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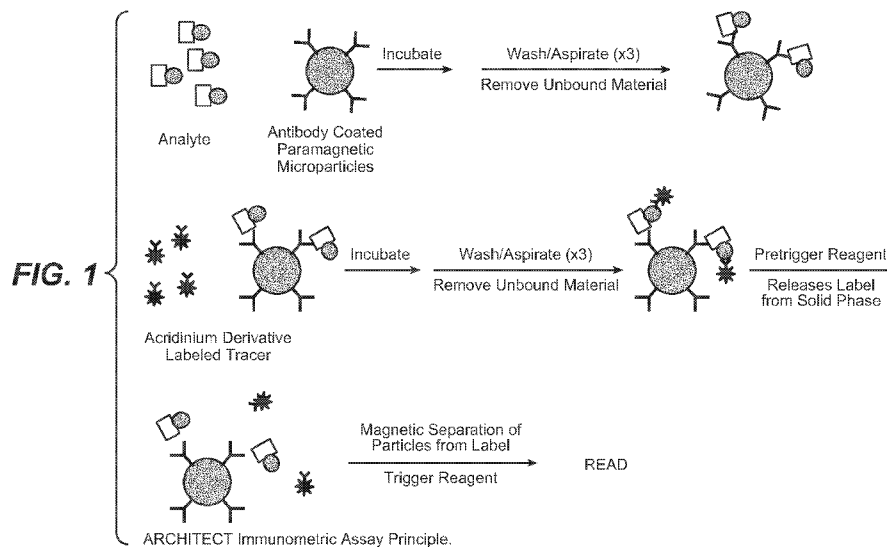(74) Agent: KOSSLAK, Renee M.; AbbVie Biotherapeutics Inc., 1500 Seaport Blvd., Redwood City, California 94063 (US).

(54) Title: SOLUBLE CMET ASSAY

FIG. 1

Analyte — Antibody Coated Paramagnetic Microparticles — Incubate — Wash/Aspirate (x3) Remove Unbound Material

Acridinium Derivative Labeled Tracer — Incubate — Wash/Aspirate (x3) Remove Unbound Material — Pretrigger Reagent Releases Label from Solid Phase

Magnetic Separation of Particles from Label — Trigger Reagent — READ

ARCHITECT Immunometric Assay Principle.

(57) Abstract: Disclosed herein are methods of using antibodies to detect soluble cMet (scMet) in a sample. The methods may be used to diagnose liver disease or cancer in a patient by detecting the presence and/or amount of scMet in a sample from the patient using anti-cMet antibodies. The methods may be used to develop an accurate prognosis for a patient having liver disease or cancer. The methods may be used to identify and/or classify a patient as a candidate for a liver disease therapy and/or cancer therapy.

SOLUBLE CMET ASSAY

[0001]   This application claims priority under 35 U.S.C. § 119(e) to United States Provisional Patent Application Serial Number 61/871,145, filed August 28, 2013, the contents of which are incorporated by reference herein in their entirety.

TECHNICAL FIELD

[0002]   The present invention relates to methods and immunoassay platforms for detecting soluble cMet and for determining a prognosis, diagnosis or risk identification of disease in a patient using anti-cMet antibodies. The anti-cMet antibodies may be used to assess the efficacy of a therapeutic/prophylactic treatment of a subject suffering from disease.

SUBMISSION OF SEQUENCE LISTING

[0003]   This application is accompanied by a sequence listing filed concurrently via EFS-Web. The file containing the sequence listing is named 11678WOO1_SL.txt and was created on July 2, 2014 and is 37,778 bytes in size. The contents of the sequence listing are hereby incorporated by reference into the specification in their entireties.

BACKGROUND

[0004]   cMet is an oncogenic receptor tyrosine kinase, with hepatocyte growth factor (HGF) being its only known ligand. The primary single chain precursor protein is post-translationally cleaved to produce the alpha (50 kDa) and beta (145 kDa) subunits, which are linked together by a disulfide bridge to form the mature receptor. Ligand dependent or independent dimerization is needed for cMet signaling. Both cMet and HGF are required for normal mammalian development. cMet is normally expressed by cells of epithelial origin, while expression of HGF is restricted to cells of mesenchymal origin. Upon HGF stimulation, cMet induces several biological responses that collectively give rise to a program known as invasive growth. cMet activation triggers mitogenesis and morphogenesis. cMet is normally expressed only in stem cells and progenitor cells, which allows these cells to grow invasively in order to generate new tissues in an embryo or regenerate damaged tissues in an adult. cMet is involved in liver regeneration and wound healing during adulthood.

[0005] In many types of cancer including kidney, liver, stomach (gastric), breast, and brain, cMet is deregulated and plays a role in tumor progression, angiogenesis, invasive growth, metastasis and resistance to therapies. Aberrantly active cMet triggers tumor growth (*i.e.*, transformation/mitogenesis), formation of new blood vessels that supply the tumor with nutrients, functions anti-apoptotically, and invasion/metastasis (*i.e.*, spread of cancer to other organs).

[0006] Accordingly, cMet may be a good biomarker for cancer. However, current cMet assays are limited by the range of detecting cMet and its sensitivity to background. There is a need in the art to use cMet to determine, monitor, and treat cancer related diseases.

SUMMARY OF INVENTION

[0007] The present invention is directed to a method for determining scMet concentration in a test sample, the method comprising contacting the test sample with at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex; contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex; and determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex. The at least one capture antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1; a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid

sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. The at least one detection antibody comprises: a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9; a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16. The method may further comprise comparing the signal generated by the detectable label as a direct or indirect indication of the scMet concentration in the test sample to a signal generated as a direct or indirect indication of the scMet concentration in a control or calibrator. The scMet concentration in the test sample may be used to determine or assess whether a subject has or is at risk of developing disease. The disease may be liver disease or cancer. An increased scMet concentration as compared to the scMet concentration in a control or calibrator indicates that the subject may have liver fibrosis or liver cancer. A decreased scMet concentration as compared to the scMet concentration in a control or calibrator indicates that the subject may have gastric cancer. The at least one capture antibody is an antibody produced by a hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under the number I-3731 and the at least one detection antibody is an antibody produced by a hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under the number I-3724.

[0008]   The present invention is also directed to a method of diagnosing and treating liver disease in a subject, the method comprising obtaining a biological sample comprising blood from

the subject; determining the scMet concentration in the biological sample from the subject using the method for determining scMet concentration in a test sample described above; comparing the scMet concentration in the biological sample with the scMet concentration in a normal control or calibrator; diagnosing the subject as having liver disease if the scMet concentration in the biological sample is greater than the scMet concentration in the normal control or calibrator; and administering a liver disease treatment regimen to the subject diagnosed as having liver disease. The biological sample of a subject is selected from a tissue sample, bodily fluid, whole blood, plasma, serum, urine, bronchoalveolar lavage fluid, and a cell culture suspension or fraction thereof. The liver disease may be liver fibrosis or liver cancer.

[0009]   The present invention is also directed to a method of diagnosing and treating cancer in a subject, the method comprising obtaining a biological sample comprising blood from the subject; determining the scMet concentration in the biological sample from the subject using the method for determining scMet concentration in a test sample described above; comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator; diagnosing the subject as having cancer if the scMet concentration in the biological sample is less than the scMet concentration in the normal control or calibrator; and administering a cancer treatment regimen to the subject diagnosed as having cancer. The biological sample of a subject is selected from a tissue sample, bodily fluid, whole blood, plasma, serum, urine, bronchoalveolar lavage fluid, and a cell culture suspension or fraction thereof. The cancer may be gastric cancer.

[0010]   The present invention is also directed to a method for determining if a subject is responding to the administration of one or more pharmaceutical compositions, the method comprising: measuring the scMet concentration in a sample from the subject using the method for determining scMet concentration in a test sample as described above; comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator, wherein an altered scMet concentration indicates that the subject is not responding to the administration of one or more pharmaceutical compositions; and adjusting the treatment of the subject if the subject is not responding to the administration of one or more pharmaceutical compositions.

[0011]   The present invention is also directed to a method of diagnosing and treating liver disease in a subject, the method comprising contacting the test sample with at least one capture

antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex; contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex; determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex formed in (b), whereupon the scMet concentration in the test sample is determined; comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator; diagnosing the subject as having liver disease if the scMet concentration in the biological sample is greater than the scMet concentration in the normal control or calibrator; and administering a liver disease treatment regimen to the subject diagnosed as having liver disease. The at least one capture antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1; a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. The at least one detection antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9; a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino

acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16. The liver disease may be liver fibrosis or liver cancer.

[0012]   The present invention is also directed to a method of diagnosing and treating cancer in a subject, the method comprising contacting the test sample with at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex; contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex; determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex formed in (b), whereupon the scMet concentration in the test sample is determined; comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator; diagnosing the subject as having cancer if the scMet concentration in the biological sample is less than the scMet concentration in the normal control or calibrator; and administering a cancer treatment regimen to the subject diagnosed as having cancer. The at least one capture antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1; a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ

ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. The at least one detection antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9; a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16. The cancer may be gastric cancer.

[0013]   The present invention is also directed to a kit for assaying a test sample for scMet. The kit comprises at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet, at least one detection antibody, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody, and instructions for assaying the test sample for scMet. The at least one capture antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1; a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the

amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. The at least one detection antibody comprises a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9; a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16. The at least detection antibody may be optionally detectably labeled. The kit may further comprise a reference standard indicating a scMet concentration in a control or calibrator. The reference standard may indicate scMet concentration levels of 200 ng/mL (High), 50 ng/mL (Medium) and 12.5 ng/mL (Low).

BRIEF DESCRIPTION OF DRAWINGS

[0014]   FIG. 1 shows a schematic diagram of the ARCHITECT® immunometric assay principle.

[0015]   FIG. 2 shows a representative sandwich array.

[0016]   FIG. 3 shows the binding curve of mAb 11E1 and cMet-A488.

[0017]   FIG. 4 shows a graph of the ARCHITECT® scMet assays using Dynal uP.

[0018]   FIG. 5 shows the complex testing of the ARCHITECT® scMet assay.

[0019]   FIG. 6 shows a standard curve of scMet assay using m224G11 uP and 11E1 conjugate where the circle on the graph represents a range of current samples tested.

[0020]   FIGS. 7A-7B show the sample stability at room temperature at 4 and 24 hrs. FIG. 7A shows the endogenous sample stability at room temperature at 4 and 24 hrs. FIG. 7B shows the spiked sample stability at room temperature at 4 and 24 hrs.

[0021]   FIGS. 8A-8B show the sample stability at 2-8°C at 24 and 72 hrs. FIG. 8A shows the endogenous sample stability at 2-8°C at 24 and 72 hrs. FIG. 8B shows the spiked sample stability at 2-8°C at 24 and 72 hrs.

[0022]   FIGS. 9A-9B show the sample stability at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles. FIG. 9A shows the endogenous sample stability at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles. FIG. 9B shows the spiked sample stability at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles.

[0023]   FIG. 10 shows the dilution linearity of the ARCHITECT® scMet assays.

[0024]   FIG. 11 shows the levels of scMet in human patients with various diseases as measured by the ARCHITECT® scMet assays using the 224G11 antibody and the 11E1 antibody.

[0025]   FIG. 12 shows a receiver operating characteristic (ROC) curve for liver fibrosis vs normal healthy subjects.

[0026]   FIG. 13 shows an ROC curve for gastric cancer vs normal healthy subjects.

[0027]   FIG. 14 shows an ROC curve for liver cancer vs normal healthy subjects.

DETAILED DESCRIPTION

[0028]   The present invention relates to an immunoassay for analyzing the levels of soluble cMet (scMet) to identify, diagnose and treat a disease in subjects in need thereof. cMet is an oncogenic receptor tyrosine kinase involved in mitogenesis and morphogenesis. cMet is found to be deregulated in many types of cancer, including liver and gastric. cMet is frequently overexpressed in cancer tissues, such as liver, however, the activity of the cMet receptor may also play a role in cancer development alone or in combination with overexpressed cMet. Table

1 shows the percentage of various types of cancers that have *MET* gene amplification (*i.e.*, increased copy number of the *MET* gene), overexpression of cMet mRNA and/or overexpression of HGF mRNA.

Table 1   MET, cMet and HGF in various cancers

Cancer	*MET* amplification	cMet overexpression	HGF overexpression
colorectal cancer	10-18% liver metastases	50%	47%
gastric cancer	10-23%	46-69%	44%
glioblastoma	17%	29-47%	44%
head and neck squamous cell carcinoma	15%	84%	45%
non-small cell lung cancer	6-22% tyrosine kinase inhibitor resistant	36%	32%
triple negative breast cancer	Rare	25%	43%

[0029]   The present invention immunoassay differs from previous scMet immunoassays by using a unique combination of antibodies to detect scMet in a sample over a wider range of concentrations, thus providing a more versatile and sensitive assay. The present invention immunoassay detects scMet in a range that is about 235 times greater than the range of commercially available assays. The increased detectable range of concentration of the disclosed immunoassay provides a more accurate and sensitive assay for diagnosing and distinguishing liver disease and cancer in a patient. By measuring scMet levels using a combination of anti-cMet antibodies, such as the combination of the 224G11 antibody and the 11E1 antibody, the present invention allows for more diseases to be more accurately diagnosed compared to scMet immunoassays that measure scMet levels using a different combination of antibodies. The disclosed immunoassay may be used to detect increased or decreased scMet concentrations in a sample compared to a control or calibrator sample and thus be used to identify in a patient various liver diseases or cancers. The wider range of detection of the disclosed immunoassay allows the determination and distinguishment of various diseases, which may vary in scMet

concentrations by tens of nanogram amounts. The use of the scMet immunoassay may provide accurate diagnosing and subsequent treatment of patients with liver disease or cancer.

[0030]   Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0031]   Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0032]   The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0033]   For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0034]   "Antibody" and "antibodies" as used herein refers to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies (fully or partially humanized), animal antibodies such as, but not limited to, a bird (for example, a duck or a goose), a shark, a whale, and a mammal, including a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, etc.) or a non-human primate (for example, a monkey, a chimpanzee, etc.), recombinant antibodies, chimeric antibodies, single-chain Fvs ("scFv"), single chain antibodies, single domain

antibodies, Fab fragments, F(ab') fragments, F(ab')2 fragments, disulfide-linked Fvs ("sdFv"), and anti-idiotypic ("anti-Id") antibodies, dual-domain antibodies, dual variable domain (DVD) or triple variable domain (TVD) antibodies (dual-variable domain immunoglobulins and methods for making them are described in Wu, C., et al., Nature Biotechnology, 25(11):1290-1297 (2007) and PCT International Application WO 2001/058956, the contents of each of which are herein incorporated by reference), and functionally active epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, namely, molecules that contain an analyte-binding site. Immunoglobulin molecules can be of any type (for example, IgG, IgE, IgM, IgD, IgA, and IgY), class (for example, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass. For simplicity sake, an antibody against an analyte is frequently referred to herein as being either an "anti-analyte antibody" or merely an "analyte antibody" (e.g., an anti-scMet antibody or a scMet antibody).

[0035]   "Antibody fragment" as used herein refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e. CH2, CH3, or CH4, depending on the antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include, but are not limited to, Fab fragments, Fab' fragments, Fab'-SH fragments, F(ab')2 fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

[0036]   The "area under curve" or "AUC" refers to area under a ROC curve. AUC under a ROC curve is a measure of accuracy. An area of 1 represents a perfect test, whereas an area of 0.5 represents an insignificant test. A preferred AUC may be at least approximately 0.700, at least approximately 0.750, at least approximately 0.800, at least approximately 0.850, at least approximately 0.900, at least approximately 0.910, at least approximately 0.920, at least approximately 0.930, at least approximately 0.940, at least approximately 0.950, at least approximately 0.960, at least approximately 0.970, at least approximately 0.980, at least approximately 0.990, or at least approximately 0.995.

[0037] "Cancer" as used herein refers to the uncontrolled and unregulated growth of abnormal cells in the body. Cancerous cells are also called malignant cells. Cancer may invade nearby parts of the body and may also spread to more distant parts of the body through the lymphatic system or bloodstream. Cancers include Adrenocortical Carcinoma, Anal Cancer, Bladder Cancer, Brain Tumor, Breast Cancer, Carcinoid Tumor, Gastrointestinal, Carcinoma of Unknown Primary, Cervical Cancer, Colon Cancer, Endometrial Cancer, Esophageal Cancer, Extrahepatic Bile Duct Cancer, Ewings Family of Tumors (PNET), Extracranial Germ Cell Tumor, Intraocular Melanoma Eye Cancer, Gallbladder Cancer, Gastric Cancer (Stomach), Extragonadal Germ Cell Tumor, Gestational Trophoblastic Tumor, Head and Neck Cancer, Hypopharyngeal Cancer, Islet Cell Carcinoma, Kidney Cancer (renal cell cancer), Laryngeal Cancer, Acute Lymphoblastic Leukemia, Leukemia, Acute Myeloid, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Hairy Cell Leukemia, Lip and Oral Cavity Cancer, Liver Cancer, Non-Small Cell Lung Cancer, Small Cell Lung Cancer, AIDS-Related Lymphoma, Central Nervous System (Primary) Lymphoma, Cutaneous T-Cell Lymphoma, Hodgkin's Disease Lymphoma, Non-Hodgkin's Disease Lymphoma, Malignant Mesothelioma, Melanoma, Merkel Cell Carcinoma, Metasatic Squamous Neck Cancer with Occult Primary, Multiple Myeloma and Other Plasma Cell Neoplasms, Mycosis Fungoides, Myelodysplastic Syndrome, Myeloproliferative Disorders, Nasopharyngeal Cancer, euroblastoma, Oral Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Pancreatic Cancer, Exocrine, Pancreatic Cancer, Islet Cell Carcinoma, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pituitary Cancer, Plasma Cell Neoplasm, Prostate Cancer, Rhabdomyosarcoma, Rectal Cancer, Renal Cell Cancer (cancer of the kidney), Transitional Cell Renal Pelvis and Ureter, Salivary Gland Cancer, Sezary Syndrome, Skin Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Testicular Cancer, Malignant Thymoma, Thyroid Cancer, Urethral Cancer, Uterine Cancer, Unusual Cancer of Childhood, Vaginal Cancer, Vulvar Cancer, and Wilms' Tumor.

[0038] "cMet" (also known as MET or MNNG HOS Transforming gene) as used herein refers to a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR). cMet proto-oncogene has a total length of 125,982 bp and is located in the 7q31 locus of chromosome 7. The primary single chain precursor protein is post-translationally cleaved to produce the alpha (50 kDa) and beta (145 kDa) subunits, which are linked together by a disulfide

bridge to form the mature receptor. The ligand for cMet is hepatocyte growth factor (HGF). "scMet" as used herein refers to soluble cMet. "scMet" and "cMet" as used herein refer to scMet and cMet in a sample regardless of whether or not the scMet or cMet is bound to HGF. The binding of HGF to proto-oncogenic cMet activate a tyrosine kinase signaling cascade that stimulates mitogenesis, cell motility, and matrix invasion. cMet plays a role in angiogenesis, tumorogenesis and tissue regeneration. cMet overexpression is typically found in most cancers, such as liver cancer. cMet activity may be more important in gastric cancer.

[0039]   "Gastric Cancer" as used herein refers to cancer originating in the stomach. Gastric cancer may be caused by infection by Helicobacter pylori, autoimmune atrophic gastritis, or intestinal metaplasia. Various genetic factors are associated with increased risk levels of gastric cancer.

[0040]   "HGF" as used herein refers to hepatocyte growth factor, which is a paracrine cellular growth, motility, and morphogenic factor. HGF is a pleotropic growth factor variously designated as scatter factor, hematopoietin A, and mammary growth factor. HGF has a central role in angiogenesis, tumorogenesis, and tissue regeneration as it stimulates mitogenesis, cell motility, and matrix invasion. HGF is secreted by mesenchymal cells and acts as a multifunctional cytokine on cells of mainly epithelial origin. HGF targets and acts primarily upon epithelial cells and endothelial cells but also haemopoietic progenitor cells. HGF plays a role in embryonic organ development, adult organ regeneration, and wound healing. HGF regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic cMet receptor. HGF may interact with copper.

[0041]   "Label" and "detectable label" as used herein refer to a moiety attached to an antibody or an analyte to render the reaction between the antibody and the analyte detectable, and the antibody or analyte so labeled is referred to as "detectably labeled." A label can produce a signal that is detectable by visual or instrumental means. Various labels include signal-producing substances, such as chromogens, fluorescent compounds, chemiluminescent compounds, radioactive compounds, and the like. Representative examples of labels include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety, itself, may not be detectable but may become detectable upon reaction with yet another moiety. Use of the term "detectably labeled" is intended to encompass such labeling.

[0042]   Any suitable detectable label as is known in the art can be used. For example, the detectable label can be a radioactive label (such as ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, *Introduction to Immunocytochemistry,* 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, *Handbook of Fluorescent Probes and Research Chemicals* (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oregon. A fluorescent label can be used in FPIA (see, e.g., U.S. Patent Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803, which are hereby incorporated by reference in their entireties). An acridinium compound can be used as a detectable label in a homogeneous chemiluminescent assay (see, e.g., Adamczyk et al., Bioorg. Med. Chem. Lett. 16: 1324-1328 (2006); Adamczyk et al., Bioorg. Med. Chem. Lett. 4: 2313-2317 (2004); Adamczyk et al., Biorg. Med. Chem. Lett. 14: 3917-3921 (2004); and Adamczyk et al., Org. Lett. 5: 3779-3782 (2003)).

[0043]   In one aspect, the acridinium compound is an acridinium-9-carboxamide. Methods for preparing acridinium 9-carboxamides are described in Mattingly, J. Biolumin. Chemilumin. 6: 107-114 (1991); Adamczyk et al., J. Org. Chem. 63: 5636-5639 (1998); Adamczyk et al., Tetrahedron 55: 10899-10914 (1999); Adamczyk et al., Org. Lett. 1: 779-781 (1999); Adamczyk et al., Bioconjugate Chem. 11: 714-724 (2000); Mattingly et al., In *Luminescence Biotechnology: Instruments and Applications;* Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77–105 (2002); Adamczyk et al., Org. Lett. 5: 3779-3782 (2003); and U.S. Pat. Nos. 5,468,646, 5,543,524 and 5,783,699 (each of which is incorporated herein by reference in its entirety for its teachings regarding same).

[0044]   Another example of an acridinium compound is an acridinium-9-carboxylate aryl ester. An example of an acridinium-9-carboxylate aryl ester of formula II is 10-methyl-9-

(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra et al., Photochem. Photobiol. 4: 1111-21 (1965); Razavi et al., Luminescence 15: 245-249 (2000); Razavi et al., Luminescence 15: 239-244 (2000); and U.S. Patent No. 5,241,070 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Such acridinium-9-carboxylate aryl esters are efficient chemiluminescent indicators for hydrogen peroxide produced in the oxidation of an analyte by at least one oxidase in terms of the intensity of the signal and/or the rapidity of the signal. The course of the chemiluminescent emission for the acridinium-9-carboxylate aryl ester is completed rapidly, i.e., in under 1 second, while the acridinium-9-carboxamide chemiluminescent emission extends over 2 seconds. Acridinium-9-carboxylate aryl ester, however, loses its chemiluminescent properties in the presence of protein. Therefore, its use requires the absence of protein during signal generation and detection. Methods for separating or removing proteins in the sample are well-known to those skilled in the art and include, but are not limited to, ultrafiltration, extraction, precipitation, dialysis, chromatography, and/or digestion (see, e.g., Wells, *High Throughput Bioanalytical Sample Preparation. Methods and Automation Strategies*, Elsevier (2003)). The amount of protein removed or separated from the test sample can be about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. Further details regarding acridinium-9-carboxylate aryl ester and its use are set forth in U.S. Pat. App. No. 11/697,835, filed April 9, 2007. Acridinium-9-carboxylate aryl esters can be dissolved in any suitable solvent, such as degassed anhydrous *N,N*-dimethylformamide (DMF) or aqueous sodium cholate.

[0045]   "Liver cancer" as used herein refers to cancer that originates in the liver. Liver cancer includes hepatocellular carcinoma and fibrolamellar carcinoma. In most cases, the cause of liver cancer is usually scarring of the liver (*i.e.*, cirrhosis).

[0046]   "Liver cirrhosis" as used herein refers to the consequence of chronic liver disease characterized by replacement of liver tissue by fibrosis, scar tissue, and regenerative nodules (lumps that occur because of a process in which damaged tissue is regenerated, leading to loss of liver function. The architectural organization of the functional units of the liver become so disrupted that blood flow through the liver and liver function become disrupted. Cirrhosis is most commonly caused by alcoholism, hepatitis B and C, and fatty liver disease, but has many

other possible causes. Some cases are idiopathic (*i.e.*, of unknown cause). Once cirrhosis has developed, the serious complications of liver disease may occur including portal hypertension, liver failure, and liver cancer. The risk of liver cancer is greatly increased once cirrhosis develops and cirrhosis should be considered to be a pre-malignant condition. Cirrhosis may be caused by alcohol abuse, autoimmune diseases of the liver, Hepatitis B or C virus infection, inflammation of the liver that is long-term (chronic), and iron overload in the body (hemochromatosis). Patients with hepatitis B or C are at risk for liver cancer, even if they have not developed cirrhosis.

[0047]   "Liver disease" as used herein refers to damage to or disease of the liver. Symptoms of liver dysfunction include both physical signs and a variety of symptoms related to digestive problems, blood sugar problems, immune disorders, abnormal absorption of fats, and metabolism problems. Liver disease includes liver fibrosis, liver cirrhosis, and liver cancer. All chronic liver diseases can lead to liver fibrosis. Chronic liver disease may be caused by chronic viral hepatitis B and alcoholic liver disease.

[0048]   "Liver fibrosis" as used herein refers to an excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. Liver fibrosis is the scarring process that represents the liver's response to injury or illness. Liver fibrosis may be cause by infections due hepatitis B and C, parasites, excessive alcohol use and exposure to toxic chemicals, including pharmaceutical drugs and blocked bile ducts. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation.

[0049]   "Monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the

chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological.

[0050]   "Predetermined cutoff" and "predetermined level" as used herein refer to an assay cutoff value that is used to assess diagnostic, prognostic, or therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (*e.g.*, presence of disease, stage of disease, severity of disease, progression, non-progression, or improvement of disease, etc.). The disclosure provides exemplary predetermined levels. However, it is well-known that cutoff values may vary depending on the nature of the immunoassay (*e.g.*, antibodies employed, reaction conditions, sample purity, etc.). It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other immunoassays based on the description provided by this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, the correlations as described herein should be generally applicable.

[0051]   "Pretreatment reagent," e.g., lysis, precipitation and/or solubilization reagent, as used in a diagnostic assay as described herein is one that lyses any cells and/or solubilizes any analyte that is/are present in a test sample. Pretreatment is not necessary for all samples, as described further herein. Among other things, solubilizing the analyte (i.e., HGF, fragments of HGF, variants of HGF or any combinations thereof) entails release of the analyte from any endogenous binding proteins present in the sample. A pretreatment reagent may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment reagent, there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay. The pretreatment reagent optionally can comprise: (a) one or more solvents and salt, (b) one or more solvents, salt and detergent, (c) detergent, (d) detergent and salt, or (e) any reagent or combination of reagents appropriate for cell lysis and/or solubilization of analyte.

[0052]   "Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (*e.g.,* one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an

antibody or an analyte. Alternatively, a single calibrator, which is near a predetermined positive/negative cutoff, can be used. Multiple calibrators (*i.e.*, more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction to comprise a "sensitivity panel."

[0053]   A "receiver operating characteristic" curve or "ROC" curve refers to a graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. It is created by plotting the fraction of true positives out of the positives (TPR = true positive rate) vs. the fraction of false positives out of the negatives (FPR = false positive rate), at various threshold settings. TPR is also known as sensitivity, and FPR is one minus the specificity or true negative rate.

[0054]   "Risk assessment," "risk classification," "risk identification," or "risk stratification" of subjects (*e.g.*, patients) as used herein refers to the evaluation of factors including biomarkers, to predict the risk of occurrence of future events including disease onset or disease progression, so that treatment decisions regarding the subject may be made on a more informed basis.

[0055]   "Sample," "test sample," "specimen," "sample from a subject," and "patient sample" as used herein may be used interchangeable and may be a sample of blood, tissue, urine, serum, plasma, amniotic fluid, cerebrospinal fluid, placental cells or tissue, endothelial cells, leukocytes, or monocytes. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art.

[0056]   Any cell type, tissue, or bodily fluid may be utilized to obtain a sample. Such cell types, tissues, and fluid may include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, blood (such as whole blood), plasma, serum, sputum, stool, tears, mucus, saliva, bronchoalveolar lavage (BAL) fluid, hair, skin, red blood cells, platelets, interstitial fluid, ocular lens fluid, cerebral spinal fluid, sweat, nasal fluid, synovial fluid, menses, amniotic fluid, semen, etc. Cell types and tissues may also include lymph fluid, ascetic fluid, gynecological fluid, urine, peritoneal fluid, cerebrospinal fluid, a fluid collected by vaginal rinsing, or a fluid collected by vaginal flushing. A tissue or cell type may be provided by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (*e.g.*, isolated by another person, at another time, and/or for another

purpose). Archival tissues, such as those having treatment or outcome history, may also be used. Protein or nucleotide isolation and/or purification may not be necessary.

[0057] "Series of calibrating compositions" refers to a plurality of compositions comprising a known concentration of scMet, wherein each of the compositions differs from the other compositions in the series by the concentration of scMet.

[0058] "Solid phase" refers to any material that is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize a capture agent. Alternatively, the solid phase can have affixed thereto a linking agent that has the ability to attract and immobilize the capture agent. For example, the linking agent can include a charged substance that is oppositely charged with respect to the capture agent itself or to a charged substance conjugated to the capture agent. In general, the linking agent can be any binding partner (preferably specific) that is immobilized on (attached to) the solid phase and that has the ability to immobilize the capture agent through a binding reaction. The linking agent enables the indirect binding of the capture agent to a solid phase material before the performance of the assay or during the performance of the assay. For example, the solid phase can be plastic, derivatized plastic, magnetic or non-magnetic metal, glass, or silicon, including, for example, a test tube, microtiter well, sheet, bead, microparticle, chip, and other configurations known to those of ordinary skill in the art.

[0059] "Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (*e.g.*, cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc) and a human). In some embodiments, the subject may be a human or a non-human. The subject or patient may be undergoing other forms of treatment.

[0060] "Treat", "treating" or "treatment" are each used interchangeably herein to describe reversing, alleviating, or inhibiting the progress of a disease, or one or more symptoms of such disease, to which such term applies. Depending on the condition of the subject, the term also refers to preventing a disease, and includes preventing the onset of a disease, or preventing the symptoms associated with a disease. A treatment may be either performed in an acute or chronic way. The term also refers to reducing the severity of a disease or symptoms associated with such disease prior to affliction with the disease. Such prevention or reduction of the severity of a

disease prior to affliction refers to administration of an antibody or pharmaceutical composition of the present invention to a subject that is not at the time of administration afflicted with the disease. "Preventing" also refers to preventing the recurrence of a disease or of one or more symptoms associated with such disease. "Treatment" and "therapeutically," refer to the act of treating, as "treating" is defined above.

[0061]   Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. scMet Detection

[0062]   The present invention is directed to method of detecting and measuring scMet in a sample from a subject using antibodies that bind to different scMet epitopes. The method includes (a) obtaining a biological sample from a subject, (b) contacting the biological sample with a capture antibody, which binds to an epitope on scMet or scMet fragment to form a capture antibody-scMet antigen complex, (c) contacting the capture antibody–scMet antigen complex with a detection antibody which includes a detectable label and binds to an epitope on scMet or scMet fragment that is not bound by the capture antibody, to form a capture antibody-scMet antigen-detection antibody, and (d) determining the presence, amount or concentration of scMet or scMet fragment in the biological sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex.

[0063]   The present invention is further directed to a method for diagnosing a disease in a subject based on the scMet levels in a sample from the subject. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having a disease if the level of scMet in the biological

sample is greater than the reference level of scMet, and (e) administering a treatment regiment to the subject identified as having disease.

[0064]   Levels of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL or 1000 ng/mL of scMet in a biological sample may be detected. Ranges of scMet detection have at least 5%, 10%, 25%, 50%, 75%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400%, or 500% improved range size compared to other commercially available scMet immunoassays. Ranges of about 0 ng/mL to about 1000 ng/mL, about 0.50 ng/mL to about 1000 ng/mL, about 1.00 ng/mL to about 1000 ng/mL, about 2.00 ng/mL to about 1000 ng/mL, about 3.00 ng/mL to about 1000 ng/mL, about 4.00 ng/mL to about 1000 ng/mL, about 5.00 ng/mL to about 1000 ng/mL, about 5.50 ng/mL to about 1000 ng/mL, about 5.75 ng/mL to about 1000 ng/mL, about 6.00 ng/mL to about 1000 ng/mL, about 6.25 ng/mL to about 1000 ng/mL, about 6.50 ng/mL to about 1000 ng/mL, about 7.00 ng/mL to about 1000 ng/mL, about 8.00 ng/mL to about 1000 ng/mL, about 9.00 ng/mL to about 1000 ng/mL, about 10.00 ng/mL to about 1000, about 0 ng/mL to about 950 ng/mL, 0.50 ng/mL to about 950 ng/mL, 1.00 ng/mL to about 950 ng/mL, 2.00 ng/mL to about 950 ng/mL, 3.00 ng/mL to about 950 ng/mL, 4.00 ng/mL to about 950 ng/mL, 5.00 ng/mL to about 950 ng/mL, 5.50 ng/mL to about 950 ng/mL, 5.75 ng/mL to about 950 ng/mL, 6.00 ng/mL to about 950 ng/mL, 6.25 ng/mL to about 950 ng/mL, 6.50 ng/mL to about 950 ng/mL, 7.00 ng/mL to about 950 ng/mL, 8.00 ng/mL to about 950 ng/mL, 9.00 ng/mL to about 950 ng/mL, 10.00 ng/mL to about 950 ng/mL, about 0 ng/mL to about 900 ng/mL, about 0.50 ng/mL to about 900 ng/mL, 1.00 ng/mL to about 900 ng/mL, 2.00 ng/mL to about 900 ng/mL, 3.00 ng/mL to about 900 ng/mL, 4.00 ng/mL to about 900 ng/mL, 5.00 ng/mL to about 900 ng/mL, 5.50 ng/mL to about 900 ng/mL, 5.75 ng/mL to about 900 ng/mL, 6.00 ng/mL to about 900 ng/mL, 6.25 ng/mL to about 900 ng/mL, 6.50 ng/mL to about 900 ng/mL, 7.00 ng/mL to about 900 ng/mL, 8.00 ng/mL to about 900 ng/mL, 9.00 ng/mL to about 900 ng/mL, 10.00 ng/mL to about 900 ng/mL, about 0 ng/mL to about 850 ng/mL, 0.50 ng/mL to about 850 ng/mL, 1.00 ng/mL to about 850 ng/mL, 2.00 ng/mL to about 850 ng/mL, 3.00 ng/mL to about 850 ng/mL, 4.00 ng/mL to about 850 ng/mL, 5.00 ng/mL to about 850 ng/mL, 5.50 ng/mL to about 850 ng/mL, 5.75 ng/mL to about 850 ng/mL, 6.00 ng/mL to about 850 ng/mL, 6.25 ng/mL to about 850 ng/mL, 6.50 ng/mL to about 850 ng/mL, 7.00

ng/mL to about 850 ng/mL, 8.00 ng/mL to about 850 ng/mL, 9.00 ng/mL to about 850 ng/mL, 10.00 ng/mL to about 850 ng/mL, about 0 ng/mL to about 800 ng/mL, 0.50 ng/mL to about 800 ng/mL, 1.00 ng/mL to about 800 ng/mL, 2.00 ng/mL to about 800 ng/mL, 3.00 ng/mL to about 800 ng/mL, 4.00 ng/mL to about 800 ng/mL, 5.00 ng/mL to about 800 ng/mL, 5.50 ng/mL to about 800 ng/mL, 5.75 ng/mL to about 800 ng/mL, 6.00 ng/mL to about 800 ng/mL, 6.25 ng/mL to about 800 ng/mL, 6.50 ng/mL to about 800 ng/mL, 7.00 ng/mL to about 800 ng/mL, 8.00 ng/mL to about 800 ng/mL, 9.00 ng/mL to about 800 ng/mL, 10.00 ng/mL to about 800 ng/mL, about 0 ng/mL to about 750 ng/mL, 0.50 ng/mL to about 750 ng/mL, 1.00 ng/mL to about 750 ng/mL, 2.00 ng/mL to about 750 ng/mL, 3.00 ng/mL to about 870 ng/mL, 4.00 ng/mL to about 750 ng/mL, 5.00 ng/mL to about 750 ng/mL, 5.50 ng/mL to about 750 ng/mL, 5.75 ng/mL to about 750 ng/mL, 6.00 ng/mL to about 750 ng/mL, 6.25 ng/mL to about 750 ng/mL, 6.50 ng/mL to about 750 ng/mL, 7.00 ng/mL to about 750 ng/mL, 8.00 ng/mL to about 750 ng/mL, 9.00 ng/mL to about 750 ng/mL, or 10.00 ng/mL to about 750 ng/mL of scMet may be detected.

A. Immunoassay

[0065]   scMet, and/or peptides or fragments thereof, i.e., scMet fragments, may be analyzed using an immunoassay. The presence or amount of scMet or scMet fragment can be determined using antibodies and detecting specific binding to scMet or scMet fragment. For example, the antibody, or antibody fragment thereof, may specifically bind to scMet or scMet fragment. If desired, one or more of the antibodies can be used in combination with one or more commercially available monoclonal/polyclonal antibodies. Such antibodies are available from companies such as R&D Systems, Inc. (Minneapolis, MN) and Enzo Life Sciences International, Inc. (Plymouth Meeting, PA).

[0066]   The presence or amount of scMet or scMet fragment present in a body sample may be readily determined using an immunoassay, such as sandwich immunoassay (e.g., monoclonal-polyclonal sandwich immunoassays, including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, MN)). A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), is an example of a preferred immunoassay. Other methods include, for example, mass spectrometry and immunohistochemistry (e.g. with sections from tissue biopsies) using scMet antibodies

(monoclonal, polyclonal, chimeric, humanized, human etc) or scMet antibody fragments against scMet. Other methods of detection include those described in, for example, U.S. Pat. Nos. 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, each of which is hereby incorporated by reference in its entirety. Specific immunological binding of the antibody to the scMet can be detected via direct labels, such as fluorescent or luminescent tags, metals and radionuclides attached to the antibody or via indirect labels, such as alkaline phosphatase or horseradish peroxidase.

[0067]   The use of immobilized antibodies or antibody fragments thereof may be incorporated into the immunoassay. The antibodies may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. An assay strip can be prepared by coating the antibody or plurality of antibodies in an array on a solid support. This strip can then be dipped into the test biological sample and processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

[0068]   A heterogeneous format may be used. For example, after the test sample is obtained from a subject, a first mixture is prepared. The mixture contains the test sample being assessed for scMet or scMet fragment and a first specific binding partner, wherein the first specific binding partner and any scMet contained in the test sample form a first specific binding partner-scMet antigen complex. The first specific binding partner may be an anti-scMet antibody that binds to an epitope having an amino acid sequence comprising at least three contiguous (3) amino acids of SEQ ID NO:33 ("cMet (25-932)-HIEGRMD-6His"; amino acid 25-932 of cMet with a HIEGRMD linker (SEQ ID NO:36) and 6-histidine tag (SEQ ID NO:37)). The order in which the test sample and the first specific binding partner are added to form the mixture is not critical. The first specific binding partner may be immobilized on a solid phase. The solid phase used in the immunoassay (for the first specific binding partner and, optionally, the second specific binding partner) can be any solid phase known in the art, such as, but not limited to, a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a film, a filter paper, a disc and a chip.

[0069]   After the mixture containing the first specific binding partner-scMet antigen complex is formed, any unbound scMet is removed from the complex using any technique known in the

art. For example, the unbound scMet can be removed by washing. Desirably, however, the first specific binding partner is present in excess of any scMet present in the test sample, such that all scMet that is present in the test sample is bound by the first specific binding partner.

[0070]   After any unbound scMet is removed, a second specific binding partner is added to the mixture to form a first specific binding partner-scMet antigen -second specific binding partner complex. The second specific binding partner may be an anti-scMet antibody that binds to an epitope having an amino acid sequence comprising at least three contiguous (3) amino acids of SEQ ID NO:33 ("cMet (25-932)-HIEGRMD-6His"). Moreover, the second specific binding partner is labeled with or contains a detectable label, as described above.

[0071]   The use of immobilized antibodies or antibody fragments thereof may be incorporated into the immunoassay. The antibodies may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. An assay strip can be prepared by coating the antibody or plurality of antibodies in an array on a solid support. This strip can then be dipped into the test biological sample and processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

(i) Sandwich ELISA

[0072]   The Sandwich ELISA measures the amount of antigen between two layers of antibodies (*i.e.,* at least one capture antibody) and a detection antibody (*i.e.* at least one detection antibody). The capture antibody and the detection antibody bind to different epitopes on the antigen, *e.g.,* scMet. Desirably, binding of the capture antibody to an epitope does not interfere with binding of the detection antibody to an epitope. Either monoclonal or polyclonal antibodies may be used as the capture and detection antibodies in the sandwich ELISA.

[0073]   Generally, at least two antibodies are employed to separate and quantify scMet or scMet fragment in a test sample. More specifically, the at least two antibodies bind to certain epitopes of scMet or a scMet fragment forming an immune complex which is referred to as a "sandwich". One or more antibodies can be used to capture the scMet or scMet fragment in the test sample (these antibodies are frequently referred to as a "capture" antibody or "capture" antibodies) and one or more antibodies is used to bind a detectable (namely, quantifiable) label to the sandwich (these antibodies are frequently referred to as the "detection" antibody or "detection" antibodies). In a sandwich assay, the binding of an antibody to its epitope desirably

is not diminished by the binding of any other antibody in the assay to its respective epitope. Antibodies are selected so that the one or more first antibodies brought into contact with a test sample suspected of containing scMet or scMet fragment do not bind to all or part of an epitope recognized by the second or subsequent antibodies, thereby interfering with the ability of the one or more second detection antibodies to bind to the scMet or scMet fragment.

[0074] The antibodies may be used as a first antibody in said immunoassay. The antibody immunospecifically binds to epitopes on scMet. In addition to the antibodies of the present invention, said immunoassay may comprise a second antibody that immunospecifically binds to epitopes that are not recognized or bound by the first antibody.

[0075] A test sample suspected of containing scMet or scMet fragment can be contacted with at least one first capture antibody (or antibodies) and at least one second detection antibodies either simultaneously or sequentially. In the sandwich assay format, a test sample suspected of containing scMet or scMet fragment is first brought into contact with the at least one first capture antibody that specifically binds to a particular epitope under conditions which allow the formation of a first antibody-scMet antigen complex. If more than one capture antibody is used, a first multiple capture antibody-scMet antigen complex is formed. In a sandwich assay, the antibodies, preferably, the at least one capture antibody, are used in molar excess amounts of the maximum amount of scMet or scMet fragment expected in the test sample. For example, from about 5 μg/ml to about 1 mg/ml of antibody per ml of microparticle coating buffer may be used.

(a) Anti-scMet Capture Antibody

[0076] Optionally, prior to contacting the test sample with the at least one first capture antibody, the at least one first capture antibody can be bound to a solid support which facilitates the separation the first antibody-scMet antigen complex from the test sample. Any solid support known in the art can be used, including but not limited to, solid supports made out of polymeric materials in the forms of wells, tubes, or beads. The antibody (or antibodies) can be bound to the solid support by adsorption, by covalent bonding using a chemical coupling agent or by other means known in the art, provided that such binding does not interfere with the ability of the antibody to bind scMet or scMet fragment. Moreover, if necessary, the solid support can be derivatized to allow reactivity with various functional groups on the antibody. Such derivatization requires the use of certain coupling agents such as, but not limited to, maleic anhydride, N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

[0077] After the test sample suspected of containing scMet or scMet fragment is brought into contact with the at least one first capture antibody, the test sample is incubated in order to allow for the formation of a first capture antibody (or multiple antibody)-scMet antigen complex. The incubation can be carried out at a pH of from about 4.5 to about 10.0, at a temperature of from about 2°C to about 45°C, and for a period from at least about one (1) minute to about eighteen (18) hours, from about 2-6 minutes, or from about 3-4 minutes.

(b) Detection Antibody

[0078] After formation of the first/multiple capture antibody-scMet antigen complex, the complex is then contacted with at least one second detection antibody (under conditions that allow for the formation of a first/multiple antibody-scMet antigen -second antibody complex). If the first antibody-scMet antigen complex is contacted with more than one detection antibody, then a first/multiple capture antibody-scMet antigen-multiple antibody detection complex is formed. As with first antibody, when the at least second (and subsequent) antibody is brought into contact with the first antibody-scMet antigen complex, a period of incubation under conditions similar to those described above is required for the formation of the first/multiple antibody-scMet antigen -second/multiple antibody complex. Preferably, at least one second antibody contains a detectable label. The detectable label can be bound to the at least one second antibody prior to, simultaneously with or after the formation of the first/multiple antibody-scMet antigen-second/multiple antibody complex. Any detectable label known in the art can be used.

[0079] Chemiluminescent assays can be performed in accordance with the methods described in Adamczyk et al., Anal. Chim. Acta 579(1): 61-67 (2006). While any suitable assay format can be used, a microplate chemiluminometer (Mithras LB-940, Berthold Technologies U.S.A., LLC, Oak Ridge, TN) enables the assay of multiple samples of small volumes rapidly. The chemiluminometer can be equipped with multiple reagent injectors using 96-well black polystyrene microplates (Costar #3792). Each sample can be added into a separate well, followed by the simultaneous/sequential addition of other reagents as determined by the type of assay employed. Desirably, the formation of pseudobases in neutral or basic solutions employing an acridinium aryl ester is avoided, such as by acidification. The chemiluminescent response is then recorded well-by-well. In this regard, the time for recording the chemiluminescent response will depend, in part, on the delay between the addition of the reagents and the particular acridinium employed.

[0080]   The order in which the test sample and the specific binding partner(s) are added to form the mixture for chemiluminescent assay is not critical. If the first specific binding partner is detectably labeled with an acridinium compound, detectably labeled first specific binding partner-scMet antigen complexes form. Alternatively, if a second specific binding partner is used and the second specific binding partner is detectably labeled with an acridinium compound, detectably labeled first specific binding partner-scMet antigen -second specific binding partner complexes form. Any unbound specific binding partner, whether labeled or unlabeled, can be removed from the mixture using any technique known in the art, such as washing.

[0081]   Hydrogen peroxide can be generated in situ in the mixture or provided or supplied to the mixture before, simultaneously with, or after the addition of an above-described acridinium compound. Hydrogen peroxide can be generated in situ in a number of ways such as would be apparent to one skilled in the art.

[0082]   Alternatively, a source of hydrogen peroxide can be simply added to the mixture. For example, the source of the hydrogen peroxide can be one or more buffers or other solutions that are known to contain hydrogen peroxide. In this regard, a solution of hydrogen peroxide can simply be added.

[0083]   Upon the simultaneous or subsequent addition of at least one basic solution to the sample, a detectable signal, namely, a chemiluminescent signal, indicative of the presence of scMet or scMet fragment is generated. The basic solution contains at least one base and has a pH greater than or equal to 10, preferably, greater than or equal to 12. Examples of basic solutions include, but are not limited to, sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium hydroxide, calcium carbonate, and calcium bicarbonate. The amount of basic solution added to the sample depends on the concentration of the basic solution. Based on the concentration of the basic solution used, one skilled in the art can easily determine the amount of basic solution to add to the sample.

[0084]   The chemiluminescent signal that is generated can be detected using routine techniques known to those skilled in the art. Based on the intensity of the signal generated, the amount of scMet or scMet fragment in the sample can be quantified. Specifically, the amount of scMet in the sample is proportional to the intensity of the signal generated. The amount of scMet present can be quantified by comparing the amount of light generated to a standard curve for

scMet or by comparison to a reference standard. The standard curve can be generated using serial dilutions or solutions of known concentrations of scMet by mass spectroscopy, gravimetric methods, and other techniques known in the art.

[0085]   In a chemiluminescent microparticle assay employing the ARCHITECT® (or its successor) analyzer, the conjugate diluent pH should be about 6.0 +/- 0.2, the microparticle coating buffer should be maintained at room temperature (*i.e.*, at about 17 to about 27 °C), the microparticle coating buffer pH should be about 6.5 +/- 0.2, and the microparticle diluent pH should be about 7.8 +/- 0.2. Solids preferably are less than about 0.2%, such as less than about 0.15%, less than about 0.14%, less than about 0.13%, less than about 0.12%, or less than about 0.11%, such as about 0.10%.

B. Antibodies

[0086]   The anti-cMet antibodies used in the methods described above may be a chimeric anti-cMet or humanized anti-cMet antibody. The antibody may inhibit binding of human hepatocyte growth factor. In one embodiment, both the humanized antibody and chimeric antibody are monovalent. In one embodiment, both the humanized antibody and chimeric antibody comprise a single Fab region linked to an Fc region. Comparison of abilities to inhibit HGF binding to its receptor can be performed according to various methods known in the art, including as described in the Examples below. In one embodiment, IC50 values are determined across an antibody concentration range from about 0.01 nM to around 1000 nM, about 0.1 nM to around 1000 nM, about 1.0 nM to around 1000 nM, about 10.0 nM to around 1000 nM, about 20.0 nM to around 1000 nM, about 50.0 nM to around 1000 nM, about 100.0 nM to around 1000 nM, about 200.0 nM to around 1000 nM, 0.01 nM to around 900 nM, about 0.1 nM to around 900 nM, about 1.0 nM to around 900 nM, about 10.0 nM to around 900 nM, about 20.0 nM to around 900 nM, about 50.0 nM to around 900 nM, about 100.0 nM to around 900 nM, about 200.0 nM to around 900 nM, 0.01 nM to around 800 nM, about 0.1 nM to around 800 nM, about 1.0 nM to around 800 nM, about 10.0 nM to around 800 nM, about 20.0 nM to around 800 nM, about 50.0 nM to around 800 nM, about 100.0 nM to around 800 nM, about 200.0 nM to around 800 nM,. 0.01 nM to around 700 nM, about 0.1 nM to around 700 nM, about 1.0 nM to around 700 nM, about 10.0 nM to around 700 nM, about 20.0 nM to around 700 nM, about 50.0 nM to around 700 nM, about 100.0 nM to around 700 nM, about 200.0 nM to around 700 nM, 0.01 nM to around 500

nM, about 0.1 nM to around 500 nM, about 1.0 nM to around 500 nM, about 10.0 nM to around 500 nM, about 20.0 nM to around 500 nM, about 50.0 nM to around 500 nM, about 100.0 nM to around 500 nM, or about 200.0 nM to around 500 nM. The anti-cMet antibody may be a 224G11 monoclonal antibody, an 11E1 antibody, or an antibody fragment thereof.

(i) 224G11 antibody

[0087]   As used herein "244G11" or "m244G11" refers to a monoclonal antibody produced by hybridoma cell line deposited on March 14, 2007 under the number CNCM I-3731 at the Collection Nationale de Cultures de Microorganismes (CNCM, National Collection of Microorganism Cultures) (Institut Pasteur, Paris, France) as described in WO 2009/007427, which is herein incorporated by reference in its entirety. 244G11 binds to an epitope on cMet that is different from the epitope that 11E1 binds. 244G11 has a heavy chain amino acid sequence of SEQ ID NO:1, which is encoded by a nucleotide sequence of SEQ ID NO:17, and a light chain amino acid sequence of SEQ ID NO:5, which is encoded by a nucleotide sequence of SEQ ID NO:21. 244G11 includes CDR-H1 (SEQ ID NO:2), CDR-H2 (SEQ ID NO:3), and CDR-H3 (SEQ ID NO:4) and CDR-L1 (SEQ ID NO:6), CDR-L2 (SEQ ID NO:7), and CDR-L3 (SEQ ID NO:8), which are encoded by nucleotide sequences of SEQ ID NOs:18-20 and 22-24, respectively.

(ii) 11E1 antibody

[0088]   As used herein "11E1" or "m11E1" refers to a monoclonal antibody produced by hybridoma cell line deposited on March 14, 2007 under the number CNCM I-3724 at the Collection Nationale de Cultures de Microorganismes (CNCM, National Collection of Microorganism Cultures) (Institut Pasteur, Paris, France) as described in WO 2009/007427, which is herein incorporated by reference in its entirety. 11E1 binds to an epitope on cMet that is different from the epitope that 224G11 binds. 11E1 has a heavy chain amino acid sequence of SEQ ID NO:9, which is encoded by a nucleotide sequence of SEQ ID NO:25, and a light chain amino acid sequence of SEQ ID NO:13, which is encoded by a nucleotide sequence of SEQ ID NO:29. 11E1 includes CDR-H1 (SEQ ID NO:10), CDR-H2 (SEQ ID NO:11), and CDR-H3 (SEQ ID NO:12) and CDR-L1 (SEQ ID NO:14), CDR-L2 (SEQ ID NO:15), and CDR-L3 (SEQ ID NO:16), which are encoded by nucleotide sequences of SEQ ID NOs:26-28 and 30-32, respectively.

(iii) Methods of Using the 224G11 Antibody and the 11E1 Antibody

[0089]   The present invention is directed to a method for determining the presence, amount, or concentration of scMet or scMet fragment in a test sample using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) contacting the test sample with a capture antibody, which binds to an epitope on scMet or scMet fragment, so as to form a capture antibody-scMet antigen complex; (b) contacting the capture antibody-scMet antigen complex with at least one detection antibody, which comprises a detectable label and binds to an epitope on scMet or scMet fragment that is not bound by the capture antibody, to form a capture antibody-scMet antigen-detection antibody complex; and (c) determining the presence, amount or concentration of scMet or scMet fragment in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex whereupon the present, amount, or concentration of scMet or scMet fragment in the test sample is determined. The capture antibody includes the 224G11 antibody or a domain or region of: a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1; a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5; a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. The detection antibody includes a 11E1 antibody or a domain or region of: a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9; a variable light domain comprising the amino acid sequence of SEQ ID NO:13; a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid

sequence of SEQ ID NO:13; a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12; a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16.

C. Controls

[0090]   It may be desirable to include a control sample. The control sample may be analyzed concurrently with the sample from the subject as described above. The results obtained from the subject sample can be compared to the results obtained from the control sample. Standard curves may be provided, with which assay results for the biological sample may be compared. Such standard curves present levels of marker as a function of assay units, *i.e.* fluorescent signal intensity, if a fluorescent label is used. Using samples taken from multiple donors, standard curves can be provided for control levels of the scMet in normal healthy tissue, as well as for "at-risk" levels of the scMet in tissue taken from donors, who may have one or more of the characteristics set forth above.

[0091]   Thus, in view of the above, a method for determining the presence, amount, or concentration of scMet or scMet fragment in a test sample is provided. The method comprises assaying the test sample for scMet by an immunoassay, for example, employing at least one capture antibody that binds to an epitope on scMet or a fragment of scMet and at least one detection antibody that binds to an epitope on scMet which is different from the epitope for the capture antibody and optionally includes a detectable label, and comprising comparing a signal generated by the detectable label as a direct or indirect indication of the presence, amount or concentration of scMet in the test sample to a signal generated as a direct or indirect indication of the presence, amount or concentration of scMet in a calibrator. The calibrator is optionally, and

is preferably, part of a series of calibrators in which each of the calibrators differs from the other calibrators in the series by the concentration of scMet.

3.   Methods of Diagnosing, Prognosticating, or Assessing the Efficacy of a Therapeutic/Prophylactic Treatment

[0092]   The method can further comprise diagnosing, prognosticating, or assessing the efficacy of a therapeutic/prophylactic treatment of a patient from whom the test sample was obtained. If the method further comprises assessing the efficacy of a therapeutic/prophylactic treatment of the patient from whom the test sample was obtained, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy. By measuring and detecting scMet or scMet fragment using a combination of anti-cMet antibody, such as the combination of the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, the method allows for diseases to be accurately diagnosed, and subsequently treated more successfully. The method can be adapted for use in an automated system or a semi-automated system.

[0093]   Generally, a predetermined level can be employed as a benchmark against which to assess results obtained upon assaying a test sample for scMet or scMet fragment. Generally, in making such a comparison, the predetermined level is obtained by running a particular assay a sufficient number of times and under appropriate conditions such that a linkage or association of analyte presence, amount or concentration with a particular stage or endpoint of a disease, disorder or condition (*e.g.*, liver disease or cancer) or with particular indicia can be made. Typically, the predetermined level is obtained with assays of reference subjects (or populations of subjects). The scMet measured can include scMet fragments thereof, degradation products thereof, and/or enzymatic cleavage products thereof.

[0094]   In particular, with respect to a predetermined level as employed for monitoring disease progression and/or treatment, the amount or concentration of scMet or scMet fragment may be "unchanged," "favorable" (or "favorably altered"), or "unfavorable" (or "unfavorably altered"). "Elevated" or "increased" refers to an amount or a concentration in a test sample that is higher or greater than a typical or normal level or range (*e.g.*, predetermined level), or is higher or greater than another reference level or range (*e.g.*, earlier or baseline sample). The term "lowered" or "reduced" refers to an amount or a concentration in a test sample that is lower or less than a typical or normal level or range (*e.g.*, predetermined level), or is lower or less than another

reference level or range (*e.g.*, earlier or baseline sample). The term "altered" refers to an amount or a concentration in a sample that is altered (increased or decreased) over a typical or normal level or range (*e.g.*, predetermined level), or over another reference level or range (*e.g.*, earlier or baseline sample).

[0095] The typical or normal level or range for scMet is defined in accordance with standard practice. A so-called altered level or alteration can be considered to have occurred when there is any net change as compared to the typical or normal level or range, or reference level or range that cannot be explained by experimental error or sample variation. Thus, the level measured in a particular sample will be compared with the level or range of levels determined in similar samples from a so-called normal subject. In this context, a "normal subject" is an individual with no detectable disease or disorder, and a "normal" (sometimes termed "control") patient or population is/are one(s) that exhibit(s) no detectable disease or disorder, respectively, for example. An "apparently normal subject" is one in which scMet has not been or is being assessed. The level of an analyte is said to be "elevated" when the analyte is normally undetectable (*e.g.*, the normal level is zero, or within a range of from about 25 to about 75 percentiles of normal populations), but is detected in a test sample, as well as when the analyte is present in the test sample at a higher than normal level. Thus, inter alia, the disclosure provides a method of screening for a subject having, or at risk of having, liver disease or cancer.

A. Methods of Providing a Diagnosis of a Subject Having Disease

[0096] The method described herein can be used to provide a diagnosis of a subject having disease by determining the levels of scMet in a subject. The method showed that scMet concentrations were higher in patients with liver disease or and lower in patients with gastric cancer. The method may be used to detect disease in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having disease if the level of scMet in the biological sample is greater than or less than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having disease.

(i) Liver Disease

[0097]   The method described herein can be used to provide a diagnosis of a subject having liver disease by determining the levels of scMet in a subject. The method showed that scMet concentrations were higher in patients with liver disease compared to healthy patients. The method may be used to detect liver disease in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having liver disease if the level of scMet in the biological sample is greater than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having liver disease.

[0098]   The reference level in this method can be the level of scMet in a patient having liver disease. Levels higher than or equal to 105 ng/mL, 106 ng/mL, 107 ng/mL, 108 ng/mL, 109 ng/mL, 110 ng/mL, 111 ng/mL, 112 ng/mL, 113 ng/mL, 114 ng/mL, 115 ng/mL, 120 ng/mL, 125 ng/mL, 130 ng/mL, 135 ng/mL, 140 ng/mL, 141 ng/mL, 142 ng/mL, 143 ng/mL, 144 ng/mL, 145 ng/mL, 146 ng/mL, 147 ng/mL, 148 ng/mL, 149 ng/mL, 150 ng/mL, 151 ng/mL, 152 ng/mL, 153 ng/mL, 154 ng/mL, 155 ng/mL, 160 ng/mL, 165 ng/mL, 170 ng/mL, 175 ng/mL, 180 ng/mL, 185 ng/mL, 190 ng/mL, 195 ng/mL, 200 ng/mL, 205 ng/mL, 210 ng/mL, 215 ng/mL, 216 ng/mL, 217 ng/mL, 218 ng/mL, 219 ng/mL, 220 ng/mL, 221 ng/mL, 222 ng/mL, 223 ng/mL, 224 ng/mL, 225 ng/mL, 226 ng/mL, 227 ng/mL, 228 ng/mL, 229 ng/mL, or 230 ng/mL in serum of scMet identify the subject as having liver disease.

(a) Liver Fibrosis

[0099]   The method described herein can be used to provide a diagnosis of a subject having liver fibrosis by determining the levels of scMet in a subject. The method showed that scMet concentrations were highest in patients with liver fibrosis compared to healthy patients and other cancer patients, including liver cancer. The method may be used to detect liver fibrosis in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c)

comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having liver fibrosis if the level of scMet in the biological sample is greater than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having liver fibrosis.

[00100] The reference level in this method can be the level of scMet in a patient having liver fibrosis. Levels higher than or equal to 145 ng/mL, 146 ng/mL, 147 ng/mL, 148 ng/mL, 149 ng/mL, 150 ng/mL, 151 ng/mL, 152 ng/mL, 153 ng/mL, 154 ng/mL, 155 ng/mL, 160 ng/mL, 165 ng/mL, 170 ng/mL, 175 ng/mL, 180 ng/mL, 185 ng/mL, 190 ng/mL, 195 ng/mL, 200 ng/mL, 205 ng/mL, 210 ng/mL, 215 ng/mL, 216 ng/mL, 217 ng/mL, 218 ng/mL, 219 ng/mL, 220 ng/mL, 221 ng/mL, 222 ng/mL, 223 ng/mL, 224 ng/mL, 225 ng/mL, 226 ng/mL, 227 ng/mL, 228 ng/mL, 229 ng/mL, or 230 ng/mL in serum of scMet identify the subject as having liver fibrosis.

(b) Liver Cancer

[00101] The method described herein can be used to provide a diagnosis of a subject having liver cancer by determining the levels of scMet in a subject. The method showed that scMet concentrations were higher in patients with liver cancer compared to healthy normal patients. The method may be used to detect liver cancer in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having liver cancer if the level of scMet in the biological sample is greater than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having liver cancer.

[00102] The reference level in this method can be the level of scMet in a patient having liver cancer. Levels higher than or equal to 105 ng/mL, 106 ng/mL, 107 ng/mL, 108 ng/mL, 109 ng/mL, 110 ng/mL, 111 ng/mL, 112 ng/mL, 113 ng/mL, 114 ng/mL, 115 ng/mL, 120 ng/mL, 125 ng/mL, 130 ng/mL, 135 ng/mL, 140 ng/mL, 141 ng/mL, 142 ng/mL, 143 ng/mL, 144 ng/mL, or 145 ng/mL in serum of scMet identify the subject as having liver cancer.

(ii) Cancer

[00103]   The method described herein can be used to provide a diagnosis of a subject having cancer by determining the levels of scMet in a subject. The method showed that scMet concentrations were lower in patients with some cancers. The method may be used to detect cancer in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having cancer if the level of scMet in the biological sample is lower than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having cancer.

[00104]   The reference level in this method can be the level of scMet in a patient having cancer. Levels less than or equal to 60 ng/mL, 61 ng/mL, 62 ng/mL, 63 ng/mL, 64 ng/mL, 65 ng/mL, 66 ng/mL, 67 ng/mL, 68 ng/mL, 69 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 81 ng/mL, 82 ng/mL, 83 ng/mL, 84 ng/mL, 85 ng/mL, 86 ng/mL, 87 ng/mL, 88 ng/mL, 89 ng/mL or 90 ng/mL in serum of scMet identify the subject as having cancer

(a) Gastric Cancer

[00105]   The method described herein can be used to provide a diagnosis of a subject having gastric cancer by determining the levels of scMet in a subject. In contrast to the increased levels of scMet found in patients with liver disease, the method showed that scMet concentrations were lower in patients with gastric cancer compared to healthy subjects and patients with liver disease. This result supports the idea that cMet activity and cMet overexpression may play a role in patients with gastric cancer. The decreased amounts of scMet detected by the combination of the 224G11 antibody and the 11E1 antibody may be due to the epitope recognized by the 224G11 antibody and may indicate that more scMet is being bound to its ligand HGF, even though there may be high levels of scMet in patients with gastric cancer, as shown when a different combination of antibodies are used to detect scMet (e.g., a combination of 11E1 antibody and 224D10 antibody (a monoclonal antibody produced by hybridoma cell line deposited on March 12, 2008 under the number I-3949 at the Collection Nationale de Cultures de Microorganismes

(CNCM, National Collection of Microorganism Cultures) (Institut Pasteur, Paris, France) as described in US 2012/0149031 (data not shown)).

[00106] The method may be used to detect gastric cancer in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, (d) identifying the subject as having gastric cancer if the level of scMet in the biological sample is less than the reference level of scMet, and (e) administering a treatment regimen to the subject identified as having liver cancer.

[00107] The reference level in this method can be the level of scMet in a patient having gastric cancer. Levels less than or equal to 60 ng/mL, 61 ng/mL, 62 ng/mL, 63 ng/mL, 64 ng/mL, 65 ng/mL, 66 ng/mL, 67 ng/mL, 68 ng/mL, 69 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 81 ng/mL, 82 ng/mL, 83 ng/mL, 84 ng/mL, 85 ng/mL, 86 ng/mL, 87 ng/mL, 88 ng/mL, 89 ng/mL or 90 ng/mL in serum of scMet identify the subject as having gastric cancer.

B. Methods for Determining the Risk of a Subject of Developing Liver Disease

[00108] The methods described herein also can be used to determine whether or not a subject has or is at risk of developing liver disease by determining the levels of scMet in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. Thus, in particular embodiments, the disclosure also provides a method for determining whether a subject having, or at risk for, liver disease, as discussed herein and known in the art, is a candidate for therapy or treatment. Generally, the subject is one who has experienced some symptom of the disease or who has actually been diagnosed as having, or being at risk for, such a disease, and/or who demonstrates an unfavorable concentration or amount of scMet or scMet fragment, as described herein.

[00109] Specifically, such a method can comprise the steps of: (a) determining the concentration or amount in a test sample from a subject of scMet or scMet fragment using the methods described herein, or methods known in the art); and (b) comparing the concentration or amount of scMet or scMet fragment determined in step (a) with a predetermined level, wherein, if the concentration or amount of scMet determined in step (a) is favorable with respect to a predetermined level, then the subject is determined not to have or be at risk for liver disease as

discussed herein and known in the art. However, if the concentration or amount of scMet determined in step (a) is unfavorable with respect to the predetermined level, then the subject is determined to have or be at risk for liver disease as discussed herein and known in the art. The liver disease may be liver fibrosis or liver cancer.

C. Methods for Determining the Risk of a Subject of Developing Cancer

[00110] The methods described herein also can be used to determine whether or not a subject has or is at risk of developing cancer by determining the levels of scMet in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. Thus, in particular embodiments, the disclosure also provides a method for determining whether a subject having, or at risk for, cancer, as discussed herein and known in the art, is a candidate for therapy or treatment. Generally, the subject is one who has experienced some symptom of the disease or who has actually been diagnosed as having, or being at risk for, such a disease, and/or who demonstrates an unfavorable concentration or amount of scMet or scMet fragment, as described herein.

[00111] Specifically, such a method can comprise the steps of: (a) determining the concentration or amount in a test sample from a subject of scMet or scMet fragment using the methods described herein, or methods known in the art), and (b) comparing the concentration or amount of scMet or scMet fragment determined in step (a) with a predetermined level, wherein, if the concentration or amount of scMet determined in step (a) is favorable with respect to a predetermined level, then the subject is determined not to have or be at risk for cancer as discussed herein and known in the art. However, if the concentration or amount of scMet determined in step (a) is unfavorable with respect to the predetermined level, then the subject is determined to have or be at risk for cancer as discussed herein and known in the art. The cancer may be gastric cancer.

D. Methods of Monitoring the Progression of Disease in a Subject

[00112] The methods described herein also can be used to monitor the progression of disease, such as liver disease or cancer, in a subject by determining the levels of scMet in a subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. Optimally, the method includes the steps of (a) determining the concentration or amount of scMet or scMet fragment in a test sample from a subject using the 224G11

antibody and the 11E1 antibody, or antibody fragments thereof, as described above, (b) determining the concentration or amount of scMet or scMet fragment in a later test sample from a subject using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, as described above, and (c) comparing the concentration or amount of scMet as determined in step (b) with the concentration or amount of scMet determined in step (a), wherein if the concentration or amount determined in step (b) is unchanged or is unfavorable when compared to the concentration or amount of scMet determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened. By comparison, if the concentration or amount of scMet as determined in step (b) is favorable when compared to the concentration or amount of scMet as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved.

[00113]   Optionally, the method further comprises comparing the concentration or amount of scMet as determined in step (b), for example, with a predetermined level. Further, optionally the method comprises treating the subject with one or more pharmaceutical compositions for a period of time if the comparison shows that the concentration or amount of scMet as determined in step (b), for example, is unfavorably altered with respect to the predetermined level.

[00114]   Still further, the methods can be used to monitor treatment in a subject receiving treatment with one or more pharmaceutical compositions. Specifically, such methods involve providing a first test sample from a subject before the subject has been administered one or more pharmaceutical compositions. Next, the concentration or amount in a first test sample from a subject of scMet is determined (e.g., using the methods described herein or as known in the art). After the concentration or amount of scMet is determined, optionally the concentration or amount of scMet is then compared with a predetermined level. If the concentration or amount of scMet as determined in the first test sample is lower than the predetermined level, then the subject is not treated with one or more pharmaceutical compositions or alternatively, the subject may be treated with one or more pharmaceutical compositions. If the concentration or amount of scMet as determined in the first test sample is higher than the predetermined level, then the subject is treated with one or more pharmaceutical compositions for a period of time or alternatively, the subject is not treated with one or more pharmaceutical compositions. The period of time that the subject is treated with the one or more pharmaceutical compositions can

be determined by one skilled in the art (for example, the period of time can be from about seven (7) days to about two years, preferably from about fourteen (14) days to about one (1) year).

[00115] During the course of treatment with the one or more pharmaceutical compositions, second and subsequent test samples are then obtained from the subject. The number of test samples and the time in which said test samples are obtained from the subject are not critical. For example, a second test sample could be obtained seven (7) days after the subject is first administered the one or more pharmaceutical compositions, a third test sample could be obtained two (2) weeks after the subject is first administered the one or more pharmaceutical compositions, a fourth test sample could be obtained three (3) weeks after the subject is first administered the one or more pharmaceutical compositions, a fifth test sample could be obtained four (4) weeks after the subject is first administered the one or more pharmaceutical compositions, etc.

[00116] After each second or subsequent test sample is obtained from the subject, the concentration or amount of scMet or scMet fragment is determined in the second or subsequent test sample is determined (*e.g.*, using the methods described herein or as known in the art). The concentration or amount of scMet as determined in each of the second and subsequent test samples is then compared with the concentration or amount of scMet as determined in the first test sample (*e.g.*, the test sample that was originally optionally compared to the predetermined level). If the concentration or amount of scMet as determined in step (c) is favorable when compared to the concentration or amount of scMet as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved, and the subject should continue to be administered the one or pharmaceutical compositions of step (b). However, if the concentration or amount determined in step (c) is unchanged or is unfavorable when compared to the concentration or amount of scMet as determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened, and the subject should be treated with a higher concentration of the one or more pharmaceutical compositions administered to the subject in step (b) or the subject should be treated with one or more pharmaceutical compositions that are different from the one or more pharmaceutical compositions administered to the subject in step (b). Specifically, the subject can be treated with one or more pharmaceutical compositions that are different from the one or more pharmaceutical compositions that the subject had previously received to decrease or lower said subject's scMet level.

[00117] Generally, for assays in which repeat testing may be done (*e.g.*, monitoring disease progression and/or response to treatment), a second or subsequent test sample is obtained at a period in time after the first test sample has been obtained from the subject. Specifically, a second test sample from the subject can be obtained minutes, hours, days, weeks or years after the first test sample has been obtained from the subject. For example, the second test sample can be obtained from the subject at a time period of about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years, about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years after the first test sample from the subject is obtained. When used to monitor disease progression, the above assay can be used to monitor the progression of disease in subjects suffering from acute conditions. Acute conditions, also known as critical care conditions, refer to acute, life-threatening diseases or other critical medical conditions involving, for example, the cardiovascular system or excretory system. Typically, critical care conditions refer to those conditions requiring acute medical intervention in a hospital-based setting (including, but not limited to, the emergency room, intensive care unit, trauma center, or other emergent care setting) or administration by a paramedic or other field-

based medical personnel. For critical care conditions, repeat monitoring is generally done within a shorter time frame, namely, minutes, hours or days (*e.g.*, about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days or about 7 days), and the initial assay likewise is generally done within a shorter timeframe, *e.g.*, about minutes, hours or days of the onset of the disease or condition.

[00118]   The assays also can be used to monitor the progression of disease in subjects suffering from chronic or non-acute conditions. Non-critical care conditions or non-acute conditions, refers to conditions other than acute, life-threatening disease or other critical medical conditions involving, for example, the cardiovascular system and/or excretory system. Typically, non-acute conditions include those of longer-term or chronic duration. For non-acute conditions, repeat monitoring generally is done with a longer timeframe, *e.g.*, hours, days, weeks, months or years (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years,

about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years), and the initial assay likewise generally is done within a longer time frame, *e.g.*, about hours, days, months or years of the onset of the disease or condition.

[00119]   Furthermore, the above assays can be performed using a first test sample obtained from a subject where the first test sample is obtained from one source, such as urine, serum, or plasma. Optionally the above assays can then be repeated using a second test sample obtained from the subject where the second test sample is obtained from another source. For example, if the first test sample was obtained from urine, the second test sample can be obtained from serum or plasma. The results obtained from the assays using the first test sample and the second test sample can be compared. The comparison can be used to assess the status of a disease or condition in the subject.

E. Methods for Determining if a Subject is Predisposed to or Suffering from a Disease

[00120]   Moreover, the methods described herein also can be used to determine whether a subject predisposed to or suffering from a disease (*e.g.*, liver disease or cancer, as discussed herein and known in the art) will benefit from treatment. In particular, the disclosure relates to scMet companion diagnostic methods and products. Thus, the method of "monitoring the treatment of disease in a subject" as described herein further optimally also can encompass selecting or identifying candidates for liver disease treatments, such as liver resection and liver transplant, or for cancer treatments, such as surgery, radiation therapy, targeted therapy, and chemotherapy.

F. Methods for Determining if a Subject is Responding to the Administration of a Pharmaceutical Composition

[00121]   The methods described herein also can be used to determine if a subject is responding to the administration of one or more pharmaceutical compositions by determining the levels of scMet in the subject using an anti-cMet antibody, such as the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof. The method optionally comprises an assay as described herein, where the level of scMet is assessed before and following treatment of the subject with one or more pharmaceutical compositions (*e.g.*, particularly with a pharmaceutical related to a mechanism of action involving scMet), or where the level of scMet is assessed following such

treatment and the concentration or the level of scMet is compared against a predetermined level. An unfavorable concentration of amount of scMet observed following treatment confirms that the subject will not benefit from receiving further or continued treatment, whereas a favorable concentration or amount of scMet observed following treatment confirms that the subject will benefit from receiving further or continued treatment. This confirmation assists with management of clinical studies, and provision of improved patient care.

[00122]   The method includes the steps of (a) obtaining a biological sample from a subject, (b) determining the level of scMet in the biological sample using the 224G11 antibody and the 11E1 antibody, or antibody fragments thereof, described above, (c) comparing the level of scMet in the biological sample to a reference level of scMet, wherein an altered concentration of scMet indicates that the subject is not responding to the administration of one or more pharmaceutical compositions, and (d) adjusting the treatment of the subject if the subject is not responding to the administration of one or more pharmaceutical compositions.

4. Combination of Biomarkers

[00123]   The antibodies and methods described above may be used to detect and measure levels and concentrations of scMet in combination with one or more biomarkers or immunoassays specific for disease. The combination of scMet with one or more biomarkers or immunoassays specific for disease may provide a greater discrimination between healthy controls and individuals with disease compared to measuring scMet alone. For example, measure a panel of scMet and HGF biomarkers provide a greater discrimination between healthy controls and individuals with disease compared to a panel of scMet alone. The combination of scMet with at least one or more biomarkers may provide greater discrimination between healthy controls and individuals who have liver disease and/or individuals who have cancer.

[00124]   Examples of the one or more biomarker and/or immunoassay include cMet, including scMet, and/or immunoassays that measure cMet and/or scMet, such as the ARCHITECT® scMet B Assay. The ARCHITECT® scMet B Assay measures scMet concentration using the 11E1 antibody as the capture antibody and an 224D10 F(ab')$_2$ fragment antibody as the detection antibody, each of which binds to cMet. The 11E1 antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under the number I-3724. The 224D10 antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 12, 2008 under the number I-3949. The 224D10

antibody recognizes and binds to an epitope that is different from the epitopes recognized by the 224G11 antibody and the 11E1 antibody. Another example includes the biomarker HGF and/or immunoassays that measure HGF, such as the ARCHITECT® HGF Assay. The ARCHITECT® HGF Assay measures HGF concentration using a 64.4.1 antibody as the capture antibody and an H98C4 antibody as the detection antibody, each of which binds to HGF. The 64.4.1 antibody is an antibody produced by hybridoma cell line that will be deposited with the American Type Culture Collection (A.T.C.C.), 10801 University Blvd., Manassas, VA 20110-2209 under the terms of the Budapest Treaty. The H98C4 antibody is an antibody produced by hybridoma cell line that will be deposited with the American Type Culture Collection (A.T.C.C.), 10801 University Blvd., Manassas, VA 20110-2209 under the terms of the Budapest Treaty.

5. Treatment of Subjects Suffering from Liver Disease

[00125] The subject identified in the methods described above having levels of scMet greater than or equal to the values discussed above is identified as a patient suffering from liver disease. The subject is then treated for the liver disease. Treatment of liver disease may include small molecule cMet inhibitors, such as K252a, SU11274, PHA-665752, SGX523, MP470, INCB28060, EMD 1214063, EMD 1214831, AMG-458, PF-04217903, crizotinib (PF-02341066), E7050, MK-2461, BMS-777607, JNJ-38877605, tivantinib (ARQ197), foretinib (GSK/1363089/XL880), and cabozantinib (XL184), anti-cMet neutralizing antibodies, such as onartuzumab, HGF inhibitors, such as NK4, and anti-HGF neutralizing antibodies, such TAK-701, rilotumumab (AMG102) and ficlatuzumab (AV-299).

A. Liver fibrosis

[00126] The subject identified in the methods described above having levels of scMet greater than or equal to the values discussed above is identified as a patient suffering from liver fibrosis. The subject is then treated for liver fibrosis. Treatment may include medications. These medications may include cMet inhibitors, HGF inhibitors, angiotensin inhibitors, corticosteroids, endothelin inhibitors, interferon-alpha, interleukin 10, pentoxifylline, peroxisome proliferator-activated receptor (PPAR) antagonists, TGF-beta1 inhibitors, antioxidants, such as S-adenosyl-methionine, phosphatidylcholine, silymarin, and tocopherol, lamivudine, or herbal compounds, such as Sho-saiko-to, glycyrrhizin, colchicine, and salvia miltiorrhiza.

B. Liver cancer

[00127] The subject identified in the methods described above having levels of scMet greater than or equal to the values discussed above is identified as a patient suffering from liver cancer. The subject is then treated for liver cancer. Treatment may include surgical removal of the cancer (liver resection) with or without liver transplant, chemotherapy, such as doxorubicin (Adriamycin), 5-fluorouracil (5 FU), tamoxifen (Nolvadex), Octreotide (Sandostatin), gemcitabine, cisplatin, and oxaliplatin, biotherapy, such as bevacizumab, chemoembolization (trans-arterial chemoembolization or TACE), radioembolization (selective internal radiotherapy; "SIRT"), ablation, such as radiofrequency ablation (RFA) therapy, percutaneous ethanol (alcohol) injection, and cryoablation, stereotactic radiosurgery, and proton beam therapy. Treatment may also include drugs that block components of the angiogenesis pathway, such as sorafenib (Nexavar). Treatment may also include cMet inhibitors and HGF inhibitors. The drugs may be delivered via the hepatic artery or portal vein.

6. Treatment of Subjects Suffering from Cancer

[00128] The subject identified in the methods described above having levels of scMet less than or equal to the values discussed above is identified as a patient suffering from cancer. The subject is then treated for the cancer. Treatment of cancer may include small molecule cMet inhibitors, such as K252a, SU11274, PHA-665752, SGX523, MP470, INCB28060, EMD 1214063, EMD 1214831, AMG-458, PF-04217903, crizotinib (PF-02341066), E7050, MK-2461, BMS-777607, JNJ-38877605, tivantinib (ARQ197), foretinib (GSK/1363089/XL880), and cabozantinib (XL184), anti-cMet neutralizing antibodies, such as onartuzumab, HGF inhibitors, such as NK4, and anti-HGF neutralizing antibodies, such TAK-701, rilotumumab (AMG102), and ficlatuzumab (AV-299).

A. Gastric Cancer

[00129] The subject identified in the methods described above having levels of scMet less than the values discussed above is identified as a patient suffering from gastric cancer. The subject is then treated for gastric cancer. Treatment may include surgery, including endoscopic mucosal resection and endoscopic submucosal dissection, radiation (X-rays), and chemotherapy, including 5-FU (fluorouracil) or its analog capecitabine, BCNU (carmustine), methyl-CCNU (Semustine), doxorubicin (Adriamycin), Mitomycin C, cisplatin and taxotere. Treatment may

also include imatinib (Gleevec) and sunitinib (Sutent). Treatment may also include small molecule cMet inhibitors, such as K252a, SU11274, PHA-665752, SGX523, MP470, INCB28060, EMD 1214063, EMD 1214831, AMG-458, PF-04217903, crizotinib (PF-02341066), E7050, MK-2461, BMS-777607, JNJ-38877605, tivantinib (ARQ197), foretinib (GSK/1363089/XL880), and cabozantinib (XL184), anti-cMet neutralizing antibodies, such as onartuzumab, HGF inhibitors, such as NK4, and anti-HGF neutralizing antibodies, such TAK-701, rilotumumab (AMG102) and ficlatuzumab (AV-299).

7. Kit

[00130] Provided herein is a kit, which may be used for assaying a test sample for scMet or scMet fragment. The kit comprises at least one component for assaying the test sample for scMet or scMet fragment and instructions for assaying the test sample for scMet or scMet fragment. For example, the kit can comprise instructions for assaying the test sample for scMet or scMet fragment by immunoassay, *e.g.*, chemiluminescent microparticle immunoassay. Instructions included in kits can be affixed to packaging material or can be included as a package insert. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site that provides the instructions.

[00131] The at least one component may include at least one composition comprising one or more isolated antibodies or antibody fragments thereof that specifically bind to scMet or scMet fragment. The antibody may be a scMet capture antibody and/or a scMet detection antibody. The antibody may include the 224G11 antibody and/or 11E1 antibody, or fragments thereof. The antibody is optionally detectably labeled.

[00132] Alternatively or additionally, the kit can comprise a calibrator or control, *e.g.*, purified, and optionally lyophilized, scMet or scMet fragment, and/or at least one container (*e.g.*, tube, microtiter plates or strips, which can be already coated with an anti-cMet monoclonal antibody) for conducting the assay, and/or a buffer, such as an assay buffer or a wash buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (*e.g.*, an enzymatic label), or a stop solution. Preferably, the kit comprises all

components, *i.e.*, reagents, standards, buffers, diluents, etc., which are necessary to perform the assay. The instructions also can include instructions for generating a standard curve.

[00133]   The kit may further comprise reference standards for quantifying scMet. The reference standards may be employed to establish standard curves for interpolation and/or extrapolation of scMet concentrations. The reference standards may include a high scMet concentration level, for example, about 100 ng/mL, about 125 ng/mL, about 150 ng/mL, about 175 ng/mL, about 200 ng/mL, about 225 ng/mL, about 250 ng/mL, about 275 ng/mL, or about 300 ng/mL; a medium scMet concentration level, for example, about 25 ng/mL, about 40 ng/mL, about 45 ng/mL, about 50 ng/mL, about 55 ng/mL, about 60 ng/mL, about 75 ng/mL or about 100 ng/mL; and/or a low scMet concentration level, for example, about 1 ng/mL, about 5 ng/mL, about 10 ng/mL, about 12.5 ng/mL, about 15 ng/mL, about 20 ng/mL, or about 25 ng/mL.

[00134]   Any antibodies, which are provided in the kit, such as recombinant antibodies specific for scMet, can incorporate a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like, or the kit can include reagents for labeling the antibodies or reagents for detecting the antibodies (*e.g.*, detection antibodies) and/or for labeling the analytes or reagents for detecting the analyte. The antibodies, calibrators, and/or controls can be provided in separate containers or pre-dispensed into an appropriate assay format, for example, into microtiter plates.

[00135]   Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays.

[00136]   The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (*e.g.*, pretreatment reagents), also can be included in the kit. The kit can additionally include one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

[00137] The various components of the kit optionally are provided in suitable containers as necessary, *e.g.*, a microtiter plate. The kit can further include containers for holding or storing a sample (*e.g.*, a container or cartridge for a urine, plasma, or serum sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instrument for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

[00138] If the detectable label is at least one acridinium compound, the kit can comprise at least one acridinium-9-carboxamide, at least one acridinium-9-carboxylate aryl ester, or any combination thereof. If the detectable label is at least one acridinium compound, the kit also can comprise a source of hydrogen peroxide, such as a buffer, solution, and/or at least one basic solution. If desired, the kit can contain a solid phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc, or chip.

[00139] If desired, the kit can further comprise one or more components, alone or in further combination with instructions, for assaying the test sample for another analyte, which can be a biomarker, such as a biomarker of liver disease or disorder.

A. Adaptation of Kit and Method

[00140] The kit (or components thereof), as well as the method for determining the concentration of scMet in a test sample by an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, *e.g.*, in U.S. Pat. Nos. 5,089,424 and 5,006,309, and as commercially marketed, *e.g.*, by Abbott Laboratories (Abbott Park, IL) as ARCHITECT®.

[00141] Some of the differences between an automated or semi-automated system as compared to a non-automated system (*e.g.*, ELISA) include the substrate to which the first specific binding partner (*e.g.*, analyte antibody or capture antibody) is attached (which can affect sandwich formation and analyte reactivity), and the length and timing of the capture, detection, and/or any optional wash steps. Whereas a non-automated format such as an ELISA may require a relatively longer incubation time with sample and capture reagent (*e.g.*, about 2 hours), an automated or semi-automated format (*e.g.*, ARCHITECT® and any successor platform, Abbott Laboratories) may have a relatively shorter incubation time (*e.g.*, approximately 18 minutes for

ARCHITECT®). Similarly, whereas a non-automated format such as an ELISA may incubate a detection antibody such as the conjugate reagent for a relatively longer incubation time (*e.g.,* about 2 hours), an automated or semi-automated format (*e.g.,* ARCHITECT® and any successor platform) may have a relatively shorter incubation time (*e.g.,* approximately 4 minutes for the ARCHITECT® and any successor platform).

[00142] Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (*see, e.g.,* U.S. Pat. No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits, and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Pat. No. 5,063,081, U.S. Pat. App. Pub. No. 2003/0170881, U.S. Pat. App. Pub. No. 2004/0018577, U.S. Pat. App. Pub. No. 2005/0054078, and U.S. Pat. App. Pub. No. 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

[00143] In particular, with regard to the adaptation of an assay to the I-STAT® system, the following configuration is preferred. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized capture antibody are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an I-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the wall of the sample-holding chamber of the cartridge there is a layer comprising the detection antibody labeled with alkaline phosphatase (or other label). Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

[00144] In operation, a sample suspected of containing scMet is added to the holding chamber of the test cartridge and the cartridge is inserted into the I-STAT® reader. After the second antibody (detection antibody) has dissolved into the sample, a pump element within the cartridge forces the sample into a conduit containing the chip. Here it is oscillated to promote formation

of the sandwich between the first capture antibody, scMet, and the labeled second detection antibody. In the penultimate step of the assay, fluid is forced out of the pouch and into the conduit to wash the sample off the chip and into a waste chamber. In the final step of the assay, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of analyte scMet in the sample by means of an embedded algorithm and factory-determined calibration curve.

[00145] The methods and kits as described herein necessarily encompass other reagents and methods for carrying out the immunoassay. For instance, encompassed are various buffers such as are known in the art and/or which can be readily prepared or optimized to be employed, *e.g.*, for washing, as a conjugate diluent, and/or as a calibrator diluent. An exemplary conjugate diluent is ARCHITECT® conjugate diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL) and containing 2-(N-morpholino)ethanesulfonic acid (MES), a salt, a protein blocker, an antimicrobial agent, and a detergent. An exemplary calibrator diluent is ARCHITECT® human calibrator diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL), which comprises a buffer containing MES, other salt, a protein blocker, and an antimicrobial agent. Additionally, as described in U.S. Pat. Application No. 61/142,048 filed December 31, 2008, improved signal generation may be obtained, *e.g.*, in an I-STAT® cartridge format, using a nucleic acid sequence linked to the signal antibody as a signal amplifier.

[00146] While certain embodiments herein are advantageous when employed to assess liver disease or cancer, the assays and kits also optionally can be employed to assess scMet in other diseases, disorders, and conditions as appropriate.

[00147] The method of assay also can be used to identify a compound that ameliorates liver disease or cancer. For example, a cell that expresses scMet can be contacted with a candidate compound. The level of expression of scMet in the cell contacted with the compound can be compared to that in a control cell using the method of assay described herein.

[00148] The present invention has multiple aspects, illustrated by the following non-limiting examples.

EXAMPLES

[00149] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples which are merely intended only to illustrate some aspects and embodiments of the disclosure, and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents and publications referred to herein are hereby incorporated by reference in their entireties.

Example 1

Antibodies for scMet assay

[00150] The ARCHITECT® sandwich immunoassay was used to determine all possible combination of antibody pairs of scMet antibodies (*see* Table 2). Briefly, as shown in FIG. 1, an antibody coated on a microparticle captures the analyte of interest, then a second antibody conjugated to acridinium binds to a second epitope on the analyte, then a separation of the particles from the label and subsequent read is performed. The antibody pairs of scMet antibodies were tested against soluble cMet (scMet) and HGF antigens using antibody arrays (*see e.g.* FIG. 2) to determine which antibodies could pair together as potential microparticles and/or conjugates. The strongest signal generated in the antibody array testing, indicated the best candidate antibody pairs to move forward for further testing. In addition to the antibody array experiments, the binding affinity (Kd) was determined for each of the individual antibodies against the cMet antigen using fluorescent resonance energy transfer (FRET) according to the method outlined in: Q. Ruan, J.P. Skinner, and S.Y. Tetin, Using nonfluorescent Forster resonance energy transfer acceptors in protein binding studies. Anal. Biochem. 393 (2009) 196-204 (*see e.g.*, FIG. 3 which shows binding curves of mAb 11E1 with free cMet (SEQ ID NO:33) bound to Alexa-488 dye ("cMet-A488")). The antibody array work and the FRET analyses were utilized to choose the best antibodies pairs for initial assay prototyping on the ARCHITECT®, where the candidate antibodies were coated on magnetic microparticles or labeled with

acridinium as conjugates and tested in a sandwich against the cMet antigen. In Table 2, the highest affinity antibodies have the lowest Kd versus the cMet antigen.

Table 2   Affinity of cMet antibodies

anti-cMet Ab	Kd (nM)
mAb 224D10	2.5
mAb h224G11	0.7
mAb223C4	0.3
mAb 11E1	0.2
mAb221C9	2
mAb227H1	0.6
mAb 205A5	7
mAb 227D3	>17
mAb 5D5	0.5

[00151] Based on antibody array and FRET analysis, anti-scMet monoclonal antibody candidates were coated on microparticles or conjugated to acridinium for further evaluation on the ARCHITECT® instrument. Combinations of antibodies were assessed for their ability to bind and produce a signal on the ARCHITECT instrument using recombinant human scMet antigen ("cMet (25-932)-HIEGRMD-6His"; SEQ ID NO:33), which includes the soluble part of cMet, *i.e.,* amino acids 25-932 of cMet, an HIEGRMD linker (SEQ ID NO:36) and a 6-histidine tag (SEQ ID NO:37) as the antigen. Table 3 shows the antibody pairs that were tested on the ARCHITECT® as either microparticles (uP) or conjugates (e.g., uP coated with mAb m224G11 was tested separately with mAb 11E1, mAb 5D5, mAb 223C4 and mAb 224D10, each conjugated to acridinium, in a sandwich fashion against the scMet antigen). There were a total of 8 combinations for the scMet assay on the ARCHITECT®. mAbs were coated onto two types of magnetic microparticles (uP) (Dynal and Polymer Labs). The number of combinations tested for all the assays was doubled due to the microparticle antibodies being coated on 2 different commercially available paramagnetic microparticles: Dynal (Dynabeads®) and Polymer Labs (LodeStars™; now Agilent). Acridinium conjugates with a low and high incorporation ratio (IR) for testing on ARCHITECT® were made.

Table 3   Antibody combinations tested in ARCHITECT® with both Dynal and Polymer Lab microparticles

scMet Combinations: 2 uP and 4 conjugates				
uP	**Conjugates**			
mAb m224G11	mAb 11E1	mAb 5D5	mAb 223C4	mAb 224D10
mAb 227H1	mAb 11E1	mAb 5D5	mAb 223C4	mAb 224D10

[00152]   The antibody pairs were tested on ARCHITECT® in all possible combinations to determine if a calibration curve was generated. Microparticle/conjugate combinations that produced a robust signal against their respective antigens were selected for further prototyping activities on the ARCHITECT®. Recombinant human scMet antigen ("cMet (25-932)-HIEGRMD-6His"; SEQ ID NO:33), which includes the soluble part of cMet, *i.e.*, amino acids 25-932 of cMet, an HIEGRMD linker (SEQ ID NO:36) and a 6-histidine tag (SEQ ID NO:37), was used for calibrators and controls. scMet (SEQ ID NO:34) was cloned into a pHybE vector generating an expression vector (SEQ ID NO:35) that was expressed in HEK293 6E cell lines. As shown in FIG. 4, two of the eight microparticle/conjugate combinations for the scMet assay produced a robust signal on the ARCHITECT®. The best uP was selected based on cMet Calibrator A calibration uses (*see e.g.*, Table 4) and accelerated stability (37°C x 7 days). Table 4 shows the background of a pair of antibodies used in the microparticle/conjugate pair of 224G11/11E1 with Calibrator A (scMet concentration of 0 ng/mL) using Dynal and Polymer Lab microparticles. The Dynal microparticles were better than the Polymer Labs because of the lower initial background when testing potential formats. All of the formats had lower background with the Dynal microparticles.

Table 4   Calibrator A Background

		Dynal Microparticles	**Polymer Labs Microparticles**
soluble cMet Assay	**Average Cal A RLUs**	2824	5011

[00153]   The robust pairs of microparticle/conjugate were uP/conjugate: 227H1/11E1 and 224G11/11E1. The final microparticle/conjugate combination of anti-cMet m224G11/anti-cMet 11E1 was used and examined for the ARCHITECT® scMet Assay ("ARCHITECT® scMet A

Assay"). The ARCHITECT® scMet A Assay demonstrated equimolarity (the ability to measure the same amount of scMet) in the presence of Hepatocyte growth factor (HGF) and mouse anticreatine kinase (MAK33), an irrelevant antibody (*see* FIG. 5).

Example 2

Comparison with commercially available kit

[00154] Table 5 shows the results of the ARCHITECT® scMet A Assay ("ARCHITECT® soluble cMet A") compared to R&D SYSTEMS® cMet ELISA (Human HGF R/c-MET, Catalog Number DY358). The time to the first test result using the ARCHITECT® scMet A Assay was 20 min and the throughput was 200 tests per hour.

Table 5   Comparison of ARCHITECT® soluble cMet A with R&D Systems cMet ELISA

	ARCHITECT® soluble cMet A	R&D Systems cMet ELISA
Calibrator Range	6.25-800 ng/mL	0.625-4 ng/mL
Sensitivity	0.011 ng/mL	not stated

Example 3

Performance of the scMet A Sample Stability

[00155] The stability of scMet A Sample was determined using recombinant human scMet antigen (amino acids 25-932) ("scMet A Sample"; SEQ ID NO:33) for calibrators and controls. Six matched serum and EDTA plasma samples were drawn and processed. Samples were tested in triplicate, per the storage conditions below, with endogenous and spiked levels at approximately 100 ng/mL. FIG.6 shows a standard curve of the ARCHITECT® scMet A Assay where the circle on the graph represents a range of current samples tested. 6 matched serum and EDTA plasma samples were drawn and processed. Samples were tested per the storage conditions in Table 6 with endogenous and spiked levels.

Table 6   Storage conditions

Timepoints tested	Room Temperature	2-8°C	-80°C	3 freeze/thaw cycles
time 0	x			

4 hrs	x			
24 hrs	x	x	x	
72 hrs		x	x	
Day 12			x	x
Day 41			x	

[00156]   As shown in FIGS. 7-9, the scMet A Samples were stable at room temperature for 24 hrs, 2-8°C for 72 hrs, and -80°C for 12 days.

[00157]   FIGS. 7A-7B show the stability of scMet A Samples at room temperature at 4 and 24 hrs. FIG. 7A shows the endogenous sample stability at room temperature at 4 and 24 hrs. FIG. 7B shows the spiked sample (100 ng) stability at room temperature at 4 and 24 hrs. The average difference between base line and room temperature at 24 hr was 1.2%.

[00158]   FIGS. 8A-8B show the stability of scMet A Samples at 2-8°C at 24 and 72 hrs. FIG. 8A shows the endogenous sample stability at 2-8°C at 24 and 72 hrs. FIG. 8B shows the spiked sample (100 ng) stability at 2-8°C at 24 and 72 hrs. The average difference between base line and 2-8°C at 72 hr was 3.0%.

[00159]   FIGS. 9A-9B show the stability of scMet A Samples at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles. FIG. 9A shows the endogenous sample stability at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles. FIG. 9B shows the spiked sample (100 ng) stability at -80°C at 24 and 72 hrs and day 12 and day 12 with 3 freeze/thaw cycles. The average difference between base line and -80°C at 12 days was 5.3%.

Example 4
Limit of Detection/Limit of Quantification

[00160]   LOD testing was performed on three days with two runs per day on two instruments each day. Six runs were performed on each of two ARCHITECT® instruments for a total of twelve runs. The LOD was determined using twelve reps of Calibrator A (0 ng/mL) and four reps of Calibrator B (6.25 ng/mL) per run. The calculation for LOD was as follows:

$$LOD = \frac{2 \times (Cal\ A\ RLU\ SD) \times (Cal\ B\ Target\ Conc)}{(Cal\ B\ RLU) - (Cal\ A\ RLU)}$$

where "Cal" was the calibrator, "RLU" was the relative light units, "SD" was the standard deviation, "Cal A" was 0 ng/mL and "Cal B target Conc" was 6.25 ng/mL. The average of each grand mean from each instrument was reported as the LOD.

[00161] LOQ testing was performed on three days with two runs per day on two instruments each day. Eight reps per run of each sample were used to calculate the LOQ. The Cal B (6.25 ng/mL) was diluted 1:2 in serum to 3.13 ng/mL, 1.56 ng/mL, 0.78 ng/mL and 0.39 ng/mL for the LOQ samples.

[00162] Table 7 shows the limit of detection and limit of quantification of the ARCHITECT® scMet A Assay. The average LOD was 0.011 ng/mL and the average LOQ was 3.13 ng/mL at 30% total error.

Table 7   Performance of scMet A assay – LOD/LOQ

two instruments	Ave LOD	Range LOD	Ave LOQ	Total Error (LOQ)
scMet A	0.011 ng/mL	(0.008-0.014)	3.13 ng/mL	30%

Example 5
5 day precision

[00163] The precision study duration was five days with two runs per day, with five reps per run each on two instruments of the low (12.5 ng/mL), medium (50 ng/mL) and high (200 ng/mL) scMet concentration controls. The overall precision (%CV) was calculated based on the mean and standard deviation of each sample tested in each run based on Clinical Laboratory Standards Institute (CLSI) guidelines found in EP5-A2 at the URL of www.CLSI.org. Table 8 shows the 5 day precision using the ARCHITECT® scMet A Assay. The total percent coefficient of variation (CV) for the Low to High controls was less than or equal to 5%.

Table 8   ARCHITECT® scMet A Overall Precision Summary (by Instrument and Level)

Instrument	Level	N	Mean	Unit	Within Run %CV	Between Run %CV	Between Day %CV	Overall %CV
i201513	LOW CTRL	53	33.351	ng/mL	4.934	0	1.068	5
iSR05997	LOW CTRL	53	35.652	ng/mL	3.127	0.66	1.051	3.4
i201513	MED CTRL	43	319.284	ng/mL	5.312	0	1.395	5.5
iSR05997	MED CTRL	54	327.302	ng/mL	3.176	1.305	0	3.4

| i201513 | HIGH CTRL | 43 | 573.733 | ng/mL | 5.345 | 2.731 | 0.944 | 6.1 |
| iSR05997 | HIGH CTRL | 54 | 578.061 | ng/mL | 3.247 | 1.128 | 0 | 3.4 |

Example 6

Spike Recovery

[00164] Known amounts of soluble cMet were spiked into five serum specimens at 50 and 400 ng/mL. The soluble cMet was then measured in the serum control and the two spike samples. The ARCHITECT® scMet A Assay used an initial 18 minute incubation time with 10 µL sample (SMP), 50 µL of microparticles (MP), 90 µL of the analyte specific diluent (ASD). The automated dilution of 1:4 was used for the specimens. After the first wash, 50 µL of conjugate was added and washed. The RLUs were then read. The percent recovery was calculated for each spiked serum sample based on the control serum without a spike. The range and the mean percent recovery for the five specimens are reported (Table 9). Table 9 shows the spike recovery using the ARCHITECT® scMet A Assay. The spike recover was calculated as follows: Spike recovery calculation = ([Spiked Serum]-[Control Serum])÷[Spiked Calibrator Matrix] * 100. The Goal was 90-100%. The mean percent recovery was 101%.

Table 9   Spike Recovery

	scMet A	
Assay File	572_003	
SMP MP ASD	10 µL 50 µL 90 µL	
Dilution	1:4	
Sample ID	ng/mL	% Rec
CAL Spike 50 ng/mL CAL Spike 400 ng/mL	40.60 351.13	
3259 Control 3259 Spike 50 ng/mL 3259 Spike 400 ng/mL	100.03 143.57 452.77	 *107* *100*
3266 Control 3266 Spiked 50 ng/mL 3266 Spike 400 ng/mL	103.43 143.70 457.00	 *99* *101*
3296 Control 3296 Spike 50 ng/mL 3296 Spike 400 ng/mL	116.27 161.23 445.43	 *111* *94*
3308 Control 3308 Spike 50 ng/mL 3308 Spike 400 ng/mL	104.17 142.63 447.40	 *95* *98*

3651 Control	85.90	
3651 Spike 50 ng/mL	127.23	*102*
3651 Spike 400 ng/mL	440.73	*101*
Range	**94 - 111**	
Mean % Recovery	**101**	

Example 7

Dilution Linearity

[00165]   Three serum specimens were spiked with known amounts of cMet starting at 1200 ng/mL. Eight 1:2 dilutions for each spiked specimen were made to a concentration of 4.6875 ng/mL. The expected versus the actual concentrations were plotted on a correlation graph to determine the slope and the R-values in Excel 2003 version 5.1 (*see e.g.*, FIG. 10). Table 10 shows the dilution linearity of the ARCHITECT® scMet A Assay. The average slope was 1.04, R=0.99.

Table 10   Dilution Linearity

Dilution Linearity		
scMet A	**Slope**	**R**
serum 1	y = 0.9939x	0.9994
serum 2	y = 1.0439x	0.9996
serum 3	y = 1.0617x	0.9996

Example 8

Interference Testing of ARCHITECT® scMet A Assay

[00166]   Interference testing was performed to screen potentially interfering substances and to quantify potential interfering effects. The levels to be tested for potential interfering substances can be found in the Clinical Laboratory Standard Institute (CLSI) guideline EP7-A2. The most common interfering substances, *i.e.*, hemoglobin, protein, bilirubin, and triglycerides, were tested. Table 11 shows the results of the interference testing of the ARCHITECT® scMet A Assay. The assay goal was to attain a mean change in measured scMet concentration of less than or equal to about 10% across the range of 90-115 ng/mL when comparing the sample control (unspiked) to the same sample spiked with the level of interferent specific in Table 11. The

ARCHITECT® scMet A Assay showed that all interferents tested in Table 11 passed the goal of less than or equal to about 10% interference.

Table 11   Interferences

Interference Study	Interferent	Result/Test	SAMPLE_ID	scMet A		
				RLUs	Conc (ng/mL)	% Diff
Hemoglobin	Hemolysate	>500 mg/dL Total Hemoglobin	Control	95479	108.53	-3.2%
			Test	92633	105.03	
Protein	Bovine gamma globulin (BGG)	>12 g/dL Total Protein	Control	93127	105.65	-5.0%
			Test	88800	100.38	
Bilirubin	Conjugated and unconjugated Bilirubin	>20 mg/dL Total Bilirubin	Control	98806	112.58	
			Unconjugate Test	94952	107.85	-4.2%
			Conjugate Test	95733	108.83	-3.3%
Triglyceride	Intralipid	>3000 mg/dL Triglyceride	Control	85174	95.98	-5.2%
			Test	81014	90.98	

Example 9

Cross-reactivity of ARCHITECT® scMet A Assay

[00167]   Proteins that may interact with cMet, such as RON, HGF-A, HAI-1, FKBP12, angiostatin, and plasminogen, were tested at twice the levels typically found in human serum to test for cross-reactivity. Table 12 shows that there was less than 0.05% cross-reactivity for all of the tested antigens using the ARCHITECT® scMet A Assay.

Table 12   Cross-reactivity

Cross-reactivity tested at:		scMet A ng/mL
RON	800 ng/mL	<0.0000%
HGF-A	500 ng/mL	<0.0000%
HAI-1	500 ng/mL	<0.0000%
FKBP12	500 ng/mL	<0.0000%
Angiostatin	3.3 µg/mL	<0.0000%
Plasminogen	900 µg/mL	<0.0000%

Example 10

HAMA/RF/Heterophilic Specimens

[00168] Interference by human anti-mouse antibodies (HAMA), rheumatoid factor (RF) and heterophilic specimens was tested. Table 13 shows the results of the ARCHITECT® scMet A Assay for ten HAMA specimens, ten RF specimens, and ten heterophilic specimens. The percentage difference was calculated without and with Mouse IgG at 0.15 mg/mL. Table 13 shows the results of the HAMA, RF, and Heterophilic Specimens. The assay goal of attaining a mean difference of less than or equal to about 10% was accomplished by the ARCHITECT® scMet A Assay.

Table 13   HAMA/RF/Heterophilic Specimens

scMet A	% Difference Mean	% Difference Range
HAMA	-2.1	(-9.5 to 4.9)
RF	0.4	(-7.3 to 7.5)
Heterophilic	3.8	(-2.2 to 10.9)

Example 11

Sample Carryover

[00169] Sample carry over is performed to determine if any cMet is carried over on the sample pipettor from a high sample of 1600 ng/mL to the next sample of Calibrator A ("Cal A"; 0 ng/mL) by comparing the detection of Cal A before and after the high sample. Table 14 shows that sample carryover of the ARCHITECT® scMet A Assay is not detectable at 1600 ng/mL.

Table 14   Carryover

scMet A within Assay Carryover		
Sample ID	AVG RLU	AVG ng/mL
High scMet (1600 ng/mL)	1067573	1218
Cal A (before high sample)	430	0
Cal A (after high sample)	647	0

[00170] Summary of the ARCHITECT® scMet A Assay evaluation is shown in Table 15.

Table 15   Summary of the ARCHITECT® scMet A assay

Research Study	Result	Conclusion
Sample Stability	Samples are stable at: RT for 24 hrs 2-8°C for 72 hrs, -80°C for 12 days	Acceptable
5 Day Precision	Total % CV for low-high control </= 5%	Acceptable
Limit of Detection (LOD)/Limit of Quantification (LOQ)	AVG LOD = 0.011 ng/mL; LOQ = 3.13 ng/mL @ 30% Total Error	Acceptable
Spike Recovery	Mean% Recovery = 101%	Acceptable
Dilution Linearity	AVG Slope 1.04, R=0.99	Acceptable
Interference	</= 10% for hemoglobin, protein, bilirubin, and triglycerides	Acceptable
Cross Reactivity	<0.05% for all	Acceptable
human anti-mouse antibodies (HAMA), rheumatoid factor (RF), Heterophilic	</= 10% for HAMA, RF, and heterophilic samples	Acceptable
Sample Carryover	Not detectable @ 1600 ng/mL	Acceptable

Example 12

Evaluation of predictive value of ARCHITECT® scMet A Assay

[00171]   The area under receiver operator curve (AUROC) was calculated using the software JMP version 9.0.0. The true positive rate (sensitivity) was plotted versus the false positive rate (1-specificity) to determine the AUROC. The higher the AUROC, the greater the discrimination between the normal healthy individuals versus the individual with cancers. FIG. 11 shows the levels of scMet in human patients with various diseases as measured by the ARCHITECT® scMet A Assay. Table 16 shows the number of normal, breast cancer, colon cancer, prostate cancer, gastric cancer, esophageal cancer, liver fibrosis, liver cirrhosis, liver non-viral disease, and liver cancer specimens used in FIG. 11. Table 17 shows the AUROC univariate summary where the results in bold have AUROC greater than 0.80.

Table 16   Specimen

Specimens	N
Normal	65
Breast cancer	53
Colon cancer	48
Prostate cancer	76
Gastric cancer	13
Esophageal cancer	7
Liver fibrosis	10
Liver cirrhosis	10
Liver non-viral disease	8
Liver cancer	10
All liver disease	38

Table 17   AUROC Univariate Summary

AUROC Univariate Table	scMet A
normal vs breast cancer	0.63
normal vs colon cancer	0.57
normal vs prostate cancer	0.67
normal vs gastric cancer	**0.85**
normal vs esophageal cancer	0.61
normal vs liver fibrosis	**0.99**
normal vs liver cirrhosis	0.50
normal vs liver non-viral disease	**0.67**
normal vs liver cancer	**0.80**
normal vs all liver disease	**0.74**

[00172]　The ARCHITECT® scMet A Assay differentiated between normal samples and liver fibrosis (*see* FIG. 12 and Table 18); normal samples and liver cancer (*see* FIG. 13 and Table 19); and normal samples and gastric cancer (*see* FIG. 14 and Table 20).

Table 18   Liver Fibrosis vs Normal AUROC

Disease	n
Liver fibrosis	10
Normal	65
Test	**Area**
scMet A	0.99

Table 19   Gastric Cancer vs Normal AUROC

Disease	n
Gastric cancer	13
Normal	65
Test	**Area**
scMet A	0.85

Table 20   Liver Cancer vs Normal AUROC

Disease	n
Liver cancer	10
Normal	65
Test	**Area**
scMet A	0.80

[00173] The ARCHITECT® scMet A Assay was analyzed with two other assays, the ARCHITECT® scMet B Assay and the ARCHITECT® HGF Assay. The ARCHITECT® scMet B Assay measures scMet concentration using the 11E1 antibody as the capture antibody and a 224D10 F(ab')$_2$ fragment antibody as the detection antibody, each of which binds to cMet. As described above, the 11E1 antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under the number I-3724. The 224D10 antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 12, 2008 under the number I-3949. The 224D10 antibody binds an epitope that is different from the 11E1 and 224G11 antibodies, hence different amounts of soluble cMet may be detected using the ARCHITECT® scMet B Assay compared to the ARCHITECT® scMet A Assay. The ARCHITECT® HGF Assay measures HGF concentration using a 64.4.1 antibody as the capture antibody and an H98C4 antibody as the detection antibody, each of which binds to HGF. The 64.4.1 antibody is an antibody produced by hybridoma cell line that will be deposited with the American Type Culture Collection (A.T.C.C.), 10801 University Blvd., Manassas, VA 20110-2209 under the terms of the Budapest Treaty. The H98C4 antibody is an antibody produced by hybridoma cell line that will be deposited with the A.T.C.C., 10801 University Blvd., Manassas, VA 20110-2209 under the terms of the Budapest Treaty.

[00174] Data from the Effects Likelihood Ratio test within Fit Model in JMP Version 9.0.0 are shown in Table 21 for the ARCHITECT® scMet A Assay, ARCHITECT® scMet B Assay and ARCHITECT® HGF Assay. Table 21 shows the multivariate summary where the p-values

shown in bold represent the biomarkers in the panel that contribute significantly (*i.e.*, less than 0.05) to the discrimination between normal healthy individuals and individuals with cancer. The multivariate analysis showed multiple markers were significant in the model for breast cancer, colon cancer, prostate cancer, gastric cancer, esophageal cancer, liver fibrosis, and liver cancer. The panel of these three biomarkers had greater discrimination than one biomarker by itself.

Table 21   AUROC Multivariate Summary

Multivariate Analysis			
Probability Table	**scMet A**	**scMet B**	**HGF**	**AUROC**
---	---	---	---	---
normal vs breast cancer	**0.0003**	**<0.0001**	**<0.0001**	0.98
normal vs colon cancer	**0.0175**	**<0.0001**	**<0.0001**	0.99
normal vs prostate cancer	**0.0138**	**<0.0001**	**0.0015**	0.93
normal vs gastric cancer	**0.0026**	**<0.0001**	>0.05	0.92
normal vs esophageal cancer	**0.0006**	>0.05	**0.0004**	0.90
normal vs liver fibrosis	**<0.0001**	>0.05	**0.0192**	0.99
normal vs liver cirrhosis	>0.05	>0.05	**<0.0001**	0.89
normal vs liver non-viral disease	>0.05	>0.05	>0.05	n/a
normal vs liver cancer	>0.05	**<0.0001**	**0.0052**	0.90
normal vs all liver disease	>0.05	>0.05	**<0.0001**	0.69

[00175]   It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[00176]   Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

Claims

What is claimed is:

1.   A method for determining scMet concentration in a test sample, the method comprising:

    (a)   contacting the test sample with at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex;

    (b)   contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex; and

    (c)   determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex formed in (b);

wherein the at least one capture antibody comprises:

    (i)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1;

    (ii)   a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

    (iii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

    (iv)   a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4;

    (v)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or

(vi)   a variable heavy chain comprising a CDR1 comprising the amino acid
         sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence
         of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of
         SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising
         the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino
         acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid
         sequence of SEQ ID NO:8;

wherein the at least one detection antibody comprises:

(vii)   a variable heavy domain comprising the amino acid sequence of SEQ ID
         NO:9;

(viii)   a variable light domain comprising the amino acid sequence of SEQ ID
         NO:13;

(ix)   a variable heavy domain comprising the amino acid sequence of SEQ ID
         NO:9 and a variable light domain comprising the amino acid sequence of
         SEQ ID NO:13;

(x)   a variable heavy chain comprising a CDR1 comprising the amino acid
         sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence
         of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of
         SEQ ID NO:12;

(xi)   a variable light chain comprising a CDR1 comprising the amino acid
         sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence
         of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of
         SEQ ID NO:16; or

(xii)   a variable heavy chain comprising a CDR1 comprising the amino acid
         sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence
         of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of
         SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising
         the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the

amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16.

2.   The method of claim 1, further comprising comparing the signal generated by the detectable label as a direct or indirect indication of the scMet concentration in the test sample to a signal generated as a direct or indirect indication of the scMet concentration in a control or calibrator.

3.   The method of claim 1, wherein the scMet concentration in the test sample is used to determine or assess whether a subject has or is at risk of developing disease.

4.   The method of claim 3, wherein the disease is liver disease.

5.   The method of claim 4, wherein an increased scMet concentration as compared to the scMet concentration in a control or calibrator indicates that the subject has liver disease.

6.   The method of claim 5, wherein the liver disease is liver fibrosis or liver cancer.

7.   The method of claim 6, wherein the increased scMet concentration indicates that the subject has liver fibrosis.

8.   The method of claim 7, wherein the scMet concentration in a control or calibrator is 150 ng/mL.

9.   The method of claim 6, wherein the increased scMet concentration indicates that the subject has liver cancer.

10.   The method of claim 9, wherein the scMet concentration in a control or calibrator is 110 ng/mL.

11.   The method of claim 3, wherein the disease is gastric cancer.

12.   The method of claim 11, wherein a decreased scMet concentration as compared to the scMet concentration in a control or calibrator indicates that the subject has gastric cancer.

13.   The method of claim 12, wherein the scMet concentration in a control or calibrator is 75 ng/mL.

14.   The method of claim 1, wherein the at least one capture antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under

the number I-3731 and the at least one detection antibody is an antibody produced by hybridoma cell line deposited at the CNCM, Institut Pasteur, Paris, on March 14, 2007 under the number I-3724.

15.   A method of diagnosing and treating liver disease in a subject, the method comprising:

    (a)   obtaining a biological sample comprising blood from the subject;

    (b)   determining the scMet concentration in the biological sample from the subject using the method of claim 1;

    (c)   comparing the scMet concentration in the biological sample with the scMet concentration in a normal control or calibrator;

    (d)   diagnosing the subject as having liver disease if the scMet concentration in the biological sample is greater than the scMet concentration in the normal control or calibrator; and

    (e)   administering a liver disease treatment regimen to the subject diagnosed as having liver disease.

16.   The method of claim 15, wherein the biological sample of a subject is selected from a tissue sample, bodily fluid, whole blood, plasma, serum, urine, bronchoalveolar lavage fluid, and a cell culture suspension or fraction thereof.

17.   The method of claim 15, wherein the biological sample of a subject is blood plasma or blood serum.

18.   A method of diagnosing and treating cancer in a subject, the method comprising:

    (a)   obtaining a biological sample comprising blood from the subject;

    (b)   determining the scMet concentration in the biological sample from the subject using the method of claim 1;

    (c)   comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator;

(d)   diagnosing the subject as having cancer if the scMet concentration in the biological sample is less than the scMet concentration in the normal control or calibrator; and

(e)   administering a cancer treatment regimen to the subject diagnosed as having cancer.

19.   The method of claim 18, wherein the biological sample of a subject is selected from a tissue sample, bodily fluid, whole blood, plasma, serum, urine, bronchoalveolar lavage fluid, and a cell culture suspension or fraction thereof.

20.   The method of claim 18, wherein the biological sample of a subject is blood plasma or blood serum.

21.   The method of claim 18, wherein the cancer is gastric cancer.

22.   A method for determining if a subject is responding to the administration of one or more pharmaceutical compositions, the method comprising:

(a)   measuring the scMet concentration in a sample from the subject using the method of claim 1;

(b)   comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator, wherein an altered scMet concentration indicates that the subject is not responding to the administration of one or more pharmaceutical compositions; and

(c)   adjusting the treatment of the subject if the subject is not responding to the administration of one or more pharmaceutical compositions.

23.   A method of diagnosing and treating liver disease in a subject, the method comprising:

(a)   contacting the test sample with at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex;

(b)   contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an

epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex;

(c)   determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex formed in (b);

(d)   comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator;

(e)   diagnosing the subject as having liver disease if the scMet concentration in the biological sample is greater than the scMet concentration in the normal control or calibrator; and

(f)   administering a liver disease treatment regimen to the subject diagnosed as having liver disease;

wherein the at least one capture antibody comprises:

(i)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1;

(ii)   a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iv)   a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4;

(v)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or

(vi)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8;

wherein the at least one detection antibody comprises:

(vii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9;

(viii)   a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(ix)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(x)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12;

(xi)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or

(xii)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the

amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16.

24. The method of claim 23, wherein the liver disease is liver fibrosis or liver cancer.

25. A method of diagnosing and treating cancer in a subject, the method comprising:

(a) contacting the test sample with at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet to form a capture antibody-scMet antigen complex;

(b) contacting the capture antibody-scMet antigen complex with at least one detection antibody comprising a detectable label, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody and forms a capture antibody-scMet antigen-detection antibody complex;

(c) determining the scMet concentration in the test sample based on the signal generated by the detectable label in the capture antibody-scMet antigen-detection antibody complex formed in (b);

(d) comparing the scMet concentration in the sample with the scMet concentration in a normal control or calibrator;

(e) diagnosing the subject as having cancer if the scMet concentration in the biological sample is less than the scMet concentration in the normal control or calibrator; and

(f) administering a cancer treatment regimen to the subject diagnosed as having cancer;

wherein the at least one capture antibody comprises:

(i) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1;

(ii) a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iv)   a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4;

(v)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or

(vi)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8;

wherein the at least one detection antibody comprises:

(vii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9;

(viii)   a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(ix)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(x)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence

of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12;

(xi)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or

(xii)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16.

26.   The method of claim 25, wherein the cancer is gastric cancer.

27.   A kit for assaying a test sample for scMet, which kit comprises at least one capture antibody, wherein the capture antibody binds to an epitope on scMet or a fragment of scMet, at least one detection antibody, wherein the detection antibody binds to an epitope on scMet that is not bound by the capture antibody, and instructions for assaying the test sample for scMet, wherein the at least one capture antibody comprises:

(i)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1;

(ii)   a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain comprising the amino acid sequence of SEQ ID NO:5;

(iv)   a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2

comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4;

(v)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8; or

(vi)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8;

wherein the at least one detection antibody comprises:

(vii)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9;

(viii) a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(ix)   a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain comprising the amino acid sequence of SEQ ID NO:13;

(x)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12;

(xi)   a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16; or

(xii)   a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16,

wherein the at least one detection antibody is optionally detectably labeled.

28.   The kit of claim 27, further comprising a reference standard indicating a scMet concentration in a control or calibrator.

29.   The kit of claim 28, wherein the reference standard indicates scMet concentration levels of 200 ng/mL (High), 50 ng/mL (Medium), and 12.5 ng/mL (Low).

FIG. 1

FIG. 2

FIG. 3

FIG. 4

Complex Testing scMet Assay A

FIG. 5

scMet A m224G11 uP, 11E1 Conjugate

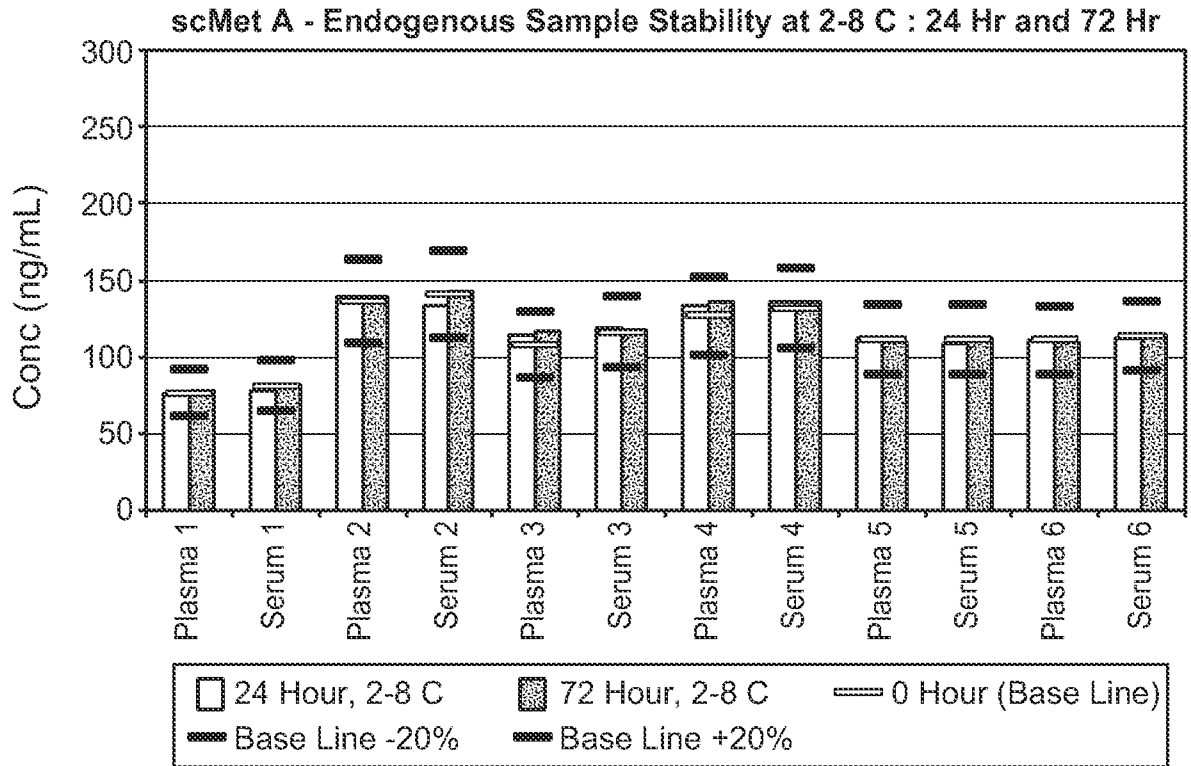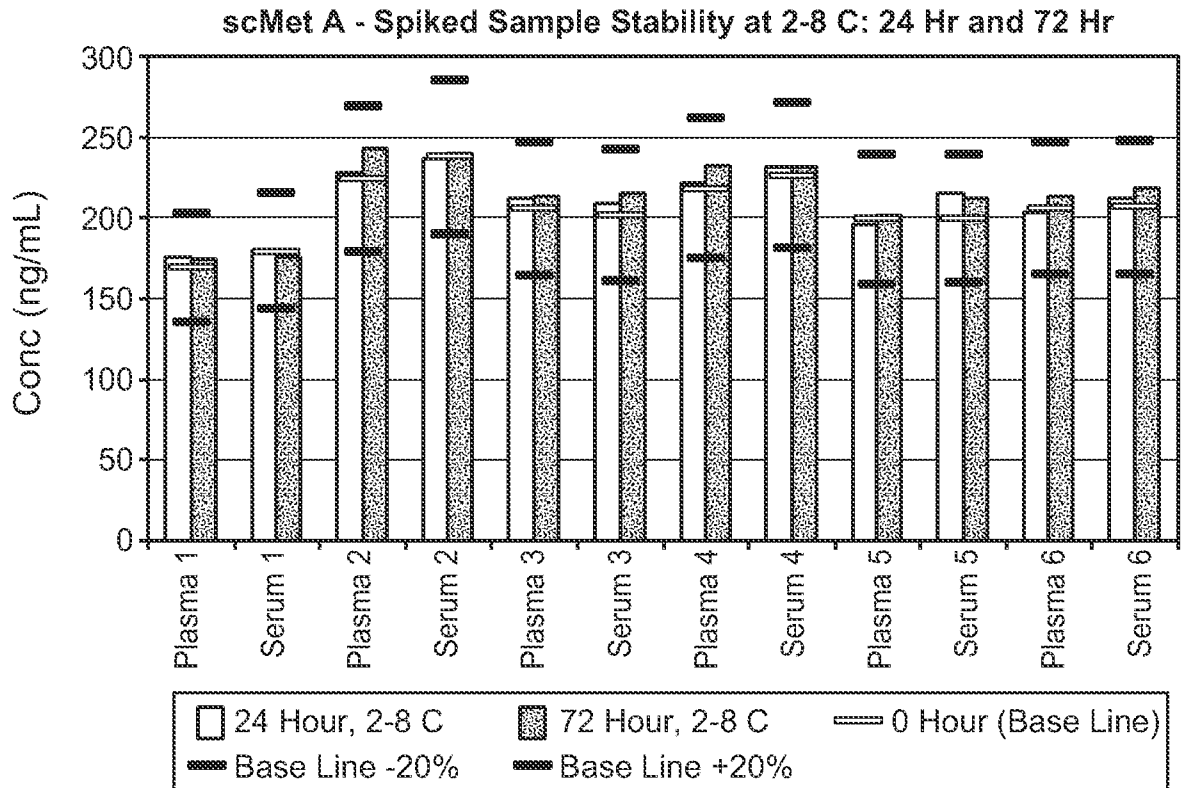| Calibrator	scMet ng/mL	Mean RLU
calA	0	89
calB	6.25	24415
calC	25	85091
calD	100	301925
calE	400	688905
calF	800	965217
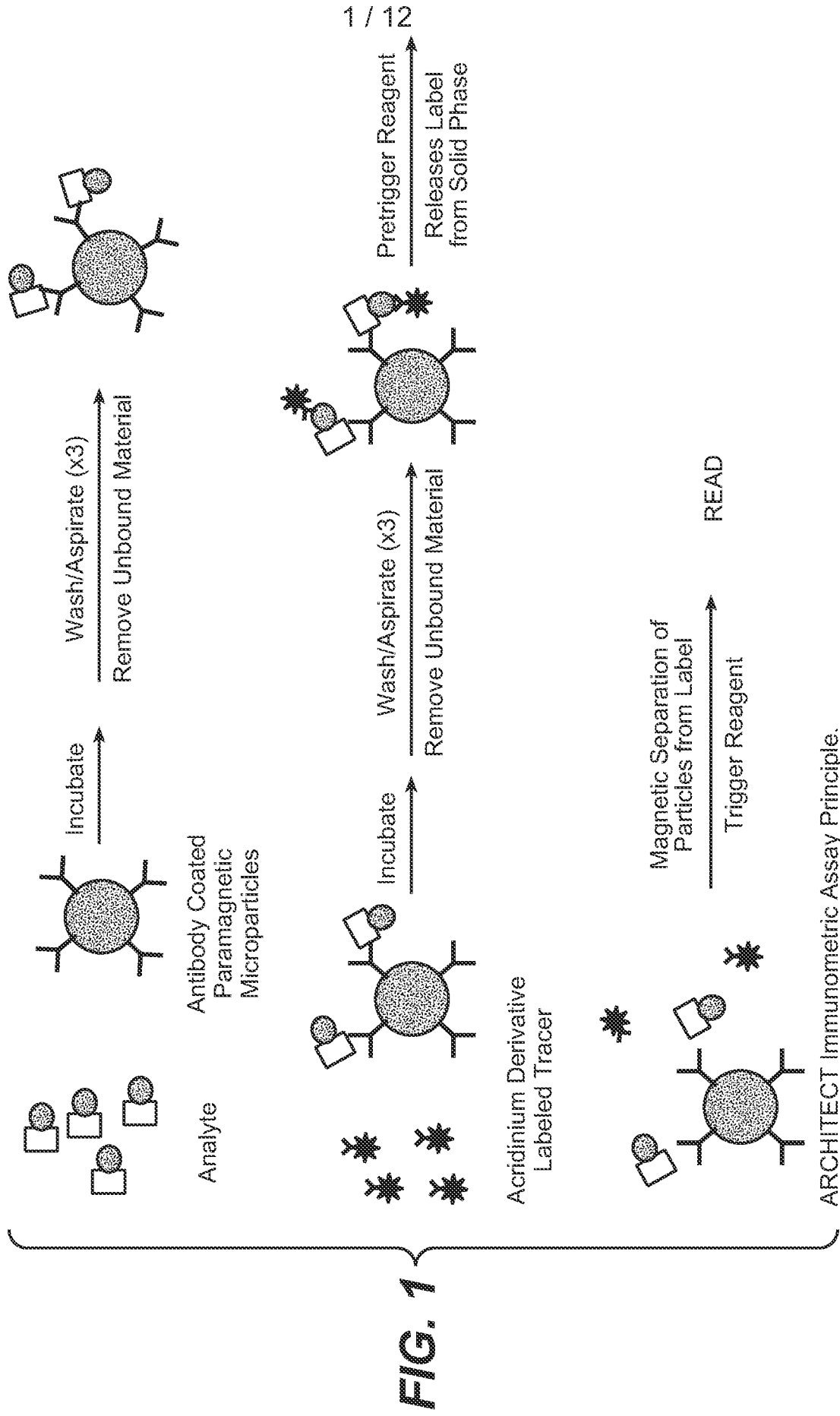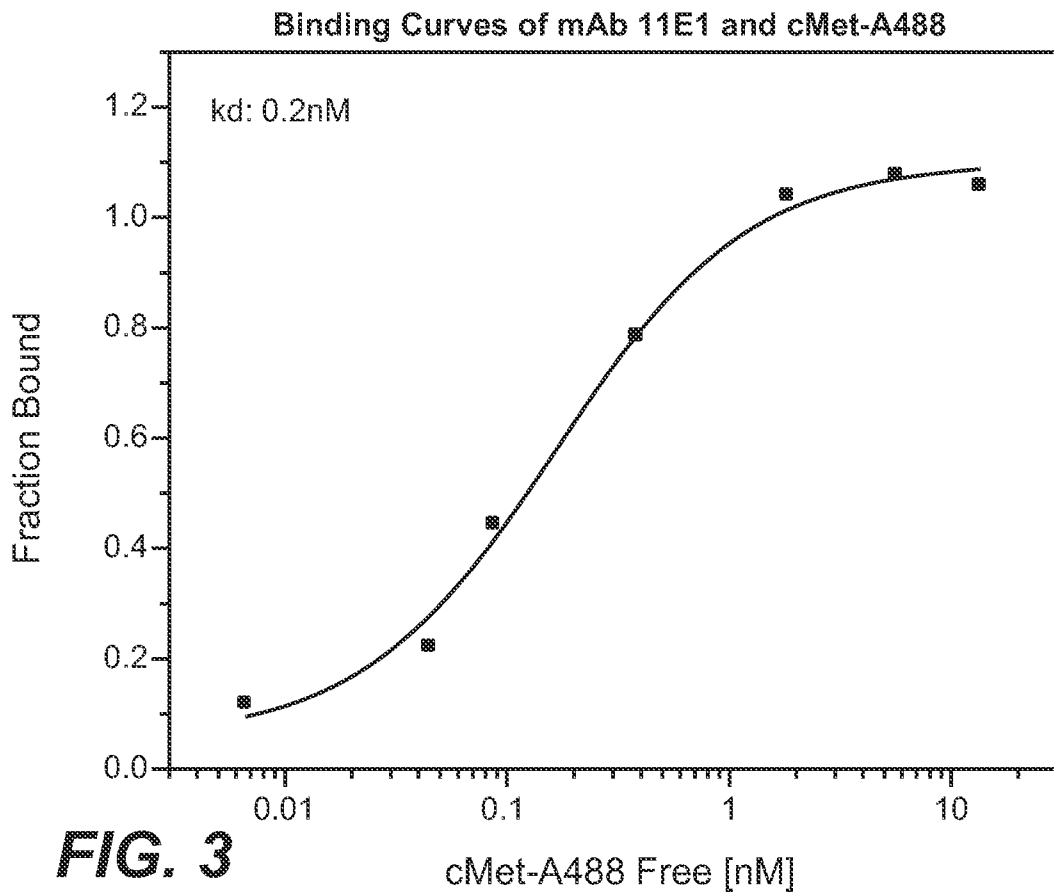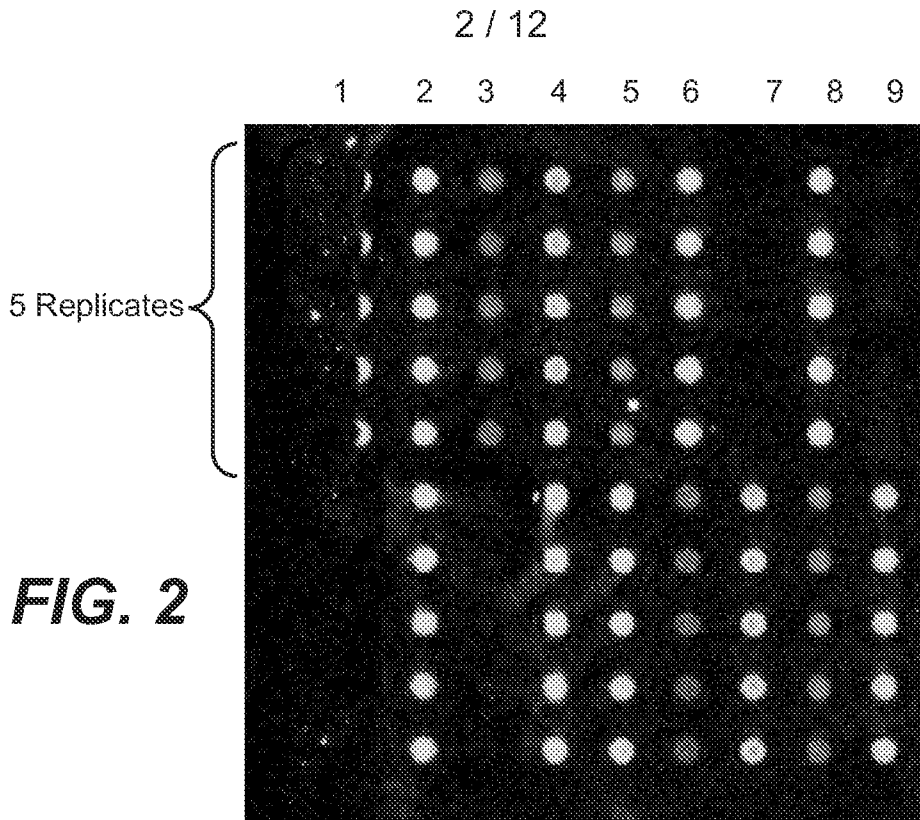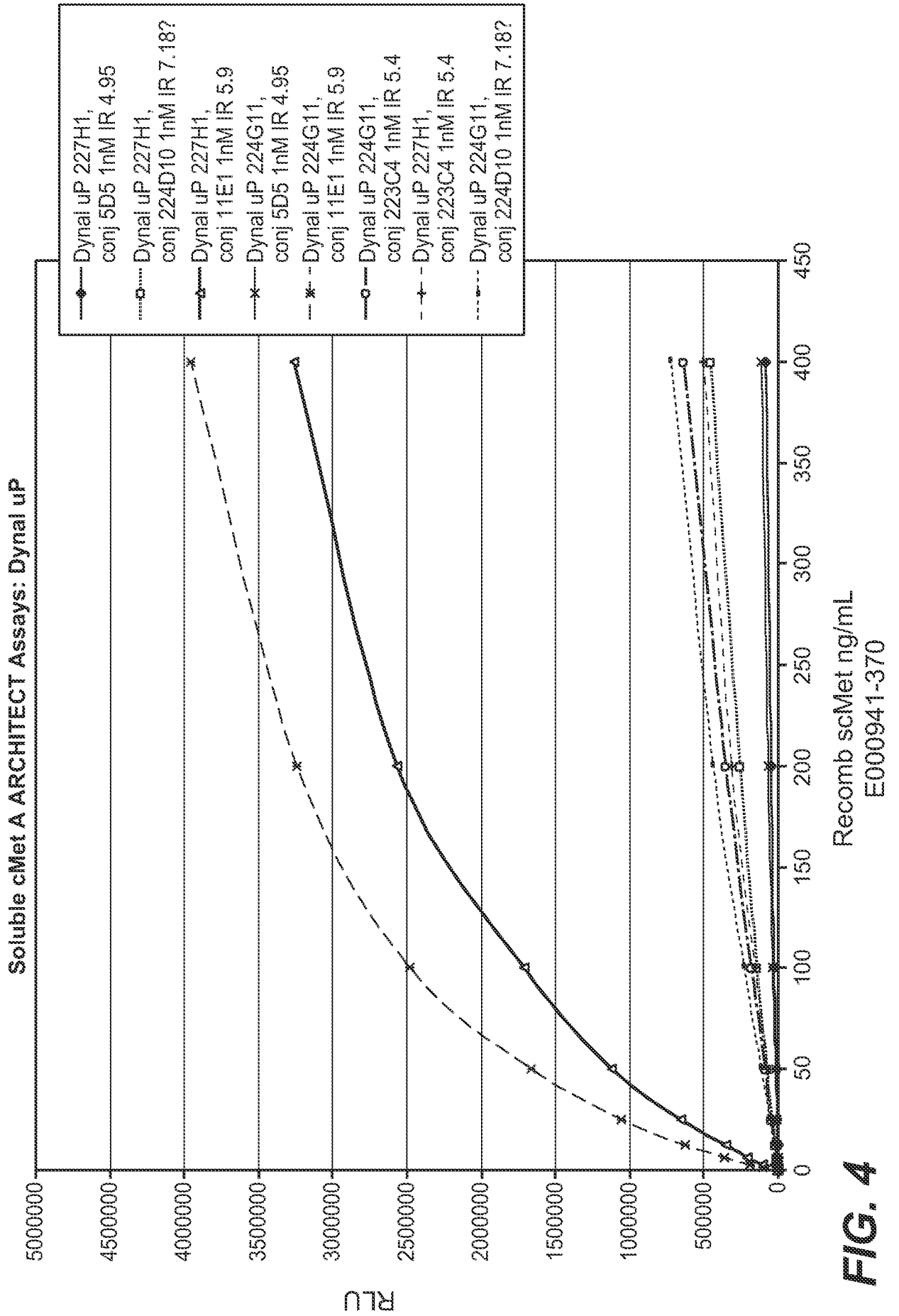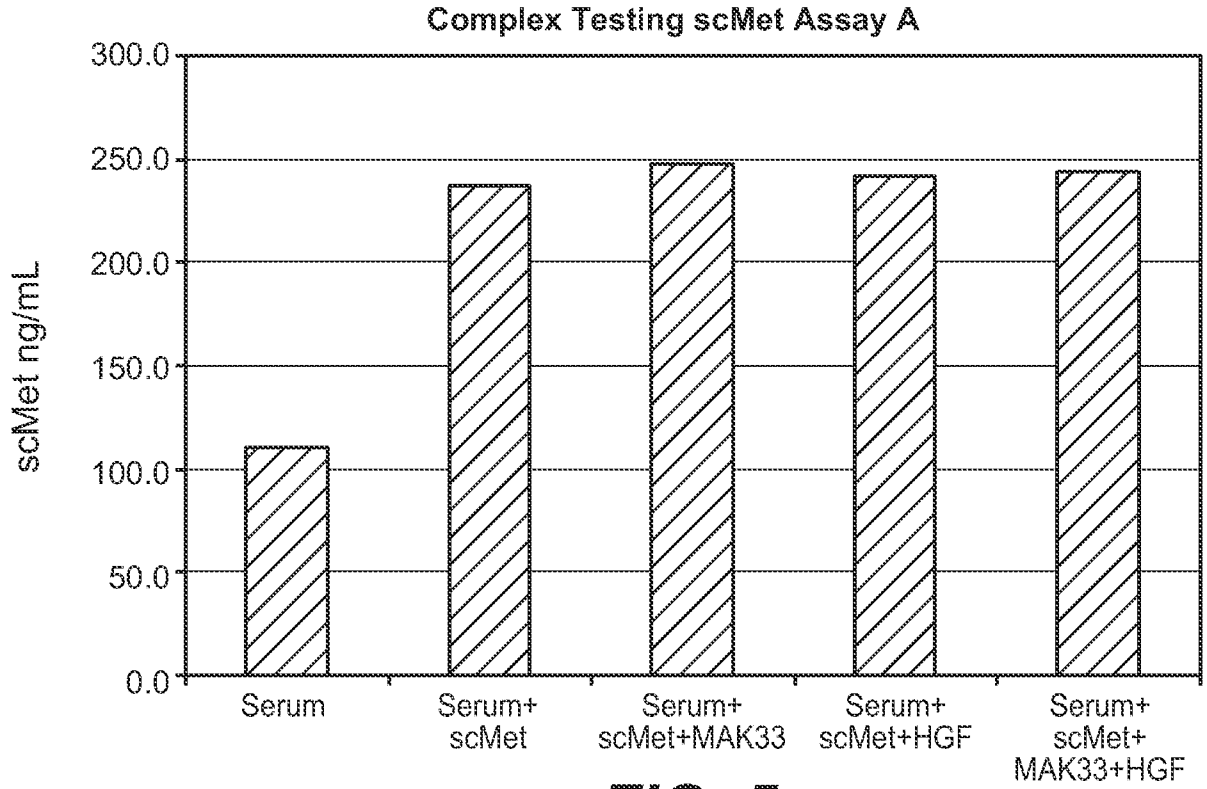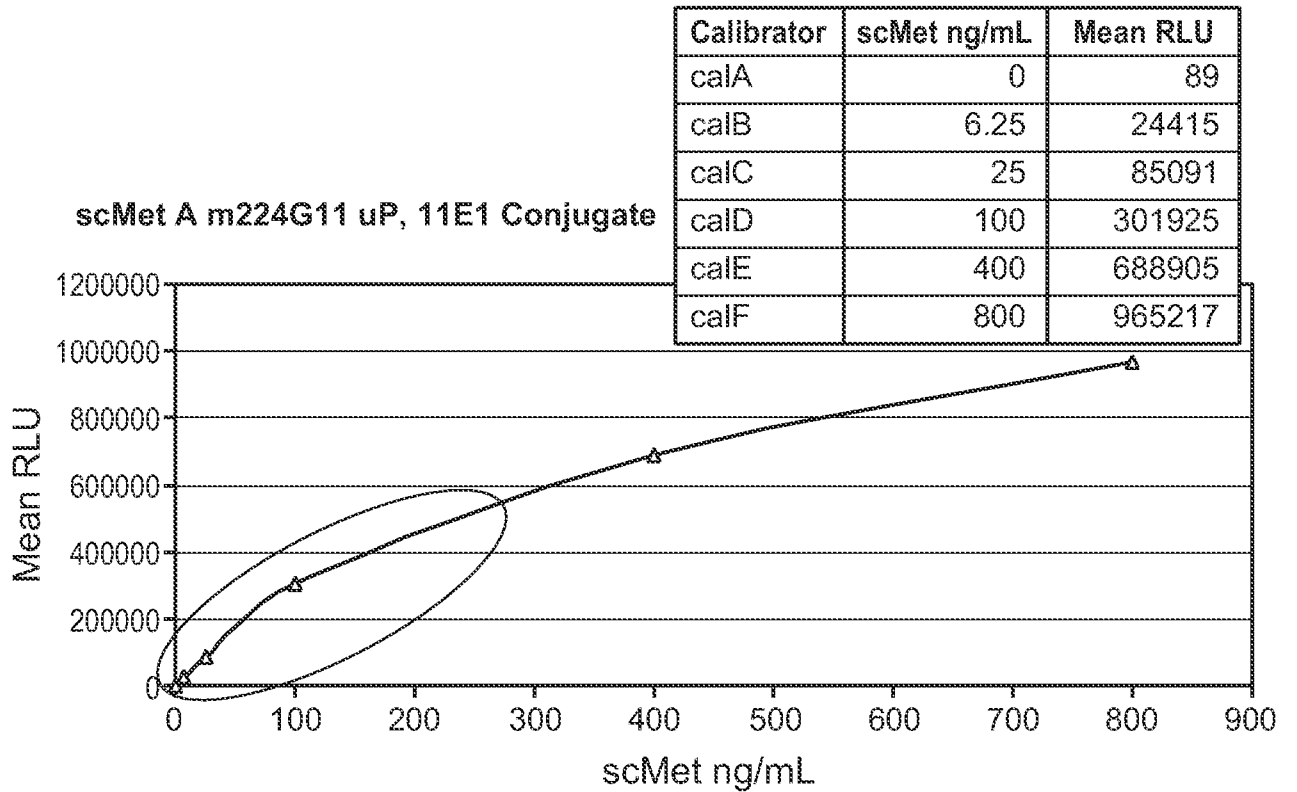
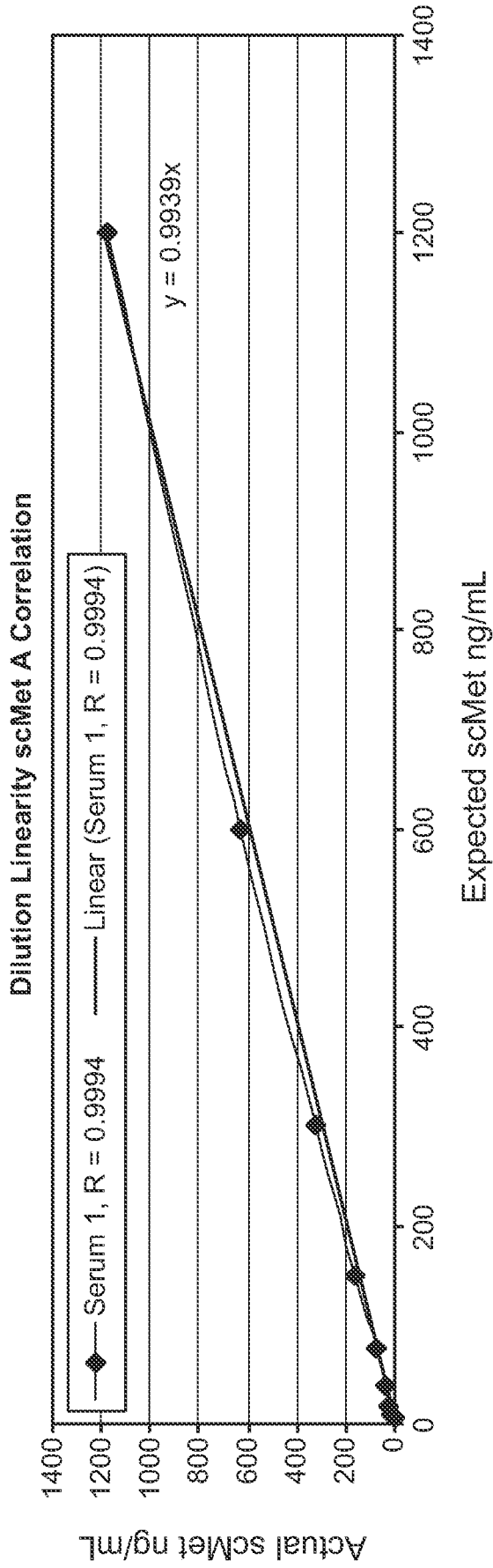**FIG. 6**
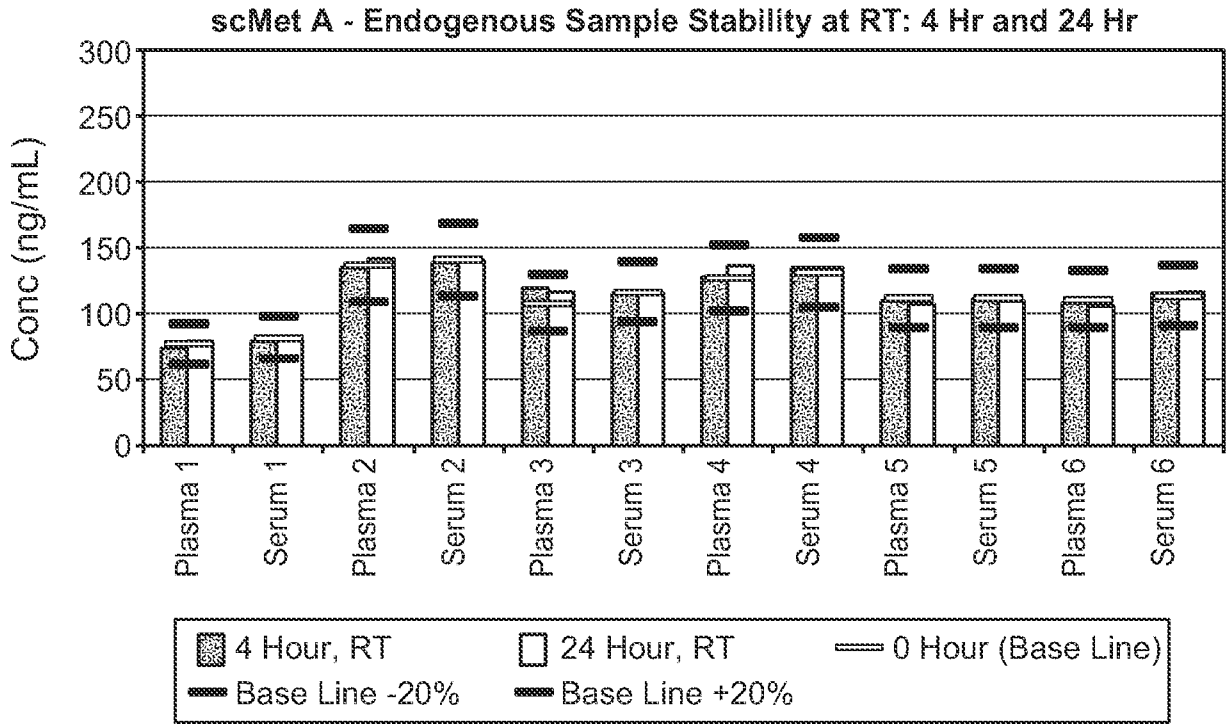
FIG. 7A
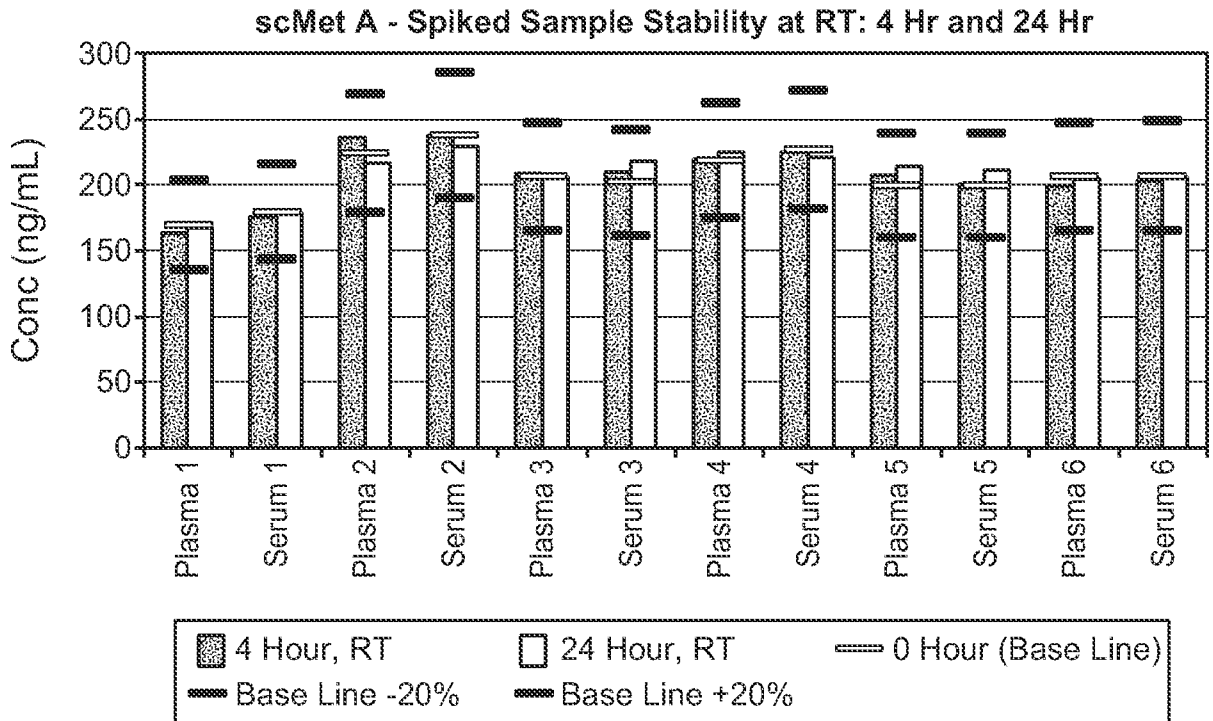
FIG. 7B

scMet A - Endogenous Sample Stability at 2-8 C : 24 Hr and 72 Hr
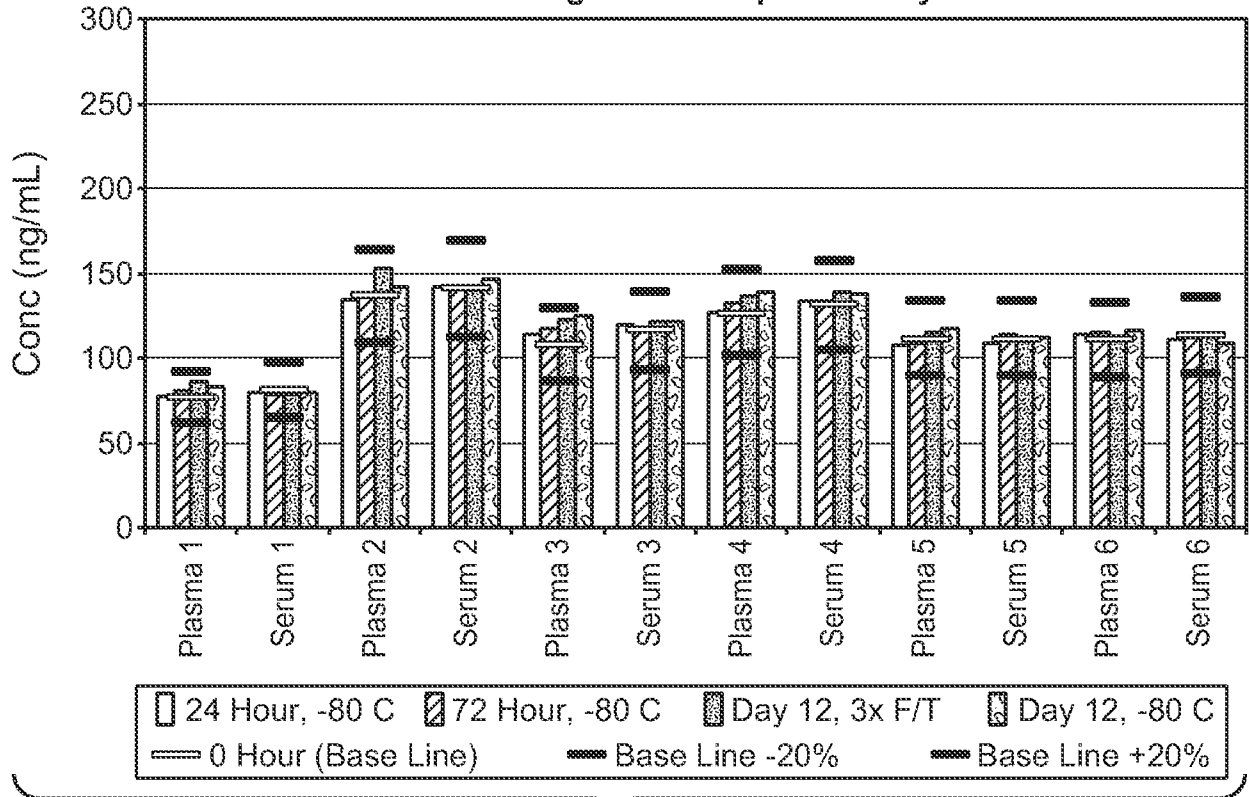
FIG. 8A

scMet A - Spiked Sample Stability at 2-8 C: 24 Hr and 72 Hr
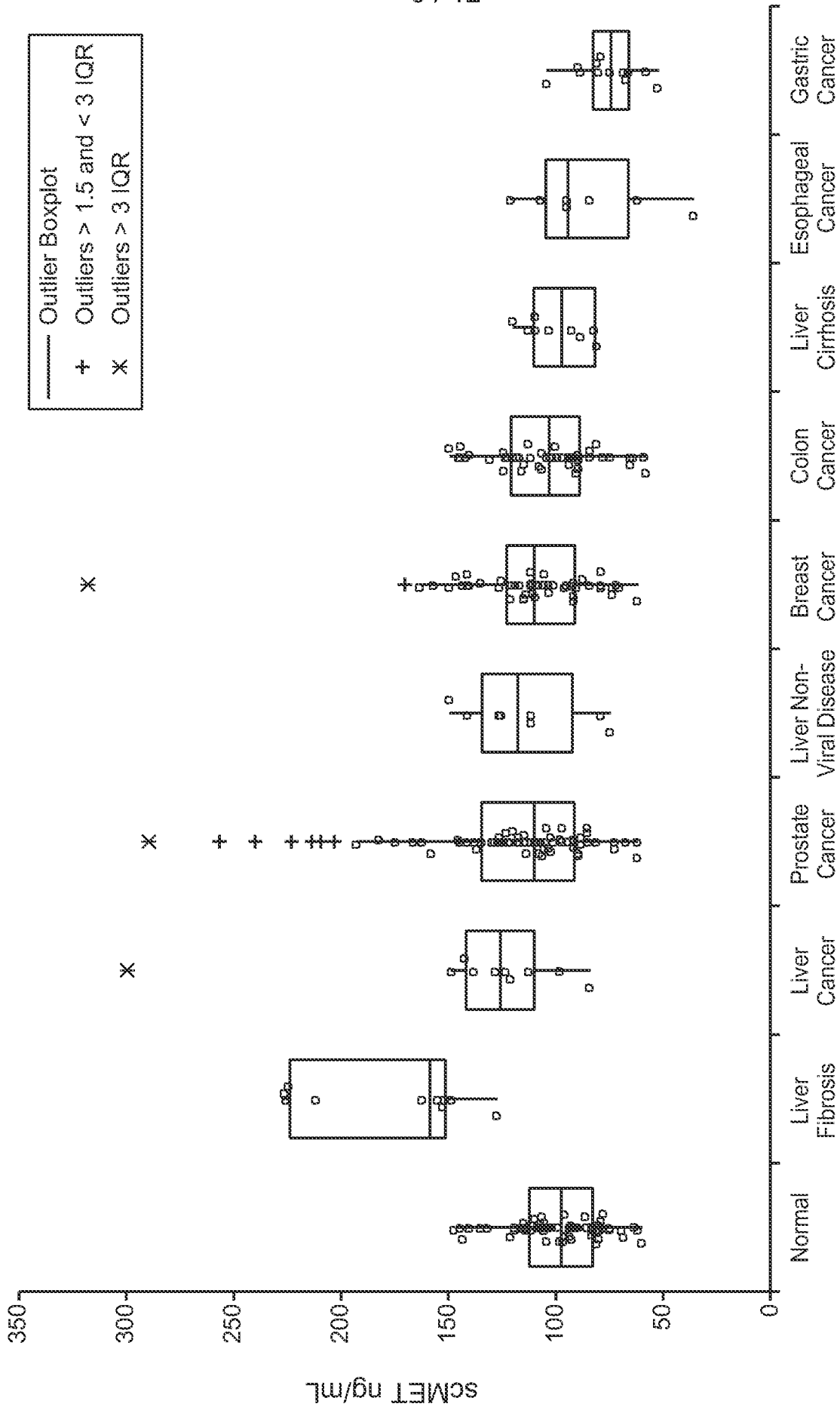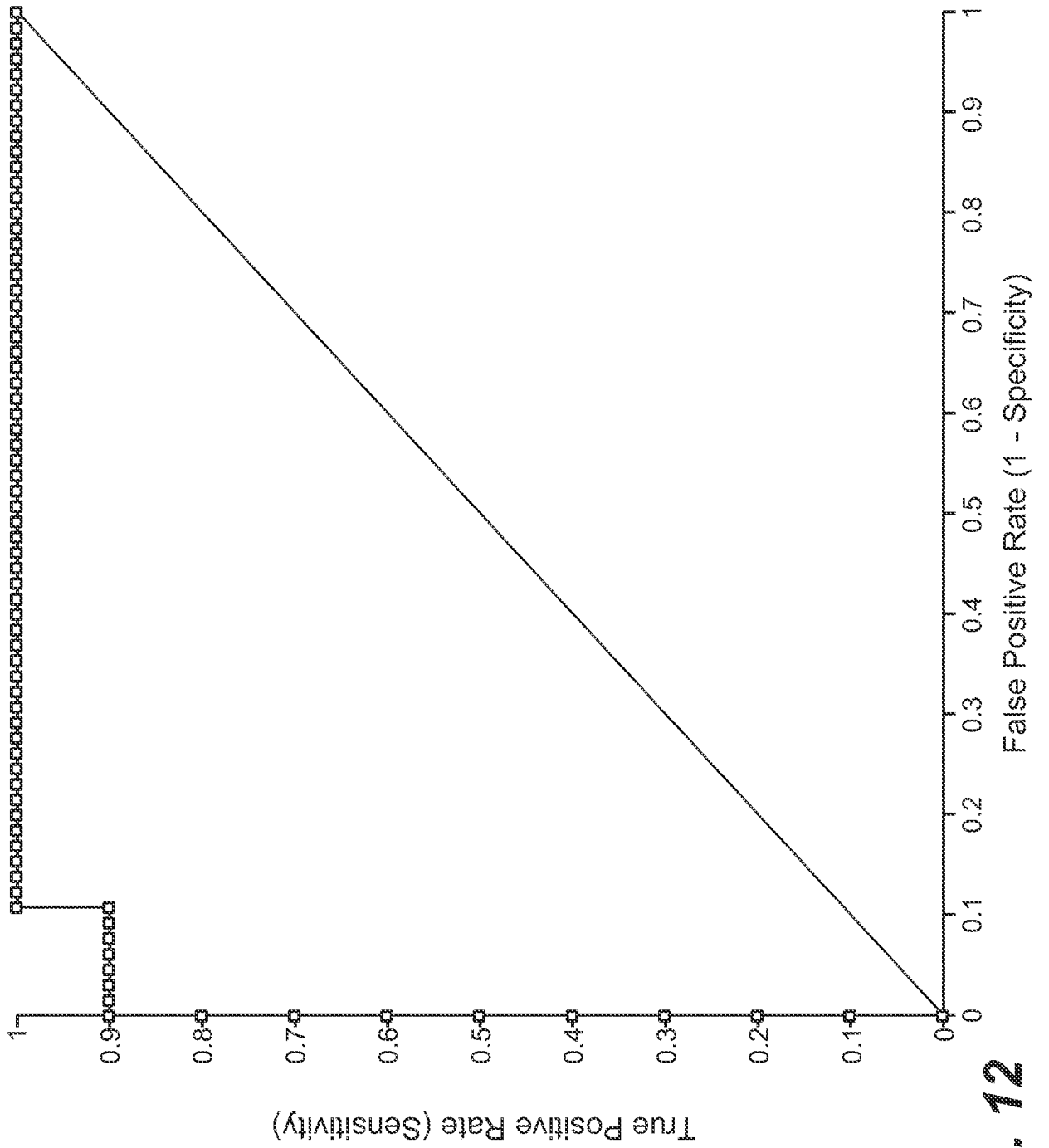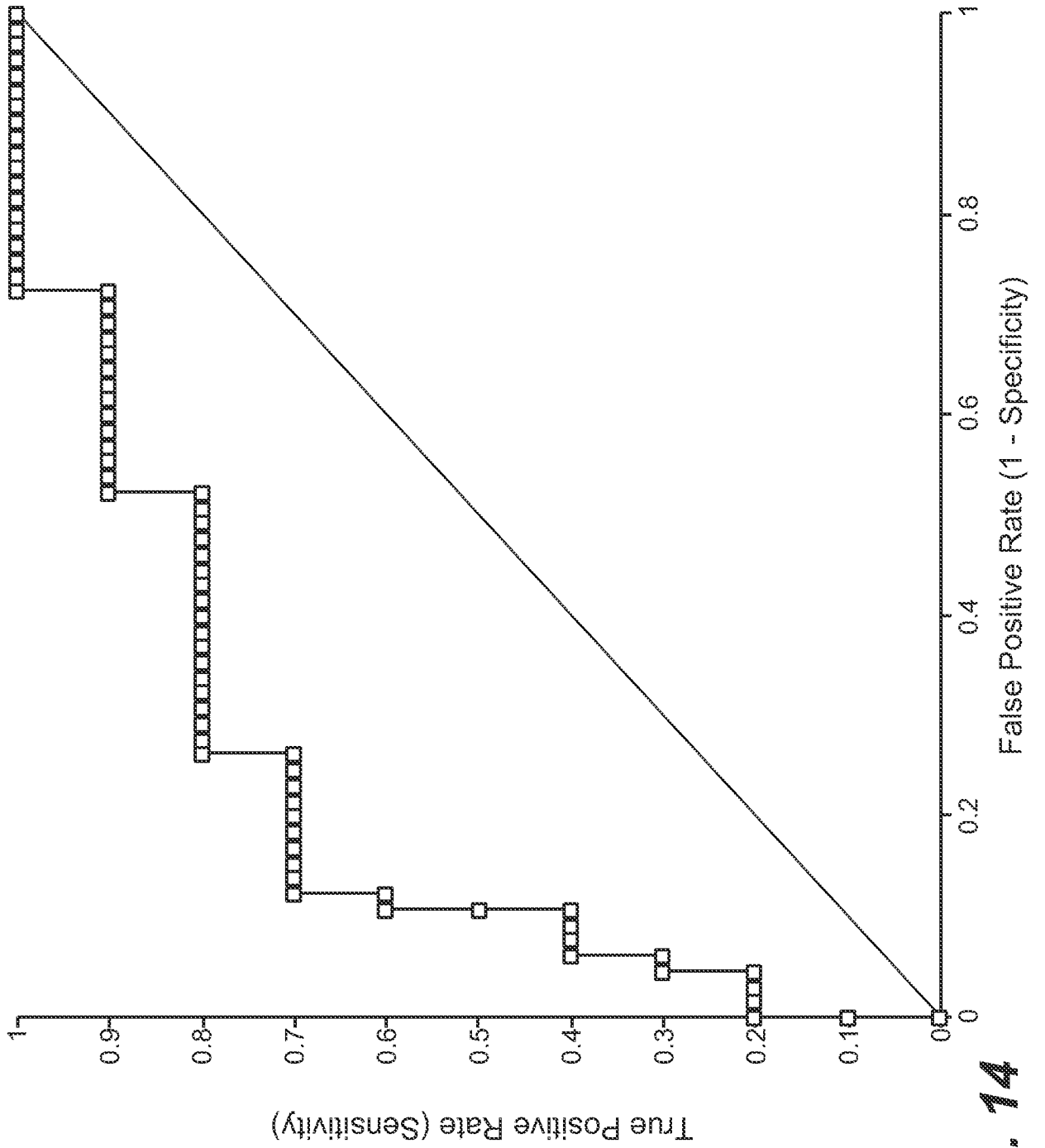
FIG. 8B

7 / 12

FIG. 9A

FIG. 9B

FIG. 10

FIG. 11

FIG. 12

FIG. 13

FIG. 14

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/574
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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

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

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/031027 A1 (GENENTECH INC [US]; PATEL PREMAL [US]; PETERSON AMY C [US]; YAUCH ROBE) 8 March 2012 (2012-03-08) claims page 21, line 16 - line 17 page 37, line 17 page 37, line 19 -----	1-29
Y	EP 2 287 197 A1 (PF MEDICAMENT [FR]) 23 February 2011 (2011-02-23) claims -----	1-29
Y	WO 2010/064089 A1 (PF MEDICAMENT [FR]; GOETSCH LILIANE [FR]; WURCH THIERRY [FR]; BES CEDR) 10 June 2010 (2010-06-10) claims page 14, line 16 - line 18 page 36, line 3 - line 6 ----- -/--	1-29

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2015	03/02/2015

| Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | Authorized officer Routledge, Brian |

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 2 143 441 A1 (PF MEDICAMENT [FR]) 13 January 2010 (2010-01-13) paragraph [0037] paragraph [0061] -----	1-29
Y	CORVAIA NATHALIE ET AL: "First bivalent fully antagonist anti-c-Met antibody targeting the c-Met receptor: I) in vitro mechanism of action", AMERICAN ASSOCIATION FOR CANCER RESEARCH. PROCEEDINGS OF THE ANNUAL MEETING; 100TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-CANCER-RESEARCH; DENVER, CA, USA; APRIL 18 -22, 2009, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 50, 22 April 2009 (2009-04-22), page 1, XP008129391, ISSN: 0197-016X the whole document -----	1-29
Y	EP 2 014 681 A1 (PF MEDICAMENT [FR]) 14 January 2009 (2009-01-14) claims paragraph [0067] paragraph [0196] -----	1-29
Y	KAMIYA: "Human c-Met ELISA For the quantitative determination of c-Met in human serum, EDTA-plasma or cell culture media", 20080201, no. KT-444, 1 February 2008 (2008-02-01), pages 1-6, XP007919726, the whole document -----	1-29
X,P	ABBOTT DIAGNOSTICS: "Architect i100SR", INTERNET CITATION, 12 January 2015 (2015-01-12), XP002734296, Retrieved from the Internet: URL:https://www.abbottdiagnostics.com/en-u s/products/ARCHITECT-i1000SR.html#test-men u [retrieved on 2015-01-12] the whole document -----	1-29

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 2012031027	A1	08-03-2012	AU	2011295919 A1	07-03-2013
			CA	2808236 A1	08-03-2012
			CN	103221825 A	24-07-2013
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