluorescently tagged target antibodies and fluorescently tagged control antibodies;
- b) allowing the sample and the antibody mixture to contact for a predetermined time thereby fluorescently tagging cells in the sample;
- c) pressing a second blister of the test cartridge thereby releasing beads comprising a fluorescent tag to contact the sample and the antibody mixture, wherein the fluorescent tag is different than those of the target antibodies and control antibodies;

d) flowing the fluorescently tagged cells and the fluorescently tagged beads through a reading zone;

e) exciting the fluorescent tags of the beads and of the antibodies;

f) measuring fluorescent signals of the fluorescently tagged cells and of the fluorescently tagged beads simultaneously;

e) analyzing fluorescent emission signatures of the fluorescently tagged cells, wherein analysis is done on individual fluorescently tagged cells; and

g) providing an indication of the biological condition in the subject, wherein the indication is provided using the analysis.

31. The CHU of claim 30, wherein the beads comprise a second fluorescent tag different from the fluorescent tag.

32. The CHU of claim 30, wherein the target antibodies are CD64 antibodies and wherein the control antibodies are CD163 antibodies.

33. The CHU of claim 30, wherein the CHU is pre-programmed to perform the steps in less than 30 minutes.

34. The CHU of claim 30, wherein the CHU is pre-programmed to press a third blister of the test cartridge thereby releasing a cell lysis reagent and permitting the cell lysis reagent to contact the sample and the antibody mixture.

35. The CHU of claim 34, wherein pressing the third blister occurs after pressing the first blister and before pressing the second blister.

36. The CHU of claim 30, wherein the CHU is pre-programmed to determine a type of the blood cells.

37. The CHU of claim 30, wherein a volume of any of the blisters is from about 1 microliter to 1000 microliters.

38. The CHU of claim 30, wherein a volume of the sample is about 50 microliters or smaller.

* * * * *

专利名称(译)	用于检测生物学病症的试剂盒，组合物和方法		
公开(公告)号	US20160146793A1	公开(公告)日	2016-05-26
申请号	US14/942921	申请日	2015-11-16
[标]申请(专利权)人(译)	LEUKODX		
申请(专利权)人(译)	LEUKODX LTD.		
当前申请(专利权)人(译)	LEUKODX LTD.		
[标]发明人	KASDAN HARVEY LEE MEISSONNIER JULIEN ZUTA YOAV DAVIS BRUCE ROSEN MICHA HIMMEL YAEL BRODER YEHOASHUA		
发明人	KASDAN, HARVEY LEE MEISSONNIER, JULIEN ZUTA, YOAV DAVIS, BRUCE ROSEN, MICHA HIMMEL, YAEL BRODER, YEHOASHUA		
IPC分类号	G01N33/53 G01N33/58		
CPC分类号	G01N33/5302 G01N2333/70596 G01N2333/70535 G01N33/582 B01L3/502 B01L3/5027 B01L3/502715 B01L3/50273 B01L2200/025 B01L2200/10 B01L2300/041 B01L2300/0654 B01L2300/0816 B01L2300/0867 B01L2300/0877 B01L2300/0883 B01L2300/123 B01L2300/18 B01L2400/0406 B01L2400/0481 G01N33/5091 G01N33/54386 G01N33/569 G01N33/56972 G01N33/68 G01N33/6872 G01N2800/26		
其他公开文献	US9989523		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供用于确定哺乳动物受试者的生物学状况的试剂盒，装置和方法，该方法包括将来自患者的样品与试剂盒中的至少一种组合物一起孵育预定的一段时间以形成至少一种反应产物，当受试者具有所述生物学状况，并且接收至少一种响应于试剂盒中的至少一种报告元件的反应产物的指示，从而提供受试者中生物学状况的指示。

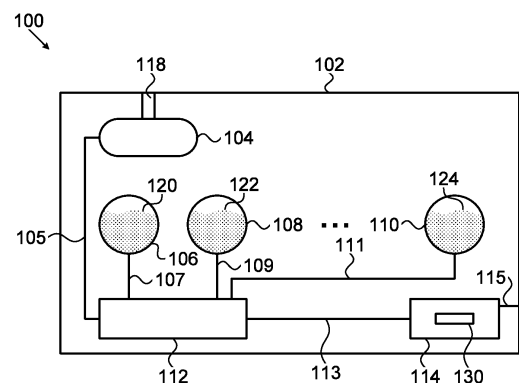


FIG. 1