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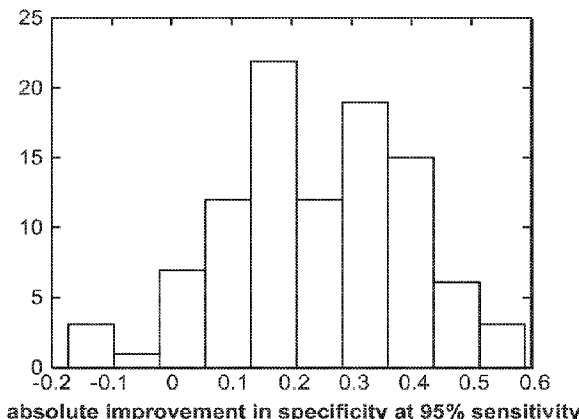
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(54) Titre : COMPOSITIONS ET METHODES D'EVALUATION DU CANCER DE L'OVAIRE A SPECIFICITE AMELIOREE

(54) Title: COMPOSITIONS AND METHODS FOR OVARIAN CANCER ASSESSMENT HAVING IMPROVED
SPECIFICITY



Panel: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1),
beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125
(CA 125 II), IGFBP2, IL6, HE4, and FSH.

(57) **Abrégé/Abstract:**

The present invention provides compositions and methods that provide a high degree of sensitivity and a high degree of specificity for the preoperative assessment of ovarian tumors in a variety of subject's (e.g., pre- and post-menopausal women) having a variety of ovarian cancer types (e.g., clear cell/mucinous, low malignant potential, high malignant potential) and at a variety of disease states (e.g., early and late stage). More particularly, the invention relates to panel to reduce a false positive of pre-operatively assessing a subject's risk of having ovarian cancer, the panel consisting of markers Cancer Antigen 125 (CA125), Prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4). The invention also relates to a method for reducing the rate of false positive pre-operative ovarian cancer assessment and to method for pre-operatively assessing a subject's risk of having ovarian cancer that involve such a panel.

ABSTRACT OF THE DISCLOSURE

The present invention provides compositions and methods that provide a high degree of sensitivity and a high degree of specificity for the preoperative assessment of ovarian tumors in a variety of subject's (e.g., pre- and post-menopausal women) having a variety of ovarian cancer types (e.g., clear cell/mucinous, low malignant potential, high malignant potential) and at a variety of disease states (e.g., early and late stage). More particularly, the invention relates to panel to reduce a false positive of pre-operatively assessing a subject's risk of having ovarian cancer, the panel consisting of markers Cancer Antigen 125 (CA125), Prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4). The invention also relates to a method for reducing the rate of false positive pre-operative ovarian cancer assessment and to method for pre-operatively assessing a subject's risk of having ovarian cancer that involve such a panel.

COMPOSITIONS AND METHODS FOR OVARIAN CANCER ASSESSMENT HAVING IMPROVED SPECIFICITY

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BACKGROUND OF THE INVENTION

Ovarian cancer is among the most lethal gynecologic malignancies in developed
10 countries. Annually in the United States alone, approximately 23,000 women are diagnosed
with the disease and almost 14,000 women die from it. Despite progress in cancer therapy,
ovarian cancer mortality has remained virtually unchanged over the past two decades. Given
the steep survival gradient relative to the stage at which the disease is diagnosed, early
detection remains the most important factor in improving long-term survival of ovarian
15 cancer patients. A second important factor is whether or not women with ovarian cancer are
treated by a surgeon that specializes in gynecological oncology.

The importance of identifying women who should be treated by a gynecological
oncologist is highlighted in a consensus statement issued by the National Institutes of Health
(NIH). In 1994, the NIH indicated that women identified preoperatively as having a
20 significant risk of ovarian cancer should have the option of having their surgery performed by
a gynecologic oncologist. To ensure that no woman who has ovarian cancer is overlooked,
current diagnostic methods optimize sensitivity at the expense of specificity. Present
diagnostic methods have an unacceptably high false positive rate. In human terms, this
means that fifty percent of women go into surgery believing that they have ovarian cancer
25 when in fact they have a benign mass. There is an urgent need for improved diagnostic
methods that not only have a high degree of sensitivity, but that also provide a high degree of

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specificity, which can be used to manage subject treatment more effectively and ensure that the appropriate patients are being promptly and properly referred to specialists.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods that provide a high degree
5 of sensitivity and a high degree of specificity for the preoperative assessment of ovarian
tumors in a variety of subject's (e.g., pre- and post-menopausal women) having a variety of
ovarian cancer types (e.g., clear cell/mucinous, low malignant potential, high malignant
potential) and at a variety of disease states (e.g., early and late stage). In particular
embodiments, compositions and methods of the invention are surprisingly effective in
10 reducing the rate of false positive diagnoses of ovarian cancer by 25%, 50%, 66%, 75% or
more.

In one aspect, the invention generally provides a panel for pre-operatively assessing a
subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125,
Prealbumin, Transferrin, and HE4.

15 In another aspect, the invention provides a method for reducing the rate of false
positive pre-operative ovarian cancer assessment, the method involving measuring the level
of a panel of markers consisting of CA125, Prealbumin, Transferrin, and HE4 in a biological
sample derived from the subject; and comparing the level of said markers to a reference level,
wherein at a fixed sensitivity of about 90% fewer than 25% of women are incorrectly
20 identified as having ovarian cancer.

In another aspect, the invention provides a method for pre-operatively assessing a
subject's risk of having ovarian cancer, the method involving measuring the level of a panel
of markers consisting of CA125, Prealbumin, Transferrin, and HE4 in a biological sample
derived from the subject; and comparing the level of said markers to a reference level,
25 wherein at a fixed sensitivity of about 90% the specificity using said panel was about 85%.

In another aspect, the invention provides a panel for pre-operatively assessing a
subject's risk of having ovarian cancer, the panel consisting of markers CA125, Prealbumin,
and HE4.

In another aspect, the invention provides a method for pre-operatively assessing a
30 subject's risk of having ovarian cancer, the method involving measuring the level of a panel
of markers consisting of CA125, Prealbumin, and HE4 in a biological sample derived from

the subject; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90% the specificity using said panel was about 80%.

In another aspect, the invention provides a method for reducing the rate of false positive pre-operative ovarian cancer assessment, the method involving measuring the level
5 of a panel of markers consisting of CA125, Prealbumin, and HE4 in a biological sample derived from the subject; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90% fewer than 25% of women are incorrectly identified as having ovarian cancer.

In another aspect, the invention provides a panel for pre-operatively assessing a
10 subject's risk of having ovarian cancer, the panel consisting of markers Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II), and HE4.

In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having ovarian cancer, the method involving measuring the level of a panel
15 of markers consisting of Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II), and HE4; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90% the specificity using said panel was about 85%.

In another aspect, the invention provides a method for reducing the rate of false
20 positive pre-operative ovarian cancer assessment, the method involving measuring the level of a panel of markers consisting of Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II), and HE4 in a biological sample derived from the subject; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90% fewer than 25% of women
25 are incorrectly identified as having ovarian cancer.

In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having ovarian cancer, the method involving measuring the level of a panel of markers consisting of Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II) and determining
30 the subject's OVA1 score or risk of cancer; measuring the level of HE4; and combining the OVA1 score with HE4 level using an algorithm wherein the fixed sensitivity is set at about 90% and the specificity using said panel is about 85%.

In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel consisting of markers Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH.

- 5 In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having ovarian cancer, the method involving
- (a) measuring the level of a panel of markers consisting of Transthyretin/prealbumin (TT), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr), Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH in a biological sample derived from
- 10 the subject; and
- (b) comparing the level of said markers to a reference level, wherein at a fixed sensitivity of 90% the specificity is at least about 75%.

- In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125-
- 15 II, transthyretin/prealbumin, IGFBP2, IL6, and FSH.

- In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having ovarian cancer, the method involving
- (a) measuring the level of a panel of markers consisting of CA125-II, transthyretin/prealbumin, IGFBP2, IL6, and FSH in a biological sample derived from the
- 20 subject; and
- (b) comparing the level of said markers to a reference level, wherein at a fixed sensitivity of 90% the specificity using said panel was greater than 75%.

- In another aspect, the invention provides a panel for assessing a subject's risk of having early stage ovarian cancer, the panel consisting of CA125, prealbumin, IGFBP2, and
- 25 IL6, and FSH.

- In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having early stage ovarian cancer, the method involving measuring the level of a panel of markers consisting of CA125, prealbumin, IGFBP2, and IL6, and FSH; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of 95%
- 30 the specificity using said panel was 65-95%.

In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125, HE4, IGFBP2, IL6, and TAG 72/CA72-4.

In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125, Transthyretin/prealbumin, TRF, and TAG 72/CA724

In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk
5 of having ovarian cancer, the panel containing or consisting of markers CA125, HE4, and TAG 72/CA724

In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125, Transthyretin/prealbumin, TRF, HE4, and TAG 72/CA724.

10 In another aspect, the invention provides a panel for pre-operatively assessing a subject's risk of having ovarian cancer, the panel containing or consisting of markers CA125, HE4, IGFBP2, IL6, and TAG 72/CA724.

In another aspect, the invention provides a method for pre-operatively assessing a subject's risk of having ovarian cancer, the method involving measuring the level of a panel
15 of markers in a biological sample derived from the subject; and comparing the level of said markers to a reference level, wherein at a fixed sensitivity of 90%, the specificity using said panel was greater than about 75%.

In another aspect, the invention provides a panel containing or consisting of Prealbumin CA125 and HE4

20 In another aspect, the invention provides a panel containing or consisting of CA125 and HE4 and any one of Prealbumin or Transferrin or ApoA1.

In various embodiments of any of the above aspects or any other aspect of the invention delineated herein, the specificity using said panel was at least about 80%, 90%, or 95%. In other embodiments of the above aspects, the method reduces the false positive rate
25 to about 25% or less (e.g., 25, 20, 15, 10, 5%) In other embodiments, the specificity is about 75%, 80%, 90%, or 95% when the sensitivity is set at about 85%, 90%, 95% or more. In other embodiments of the above aspects, the method further involves assessing clinical markers of ovarian cancer risk involving age, pre-menopausal status, post-menopausal status, family history, physical examination, imaging results, and history of smoking. In other
30 embodiments of the above aspects, the comparing comprises using a linear model or a non-linear model. In other embodiments of the above aspects, the method further involves detecting CA 125. In other embodiments of the above aspects, the detecting step is by immunoassay or affinity capture. In other embodiments of the above aspects, the

immunoassay comprises affinity capture assay, immunometric assay, heterogeneous chemiluminescence immunometric assay, homogeneous chemiluminescence immunometric assay, ELISA, western blotting, radioimmunoassay, magnetic immunoassay, real-time immunoquantitative PCR (iqPCR) and SERS label free assay. In other embodiments of the
 5 above aspects, the method is carried out in a plate, chip, beads, microfluidic platform, membrane, planar microarray, or suspension array. In still other embodiments, a panel of the invention further contains IL6, IGFBP2 and/or CA 724. In other embodiments of the above aspects, the method reduces the false positive rate to about 25% or less.

The measurement of the panel of biomarkers set forth herein in subject samples
 10 provides information that can be used to characterize an adnexal mass (e.g., an ovarian tumor) and determine whether the subject should be referred to a gynecologic oncologist. In various embodiments, the markers are identified and quantified by immunoassay (e.g., ELISA).

Specifically, biomarker panels of the invention comprise one or more of insulin-like
 15 growth factor binding protein 2 (IGFBP2), Interleukin 6 (IL6), Follicle-stimulating hormone (FSH), and/or Human Epididymis Protein 4 (HE4) polypeptides and fragments thereof as set forth in Table 1. In further embodiments, biomarker panels of the invention further comprise one or more of CA-125-II, Transthyretin/prealbumin, Apolipoprotein A-1, β 2-microglobulin, and/or Transferrin. As described herein, the following particular combinations of biomarkers
 20 were shown to be surprisingly effective in assessing ovarian malignancy of an adnexal mass:

CA125, Prealbumin, Transferrin, and HE4;

CA125, Prealbumin, and HE4;

Insulin-like growth factor binding protein 2 (IGFBP2), Interleukin 6 (IL6), follicle-stimulating hormone (FSH), CA-125-II, and

25 Transthyretin/prealbumin;

Insulin-like growth factor binding protein 2 (IGFBP2), Interleukin 6 (IL6), Human Epididymis Protein 4 (HE4), CA-125-II, and Transthyretin/prealbumin;

CA-125-II, Transthyretin/prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4);

5 CA-125-II, Transthyretin/prealbumin, and Human Epididymis Protein 4 (HE4);

CA 125, Transthyretin/prealbumin, Apolipoprotein A1, β -2-microglobulin, and Transferrin, and Human Epididymis Protein 4 (HE4);

CA-125, HE4, IGFBP2, IL6, and TAG72/ CA72-4;

10 CA-125, APOA1, Transthyretin/prealbumin, B2M, TRF, HE4, IGFBP2, IL6, FSH, CA724;

CA125, APOA1, Transthyretin/prealbumin, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG 72/CA72-4.

CA125, HE4, IGFBP2, IL6, and CA724

CA125, Prealb, TRF, HE4, and CA 72-4;

15 CA125, HE4, CA724;

CA125, Prealb, TRF, and CA724; and

CA125, Prealb, IGFBP2, IL6, and CA724.

20 In particular embodiments, use of these panels unexpectedly increased specificity, increased sensitivity, and/or reduced the rate of false positives identified by conventional panels of biomarkers.

As described in detail herein, any method known in the art can be used to measure a panel of biomarkers. In aspects of the invention, the panel of biomarkers are measured using any immunoassay well known in the art. In embodiments, the immunoassay can be, but is not limited to, ELISA, western blotting, and radioimmunoassay.

25

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology
 5 (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

A “biomarker” or “marker” as used herein generally refers to a protein, nucleic acid
 10 molecule, clinical indicator, or other analyte that is associated with a disease. In one embodiment, a marker of ovarian cancer is differentially present in a biological sample obtained from a subject having or at risk of developing ovarian cancer relative to a reference. A marker is differentially present if the mean or median level of the biomarker present in the sample is statistically different from the level present in a reference. A reference level may
 15 be, for example, the level present in a sample obtained from a healthy control subject or the level obtained from the subject at an earlier timepoint, i.e., prior to treatment. Common tests for statistical significance include, among others, t-test, ANOVA, Kruskal-Wallis, Wilcoxon, Mann-Whitney and odds ratio. Biomarkers, alone or in combination, provide measures of relative likelihood that a subject belongs to a phenotypic status of interest. The differential
 20 presence of a marker of the invention in a subject sample can be useful in characterizing the subject as having or at risk of developing ovarian cancer, for determining the prognosis of the subject, for evaluating therapeutic efficacy, or for selecting a treatment regimen (e.g., selecting that the subject be evaluated and/or treated by a surgeon that specializes in gynecologic oncology).

25 Markers useful in the panels of the invention include, for example, IGFBP2, IL6, FSH, HE4, CA125, transthyretin, transferrin, ApoA1, β 2 microglobulin and CA72-4 proteins, as well as the nucleic acid molecules encoding such proteins. Fragments useful in the methods of the invention are sufficient to bind an antibody that specifically recognizes the protein from which the fragment is derived. The invention includes markers that are
 30 substantially identical to the following sequences. Preferably, such a sequence is at least 85%, 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

By "Insulin-like growth factor binding protein (IGFBP2) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_000588.

5 By "Interleukin 6 (IL6) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_000591.

By "Follicle-stimulating hormone (FSH) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_000501.

10 By "Human Epididymis Protein 4 (HE4) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_006094.

By "Cancer Antigen 125 (CA 125) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to Swiss-Prot Accession number Q8WXI7.

15 By "Transthyretin (Prealbumin) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to Swiss Prot Accession number P02766.

By "Transferrin polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to UniProtKB/TrEMBL Accession number Q06AH7.

20 By "Apolipoprotein A1 (ApoA1) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to Swiss Prot Accession number P02647.

By " β -2 microglobulin polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to SwissProt Accession No. P61769.

25 Select exemplary sequences delineated herein are shown at Figure 19.

By "tumor associated glycoprotein (TAG)-72" is meant the antigen measured using the CA72-4 assay. For example, Guadagni, et al., CANCER RESEARCH 52, 1222-1227, March 1, 1992, describes the measurement of TAG-72 using an immunoradiometric assay kit, CA 72-4, supplied by Centocor (Malvern, PA). Either "TAG-72" or "CA72-4" may be used to refer to the antigen identified by CA72-4 as indicated by the term "TAG-72/CA72-4."

30 By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "alteration" or "change" is meant an increase or decrease. An alteration may be by as little as 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, or by 40%, 50%, 60%, or even by as much as 70%, 75%, 80%, 90%, or 100%.

By "biologic sample" is meant any tissue, cell, fluid, or other material derived from an
5 organism.

By "capture reagent" is meant a reagent that specifically binds a nucleic acid molecule or polypeptide to select or isolate the nucleic acid molecule or polypeptide.

By "clinical aggressiveness" is meant the severity of the neoplasia. Aggressive neoplasias are more likely to metastasize than less aggressive neoplasias. While conservative
10 methods of treatment are appropriate for less aggressive neoplasias, more aggressive neoplasias require more aggressive therapeutic regimens.

As used herein, the terms "determining", "assessing", "assaying", "measuring" and "detecting" refer to both quantitative and qualitative determinations, and as such, the term "determining" is used interchangeably herein with "assaying," "measuring," and the like.
15 Where a quantitative determination is intended, the phrase "determining an amount" of an analyte and the like is used. Where a qualitative and/or quantitative determination is intended, the phrase "determining a level" of an analyte or "detecting" an analyte is used.

The term "subject" or "patient" refers to an animal which is the object of treatment, observation, or experiment. By way of example only, a subject includes, but is not limited to,
20 a mammal, including, but not limited to, a human or a non-human mammal, such as a non-human primate, murine, bovine, equine, canine, ovine, or feline.

By "Marker profile" is meant a characterization of the expression or expression level of two or more polypeptides or polynucleotides.

By "neoplasia" is meant any disease that is caused by or results in inappropriately
25 high levels of cell division, inappropriately low levels of apoptosis, or both. Examples of cancers include, without limitation, prostate cancer, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic
30 leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma,

lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, 5 hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, 10 oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). Lymphoproliferative disorders are also considered to be proliferative diseases.

The term "ovarian cancer" refers to both primary ovarian tumors as well as metastases of the primary ovarian tumors that may have settled anywhere in the body.

15 The term "ovarian cancer status" refers to the status of the disease in the patient. Examples of types of ovarian cancer statuses include, but are not limited to, the subject's risk of cancer, the presence or absence of disease, the stage of disease in a patient, and the effectiveness of treatment of disease. In embodiments, a subject identified as having a pelvic mass is assessed to identify if their ovarian cancer status is benign or malignant.

20 Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a 25 double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

30 For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g.,

formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying
5 additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment,
10 hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

15 For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably
20 less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42° C in 15
25 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl.
30 Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%,
 5 more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705,
 10 BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and
 15 phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "reference" is meant a standard of comparison. For example, the marker level(s) present in a patient sample may be compared to the level of the marker in a corresponding
 20 healthy cell or tissue or in a diseased cell or tissue (e.g., a cell or tissue derived from a subject having ovarian cancer). In particular embodiments, the IGFBP2, IL6, FSH, HE4, CA 125; Transthyretin, Transferrin, TAG-72/CA 72-4 polypeptide level present in a patient sample may be compared to the level of said polypeptide present in a corresponding sample obtained at an earlier time point (i.e., prior to treatment), to a healthy cell or tissue or a neoplastic cell
 25 or tissue that lacks a propensity to metastasize. As used herein, the term "sample" includes a biologic sample such as any tissue, cell, fluid, or other material derived from an organism.

By "specifically binds" is meant a compound (e.g., antibody) that recognizes and binds a molecule (e.g., polypeptide), but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample.

30 The accuracy of a diagnostic test can be characterized using any method well known in the art, including, but not limited to, a Receiver Operating Characteristic curve ("ROC curve"). An ROC curve shows the relationship between sensitivity and specificity. Sensitivity is the percentage of true positives that are predicted by a test to be positive, while

specificity is the percentage of true negatives that are predicted by a test to be negative. An ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. Thus, an increase in sensitivity will be accompanied by a decrease in specificity. The closer the curve follows the left axis and then the top edge of the ROC space, the more accurate the test. Conversely, the closer the curve comes to the 45-degree diagonal of the ROC graph, the less accurate the test. The area under the ROC is a measure of test accuracy. The accuracy of the test depends on how well the test separates the group being tested into those with and without the disease in question. An area under the curve (referred to as "AUC") of 1 represents a perfect test. In embodiments, biomarkers and diagnostic methods of the present invention have an AUC greater than 0.50, greater than 0.60, greater than 0.70, greater than 0.80, or greater than 0.9.

Other useful measures of the utility of a test are positive predictive value ("PPV") and negative predictive value ("NPV"). PPV is the percentage of actual positives who test as positive. NPV is the percentage of actual negatives that test as negative.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

Any compounds, compositions, or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

As used herein, the singular forms "a", "an", and "the" include plural forms unless the context clearly dictates otherwise. Thus, for example, reference to "a biomarker" includes reference to more than one biomarker.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to."

As used herein, the terms “comprises,” “comprising,” “containing,” “having” and the like can have the meaning ascribed to them in U.S. Patent law and can mean “includes,” “including,” and the like; “consisting essentially of” or “consists essentially” likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of
 5 more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Figure 1 is a plot depicting distribution of absolute improvement in specificity at fixed 95% sensitivity in testing over 100 rounds of cross-validation of multivariate models using nine markers Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), insulin growth factor binding protein 2 (IGFBP2), interleukin-6 (IL6), Human Epididymis Protein 4
 15 (HE4), and follicle stimulating hormone (FSH). Mean and median absolute improvement were 24.1% (95% CI: 21.0-27.2%) and 24.6%, respectively, over Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II).

Figure 2 is a plot depicting distribution of absolute improvement in specificity for a
 20 model using 8 biomarkers: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, and FSH over the specificity provided by the following markers: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) at fixed 95% sensitivity in testing over
 25 100 rounds of cross-validation of multivariate models. Mean and median absolute improvement were 22.0% (95% CI: 18.8-25.3%) and 23.3%, respectively.

Figure 3 shows plots depicting Distribution of Specificity in linear models at 90% Sensitivity in 30 cross-validation runs – (50/50 split between training (left panel) and testing (right panel). Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH.

30 Figures 4A and 4B show plots depicting Distribution of Specificity in nonlinear models. Figure 4A shows plots depicting Distribution of Specificity at 90% Sensitivity in 30 cross-validation runs – (50/50 split between training (left panel) and testing (right panel).

Figure 4B shows plots depicting Distribution of Specificity at 95% Sensitivity in 100 cross-validation runs – (50/50 split between training (left panel) and testing (right panel). Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

Figure 5 shows plots depicting Distribution of Sensitivity in nonlinear models for stage 1 cases at 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between training (left panel) and testing (right panel). Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

Figure 6 shows plots depicting comparison of distributions of Sensitivity (nonlinear models) between invasive (red) and LMP (blue) ovarian tumors at 95% overall Sensitivity (on training data) in 100 cross-validation runs – (50/50 split between training (left panel) and testing (right panel). Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

Figures 7A-7D show plots depicting comparison of distributions of Sensitivity in nonlinear models among ovarian cancer histologic subtypes. Figure 7A is a plot depicting Distribution of Specificity at 95% overall Sensitivity (on training data) in 100 cross-validation runs – (50/50 split between training and testing) for serous ovarian cancer. Figure 7B is a plot depicting Distribution of Specificity at 95% overall Sensitivity (on training data) in 100 cross-validation runs – (50/50 split between training and testing) for endometrioid ovarian cancer. Figure 7C is a plot depicting Distribution of Specificity at 95% overall Sensitivity (on training data) in 100 cross-validation runs – (50/50 split between training and testing) for mucinous ovarian cancer. Figure 7D is a plot depicting Distribution of Specificity at 95% overall Sensitivity (on training data) in 100 cross-validation runs – (50/50 split between training and testing) for clear cell ovarian cancer. Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH.

Figures 8A-8D show plots depicting Distributions of Sensitivity and Specificity in training and testing sets between Pre- (red) and Post- (blue) Menopausal patients. Figure 8A is a plot depicting Distributions of Sensitivity between Pre- (red) and Post- (blue) Menopausal patients at cutoff corresponding to 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between training and testing) in the training set. Figure 8B is a plot depicting Distributions of Specificity between Pre- (red) and Post- (blue) Menopausal patients at cutoff corresponding to 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between training and testing) in the training set. Figure 8C is a plot depicting Distributions of Sensitivity between Pre- (red) and Post- (blue) Menopausal patients at cutoff corresponding to 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between

training and testing) in the testing set. Figure 8D is a plot depicting Distributions of Specificity between Pre- (red) and Post- (blue) Menopausal patients at cutoff corresponding to 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between training and testing) in the testing set. Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH.

5 Figure 9 shows plots depicting Distribution of improvement in Specificity of nonlinear models over the following panel of markers: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), both computed using all benign controls at cutoffs corresponding to 95% overall Sensitivity in 100 cross-validation runs – (50/50 split between training and
10 testing). Red hollow bars: evaluated on combined training and testing sets, blue solid bars: evaluated on test sets only. Panel: CA125, prealbumin, IGFBP2, IL6, and FSH.

Figure 10 shows plots depicting Distribution of ROC/AUC for a nonlinear model in 100 cross-validation runs – (50/50 split between training (left panel) and testing (right panel). Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

15 Figure 11 shows plots depicting Distribution of Specificity for a nonlinear model at 95% Sensitivity in 100 cross-validation runs (50/50 split between training (left panel) and testing (right panel). Red hollow bars: HE4 replacing FSH. Blue: Panel: CA125, prealbumin, IGFBP2, IL6, and FSH; Red Outline: CA125, prealbumin, IGFBP2, IL6, and HE4

20 Figure 12 shows plots depicting Distribution of Specificity for a nonlinear model at 90% Sensitivity in 100 cross-validation runs (50/50 split between training (left panel) and testing (right panel). Red hollow bars: Red Outline: CA125, prealbumin, IGFBP2, IL6, and HE4. Blue: Panel: CA125, prealbumin, IGFBP2, IL6, and FSH

Figure 13 shows plots depicting Distribution of Specificity for panel of markers
25 described in the prior art and in clinical use for characterizing ovarian cancer: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) +HE4 linear model at 90% Sensitivity in 100 cross-validation runs (50/50 split between training (left panel) and testing (right panel). Red hollow bars: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1),
30 beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) +HE4 linear model; Solid blue: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II). Cutoffs are selected to maintain 90% sensitivity.

Figure 14 shows plots depicting Distributions of Specificities for CA125+Preabl+TRF+HE4 and CA125+Preabl+TRF+HE4 nonlinear models at 90% Sensitivity in 100 cross-validation runs (50/50 split between training (left panel) and testing (right panel). Red: CA125+Preabl+TRF+HE4; Blue: CA125+Preabl+TRF+HE4; Green: 5 Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II).

Figure 15 shows plots depicting Distributions of ROC/AUCs for CA125+Preabl+TRF+HE4 and CA125+Preabl+TRF+HE4 nonlinear models at 90% Sensitivity in 100 cross-validation runs (50/50 split between training (left panel) and testing 10 (right panel). Red: CA125+Preabl+TRF+HE4; Blue: CA125+Preabl+TRF+HE4; Green: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II).

Figures 16A-16C shows plots depicting Distribution of biomarkers before and after preprocessing and normalization. Figure 16A is a plot depicting Distribution of biomarkers 15 prior to transformation and normalization. Figure 16B is a plot depicting Distribution of biomarkers after transformation. Figure 16C is a plot depicting Distribution of biomarkers after transformation and normalization to z-scores.

Figures 17A-17D shows plots depicting Bootstrap estimated ROC/AUC. Figure 17A is a plot depicting Bootstrap estimated ROC/AUC for IGFBP2, AUC=0.8341. Figure 17B is 20 a plot depicting Bootstrap estimated ROC/AUC for IL6, AUC=0.8039. Figure 17C is a plot depicting Bootstrap estimated ROC/AUC for FSH, AUC=0.6101. Figure 17D is a plot depicting Bootstrap estimated ROC/AUC for HE4, AUC=0.9069.

Figures 18A and 18B depict receiver operating characteristic curves for a single training/test run using a linear model. Figure 18A depicts receiver operating characteristic 25 curves for a training run using a linear model. Figure 18B depicts receiver operating characteristic curves for a test run using a linear model.

Figure 19 provides exemplary sequences of human Insulin-like growth factor binding protein (IGFBP2); Interleukin 6 (IL6); Follicle-stimulating hormone (FSH); Human Epididymis Protein 4 (HE4); Cancer Antigen 125 (CA 125); Transthyretin (prealbumin); 30 Transferrin; apolipoprotein A-1 (ApoA1), and β 2-microglobulin (B2MG) polypeptides.

Figure 20 is a biplot of supervised component analysis results using all ten (10) biomarkers (CA125, APOA1, PREALB, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG 72/CA724). Hollow red circles: training samples for cancer; hollow green circles: training

samples for benign; Solid red circles: testing samples for cancer; solid green circles: testing samples for benign. Markers that are to the right of the center vertical axis (First component) are upregulated in ovarian cancer. Markers to left of the center axis are down regulated. The length of the line is an indication of the contribution of each marker to the characterization of ovarian cancer. Because markers CA724, IGFBP2, and IL6 have equal contributions and are all upregulated, these markers are typically interchangeable in any of the panels where they are listed. As shown, Prealbumin, CA125 and HE4 provide one preferred panel for characterizing ovarian cancer. Any one of IL6, IGFBP2 or CA 724 may be added to this panel.

Figure 21 shows receiver-operating characteristic (ROC) curves of a linear classifier (represented by the x-axis in figure 1) on the training data (left) and testing data (right) set forth in Figure 20.

Figure 22 is a graph showing results using CA125, HE4, IGFBP2, IL6, CA724 (biomarkers with the best ranking in bootstrap feature selection). These results were obtained using a Linear Model. Shown is the distribution of Specificity at 90% Sensitivity in 100 cross-validation runs. (50/50 split between training and testing). Blue: training, Red: cross-validation.

Figure 23 is a graph showing results using CA125, Prealb, TRF, HE4, CA724. These results were obtained using a Linear Model. Shown is the distribution of Specificity at 90% Sensitivity in 100 cross-validation runs. (50/50 split between training and testing). Blue: training, Red: cross-validation.

Figure 24 is a graph showing results using markers CA125, HE4, CA724. These results were obtained using a Linear Model. Shown is the distribution of Specificity at 90% Sensitivity in 100 cross-validation runs. (50/50 split between training and testing). Blue: training, Red: cross-validation.

Figure 25 is a graph showing results using markers CA125, Prealb, TRF, CA724 (without HE4). These results were obtained using a Linear Model. Shown is the distribution of Specificity at 90% Sensitivity in 100 cross-validation runs. (50/50 split between training and testing). Blue: training, Red: cross-validation.

Figure 26 is a graph showing results using markers CA125, Prealb, IGFBP2, IL6, CA724 (without HE4). These results were obtained using a Linear Model. Shown is the distribution of Specificity at 90% Sensitivity in 100 cross-validation runs, (50/50 split between training and testing). Blue: training, Red: cross-validation.

5

DETAILED DESCRIPTION OF THE INVENTION

The invention comprises panels of biomarkers and the use of such panels for characterizing ovarian cancer.

The invention is based, at least in part, on the discovery that panels of the invention advantageously enhance specificity (e.g., to about mean/median 70%, 75%, 80%, 85%, 90%) at a sensitivity of 90% or 95% and reduce false positives identified by conventional panels of biomarkers.

In particular, the invention provides panels comprising or consisting of the following sets of markers:

15 CA125, Prealbumin, Transferrin, and HE4;

CA125, Prealbumin, and HE4;

Insulin-like growth factor binding protein 2 (IGFBP2), Interleukin 6 (IL6), follicle-stimulating hormone (FSH), CA-125-II, and Transthyretin/prealbumin;

20

Insulin-like growth factor binding protein 2 (IGFBP2), Interleukin 6 (IL6), Human Epididymis Protein 4 (HE4), CA-125-II, and Transthyretin/prealbumin;

CA-125-II, Transthyretin/prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4);

25 CA-125-II, Transthyretin/prealbumin, and Human Epididymis Protein 4 (HE4);

CA 125, Transthyretin/prealbumin, Apolipoprotein A1, β -2-microglobulin, and Transferrin, and Human Epididymis Protein 4 (HE4);

CA-125, HE4, IGFBP2, IL6, and TAG72/ CA72-4;

30 CA-125, APOA1, Transthyretin/prealbumin, B2M, TRF, HE4, IGFBP2, IL6, FSH, CA724;

CA125, APOA1, Transthyretin/prealbumin, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG 72/CA72-4.

CA125, HE4, IGFBP2, IL6, and CA724

CA125, Prealb, TRF, HE4, and CA 72-4;

5 CA125, HE4, CA724;

CA125, Prealb, TRF, and CA724; and

CA125, Prealb, IGFBP2, IL6, and CA724.

The invention further features the use of such panels for characterizing ovarian cancer (e.g., assessing risk of malignancy, diagnosis, prognosis). In particular, the use of such
10 panels provides methods for pre-surgically characterizing a pelvic mass in a subject and identifying subjects that might benefit from evaluation/treatment by a gynecological oncologist.

Ovarian Cancer

Ovarian tumors are being detected with increasing frequency in women of all ages,
15 yet there is no standardized or reliable method to determine which are malignant prior to surgery. In 1994, the National Institutes of Health (NIH) released a consensus statement indicating that women with ovarian masses having been identified preoperatively as having a significant risk of ovarian cancer should be given the option of having their surgery performed by a gynecologic oncologist. At present, the National Comprehensive Cancer
20 Network (NCCN), the Society of Gynecologic Oncologists (SGO), SOGC clinical practice guidelines, Standing Subcommittee on Cancer of the Medical Advisory Committee, and several other published statements, all recommend that women with ovarian cancer be under the care of a gynecologic oncologist (GO).

Recent publications on breast, bladder, gastrointestinal, and ovarian cancers have
25 reported improved outcome when cancer management involves a surgical specialist. In addition, a recent meta-analysis of 18 ovarian cancer studies found that the early involvement of a gynecologic oncologist, rather than a general surgeon or general gynecologist, improved patient outcomes. The authors concluded: 1) subjects with early stage disease are more likely to have comprehensive surgical staging, facilitating appropriate adjuvant chemotherapy, 2)

subjects with advanced disease are more likely to receive optimal cytoreductive surgery, and
 3) subjects with advanced disease have an improved median and overall 5-year survival.
 Despite the availability of this important information, only a fraction of women with
 malignant ovarian tumors (an estimated 33%) are referred to a gynecologic oncologist for the
 5 primary surgery. Based on reported patterns of care for ovarian cancer management, the
 majority of women in the United States may not be receiving optimal care for this disease.

The decision for operative removal of an ovarian tumor, and whether a generalist or
 specialist should perform the surgery, is based on interpretations of physical examination,
 imaging studies, laboratory tests, and clinical judgment. Pelvic examination alone is
 10 inadequate to reliably detect or differentiate ovarian tumors, particularly in early stages when
 ovarian cancer treatment is most successful. Examination has also been eliminated from the
 Prostate, Lung, Colorectal and Ovarian cancer screening trial algorithm. Pelvic ultrasound is
 clinically useful and the least expensive imaging modality, but has limitations in consistently
 identifying malignant tumors. In general, nearly all unilocular cysts are benign, whereas
 15 complex cystic tumors with solid components or internal papillary projections are more likely
 to be malignant. CA 125 has been used alone or in conjunction with other tests in an effort to
 establish risk of malignancy. Unfortunately, CA 125 has low sensitivity (50%) in early stage
 ovarian cancers, and low specificity resultant from numerous false positives in both pre- and
 postmenopausal women.

20 The American College of Obstetrics and Gynecology (ACOG) and the SGO have
 published referral guidelines for patients with a pelvic mass. These guidelines include:
 patient age, serum CA 125 level, physical examination, imaging results, and family history.
 This referral strategy has been evaluated both retrospectively and prospectively. In a single
 institution review, Dearing and colleagues concluded that the guidelines were useful in
 25 predicting advanced stage ovarian cancer, but “performed poorly in identifying early-stage
 disease, especially in premenopausal women, primarily due to lack of early markers and signs
 of ovarian cancer”.

BIOMARKERS

30 In particular embodiments, a biomarker is an organic biomolecule that is differentially
 present in a sample taken from a subject of one phenotypic status (*e.g.*, having a disease) as
 compared with another phenotypic status (*e.g.*, not having the disease). A biomarker is

differentially present between different phenotypic statuses if the mean or median expression level of the biomarker in the different groups is calculated to be statistically significant. Common tests for statistical significance include, among others, t-test, ANOVA, Kruskal-Wallis, Wilcoxon, Mann-Whitney and odds ratio. Biomarkers, alone or in combination, provide measures of relative risk that a subject belongs to one phenotypic status or another. Therefore, they are useful as markers for characterizing a disease.

BIOMARKERS FOR OVARIAN CANCER

The invention provides a panel of polypeptide biomarkers that are differentially present in subjects having ovarian cancer, in particular, a benign vs. malignant pelvic mass. The biomarkers of this invention are differentially present depending on ovarian cancer status, including, subjects having ovarian cancer vs. subjects that do not have ovarian cancer.

The biomarker panel of the invention comprises one or more of the biomarkers presented in the following Table 1.

15

Table 1

Biomarker	Differential Regulation in ovarian cancer
ApoA1	Decreased
Beta2 Microglobulin (B2M)	Increased
Insulin-like growth factor binding protein (IGFBP2)	Increased
Interleukin 6 (IL6)	Increased
Follicle-stimulating hormone (FSH)	Increased
Human Epididymis Protein 4 (HE4)	Increased
Cancer Antigen 125 (CA 125)	Increased
Transthyretin (prealbumin)	Decreased
Transferrin	Decreased
TAG 72/CA 72-4	Increased

As would be understood, references herein to a biomarker of Table 1, a panel of biomarkers, or other similar phrase indicates one or more of the biomarkers set forth in Table 1 or otherwise described herein.

5 A biomarker of the invention may be detected in a biological sample of the subject (e.g., tissue, fluid), including, but not limited to, blood, blood serum, plasma, saliva, urine, ascites, cyst fluid, a homogenized tissue sample (e.g., a tissue sample obtained by biopsy), a cell isolated from a patient sample, and the like.

The invention provides panels comprising isolated biomarkers. The biomarkers can be isolated from biological fluids, such as urine or serum. They can be
10 isolated by any method known in the art. In certain embodiments, this isolation is accomplished using the mass and/or binding characteristics of the markers. For example, a sample comprising the biomolecules can be subject to chromatographic fractionation and subject to further separation by, e.g., acrylamide gel electrophoresis. Knowledge of the identity of the biomarker also allows their isolation by immunoaffinity chromatography. By
15 “isolated biomarker” is meant at least 60%, by weight, free from proteins and naturally-occurring organic molecules with which the marker is naturally associated. Preferably, the preparation is at least 75%, more preferably 80, 85, 90 or 95% pure or at least 99%, by weight, a purified marker.

20 ***Insulin-like growth factor binding protein (IGFBP2)***

One exemplary biomarker present in the panel of the invention is IGFBP2. IGFBP2 is a 328 amino acid protein (NCBI Accession number NP_000588). The amino acid sequence of an exemplary IGFBP2 polypeptide is set forth in Figure 19. Antibodies to IGFBP2 can be made using any method well known in the art, or can be purchased from, for
25 example, Santa Cruz Biotechnology, Inc. (e.g., Catalog Number sc-130070). In aspects of the invention, IGFBP2 is upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

Interleukin 6 (IL6)

One exemplary biomarker present in the panel of the invention is IL6. IL6 is a 212
30 amino acid protein (NCBI Accession number NP_000591). The amino acid sequence of an exemplary IL6 polypeptide is set forth in Figure 19. Antibodies to IL6 can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (e.g., Catalog Number sc-1265). In

aspects of the invention, IL6 is upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

Follicle-stimulating hormone (FSH)

5 One exemplary biomarker present in the panel of the invention is FSH. FSH is a 128 amino acid protein (NCBI Accession number NP_000501). The amino acid sequence of an exemplary FSH polypeptide is set forth in Figure 19. Antibodies to FSH can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (e.g., Catalog Number sc-57149). In aspects of the invention, FSH is
10 upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

Human Epididymis Protein 4 (HE4)

 One exemplary biomarker present in the panel of the invention is HE4. HE4 is a 124
15 amino acid protein (NCBI Accession number NP_006094). The amino acid sequence of an exemplary HE4 polypeptide is set forth in Figure 19. Antibodies to HE4 can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (Catalog Number sc-27570). In aspects of the invention, HE4 is
20 upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

Cancer Antigen 125 (CA 125)

 One exemplary biomarker present in the panel of the invention is CA 125. CA 125 is a 22152 amino acid protein (Swiss-Prot Accession number Q8WXI7). The amino acid
25 sequence of an exemplary CA 125 polypeptide is set forth in Figure 19. Antibodies to CA 125 can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (Catalog Number sc-52095). In aspects of the invention, CA 125 is upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

30

Transthyretin (Prealbumin)

Another exemplary biomarker present in the panel of the invention is a form of pre-albumin, also referred to herein as transthyretin. Transthyretin is a 147 amino acid protein (Swiss Prot Accession number P02766). The amino acid sequence of an exemplary transthyretin polypeptide is set forth in Figure 19. Antibodies to transthyretin can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (Catalog Number sc-13098). In aspects of the invention, transthyretin is downregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

10 ***Transferrin***

Transferrin is another exemplary biomarker of the panel of biomarkers of the invention. Transferrin is a 698 amino acid protein (UniProtKB/TrEMBL Accession number Q06AH7). The amino acid sequence of an exemplary transferring polypeptide is set forth in Figure 19. Antibodies to transferrin can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (Catalog Number sc-52256). In aspects of the invention, transferrin is downregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

Apolipoprotein A1

20 Apolipoprotein A1, also referred to herein as “Apo A1” is another exemplary biomarker in the panel of biomarkers of the invention. Apo A1 is a 267 amino acid protein (Swiss Prot Accession number P02647). The amino acid sequence of an exemplary Apo A1 is set forth in Figure 19. Antibodies to Apolipoprotein A1 can be made using any method well known in the art, or can be purchased from, for example, Santa Cruz Biotechnology, Inc. (Catalog Number sc-130503). In aspects of the invention, Apo A1 is downregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

β-2 microglobulin

30 One exemplary biomarker that is useful in the methods of the present invention is β2-microglobulin. β2-microglobulin is described as a biomarker for ovarian cancer in US provisional patent publication 60/693,679, filed June 24, 2005 (Fung et al.). The mature form

of β 2-microglobulin is a 99 amino acid protein derived from an 119 amino acid precursor (GI:179318; SwissProt Accession No. P61769). The amino acid sequence of an exemplary β 2-microglobulin polypeptide is set forth in Figure 19. The mature form of β 2-microglobulin consist of residues 21-119 of the β 2-microglobulin set forth in Figure 19. β 2-microglobulin is recognized by antibodies. Such antibodies can be made using any method well known in the art, and can also be commercially purchased from, e.g., Abcam (catalog AB759). In aspects of the invention, β 2-microglobulin is upregulated in subjects with ovarian cancer as compared to subjects that do not have ovarian cancer.

10 BIOMARKERS AND DIFFERENT FORMS OF A PROTEIN

Proteins frequently exist in a sample in a plurality of different forms. These forms can result from pre- and/or post-translational modification. Pre-translational modified forms include allelic variants, splice variants and RNA editing forms. Post-translationally modified forms include forms resulting from proteolytic cleavage (e.g., cleavage of a signal sequence or fragments of a parent protein), glycosylation, phosphorylation, lipidation, oxidation, methylation, cysteinylolation, sulphonation and acetylation. When detecting or measuring a protein in a sample, any or all of the forms may be measured to determine the level of biomarker or a form of interest is measured. The ability to differentiate between different forms of a protein depends upon the nature of the difference and the method used to detect or measure the protein. For example, an immunoassay using a monoclonal antibody will detect all forms of a protein containing the epitope and will not distinguish between them. However, a sandwich immunoassay that uses two antibodies directed against different epitopes on a protein will detect all forms of the protein that contain both epitopes and will not detect those forms that contain only one of the epitopes. Distinguishing different forms of an analyte or specifically detecting a particular form of an analyte is referred to as “resolving” the analyte.

Mass spectrometry is a particularly powerful methodology to resolve different forms of a protein because the different forms typically have different masses that can be resolved by mass spectrometry. Accordingly, if one form of a protein is a superior biomarker for a disease than another form of the biomarker, mass spectrometry may be able to specifically

detect and measure the useful form where traditional immunoassay fails to distinguish the forms and fails to specifically detect to useful biomarker.

One useful methodology combines mass spectrometry with immunoassay. For example, a biospecific capture reagent (e.g., an antibody, aptamer, Affibody, and the like that
5 recognizes the biomarker and other forms of it) is used to capture the biomarker of interest. In embodiments, the biospecific capture reagent is bound to a solid phase, such as a bead, a plate, a membrane or an array. After unbound materials are washed away, the captured analytes are detected and/or measured by mass spectrometry. This method will also result in the capture of protein interactors that are bound to the proteins or that are otherwise
10 recognized by antibodies and that, themselves, can be biomarkers. Various forms of mass spectrometry are useful for detecting the protein forms, including laser desorption approaches, such as traditional MALDI or SELDI, electrospray ionization, and the like.

Thus, when reference is made herein to detecting a particular protein or to measuring the amount of a particular protein, it means detecting and measuring the protein with or
15 without resolving various forms of protein. For example, the step of “detecting β -2 microglobulin” includes measuring β -2 microglobulin by means that do not differentiate between various forms of the protein (e.g., certain immunoassays) as well as by means that differentiate some forms from other forms or that measure a specific form of the protein.

20 DETECTION OF BIOMARKERS FOR OVARIAN CANCER

The biomarkers of this invention can be detected by any suitable method. The methods described herein can be used individually or in combination for a more accurate detection of the biomarkers (e.g., biochip in combination with mass spectrometry, immunoassay in combination with mass spectrometry, and the like).

25 Detection paradigms that can be employed in the invention include, but are not limited to, optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, *e.g.*, multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence,
30 absorbance, reflectance, transmittance, and birefringence or refractive index (*e.g.*, surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

These and additional methods are described *infra*.

Detection by Immunoassay

In particular embodiments, the biomarkers of the invention are measured by immunoassay. Immunoassay typically utilizes an antibody (or other agent that specifically binds the marker) to detect the presence or level of a biomarker in a sample. Antibodies can be produced by methods well known in the art, *e.g.*, by immunizing animals with the biomarkers. Biomarkers can be isolated from samples based on their binding characteristics. Alternatively, if the amino acid sequence of a polypeptide biomarker is known, the polypeptide can be synthesized and used to generate antibodies by methods well known in the art.

This invention contemplates traditional immunoassays including, for example, Western blot, sandwich immunoassays including ELISA and other enzyme immunoassays, fluorescence-based immunoassays, chemiluminescence,. Nephelometry is an assay done in liquid phase, in which antibodies are in solution. Binding of the antigen to the antibody results in changes in absorbance, which is measured. Other forms of immunoassay include magnetic immunoassay, radioimmunoassay, and real-time immunoquantitative PCR (iqPCR).

Immunoassays can be carried out on solid substrates (*e.g.*, chips, beads, microfluidic platforms, membranes) or on any other forms that supports binding of the antibody to the marker and subsequent detection. A single marker may be detected at a time or a multiplex format may be used. Multiplex immunoanalysis may involve planar microarrays (protein chips) and bead-based microarrays (suspension arrays).

In a SELDI-based immunoassay, a biospecific capture reagent for the biomarker is attached to the surface of an MS probe, such as a pre-activated ProteinChip array. The biomarker is then specifically captured on the biochip through this reagent, and the captured biomarker is detected by mass spectrometry.

Detection by Biochip

In aspects of the invention, a sample is analyzed by means of a biochip (also known as a microarray). The polypeptides and nucleic acid molecules of the invention are useful as hybridizable array elements in a biochip. Biochips generally comprise solid substrates and have a generally planar surface, to which a capture reagent (also called an adsorbent or

affinity reagent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there.

The array elements are organized in an ordered fashion such that each element is present at a specified location on the substrate. Useful substrate materials include
5 membranes, composed of paper, nylon or other materials, filters, chips, glass slides, and other solid supports. The ordered arrangement of the array elements allows hybridization patterns and intensities to be interpreted as expression levels of particular genes or proteins. Methods for making nucleic acid microarrays are known to the skilled artisan and are described, for example, in U.S. Pat. No. 5,837,832, Lockhart, et al. (Nat. Biotech. 14:1675-1680, 1996), and
10 Schena, et al. (Proc. Natl. Acad. Sci. 93:10614-10619, 1996). Methods for making polypeptide microarrays are described, for example, by Ge (Nucleic Acids Res. 28: e3. i-e3. vii, 2000), MacBeath et al., (Science 289:1760-1763, 2000), Zhu et al. (Nature Genet. 26:283-289), and in U.S. Pat. No. 6,436,665.

15 ***Detection by Protein Biochip***

In aspects of the invention, a sample is analyzed by means of a protein biochip (also known as a protein microarray). Such biochips are useful in high-throughput low-cost screens to identify alterations in the expression or post-translation modification of a polypeptide of the invention, or a fragment thereof. In embodiments, a protein biochip of the
20 invention binds a biomarker present in a subject sample and detects an alteration in the level of the biomarker. Typically, a protein biochip features a protein, or fragment thereof, bound to a solid support. Suitable solid supports include membranes (e.g., membranes composed of nitrocellulose, paper, or other material), polymer-based films (e.g., polystyrene), beads, or glass slides. For some applications, proteins (e.g., antibodies that bind a marker of the
25 invention) are spotted on a substrate using any convenient method known to the skilled artisan (e.g., by hand or by inkjet printer).

In embodiments, the protein biochip is hybridized with a detectable probe. Such probes can be polypeptide, nucleic acid molecules, antibodies, or small molecules. For some applications, polypeptide and nucleic acid molecule probes are derived from a biological
30 sample taken from a patient, such as a bodily fluid (such as blood, blood serum, plasma, saliva, urine, ascites, cyst fluid, and the like); a homogenized tissue sample (e.g., a tissue sample obtained by biopsy); or a cell isolated from a patient sample. Probes can also include

- antibodies, candidate peptides, nucleic acids, or small molecule compounds derived from a peptide, nucleic acid, or chemical library. Hybridization conditions (e.g., temperature, pH, protein concentration, and ionic strength) are optimized to promote specific interactions. Such conditions are known to the skilled artisan and are described, for example, in Harlow,
- 5 E. and Lane, D., *Using Antibodies : A Laboratory Manual*. 1998, New York: Cold Spring Harbor Laboratories. After removal of non-specific probes, specifically bound probes are detected, for example, by fluorescence, enzyme activity (e.g., an enzyme-linked calorimetric assay), direct immunoassay, radiometric assay, or any other suitable detectable method known to the skilled artisan.
- 10 Many protein biochips are described in the art. These include, for example, protein biochips produced by CIPHERGEN Biosystems, Inc. (Fremont, CA), Zyomyx (Hayward, CA), Packard BioScience Company (Meriden, CT), Phyllos (Lexington, MA), Invitrogen (Carlsbad, CA), Biacore (Uppsala, Sweden) and Procognia (Berkshire, UK). Examples of such protein biochips are described in the following patents or published patent applications:
- 15 U.S. Patent Nos. 6,225,047; 6,537,749; 6,329,209; and 5,242,828; PCT International Publication Nos. WO 00/56934; WO 03/048768; and WO 99/51773.

Detection by Nucleic Acid Biochip

- In aspects of the invention, a sample is analyzed by means of a nucleic acid biochip
- 20 (also known as a nucleic acid microarray). To produce a nucleic acid biochip, oligonucleotides may be synthesized or bound to the surface of a substrate using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/251116 (Balteschweiler et al.). Alternatively, a gridded array may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum
- 25 system, thermal, UV, mechanical or chemical bonding procedure.

- A nucleic acid molecule (e.g. RNA or DNA) derived from a biological sample may be used to produce a hybridization probe as described herein. The biological samples are generally derived from a patient, e.g., as a bodily fluid (such as blood, blood serum, plasma, saliva, urine, ascites, cyst fluid, and the like); a homogenized tissue sample (e.g., a tissue
- 30 sample obtained by biopsy); or a cell isolated from a patient sample. For some applications, cultured cells or other tissue preparations may be used. The mRNA is isolated according to standard methods, and cDNA is produced and used as a template to make complementary RNA suitable for hybridization. Such methods are well known in the art. The RNA is

amplified in the presence of fluorescent nucleotides, and the labeled probes are then incubated with the microarray to allow the probe sequence to hybridize to complementary oligonucleotides bound to the biochip.

Incubation conditions are adjusted such that hybridization occurs with precise
5 complementary matches or with various degrees of less complementarity depending on the degree of stringency employed. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g.,
10 formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, of at least about 37°C., or of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or
15 exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In embodiments, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA
20 (ssDNA). In other embodiments, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The removal of nonhybridized probes may be accomplished, for example, by washing. The washing steps that follow hybridization can also vary in stringency. Wash
25 stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash
30 steps will ordinarily include a temperature of at least about 25°C, of at least about 42°C, or of at least about 68°C. In embodiments, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at

42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In other embodiments, wash steps will occur at 68 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art.

Detection system for measuring the absence, presence, and amount of hybridization
5 for all of the distinct nucleic acid sequences are well known in the art. For example, simultaneous detection is described in Heller et al., Proc. Natl. Acad. Sci. 94:2150-2155, 1997. In embodiments, a scanner is used to determine the levels and patterns of fluorescence.

Detection by Mass Spectrometry

10 In aspects of the invention, the biomarkers of this invention are detected by mass spectrometry (MS). Mass spectrometry is a well known tool for analyzing chemical compounds that employs a mass spectrometer to detect gas phase ions. Mass spectrometers are well known in the art and include, but are not limited to, time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids
15 of these. The method may be performed in an automated (Villanueva, *et al.*, *Nature Protocols* (2006) 1(2):880-891) or semi-automated format. This can be accomplished, for example with the mass spectrometer operably linked to a liquid chromatography device (LC-MS/MS or LC-MS) or gas chromatography device (GC-MS or GC-MS/MS). Methods for performing mass spectrometry are well known and have been disclosed, for example, in US
20 Patent Application Publication Nos: 20050023454; 20050035286; US Patent No. 5,800,979 and the references disclosed therein.

Laser Desorption/Ionization

In embodiments, the mass spectrometer is a laser desorption/ionization mass
25 spectrometer. In laser desorption/ionization mass spectrometry, the analytes are placed on the surface of a mass spectrometry probe, a device adapted to engage a probe interface of the mass spectrometer and to present an analyte to ionizing energy for ionization and introduction into a mass spectrometer. A laser desorption mass spectrometer employs laser energy, typically from an ultraviolet laser, but also from an infrared laser, to desorb analytes
30 from a surface, to volatilize and ionize them and make them available to the ion optics of the mass spectrometer. The analysis of proteins by LDI can take the form of MALDI or of SELDI. The analysis of proteins by LDI can take the form of MALDI or of SELDI.

Laser desorption/ionization in a single time of flight instrument typically is performed in linear extraction mode. Tandem mass spectrometers can employ orthogonal extraction modes.

5 ***Matrix-assisted Laser Desorption/Ionization (MALDI) and Electrospray Ionization (ESI)***

In embodiments, the mass spectrometric technique for use in the invention is matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI). In related embodiments, the procedure is MALDI with time of flight (TOF) analysis, known as MALDI-TOF MS. This involves forming a matrix on a membrane with an agent that absorbs
10 the incident light strongly at the particular wavelength employed. The sample is excited by UV or IR laser light into the vapor phase in the MALDI mass spectrometer. Ions are generated by the vaporization and form an ion plume. The ions are accelerated in an electric field and separated according to their time of travel along a given distance, giving a mass/charge (m/z) reading which is very accurate and sensitive. MALDI spectrometers are
15 well known in the art and are commercially available from, for example, PerSeptive Biosystems, Inc. (Framingham, Mass., USA).

Magnetic-based serum processing can be combined with traditional MALDI-TOF. Through this approach, improved peptide capture is achieved prior to matrix mixture and deposition of the sample on MALDI target plates. Accordingly, in embodiments, methods of
20 peptide capture are enhanced through the use of derivatized magnetic bead based sample processing.

MALDI-TOF MS allows scanning of the fragments of many proteins at once. Thus, many proteins can be run simultaneously on a polyacrylamide gel, subjected to a method of the invention to produce an array of spots on a collecting membrane, and the array may be
25 analyzed. Subsequently, automated output of the results is provided by using a server (e.g., ExPASy) to generate the data in a form suitable for computers.

Other techniques for improving the mass accuracy and sensitivity of the MALDI-TOF MS can be used to analyze the fragments of protein obtained on a collection membrane. These include, but are not limited to, the use of delayed ion extraction, energy reflectors, ion-
30 trap modules, and the like. In addition, post source decay and MS-MS analysis are useful to provide further structural analysis. With ESI, the sample is in the liquid phase and the analysis can be by ion-trap, TOF, single quadrupole, multi-quadrupole mass spectrometers, and the like. The use of such devices (other than a single quadrupole) allows MS-MS or MSⁿ

analysis to be performed. Tandem mass spectrometry allows multiple reactions to be monitored at the same time.

Capillary infusion may be employed to introduce the marker to a desired mass spectrometer implementation, for instance, because it can efficiently introduce small quantities of a sample into a mass spectrometer without destroying the vacuum. Capillary columns are routinely used to interface the ionization source of a mass spectrometer with other separation techniques including, but not limited to, gas chromatography (GC) and liquid chromatography (LC). GC and LC can serve to separate a solution into its different components prior to mass analysis. Such techniques are readily combined with mass spectrometry. One variation of the technique is the coupling of high performance liquid chromatography (HPLC) to a mass spectrometer for integrated sample separation/and mass spectrometer analysis.

Quadrupole mass analyzers may also be employed as needed to practice the invention. Fourier-transform ion cyclotron resonance (FTMS) can also be used for some invention embodiments. It offers high resolution and the ability of tandem mass spectrometry experiments. FTMS is based on the principle of a charged particle orbiting in the presence of a magnetic field. Coupled to ESI and MALDI, FTMS offers high accuracy with errors as low as 0.001%.

20 ***Surface-enhanced laser desorption/ionization (SELDI)***

In embodiments, the mass spectrometric technique for use in the invention is "Surface Enhanced Laser Desorption and Ionization" or "SELDI," as described, for example, in U.S. Patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip. This refers to a method of desorption/ionization gas phase ion spectrometry (*e.g.*, mass spectrometry) in which an analyte (here, one or more of the biomarkers) is captured on the surface of a SELDI mass spectrometry probe.

SELDI has also been called "affinity capture mass spectrometry." It also is called "Surface-Enhanced Affinity Capture" or "SEAC". This version involves the use of probes that have a material on the probe surface that captures analytes through a non-covalent affinity interaction (adsorption) between the material and the analyte. The material is variously called an "adsorbent," a "capture reagent," an "affinity reagent" or a "binding moiety." Such probes can be referred to as "affinity capture probes" and as having an "adsorbent surface." The capture reagent can be any material capable of binding an analyte.

The capture reagent is attached to the probe surface by physisorption or chemisorption. In certain embodiments the probes have the capture reagent already attached to the surface. In other embodiments, the probes are pre-activated and include a reactive moiety that is capable of binding the capture reagent, *e.g.*, through a reaction forming a covalent or coordinate covalent bond. Epoxide and acyl-imidazole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitrilotriacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. Adsorbents are generally classified as chromatographic adsorbents and biospecific adsorbents.

“Chromatographic adsorbent” refers to an adsorbent material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (*e.g.*, nitrilotriacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (*e.g.*, nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents).

“Biospecific adsorbent” refers to an adsorbent comprising a biomolecule, *e.g.*, a nucleic acid molecule (*e.g.*, an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (*e.g.*, DNA)-protein conjugate). In certain instances, the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent No. 6,225,047. A “bioselective adsorbent” refers to an adsorbent that binds to an analyte with an affinity of at least 10^{-8} M.

Protein biochips produced by CIPHERGEN comprise surfaces having chromatographic or biospecific adsorbents attached thereto at addressable locations. CIPHERGEN’s ProteinChip® arrays include NP20 (hydrophilic); H4 and H50 (hydrophobic); SAX-2, Q-10 and (anion exchange); WCX-2 and CM-10 (cation exchange); IMAC-3, IMAC-30 and IMAC-50 (metal chelate); and PS-10, PS-20 (reactive surface with acyl-imidazole, epoxide) and PG-20 (protein G coupled through acyl-imidazole). Hydrophobic ProteinChip arrays have isopropyl or nonylphenoxy-poly(ethylene glycol)methacrylate functionalities. Anion exchange ProteinChip arrays have quaternary ammonium functionalities. Cation exchange ProteinChip

arrays have carboxylate functionalities. Immobilized metal chelate ProteinChip arrays have nitrilotriacetic acid functionalities (IMAC 3 and IMAC 30) or O-methacryloyl-N,N-bis-carboxymethyl tyrosine functionalities (IMAC 50) that adsorb transition metal ions, such as copper, nickel, zinc, and gallium, by chelation. Preactivated ProteinChip arrays have acyl-
5 imidazole or epoxide functional groups that can react with groups on proteins for covalent binding.

Such biochips are further described in: U.S. Patent No. 6,579,719 (Hutchens and Yip, "Retentate Chromatography," June 17, 2003); U.S. Patent 6,897,072 (Rich *et al.*, "Probes for a Gas Phase Ion Spectrometer," May 24, 2005); U.S. Patent No. 6,555,813 (Beecher *et al.*,
10 "Sample Holder with Hydrophobic Coating for Gas Phase Mass Spectrometer," April 29, 2003); U.S. Patent Publication No. U.S. 2003 -0032043 A1 (Pohl and Papanu, "Latex Based Adsorbent Chip," July 16, 2002); and PCT International Publication No. WO 03/040700 (Um *et al.*, "Hydrophobic Surface Chip," May 15, 2003); U.S. Patent Application Publication No. US 2003/0218130 A1 (Boschetti *et al.*, "Biochips With Surfaces Coated With
15 Polysaccharide-Based Hydrogels," April 14, 2003) and U.S. Patent 7,045,366 (Huang *et al.*, "Photocrosslinked Hydrogel Blend Surface Coatings" May 16, 2006).

In general, a probe with an adsorbent surface is contacted with the sample for a period of time sufficient to allow the biomarker or biomarkers that may be present in the sample to bind to the adsorbent. After an incubation period, the substrate is washed to remove unbound
20 material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed. The extent to which molecules remain bound can be manipulated by adjusting the stringency of the wash. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature. Unless the probe has both SEAC and SEND properties (as described
25 herein), an energy absorbing molecule then is applied to the substrate with the bound biomarkers.

In yet another method, one can capture the biomarkers with a solid-phase bound immuno-adsorbent that has antibodies that bind the biomarkers. After washing the adsorbent to remove unbound material, the biomarkers are eluted from the solid phase and detected by
30 applying to a SELDI biochip that binds the biomarkers and analyzing by SELDI.

The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a

mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

5

METHODS OF THE INVENTION

Panels comprising biomarkers of the invention are used to characterize a pelvic mass in a subject to determine whether the subject should be seen by a general surgeon or should be evaluated and/or treated by a gynecologic oncologist. In other embodiments, a panel of the invention is used to diagnose or stage an ovarian cancer by determining the molecular profile of the cancer. In certain embodiments, panels of the invention are used to select a course of treatment for a subject. The phrase "ovarian cancer status" includes any distinguishable manifestation of the disease, including non-disease. For example, ovarian cancer status includes, without limitation, the presence or absence of disease (e.g., ovarian cancer v. non-ovarian cancer), the risk of developing disease, the stage of the disease, the progression of disease (e.g., progress of disease or remission of disease over time), prognosis, the effectiveness or response to treatment of disease, and the determination of whether a pelvic mass is malignant or benign. Based on this status, further procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens.

20 In aspects of the invention, the biomarkers of the invention can be used in diagnostic tests to identify early stage ovarian cancer in a subject.

The correlation of test results with ovarian cancer involves applying a classification algorithm of some kind to the results to generate the status. The classification algorithm may be as simple as determining whether or not the amounts of the markers listed in Table 1 are above or below a particular cut-off number. When multiple biomarkers are used, the classification algorithm may be a linear regression formula. Alternatively, the classification algorithm may be the product of any of a number of learning algorithms described herein.

25 In the case of complex classification algorithms, it may be necessary to perform the algorithm on the data, thereby determining the classification, using a computer, e.g., a programmable digital computer. In either case, one can then record the status on tangible medium, for example, in computer-readable format such as a memory drive or disk or simply printed on paper. The result also could be reported on a computer screen.

Biomarkers of the Invention

Individual biomarkers are useful diagnostic biomarkers. In addition, as described in the examples, it has been found that a specific combination of biomarkers provides greater
 5 predictive value of a particular status than any single biomarker alone, or any other combination of previously identified biomarkers. Specifically, the detection of a plurality of biomarkers in a sample can increase the sensitivity, accuracy and specificity of the test.

Each biomarkers described herein can be differentially present in ovarian cancer, and, therefore, each is individually useful in aiding in the determination of ovarian cancer status.
 10 The method involves, first, measuring the selected biomarker in a subject, sample using any method well known in the art, including but not limited to the methods described herein, e.g. capture on a SELDI biochip followed by detection by mass spectrometry and, second, comparing the measurement with a diagnostic amount or cut-off that distinguishes a positive ovarian cancer status from a negative ovarian cancer status. The diagnostic amount represents
 15 a measured amount of a biomarker above which or below which a subject is classified as having a particular ovarian cancer status. For example, if the biomarker is up-regulated compared to normal during ovarian cancer, then a measured amount above the diagnostic cutoff provides a diagnosis of ovarian cancer. Alternatively, if the biomarker is down-regulated during ovarian cancer, then a measured amount below the diagnostic cutoff
 20 provides a diagnosis of ovarian cancer. As is well understood in the art, by adjusting the particular diagnostic cut-off used in an assay, one can increase sensitivity or specificity of the diagnostic assay depending on the preference of the diagnostician. The particular diagnostic cut-off can be determined, for example, by measuring the amount of the biomarker in a statistically significant number of samples from subjects with the different ovarian cancer
 25 statuses, as was done here, and drawing the cut-off to suit the diagnostician's desired levels of specificity and sensitivity.

The biomarkers of this invention (used alone or in combination) show a statistical difference in different ovarian cancer statuses of at least $p \leq 0.05$, $p \leq 10^{-2}$, $p \leq 10^{-3}$, $p \leq 10^{-4}$, or $p \leq 10^{-5}$. Diagnostic tests that use these biomarkers alone or in combination show a
 30 sensitivity and specificity of at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or about 100%.

Determining Course (Progression/Remission) of Disease

In one embodiment, this invention provides methods for determining the course of disease in a subject. Disease course refers to changes in disease status over time, including
5 disease progression (worsening) and disease regression (improvement). Over time, the amounts or relative amounts (e.g., the pattern) of the biomarkers change. Accordingly, this method involves measuring the panel of biomarkers in a subject at least two different time points, e.g., a first time and a second time, and comparing the change in amounts, if any. The course of disease (e.g., during treatment) is determined based on these comparisons.

10

Reporting the Status

Additional embodiments of the invention relate to the communication of assay results or diagnoses or both to technicians, physicians or patients, for example. In certain
15 embodiments, computers will be used to communicate assay results or diagnoses or both to interested parties, e.g., physicians and their patients. In some embodiments, the assays will be performed or the assay results analyzed in a country or jurisdiction which differs from the country or jurisdiction to which the results or diagnoses are communicated.

In a preferred embodiment of the invention, a diagnosis based on the differential presence or absence in a test subject of the biomarkers of Table 1 is communicated to the
20 subject as soon as possible after the diagnosis is obtained. The diagnosis may be communicated to the subject by the subject's treating physician. Alternatively, the diagnosis may be sent to a test subject by email or communicated to the subject by phone. A computer may be used to communicate the diagnosis by email or phone. In certain embodiments, the message containing results of a diagnostic test may be generated and delivered automatically
25 to the subject using a combination of computer hardware and software which will be familiar to artisans skilled in telecommunications. One example of a healthcare-oriented communications system is described in U.S. Patent Number 6,283,761; however, the present invention is not limited to methods which utilize this particular communications system. In certain embodiments of the methods of the invention, all or some of the method steps,
30 including the assaying of samples, diagnosing of diseases, and communicating of assay results or diagnoses, may be carried out in diverse (e.g., foreign) jurisdictions.

Subject Management

In certain embodiments, the methods of the invention involve managing subject treatment based on the status. Such management includes referral, for example, to a
5 gynecologic oncologist, or other actions of the physician or clinician subsequent to determining ovarian cancer status. For example, if a physician makes a diagnosis of ovarian cancer, then a certain regime of treatment, such as prescription or administration of therapeutic agent might follow. Alternatively, a diagnosis of non-ovarian cancer or non-ovarian cancer might be followed with further testing to determine a specific disease that
10 might the patient might be suffering from. Also, if the diagnostic test gives an inconclusive result on ovarian cancer status, further tests may be called for.

In one embodiment, the diagnosis may be determining if a pelvic mass is benign or malignant. If the diagnosis is malignant, a gynecologic oncologist may be chosen to perform the surgery. In contrast, if the diagnosis is benign, a general surgeon or a gynecologist may
15 be chosen to perform the surgery.

Additional embodiments of the invention relate to the communication of assay results or diagnoses or both to technicians, physicians or patients, for example. In certain embodiments, computers will be used to communicate assay results or diagnoses or both to interested parties, *e.g.*, physicians and their patients. In some embodiments, the assays will
20 be performed or the assay results analyzed in a country or jurisdiction which differs from the country or jurisdiction to which the results or diagnoses are communicated.

HARDWARE AND SOFTWARE

The any of the methods described herein, the step of correlating the measurement of
25 the biomarker(s) with ovarian cancer can be performed on general-purpose or specially-programmed hardware or software.

In aspects, the analysis is performed by a software classification algorithm. The analysis of analytes by any detection method well known in the art, including, but not limited to the methods described herein, will generate results that are subject to data processing.
30 Data processing can be performed by the software classification algorithm. Such software classification algorithms are well known in the art and one of ordinary skill can readily select

and use the appropriate software to analyze the results obtained from a specific detection method.

In aspects, the analysis is performed by a computer-readable medium. The computer-readable medium can be non-transitory and/or tangible. For example, the computer readable
5 medium can be volatile memory (*e.g.*, random access memory and the like) or non-volatile memory (*e.g.*, read-only memory, hard disks, floppy discs, magnetic tape, optical discs, paper table, punch cards, and the like).

For example, analysis of analytes by time-of-flight mass spectrometry generates a time-of-flight spectrum. The time-of-flight spectrum ultimately analyzed typically does not
10 represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. Exemplary software includes, but is not limited to, Ciphergen's ProteinChip[®] software, in which data processing typically includes TOF-to-M/Z transformation to generate a mass spectrum, baseline subtraction to
15 eliminate instrument offsets and high frequency noise filtering to reduce high frequency noise.

Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of biomarkers detected, and optionally the strength of the signal and the
20 determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference. The reference can be background noise generated by the instrument and chemicals such as the energy absorbing
25 molecule which is set at zero in the scale.

The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling
biomarkers with nearly identical molecular weights to be more easily seen. In another useful
30 format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

Analysis generally involves the identification of peaks in the spectrum that represent signal from an analyte. Peak selection can be done visually, but software is available, for example, as part of Ciphergen's ProteinChip® software package, that can automate the detection of peaks. This software functions by identifying signals having a signal-to-noise ratio above a selected threshold and labeling the mass of the peak at the centroid of the peak signal. In embodiments, many spectra are compared to identify identical peaks present in some selected percentage of the mass spectra. One version of this software clusters all peaks appearing in the various spectra within a defined mass range, and assigns a mass (N/Z) to all the peaks that are near the mid-point of the mass (M/Z) cluster.

In aspects, software used to analyze the data can include code that applies an algorithm to the analysis of the results (e.g., signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention). The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates the status of the particular clinical parameter under examination. Analysis of the data may be "keyed" to a variety of parameters that are obtained, either directly or indirectly, from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the shape of a peak or group of peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

CLASSIFICATION ALGORITHMS FOR QUALIFYING OVARIAN CANCER STATUS

In some embodiments, data derived from the assays (e.g., ELISA assays) that are generated using samples such as "known samples" can then be used to "train" a classification model. A "known sample" is a sample that has been pre-classified. The data that are derived from the spectra and are used to form the classification model can be referred to as a "training data set." Once trained, the classification model can recognize patterns in data derived from spectra generated using unknown samples. The classification model can then be used to classify the unknown samples into classes. This can be useful, for example, in predicting whether or not a particular biological sample is associated with a certain biological condition (e.g., diseased versus non-diseased).

The training data set that is used to form the classification model may comprise raw data or pre-processed data. In some embodiments, raw data can be obtained directly from time-of-flight spectra or mass spectra, and then may be optionally “pre-processed” as described above.

5 Classification models can be formed using any suitable statistical classification (or “learning”) method that attempts to segregate bodies of data into classes based on objective parameters present in the data. Classification methods may be either supervised or unsupervised. Examples of supervised and unsupervised classification processes are described in Jain, “Statistical Pattern Recognition: A Review”, *IEEE Transactions on*
10 *Pattern Analysis and Machine Intelligence*, Vol. 22, No. 1, January 2000.

 In supervised classification, training data containing examples of known categories are presented to a learning mechanism, which learns one or more sets of relationships that define each of the known classes. New data may then be applied to the learning mechanism, which then classifies the new data using the learned relationships. Examples of supervised
15 classification processes include linear regression processes (e.g., multiple linear regression (MLR), partial least squares (PLS) regression and principal components regression (PCR)), binary decision trees (e.g., recursive partitioning processes such as CART - classification and regression trees), artificial neural networks such as back propagation networks, discriminant analyses (e.g., Bayesian classifier or Fischer analysis), logistic classifiers, and support vector
20 classifiers (support vector machines).

 In embodiments, a supervised classification method is a recursive partitioning process. Recursive partitioning processes use recursive partitioning trees to classify spectra derived from unknown samples. Further details about recursive partitioning processes are provided in U.S. Patent Application No. 2002 0138208 A1 to Paulse *et al.*, “Method for
25 analyzing mass spectra.”

 In other embodiments, the classification models that are created can be formed using unsupervised learning methods. Unsupervised classification attempts to learn classifications based on similarities in the training data set, without pre-classifying the spectra from which the training data set was derived. Unsupervised learning methods include cluster analyses. A
30 cluster analysis attempts to divide the data into “clusters” or groups that ideally should have members that are very similar to each other, and very dissimilar to members of other clusters. Similarity is then measured using some distance metric, which measures the distance between

data items, and clusters together data items that are closer to each other. Clustering techniques include the MacQueen's K-means algorithm and the Kohonen's Self-Organizing Map algorithm.

Learning algorithms asserted for use in classifying biological information are described, for example, in PCT International Publication No. WO 01/31580 (Barnhill *et al.*, "Methods and devices for identifying patterns in biological systems and methods of use thereof"), U.S. Patent Application No. 2002 0193950 A1 (Gavin *et al.*, "Method or analyzing mass spectra"), U.S. Patent Application No. 2003 0004402 A1 (Hitt *et al.*, "Process for discriminating between biological states based on hidden patterns from biological data"), and U.S. Patent Application No. 2003 0055615 A1 (Zhang and Zhang, "Systems and methods for processing biological expression data").

The classification models can be formed on and used on any suitable digital computer. Suitable digital computers include micro, mini, or large computers using any standard or specialized operating system, such as a Unix, Windows™ or Linux™ based operating system. The digital computer that is used may be physically separate from the mass spectrometer that is used to create the spectra of interest, or it may be coupled to the mass spectrometer.

The training data set and the classification models according to embodiments of the invention can be embodied by computer code that is executed or used by a digital computer. The computer code can be stored on any suitable computer readable media including optical or magnetic disks, sticks, tapes, etc., and can be written in any suitable computer programming language including C, C++, visual basic, etc.

The learning algorithms described above are useful both for developing classification algorithms for the biomarkers already discovered, or for finding new biomarkers for ovarian cancer. The classification algorithms, in turn, form the base for diagnostic tests by providing diagnostic values (*e.g.*, cut-off points) for biomarkers used singly or in combination.

KITS FOR DETECTION OF BIOMARKERS FOR OVARIAN CANCER

In another aspect, the invention provides kits for aiding in the diagnosis of ovarian cancer (*e.g.*, identifying ovarian cancer status, detecting ovarian cancer, identifying early stage ovarian cancer, selecting a treatment method for a subject at risk of having ovarian cancer, and the like), which kits are used to detect biomarkers according to the invention. In

one embodiment, the kit comprises agents that specifically recognize the biomarkers identified in Table 1. In related embodiments, the agents are antibodies. The kit may contain 1, 2, 3, 4, 5, or more different antibodies that each specifically recognize one of the biomarkers set forth in Table 1.

5 In another embodiment, the kit comprises a solid support, such as a chip, a microtiter plate or a bead or resin having capture reagents attached thereon, wherein the capture reagents bind the biomarkers of the invention. Thus, for example, the kits of the present invention can comprise mass spectrometry probes for SELDI, such as ProteinChip® arrays. In the case of biospecific capture reagents, the kit can comprise a solid support with a reactive
10 surface, and a container comprising the biospecific capture reagents.

The kit can also comprise a washing solution or instructions for making a washing solution, in which the combination of the capture reagent and the washing solution allows capture of the biomarker or biomarkers on the solid support for subsequent detection by, *e.g.*, mass spectrometry. The kit may include more than type of adsorbent, each present on a
15 different solid support.

In a further embodiment, such a kit can comprise instructions for use in any of the methods described herein. In embodiments, the instructions provide suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform a consumer about how to collect the sample, how to wash the probe or the particular
20 biomarkers to be detected.

In yet another embodiment, the kit can comprise one or more containers with controls (*e.g.*, biomarker samples) to be used as standard(s) for calibration.

EXAMPLES

25 **Example 1: Panels of biomarkers that included IGFBP2, Interleukin 6 (IL-6), and FSH and/or HE4 improved the preoperative assessment of ovarian tumors compared to a diagnostic panel comprising** Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II)

30 OVA1 provides an important method for characterizing ovarian cancer. OVA1 involves measuring biomarkers Apolipoprotein A1 (ApoA1), Beta2 microglobulin (B2MG), CA-125, Transthyretin/prealbumin, and Transferrin for pre-surgical assessment of adnexal masses for risk of ovarian malignancy. To further improve the specificity of these markers, potential biomarkers and panels of biomarkers were evaluated using a set of clinical samples

enriched for OVA1 false positive benign patients to identify panels that would improve the specificity of the test and properly exclude these false positive subjects. This testing identified a panel comprising or consisting of CA125-II, transthyretin/prealbumin, IGFBP2, IL6, and FSH. At a fixed sensitivity of 90%, the mean and median specificity of models
 5 using the new panel in testing were 78.2% (95% CI: 76.7 - 79.8%), and 80.6%, respectively. The mean and median absolute improvements over that of Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) were 18.6% (95% CI: 16.4% - 20.9%) and 20.3%, respectively.

A list of 81 potential biomarkers was initially looked at for value in increasing
 10 specificity. From this list, 30 potential biomarkers were selected. A screening panel of serum samples from 19 patients with ovarian cancer and 22 patients with benign pelvic masses was designed and used to perform initial assessment of candidate biomarkers. The benign patients were specifically selected so that the majority of them would have false positive scores when evaluated with biomarkers: Transthyretin (TT or prealbumin),
 15 Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) markers.

Based on availability and quality of ELISA assays, a total of 13 candidate biomarkers were evaluated using the designed screening sample panel. When possible, ELISA kits from different vendors were evaluated to select those with better analytical performance. From
 20 results of this screening sample set, insulin-like growth factor-binding protein 2 (IGFBP2) and interleukin 6 (IL6) were selected to be evaluated on the study sample set, denoted as "OVA500." In addition, Human Epididymis Protein 4 (HE4) and Follicle-stimulating hormone (FSH) were also evaluated on the OVA500 study sample set. The inclusion of FSH potentially eliminates the need to have separate menopausal status-dependent cutoffs (In
 25 Vitro Diagnostic Multivariate Index Assay (IVDMIA) values can be adjusted for menopausal status internally at the algorithm level based on serum FSH level). In all, a set of 9 biomarkers have been evaluated on the OVA500 study samples, including those for OVA1 calculation: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2,
 30 IL6, HE4, and FSH.

Insulin-like growth factor binding protein 2 (IGFBP2), interleukin 6 (IL6), and follicle stimulating hormone (FSH) were among the biomarkers selected to be further evaluated on a prospectively collected clinical sample set, along with the original five

biomarkers of OVA1. Consecutive patients with a documented pelvic mass planned for surgical intervention were prospectively enrolled at 27 sites. Exclusion criteria included a diagnosis of malignancy in the previous 5 years or initial enrollment by a gynecologic oncologist. At the time of analysis, 384 subjects had all biomarker values. Among them 69
5 were ovarian cancer cases (13 low malignancy potential (LMP), 27 stages 1/2, 19 serous, 11 endometrioid, 5 mucinous, and 4 clear cell). Biomarkers were tested by ELISA and reported as continuous values. Using a subset of the samples, the biomarkers were first selected for inclusion in a final panel based on contributions in multivariate models estimated by bootstrap. The selected biomarkers were further assessed for ability to improve specificity of
10 risk stratification at a fixed sensitivity over that of biomarkers Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) using the full data set. This was done by cross-validation of multivariate models with 50/50 split between training and testing.

In models using all 9 biomarkers (i.e., Transthyretin (TT or prealbumin),
15 Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH), distribution of absolute improvement by in specificity over that of biomarkers Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) at fixed 95% sensitivity in testing over 100 rounds of cross-
20 validation of multivariate models was observed. Mean and median absolute improvement were 24.1% (95% Confidence Interval (CI): 21.0-27.2%) and 24.6%, respectively (Figure 1).

In models using 8 of the 9 biomarkers (Transthyretin (TT or prealbumin),
Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, and FSH, without HE4), distribution of absolute
25 improvement in specificity over that of Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) at fixed 95% sensitivity in testing over 100 rounds of cross-validation of multivariate models was also observed. Mean and median absolute improvement were 22.0% (95% Confidence Interval (CI): 18.8-25.3%) and 23.3%, respectively (Figure 2).

30 Bootstrap analysis of linear models of each of the markers was performed, and ranked accordingly (Table 2).

Table 2. Rankings of Ovarian Cancer Biomarkers

Biomarker	Mean Rank	STD of Rank	Median Rank
CA125	1.0	1.1	1
ApoA1	5.6	1.1	6
PreAlb	4.6	0.9	4
B2M	5.7	1.1	6
TRF	6.0	0.5	3
IGFBP2	2.8	0.5	3
IL6	2.3	0.2	2

The top ranked (mean rank and median rank) four biomarkers from bootstrap analysis of linear models were selected for a panel of biomarkers for further study. Additionally, FSH was “artificially” added to the panel mainly for its high correlation with menopausal status which can be used in place of menopausal status-dependent cutoffs (as in other ovarian cancer diagnostics). Thus, the panel of biomarkers selected for further study included: CA125, PREALB, IGFBP2, IL6, and FSH.

The performance of multivariate index assays was assessed. Distribution of Specificity at 90% Sensitivity in 30 cross-validation runs (50/50 split between training and testing) showed that for both linear and nonlinear models, the majority of the models had specificity > 70% at the fixed sensitivity of 90% (Figures 3 and 4A).

Distribution of Specificity at 95% Sensitivity in 100 cross-validation runs (50/50 split between training and testing) for nonlinear models only, indicated that the majority of the models have specificity > 60% (Figure 4B).

At cutoffs corresponding to overall sensitivity of 95%, the majority of models have sensitivities ranging from ~85-95% for stage 1 cases (Figure 5). The distribution of specificity was the same as in Figure 4.

Distribution of sensitivity was also evaluated between invasive and low malignancy potential (LMP) ovarian tumor, and among the four major histologic subtypes. At a cutoff

corresponding to 95% overall sensitivity (on training data), as expected, the models offered higher sensitivity for invasive cases than those with LMP ovarian tumors (Figure 6).

The breakdown comparison of sensitivities (training: red, testing blue) among the four major histologic subtypes of ovarian cancer was statistically not very meaningful due to the
5 small number of cases in each of the subtypes (Figures 7A-7D).

Distributions of sensitivity and specificity were evaluated for pre-/post-menopausal groups and training/test using cutoffs for 95% overall Sensitivity in 100 cross-validation runs. From the distributions, specificities were somewhat higher among the pre-menopausal than that among the post-menopausal benign patients (Figures 8A-8D).

10 The distribution of improvement of models with the new panel comprising or consisting of CA125, prealbumin, IGFBP2, IL6, and FSH (Figure 9) over a panel comprising Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) showed that the selected panels of biomarkers had absolute improvement > 15% when evaluated on combined training and
15 test sets (Figure 9). Moreover, the null hypothesis that the absolute improvement in specificity is $\leq 15\%$ was rejected with statistical significance ($p\text{-value} < 2.5e-017$). When evaluated on the test sets only, the distribution of the absolute improvement had a wider spread, partially due to smaller sample sizes of the test sets and possible “shrinkage in performance” when models are generalized on independent sets. However, the majority still
20 had an absolute improvement > 12%. Again, the null hypothesis that the absolute improvement in specificity is $\leq 12\%$ is rejected with statistical significance ($p\text{-value} = 0.0010$).

Distribution of area-under-curve (AUC) from receiver-operating-characteristic (ROC) analysis from 100 cross-validation runs – nonlinear models only, confirmed that the majority
25 of models have very high AUC values (Figure 10). However, for study purposes, the improvement in specificity at extremely high sensitivity may not be reflected in the overall AUCs. In actual model development, “partial AUC” may be chosen to access model performance and drive model training.

An improvement in specificity of the 5-biomarker panel: CA125, prealbumin,
30 IGFBP2, IL6, and FSH was observed after replacing FSH with HE4. Thus HE4, with its relatively high specificity among benign ovarian tumor patients, further improved the performance of multivariate models (Figures 11 and 12). The performance of the 5-

biomarker panels comprising CA125, prealbumin, IGFBP2, IL6, and FSH are summarized at Table 3.

Table 3

	New Panel		New Panel, FSH replaced by HE4	
	Training	Testing	Training	Testing
Mean	78.3%	78.2%	84.1%	83.8%
99%C1	(76.2, 79.8)	(76.5, 80.8)	(83.3, 84.9)	(82.8, 84.8)
Median	80.3%	80.6%	84.6%	84.9%
95%C1	(78.5, 81.1)	(77.4, 81.4)	(83.4, 85.3)	(83.7, 85.6)

5

The performance estimates are based on 50/50 cross-validation, and actual performance on a totally independent test set may have a certain degree of “shrinkage.” Additionally, the mean is not as informative as the median, as the distributions are not very symmetric.

10

Example 2: 3- and 4-marker panels dramatically improve the specificity of ovarian cancer assessment

Comparison of the following panel: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) +HE4 (combination) using a linear model against HE4 alone and Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) also showed improved specificity using the combination model (at 90% Sensitivity in 100 cross-validation runs; 50/50 split between training and testing) (Figure 13). In particular, the combination model performance was not calculated using menopausal status dependent cutoffs.

20

As shown in Figure 13, right hand panel, the median for specificity in the set of markers including HE4 is between 75-80% at 90% sensitivity. This represents a dramatic improvement over all panels currently in clinical use to characterize ovarian cancer. An exemplary panel is shown in solid bars in the right hand panel, where specificity is between 55 and 60%.

25

Bootstrap estimated rank and standard rank of OVA1 component markers and HE4 using linear models showed that the 4 highest ranked biomarkers were CA125, Transthyretin/Prealbumin, Transferrin, and HE4; and the 3 highest ranked biomarkers were CA125, Transthyretin/Prealbumin, and HE4 (Table 4).

5

Table 4

Biomarker	Mean Rank	STD of Rank	Median Rank
CA125	1.8	0.43	2
ApoA1	5.8	0.46	6
PreAlb	3.4	0.63	3
B2M	5.0	0.53	5
TRF	3.7	0.64	4
HE4	1.2	0.43	1

Panels having the 4 and 3 highest ranked biomarkers were evaluated for distributions of specificity at 90% sensitivity (100 cross-validation runs, 50/50 split between training and testing), and compared to OVA1 (OVA1 performances were not calculated using product cutoffs).

Comparison of CA125+PreAlb+TRF+HE4 and CA125+PreAlb+HE4 non-linear models against Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) also showed improved specificity over Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) (at 90% Sensitivity in 100 cross-validation runs; 50/50 split between training and testing) (Figure 14). The performance of a panel comprising Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) was computed on the same training/test sets used for cross-validation of other models, and product cut-offs were not used.

As shown in Figure 14, right hand panel, the specificity of the four marker panel including HE4 showed more than 90% specificity. This represents a dramatic improvement over all panels currently in clinical use to characterize ovarian cancer. An exemplary panel is shown in solid bars in the right hand panel, where specificity is between 55 and 60%.

5 Both the 3-marker (CA125, prealbumin, HE4) panel and the 4-marker (CA125, prealbumin, transferrin, HE4) panel show unexpectedly superior specificity relative to the prior art—without sacrificing sensitivity. Currently, 1 out of every 2 women evaluated with state of the art diagnostic tests are needlessly referred to a gynecological oncologist. When adnexal masses are assessed using the 3 or 4 marker panels of the invention, the number of
10 false positives is greatly reduced. The 3 marker panel provides a 19% false positive rate, which is less than half the false positive rate provided by ovarian cancer panels present in the prior art. The 4 marker panel provides a 13-14% false positive rate—cutting the false positive rate provided by prior art diagnostics by two thirds. The 3- and 4-marker panels of the invention ensure that all women receive the correct diagnosis and a referral to the
15 appropriate physician.

The performance of CA125+PreAlb+TRF+HE4, CA125+PreAlb+HE4, and Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) (bootstrap estimated Means, Medians and 95%CIs of specificity at 90% sensitivity) are summarized at Table 6.

20

Table 5

	OVA1* (did not use product cutoffs)		CA125+Prealb+TRF+HE4		CA125+Prealb+HE4	
	Training	Testing	Training	Testing	Training	Testing
Mean	58.8%	59.6%	85.3%	85.4%	80.6%	80.9%
95%CI	(57.3, 61.3)	(57.7, 61.7)	(84.0, 86.6)	(83.9, 86.3)	(78.7, 81.9)	(79.0, 81.8)
Median	56.5%	58.0%	86.3%	86.3%	80.8%	81.3%
95%CI	(55.1, 60.7)	(55.2, 61.3)	(85.3, 87.3)	(85.9, 87.2)	(80.4, 82.5)	(80.3, 81.8)

Comparison of CA125+PreAlb+TRF+HE4 and CA125+PreAlb+HE4 non-linear
25 models against Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) also showed an improvement in ROC/AUCs over Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA

125 II) (100 cross-validation runs; 50/50 split between training and testing) (Figure 15).
 Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta
 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) performance was computed on
 the same training/test sets used for cross-validation of other models, and product cut-offs
 5 were not used. The performance of CA125+PreAlb+TRF+HE4, CA125+PreAlb+HE4, and
 Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta
 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II) (bootstrap estimated Means,
 Medians and 95%CI of ROC/AUCs) are summarized at Table 7.

Table 6

10

	OVA1* (did not use product cutoffs)		CA125+Prealb+TRF+HE4		CA125+Prealb+HE4	
	Training	Testing	Training	Testing	Training	Testing
Mean	88.4%	89.0%	94.1%	94.1%	93.5%	93.4%
95%CI	(88.0, 88.9)	(88.5, 89.5)	(93.4, 94.5)	(93.6, 94.4)	(92.8, 93.9)	(93.0, 93.8)
Median	88.5%	88.9%	94.3%	94.1%	93.9%	93.5%
95%CI	(88.0, 88.8)	(88.7, 89.7)	(93.6, 94.8)	(93.6, 94.4)	(93.3, 94.1)	(93.1, 93.9)

There was no significant difference in distributions of AUCs between the 3-biomarker
 model and 4-biomarker model, as both had reached fairly high values. However, the
 difference between the two models comparing specificity at 90% sensitivity (Figure 14)
 15 indicated that the addition of the 4th marker mainly contributed to the shape of the ROC
 rather than its AUC.

In summary, the identified panels demonstrated the potential to significantly improve
 specificity over that of the first-generation of ovarian cancer diagnostics that rely on a panel
 that includes Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-
 20 Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II). The
 performance estimation by leave-k-out cross-validation indicated that at cutoffs
 corresponding to a sensitivity of 95% for all malignant cases (~90% for stage I cases), the
 panel of biomarkers with nonlinear classification models demonstrated a significantly
 improved specificity over a panel comprising Transthyretin (TT or prealbumin),
 25 Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer
 Antigen 125 (CA 125 II). The overall improvement in ROC/AUC was mostly from better
 specificity at ROC curve range >90% sensitivity. The observed distribution and variation of
 cross-validation performance indicated that a IVDMLA model will have clinically meaningful

improvement (~15% or more) in specificity over OVA1. In particular, the null hypothesis that “the absolute improvement in specificity is $\leq 15\%$ ” was rejected with statistical significance (p-value $< 2.5e-017$). Thus, the identified panels of biomarkers have improved specificity over the first-generation ovarian cancer diagnostics, while maintaining a high
 5 sensitivity in pre-surgical assessment of adnexal masses for risk of malignancy.

Example 3: Panels comprising CA125, APOA1, PREALB, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG-72/CA724 are useful in characterizing ovarian cancer.

Serum samples from patients (n=381) were obtained. These patients included 69
 10 ovarian cancer cases and 312 patients with benign ovarian tumor as controls. The patient diagnoses were confirmed by surgery. Serum samples from these patients were divided into roughly equal proportions and used as a training set and a test set for plotting purposes. Figure 20A shows a biplot of supervised component analysis results using all ten (10) biomarkers: CA125, APOA1, PREALB, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG-
 15 72/CA724. A biplot is a generalization of the simple two-variable scatterplot. A biplot allows information on both samples and variables of a data matrix to be displayed graphically. Samples are displayed as points while variables are displayed either as vectors, linear axes or nonlinear trajectories.

The plot indicates that the ovarian cancer cases and benign controls were fairly well
 20 separated along the x-axis which is a linear combination of the 10 biomarkers derived using a supervised learning algorithm. The contributions of the 10 biomarkers are indicated as vectors from the origin of the plot. In this plot, the length of the projection of a vector to the x-axis indicates its contribution to the two group separation. The relative angular position provides information on whether the biomarkers are similar or complementary for the group
 25 separation.

Figure 21 provides an ROC analysis of the results using biomarkers CA125, APOA1, PREALB, B2M, TRF, HE4, IGFBP2, IL6, FSH, TAG 72/CA72-4.

A bootstrap analysis featuring ranking and selection was performed next. A total of 30 bootstrap runs were performed. In each run, a bootstrap sample which has the same
 30 sample size as the original sample were randomly selected with replacement from the original sample set. Using the same training parameters, a linearly classifier was derived, the individual features (biomarkers) were ranked according to their contribution to the linear classifier as indicated by their respective weights in the linear combination formula. In Table

8, the estimated mean and median rank of the features along with the standard deviations were listed in sorted order. A smaller rank indicates a more informative biomarker.

TABLE 7. MEAN AND MEDIAN RANK OF BIOMARKERS FROM BOOTSTRAP FEATURE SELECTION				
	Biomarker	MeanRank	MedianRank	StdRank
	HE4	1.06667	1	0.25371
	CA125	1.96667	2	0.31984
	IGFBP2	3.3	3	0.46609
	CA724	3.83333	4	0.74664
	IL6	5.16667	5	0.69893
	PREALB	6.2	6	0.99655
	TRF	7.73333	8	1.38796
	B2M	7.83333	8	1.23409
	FSH	8.93333	9	1.20153
	APOA1	8.96667	9	0.92786

5 Cross-validation assessment of specificity at fixed 90% sensitivity was carried out. The other basic settings remained the same. The total available sample set (n=381) included 69 ovarian cancer cases and 312 patients with benign ovarian tumor as controls, all conformed by surgery. With these samples, cross-validation was used to assess the various models' potential performance in future samples. In each training session, the samples were

10 randomly divided into a training set and a set-aside testing set at roughly 50/50 division. A model was derived on the training set and then tested (cross-validated) on the set-aside set. For both training and test sets, specificities were set at a fixed sensitivity of 90% were estimated and recorded. The same training sessions were repeated 100 times, each time with a new random division of the sample set. At the end of these analyses, the histograms

15 (distributions) of the 100 estimated specificities (at fixed 90% sensitivity) from training and from cross-validation, respectively. In general, the histograms from cross-validation would have a lightly wide spread, due to the relatively small sample size and possible "shrinkage" in generalization. However, once the training parameters were optimized, the differences in results from training and cross-validation were typically not significant.

Table 8 shows feature ranks from Bootstrap Selection.

Table 8: Feature Ranks from Bootstrap Selection

meanRank, seRank, medianRank							
'CA125'	2.2333	1.6955	2.000				
'APO'	9.2333	1.8134	10.0000	Biomarker	Mean Rank	Median Rank	Std Rank
'PREALB'	6.2333	1.6333	7.0000	CA125	1.96667	2	0.31984
'B2M'	7.4333	1.2507	8.0000	APOA1	8.96667	9	0.92786
'TRF'	6.2000	1.7301	6.5000	PREALB	6.2	6	0.99655
'HE4'	1.4667	1.1666	1.0000	B2M	7.83333	8	1.23409
'IGFBP2'	4.5000	1.7171	4.0000	TRF	7.73333	8	1.38796
'IL6'	4.8667	1.2521	5.0000	HE4	1.06667	1	0.25371
'FSH'	8.9333	0.8277	9.0000	IGFBP2	3.3	3	0.46609
'CA724'	3.9000	1.3734	3.5000	IL6	5.16667	5	0.69893
				FSH	8.93333	9	1.20153
				CA724	3.83333	4	0.74664

5

Biomarkers with the best ranking in bootstrap feature selection included CA125, HE4, IGFBP2, IL6, CA724 (Figure 22). Results using a panel comprising CA125, Prealb, TRF, HE4, and TAG-72/CA 72-4 are shown in Figure 23. Results obtained using a linear model with a panel comprising CA125, HE4, CA724 are shown in Figure 24. A linear model was also used to obtain the results shown in Figure 25, which analyzes markers CA125, Prealb, TRF, and CA724. Figure 26 shows results obtained using markers CA125, Prealb, IGFBP2, IL6, and CA724.

10

The results described herein were obtained using the following materials and methods.

15

Study Population for Example 1 and Example 2

A multicenter prospective collection from non-gynecologic oncologist practices was obtained. Consecutive patients who met inclusion criteria were prospectively enrolled at 27 sites throughout the United States, with Institutional Review Board approval from each site. All clinicians initially enrolling patients were from non-gynecologic oncology specialty practices, although patients may ultimately have had consultation with or undergone surgery by a gynecologic oncologist. Inclusion criteria were: females age ≥ 18 years, signed informed consent and agreeable to phlebotomy, documented pelvic mass planned for surgical

20

intervention within 3 months of imaging. A pelvic mass was confirmed by imaging (computed tomography, ultrasonography, or magnetic resonance imaging) prior to enrollment. Exclusion criteria included a diagnosis of malignancy in the previous 5 years (except of non-melanoma skin cancers) or enrollment by a gynecologic oncologist.

- 5 Menopause was defined as the absence of menses for ≥ 12 months, or age ≥ 50 . Demographic and clinic-pathologic information were collected on case report forms. A total of 494 subjects were evaluable (Table 9). Currently, 491 samples have values for all 9 biomarkers. Among them, 107 had IGFBP2 and IL6 analyzed using different batches of kits. With observed batch variations, data for these samples were not used in the current analysis.

10

Table 10. Demographics of evaluable subjects of OVA500 study

	All Evaluable Subjects (N=494)	Premenopausal Women (N=277)	Postmenopausal Women (N=217)
Age, years			
N	494	277	217
Mean (SD)	48.6 (14.15)	39.6 (8.95)	60.2(10.74)
Median	48	41	60
Range(min to max)	18 to 87	18 to 60	33 to 87
Ethnicity/race, n (%)			
White	348 (70.4)	174 (62.8)	174 (80.2)
African-American	81 (16.4)	54 (19.5)	27 (12.4)
Hispanic or Latino	46 (9.3)	36 (13.0)	10 (4.6)
Asian	13 (2.6)	8 (2.9)	5 (2.3)
Native Hawaiian/Pacific Islander	1 (0.2)	1 (0.4)	0 (0.0)
Other	5 (1.0)	4 (1.4)	1 (0.5)
No. of pregnancies, n (%)			
None	80 (16.2)	56 (20.2)	24 (11.1)
1	87 (17.6)	53 (19.1)	34 (15.7)
2	131 (26.5)	70 (25.3)	61 (28.1)
3	94 (19.0)	50 (18.1)	44 (20.3)
4 or more	102 (20.6)	48 (17.3)	54 (24.9)
Physician's Assessment, n (%)			
Malignant	98 (19.8)	39 (14.1)	59 (27.2)
Benign	396 (80.2)	238 (85.9)	158 (72.8)
Pathology diagnosis, n (%)			
Benign	402 (81.4)	246 (88.8)	156 (71.9)
Non-ovarian primary malignancy with no involvement of the ovaries	4 (0.8)	1 (0.4)	3 (1.4)
Non-ovarian primary malignancy with involvement of the ovaries	6 (1.2)	2 (0.7)	4 (1.8)
Ovarian low malignant potential (Borderline)	17 (3.4)	5 (1.8)	12 (5.5)
Primary malignant ovarian tumor	65 (13.2)	23 (8.3)	42 (19.4)
Epithelial ovarian cancer	60 (12.1)	18 (6.5)	42 (19.4)
Serous	24 (4.9)	8 (2.9)	16 (7.4)
Mucinous	9 (1.8)	1 (0.4)	8 (3.7)
Endometrioid	13 (2.6)	5 (1.8)	8 (3.7)
Clear cell	5 (1.0)	1 (0.4)	4 (1.8)
Carcinosarcoma	1 (0.2)	1 (0.4)	0 (0.0)
Mixed	1 (0.2)	0 (0.0)	1 (0.5)
Other	7 (1.4)	2 (0.7)	5 (2.3)
Non-Epithelial; Other	5 (1.0)	5 (1.8)	0 (0.0)
Tumor Stage, n (% of all primary malignant ovarian tumor)			
Stage 1	28 (43.1)	9 (39.1)	19 (45.2)
Stage 2	7 (10.8)	2 (8.7)	5 (11.9)
Stage 3	25 (38.5)	10 (43.5)	15 (35.7)
Stage 4	5 (7.7)	2 (8.7)	3 (7.1)

More than 30 potential biomarkers from an initial list of 81 were considered based on their reported performance on clinical samples and biological relevance to ovarian cancer. A “designed” screening panel of serum samples from 19 patients with ovarian cancer and 22 patients with benign pelvic masses were used to perform initial assessment of candidate biomarkers. The benign patients were specifically selected such that the majority of them would have false positive scores using an ovarian cancer panel comprising Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II). The primary objective of the study was to assess additional biomarkers’ ability to further improve specificity over that of Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II).

Based on the availability and quality of ELISA assays, a total of 13 candidate biomarkers were evaluated using the designed screening sample panel. When possible, ELISA kits from different vendors were evaluated to select those with better analytical performance.

From results of this screening sample set, insulin-like growth factor-binding protein 2 (IGFBP2) and interleukin 6 (IL6) were selected to be evaluated on the OVA500 study sample set.

In addition, Human Epididymis Protein 4 (HE4) and Follicle-stimulating hormone (FSH) were also evaluated on the OVA500 study sample set. The inclusion of FSH potentially eliminates the need to have separate menopausal status-dependent cutoffs (IVDMIA values can be adjusted for menopausal status internally at the algorithm level based on serum FSH level).

Currently, a set of 9 biomarkers (which includes those for OVA1 calculation) have been evaluated on the OVA500 study samples: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), β 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH. The kit/system information of these biomarkers is provided at Table 9.

Table 9

Biomarker	Manufacturer/Kit	System
PreAlbumin	Siemens Healthcare Diagnostics, #OU1F09	Siemens BNII
CA125 II	Roche Diagnostics, #11776223	Elecsys 2010
B2M	The Binding Site, #LK043.T	Siemens BNII
ApoA1	Siemens Healthcare Diagnostics, #OUED15	Siemens BNII
TRF	Siemens Healthcare Diagnostics, #OSAX15	Siemens BNII
IGFBP2*	RayBiotech, IGFBP-2 ELISA Kit #ELH-IGFBP2-001	
IL6*	R&D Systems, Human IL-6 Quantikine HS SixPak #SS600B	
FSH	Roche Diagnostics, #11775863	Elecsys 2010
HE4	Abbott Diagnostics, #2P54-Z7	Architect i2000

Data preprocessing and normalization

- 5 In addition to the 9 biomarkers, OVA1 scores for the 491 samples were computed using the raw test values of the OVA1 five-biomarker panel. Individual biomarkers were first evaluated for their distribution patterns to decide whether numerical transformations were required to achieve reasonable symmetric distribution patterns. (Figure 16A). For further analysis, the individual biomarkers were transformed as provided in Table 10.

10

Table 10. Transformation of biomarker to correct for skewness.

Biomarker	Transformation
CA125	$\log_{10}(\text{CA125} + 90.001)$;
B2M	$\log_{10}(\text{B2M} + 0.001)$;
IGFBP2	$\log_{10}(\text{IGFBP2} + 1)$;
IL6	$\log_{10}(\text{IL6} + 1)$;
HE4	$\log_{10}(\log_{10}(\text{HE4}) + 0.001)$;
FSH	$\log_{10}(\text{FSH} + 1)$;

- As shown in Figure 16B, the transformations provided reasonable symmetric distribution patterns for the selected biomarkers. After transformation, the results were further converted to z-scores using each biomarker's population mean and standard deviation (Figure 16C). X-score conversion was performed to ensure that the biomarkers have comparable ranges in algorithm/model development.

- 20 *Selection and construction of biomarker panel*

Selection and construction of the panel of biomarkers were done using a randomly selected training subset of the samples, involving extensive use of statistical resampling – bootstrap and leave-k-out cross-validation. Univariate assessment was performed, including ROC curve analysis, AUCs, and standard deviation of AUCs, estimated by bootstrap analysis. Examples of bootstrap estimated ROC/AUC of IGFBP2, IL6, FSH, and HE4 are provided at Figures 17A-17D.

Multivariate assessment was performed using linear and nonlinear multivariate models, including comparison of difference in AUC, and sensitivity at fixed specificity of 90% and 95%. The total number of biomarkers evaluated in the panels were ≤ 5 . FSH was added to panels as a biomarker to eliminate the need for menopausal status-dependent cutoffs. An example of a single training/testing run is shown at (Figures 18A and 18B).

Derivation of multivariate index assay models

Linear and non-linear multivariate index assay models were derived, using extensive statistical resampling. Performance of the models were compared to Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), CA125 alone, CA125+HE4, using criteria including AUC and sensitivity at fixed specificity of 90% and 95%.

Other Embodiments

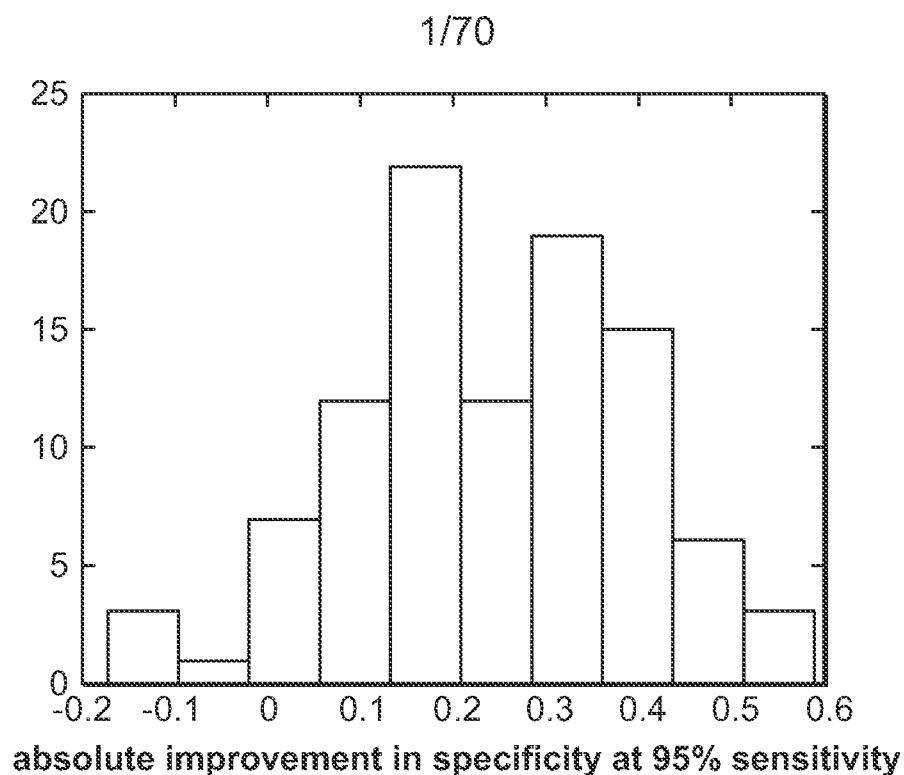
From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

WHAT IS CLAIMED IS:

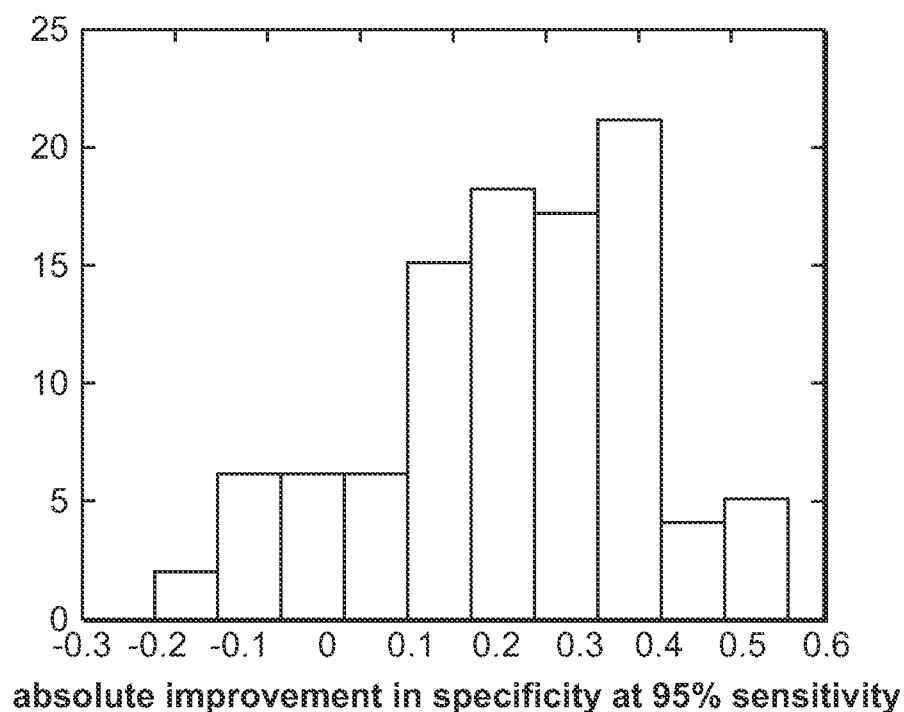
1. Use of a panel to reduce a false positive of pre-operatively assessing a risk of a subject having an adnexal mass as having ovarian cancer, the panel comprising markers Cancer Antigen 125 (CA125), Prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4), comprising measuring levels of markers in a serum sample, and comparing the level of markers in the panel to reference levels, wherein the markers are at a fixed sensitivity of about 90%, thereby reducing the false positive rate to fewer than 25% of women incorrectly identified as having ovarian cancer.
2. A method for reducing the rate of false positive pre-operative ovarian cancer assessments in subjects having an adnexal mass, the method comprising,
 - (a) measuring the level of a panel of markers in a serum sample comprising Cancer Antigen 125 (CA125), Prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4) in a biological sample derived from a subject; and
 - (b) comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90%, fewer than 25% of women are incorrectly identified as having ovarian cancer.
3. A method for pre-operatively assessing a risk of a subject having an adnexal mass as having ovarian cancer, the method comprising
 - (a) measuring the level of a panel of markers in a serum sample comprising Cancer Antigen 125 (CA125), Prealbumin, Transferrin, and Human Epididymis Protein 4 (HE4) in a biological sample derived from the subject; and
 - (b) comparing the level of said markers to a reference level, wherein at a fixed sensitivity of about 90% the specificity used by said panel is about 85%.
4. The method of claim 2, wherein the specificity is about 75%, about 80%, about 90%, or about 95%.
5. The method of claim 2 or 3, wherein the method further involves assessing clinical markers of ovarian cancer risk comprising age, pre-menopausal status, post-menopausal status, family history, physical examination, imaging results, and history of smoking.
6. The method of claim 2 or 3, wherein the comparing comprises using a linear model.

7. The method of claim 2 or 3, wherein the comparing comprises using a non-linear model.
8. The method of claim 2 or 3, wherein the measuring step is conducted by immunoassay or affinity capture.
9. The method of claim 8, wherein the immunoassay comprises affinity capture assay, immunometric assay, heterogeneous chemiluminescence immunometric assay, homogeneous chemiluminescence immunometric assay, enzyme-linked immunosorbent assay (ELISA), western blotting, radioimmunoassay, magnetic immunoassay, real-time immunoquantitative polymerase chain reaction (iqPCR) or Surface-enhanced Raman spectroscopy (SERS) label free assay.
10. The method of claim 2 or 3, wherein the method is carried out with a plate, chip, beads, microfluidic platform, membrane, planar microarray, or suspension array.



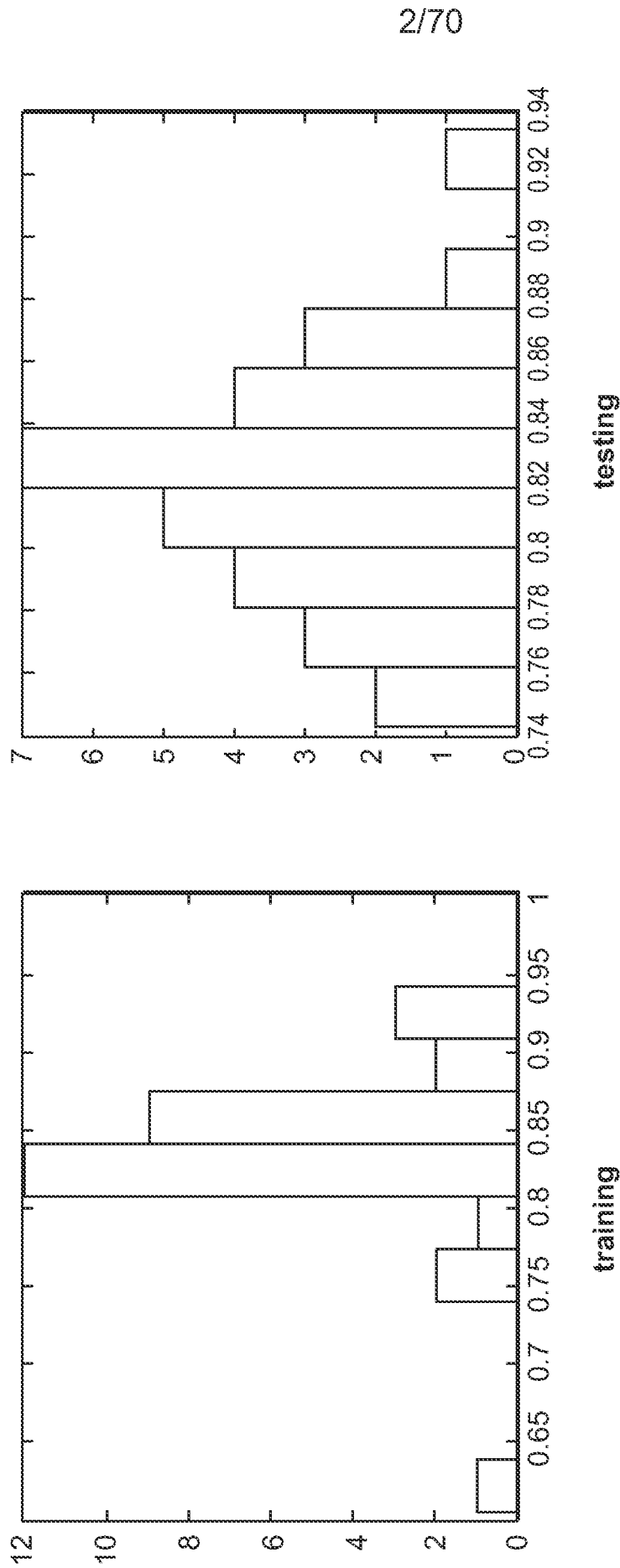
Panel: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH.

FIG. 1



Panel: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, and FSH

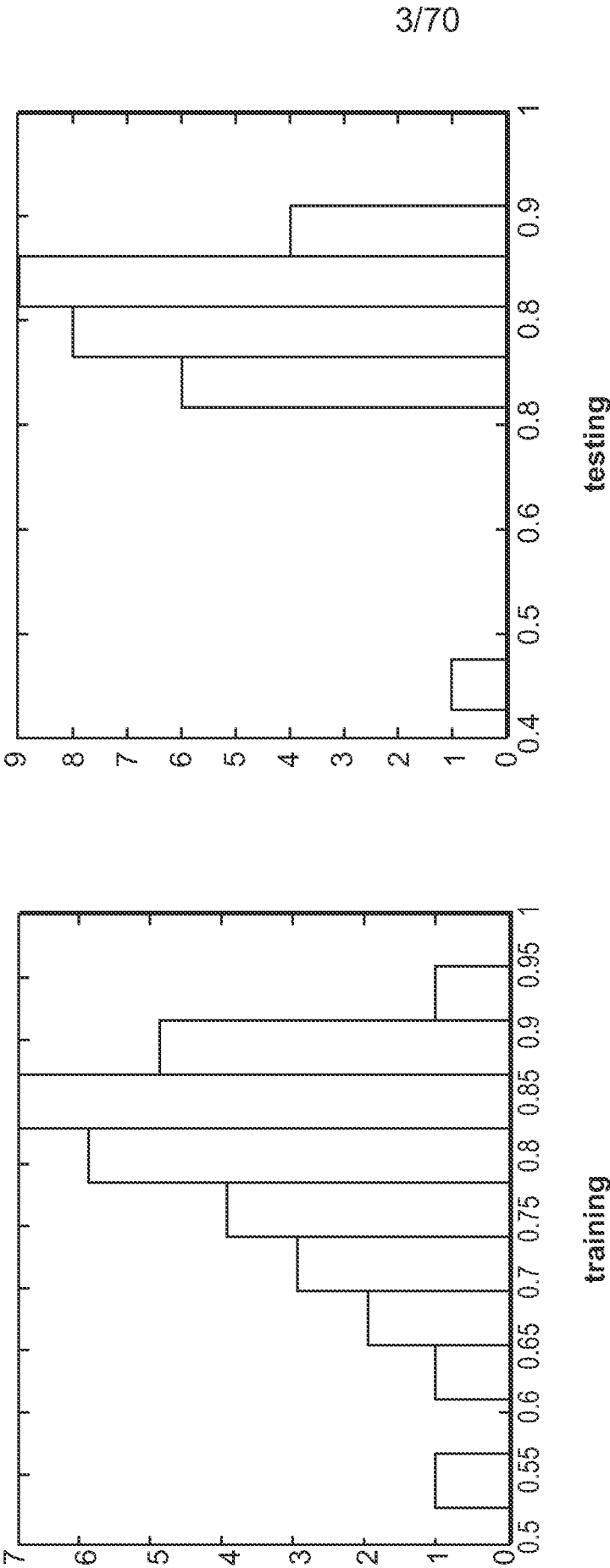
FIG. 2



Linear Models: Distribution of Specificity at 90% Sensitivity in 30 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

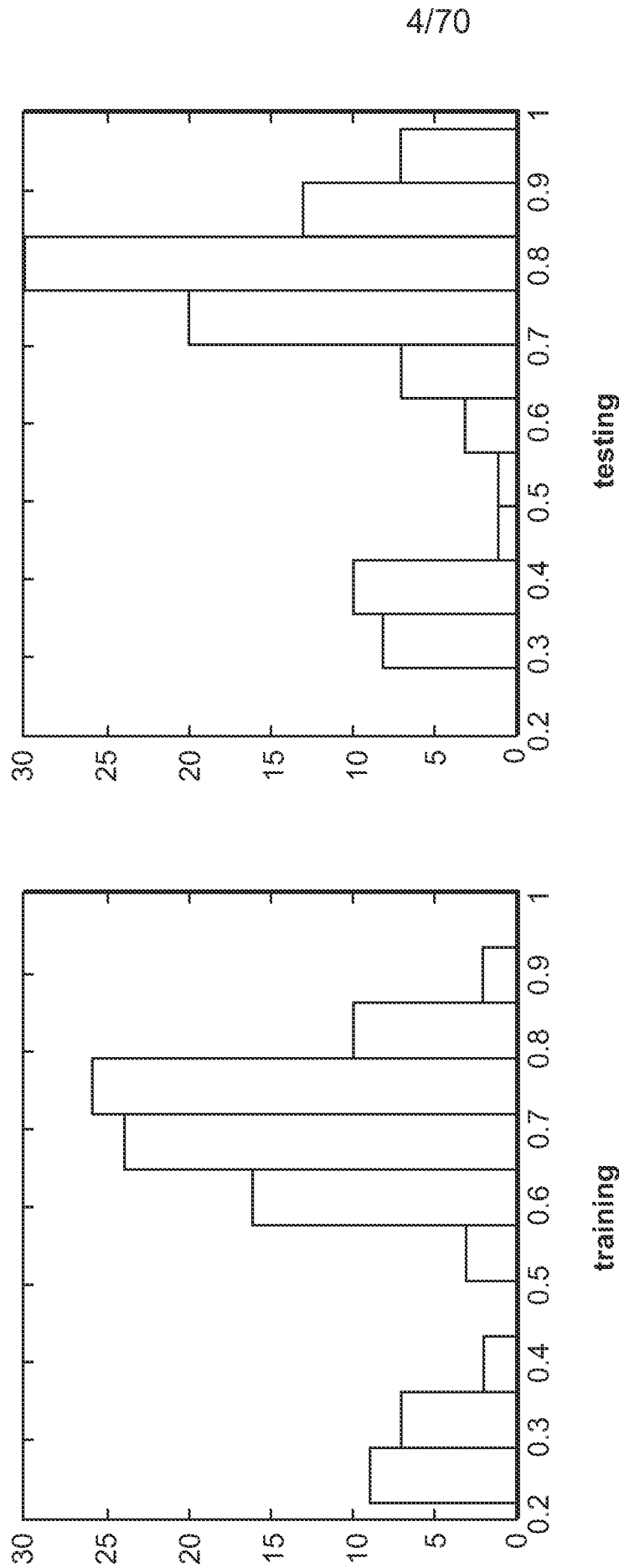
FIG. 3



Nonlinear Models: Distribution of Specificity at 90% Sensitivity in 30 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

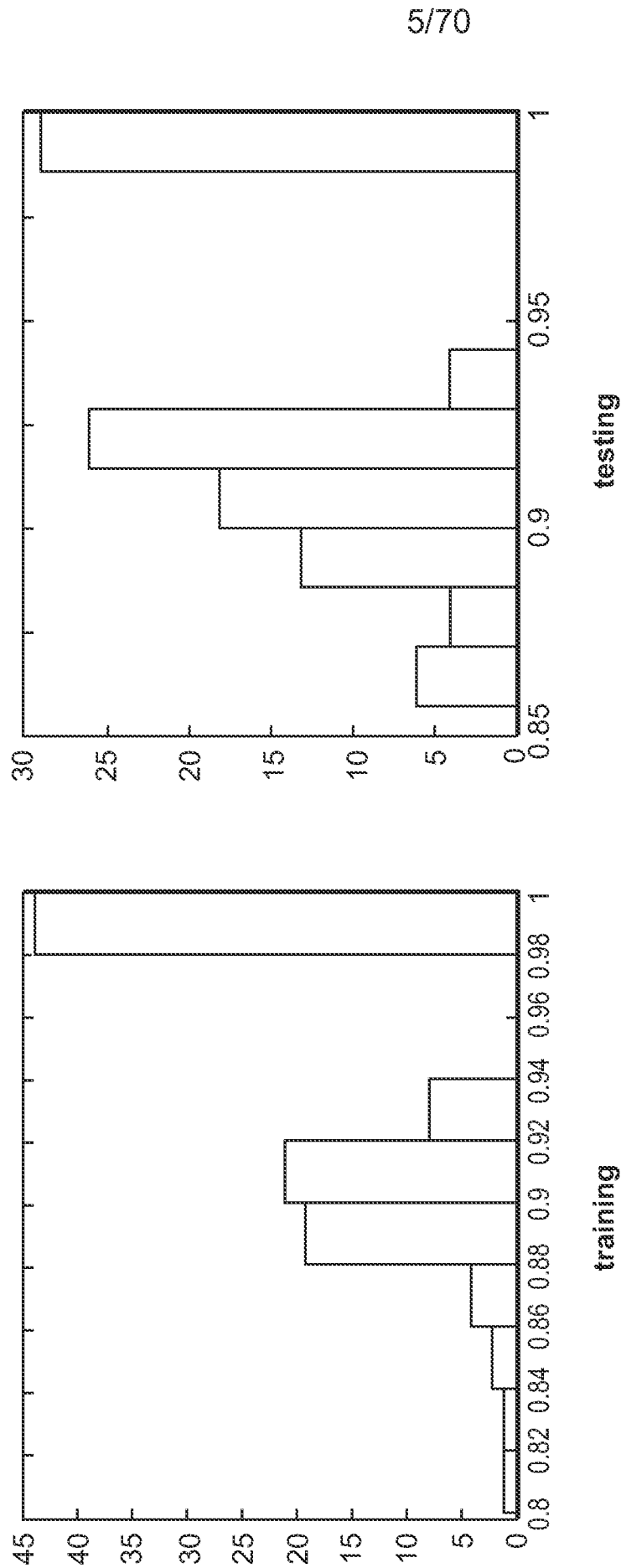
FIG. 4A



Nonlinear Models: Distribution of Specificity at 95% Sensitivity in 100 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

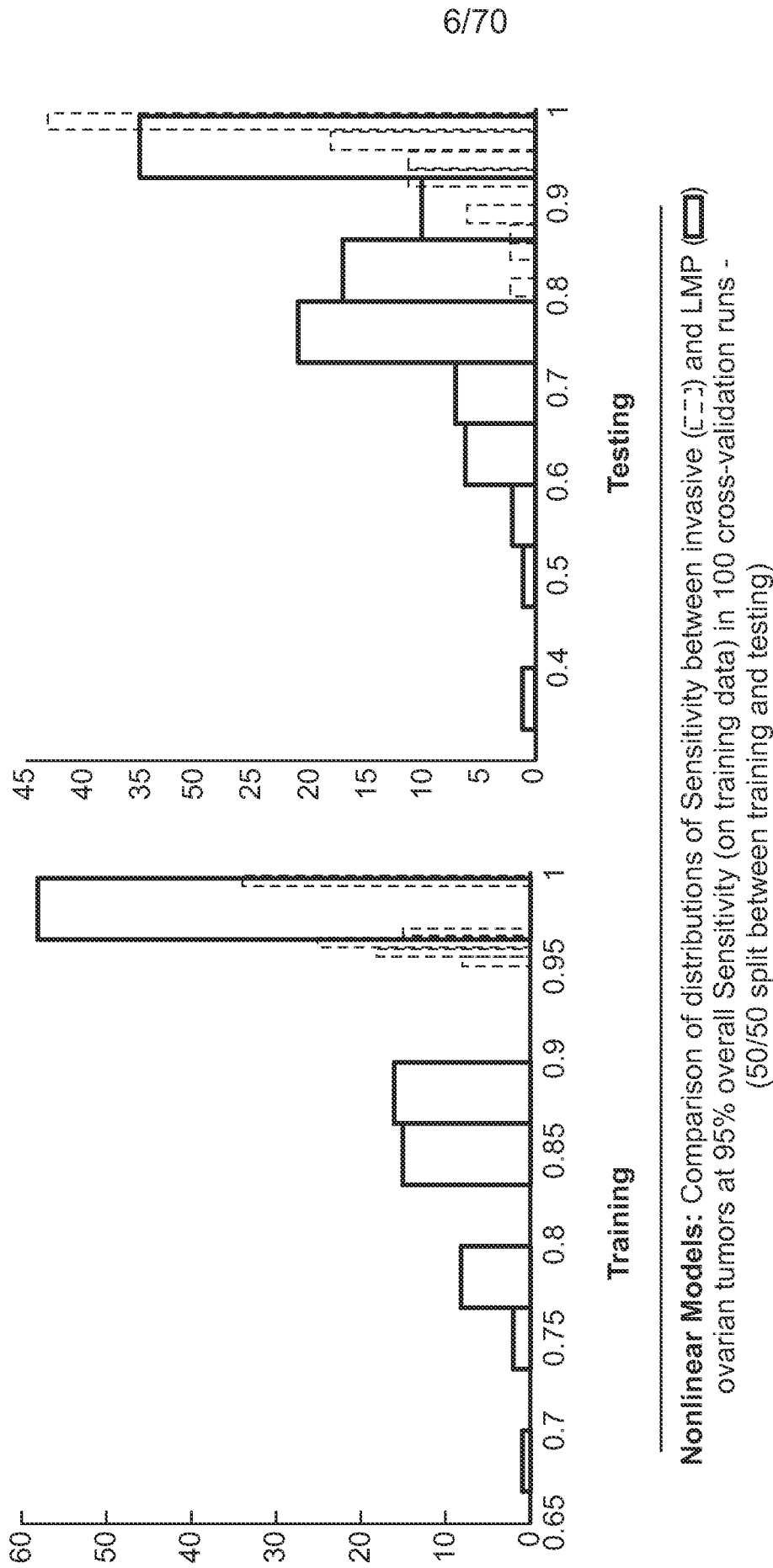
FIG. 4B



Nonlinear Models: Distribution of Sensitivity for stage 1 cases at 95% overall Sensitivity in 100 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

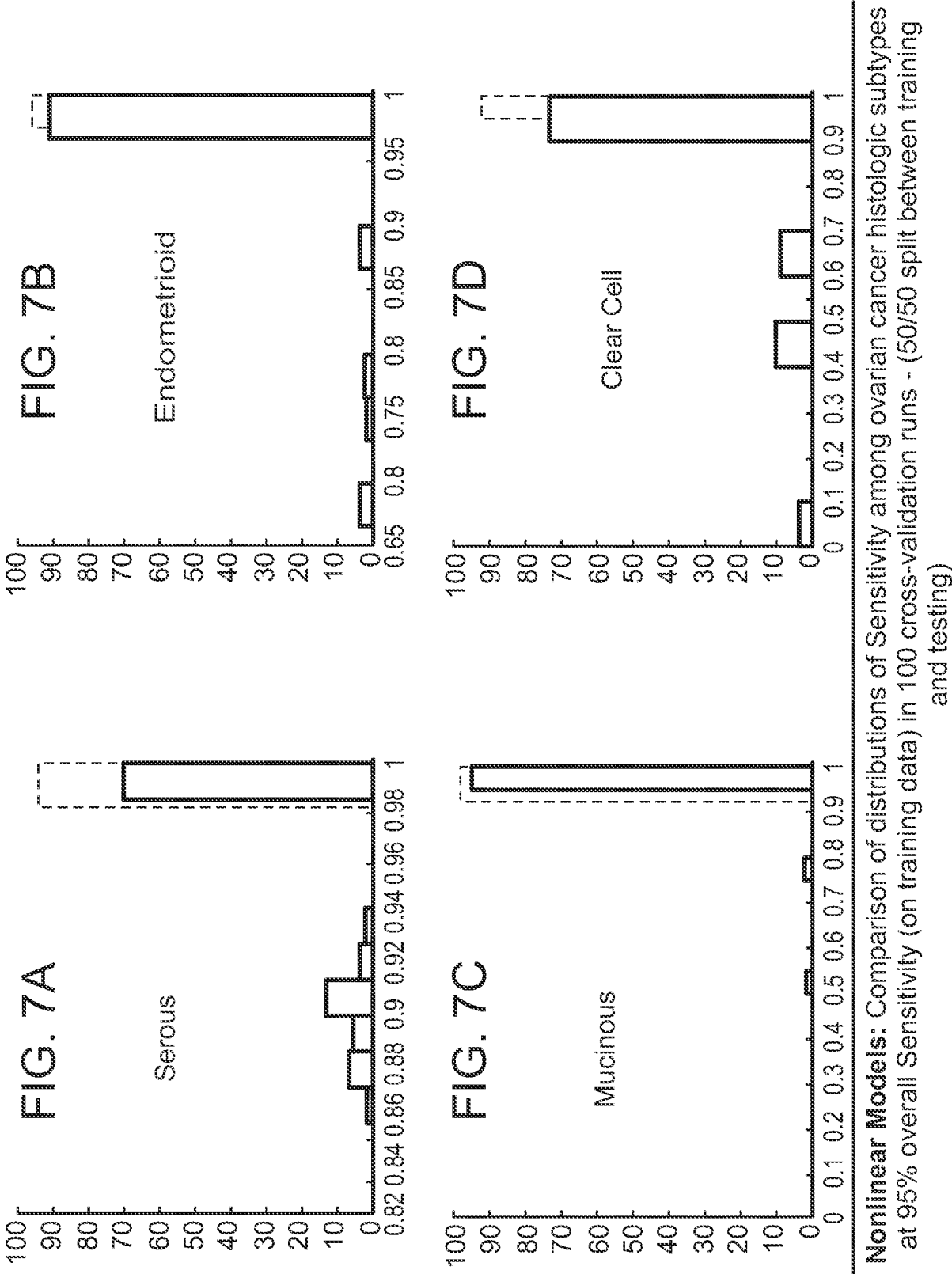
FIG. 5

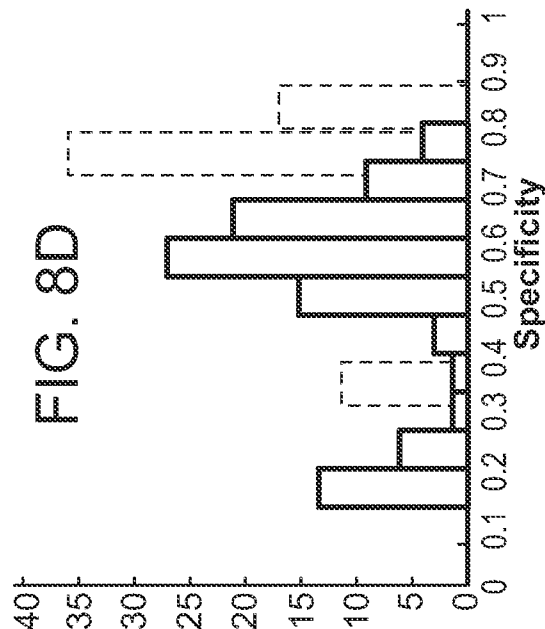
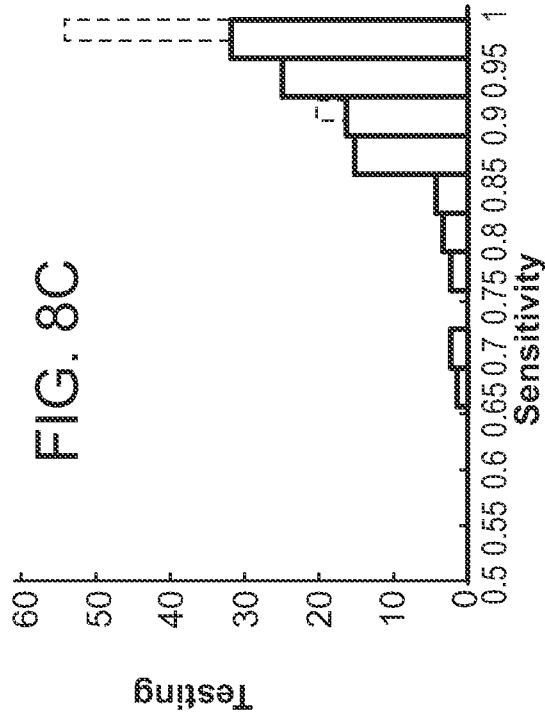
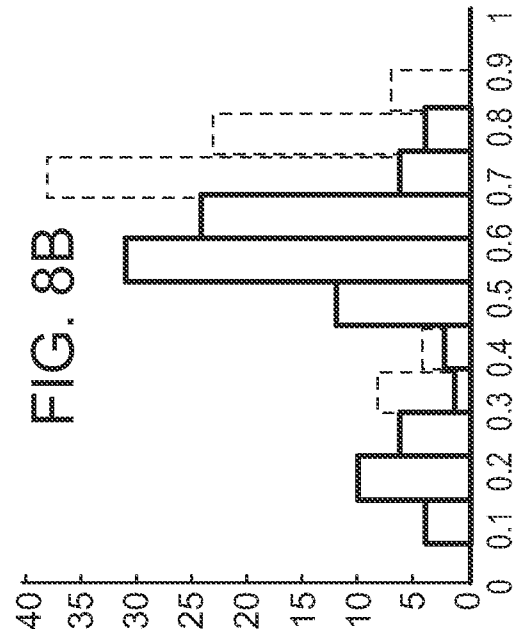
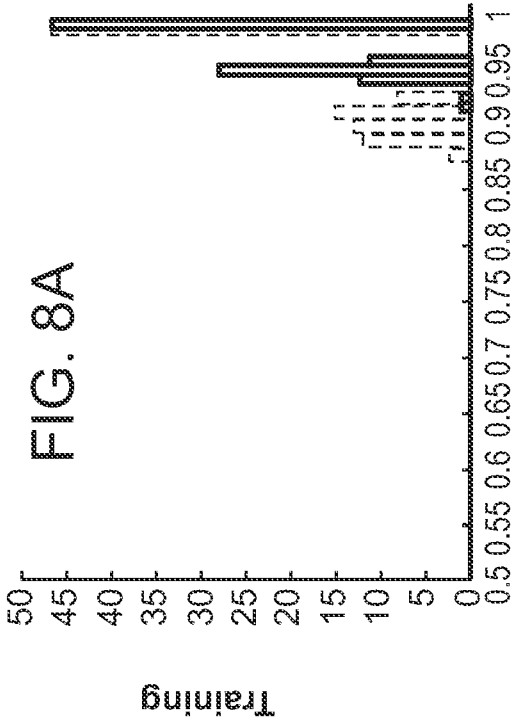


Nonlinear Models: Comparison of distributions of Sensitivity between invasive (—) and LMP (---) ovarian tumors at 95% overall Sensitivity (on training data) in 100 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

FIG. 6

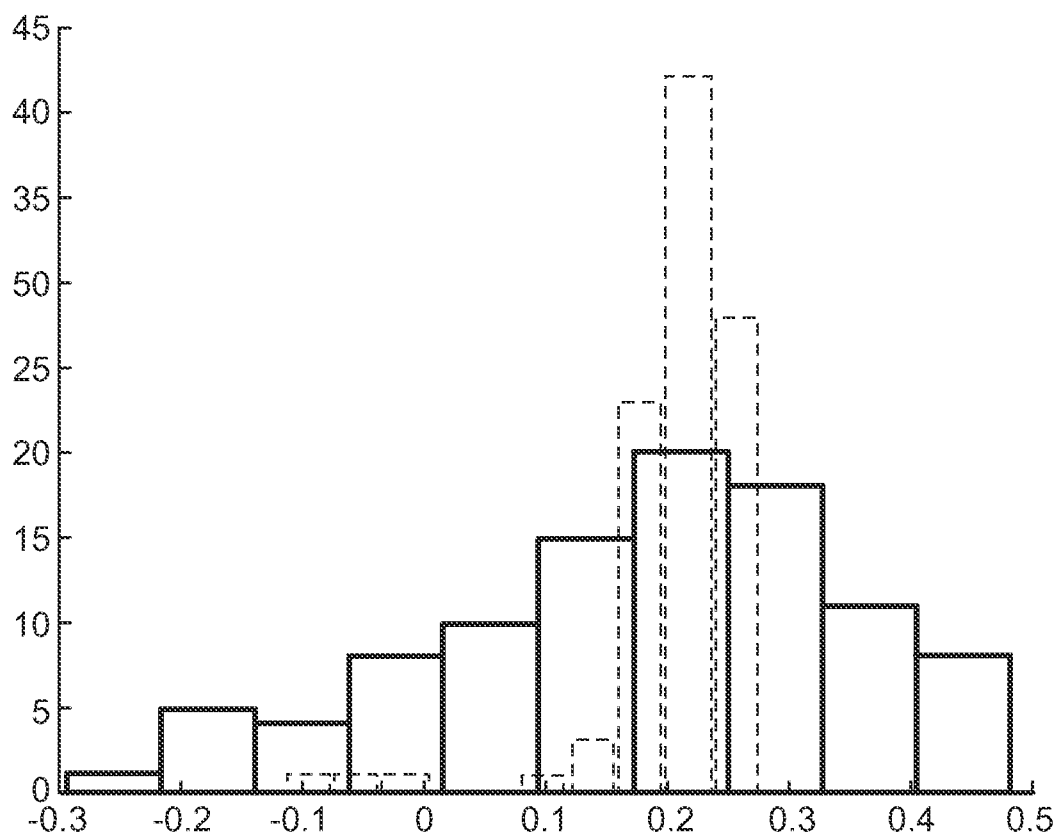




Nonlinear Models: Breakdown of Sensitivity and Specificity between Pre- (---) and Post- (—) Menopausal patients at cutoff corresponding to 95% overall Sensitivity in 100 cross-validation runs -

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

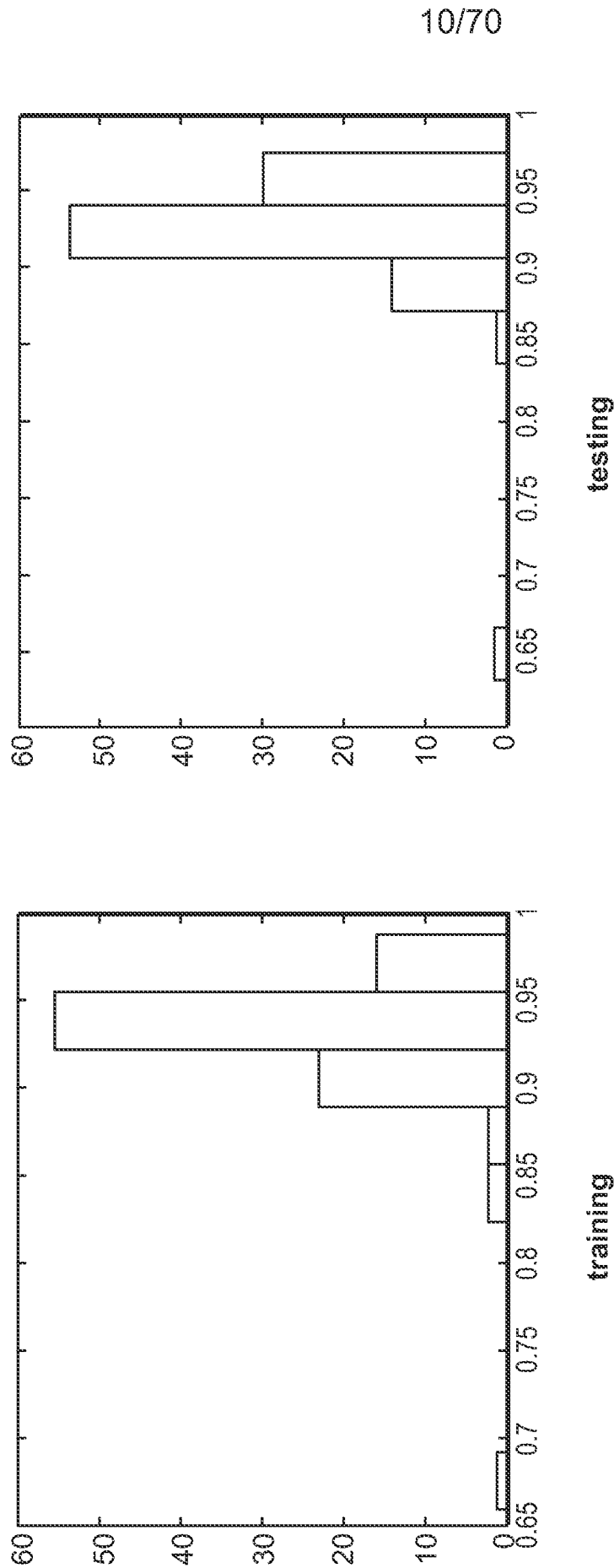
9/70



Distribution of improvement in Specificity of non-linear models over OVA1, both computed using all benign controls at cutoffs corresponding to 95% overall Sensitivity in 100 cross-validation runs - (50/50 split between training and testing).
□ : evaluated on combined training and testing sets
▣ : evaluated on test sets only

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

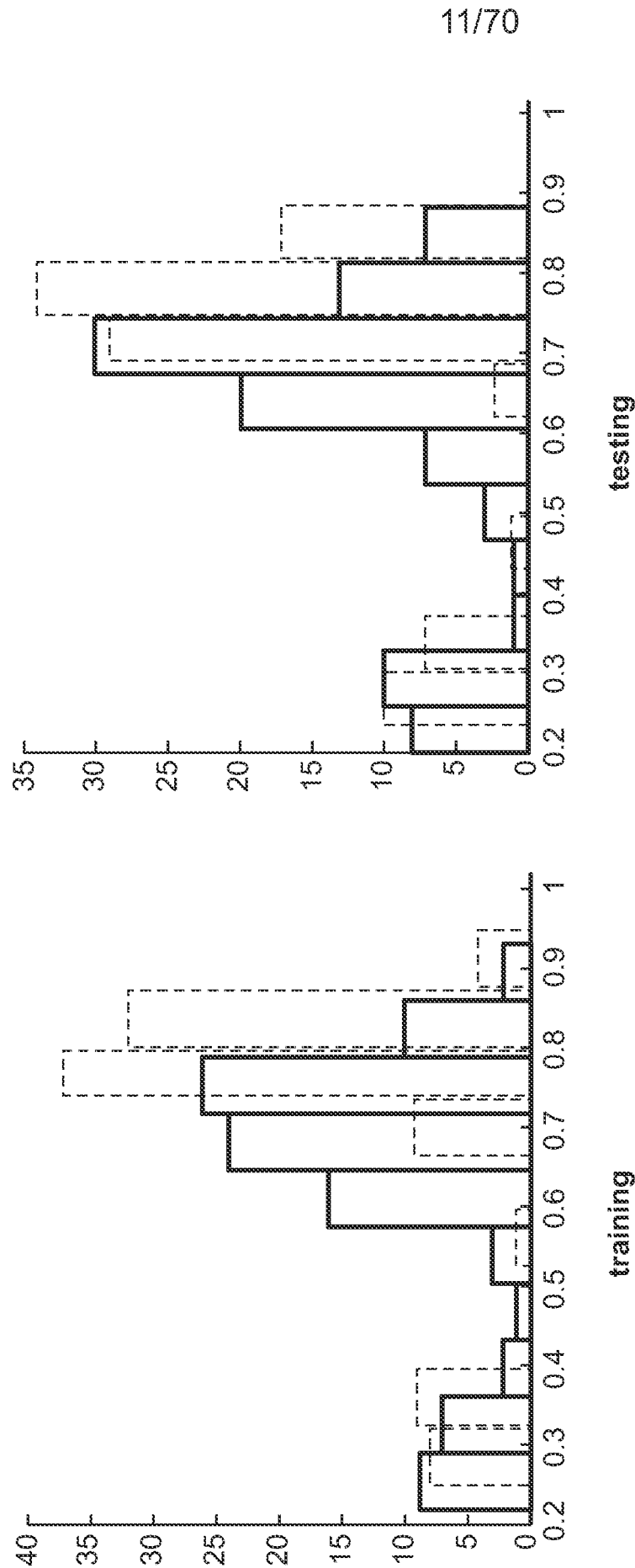
FIG. 9



Nonlinear Models: Distribution of ROC/AUC in 100 cross-validation runs - (50/50 split between training and testing)

Panel: CA125, prealbumin, IGFBP2, and IL6, and FSH

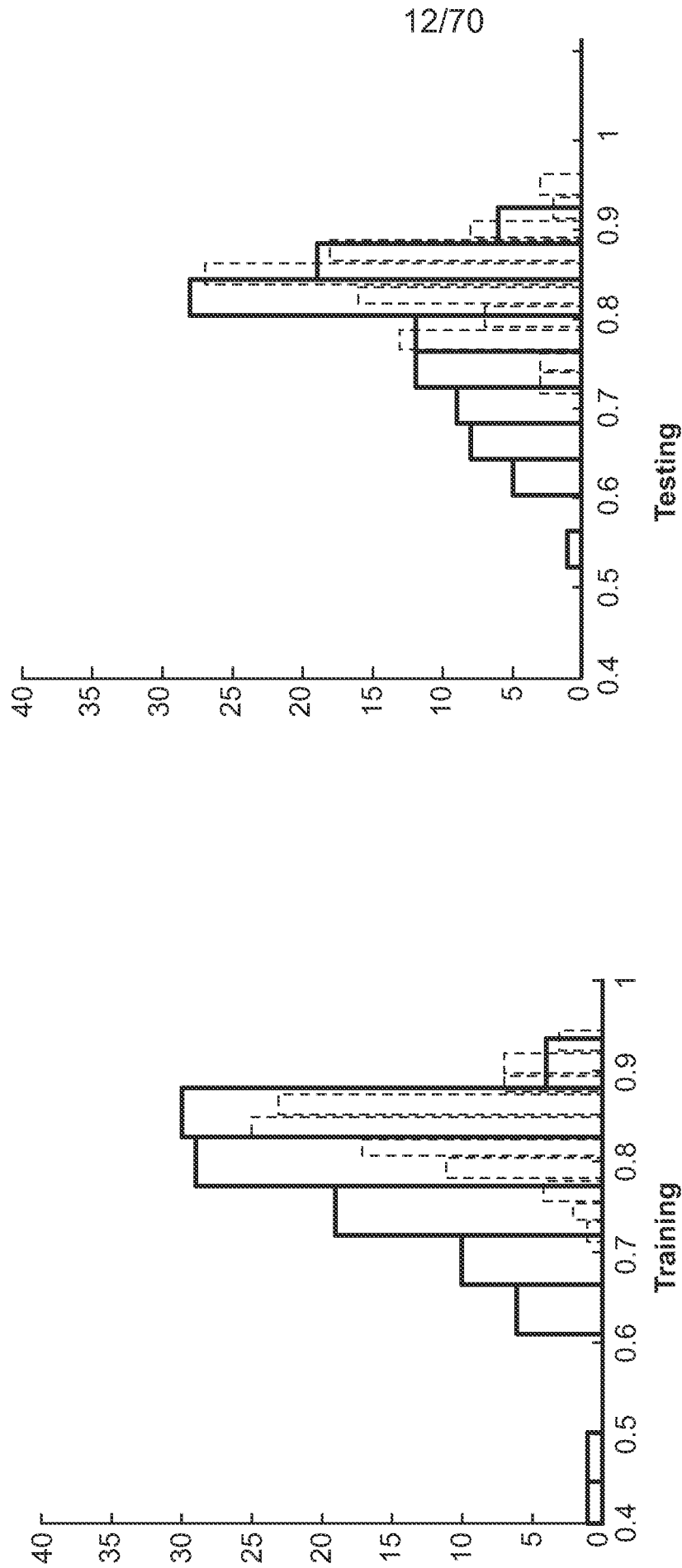
FIG. 10



Nonlinear Models: Distribution of Specificity at 95% Sensitivity in 100 cross-validation runs (50/50 split between training and testing). Dashed outlines: replacing FSH with HE4

Panel: CA125, prealbumin, IGFBP2, IL6, and FSH
CA125, prealbumin, IGFBP2, IL6, and HE4

FIG. 11



Nonlinear Model: Distribution of Specificity at 90% Sensitivity in 100 cross-validation runs. (50/50 split between training and testing). Dashed outlines: replacing FSH with HE4.

FIG. 12

Comparison of OVA1+HE4 linear model (---) against OVA1 alone (■).
 Notice that OVA1 and combo model performances were not calculated using
 menopausal status dependent cutoffs.

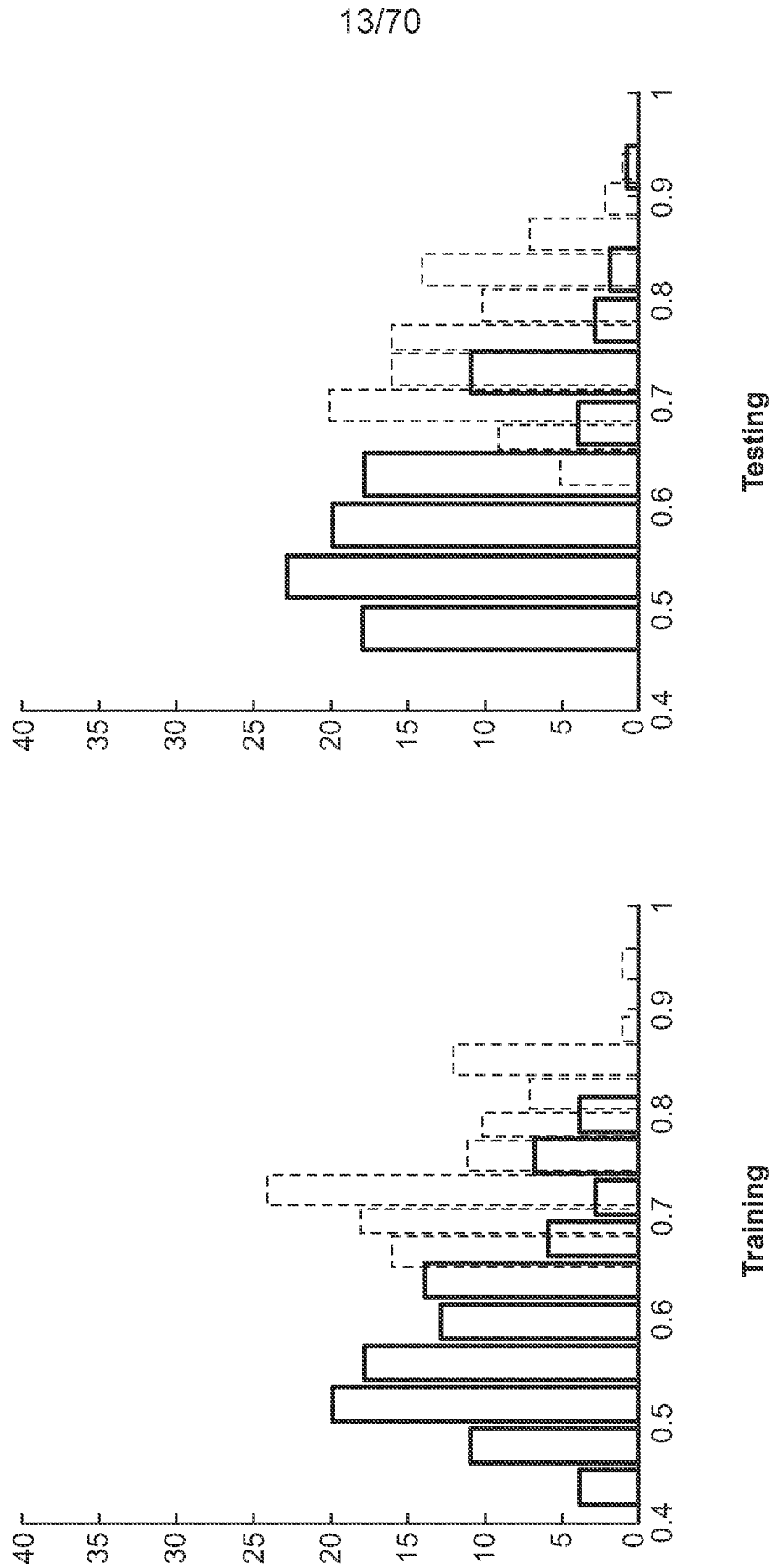


FIG. 13

Nonlinear Models: Distribution of specificity at 90% sensitivity among
 1) CA125+Prealb+TRF+HE4 (---), 2) CA125+Prealb+HE4 (□), and 3) OVA1 (■).
 Notice that OVA1 performances were not calculated using product cutoffs.
 100 cross-validation runs, 50/50 split between training and testing.

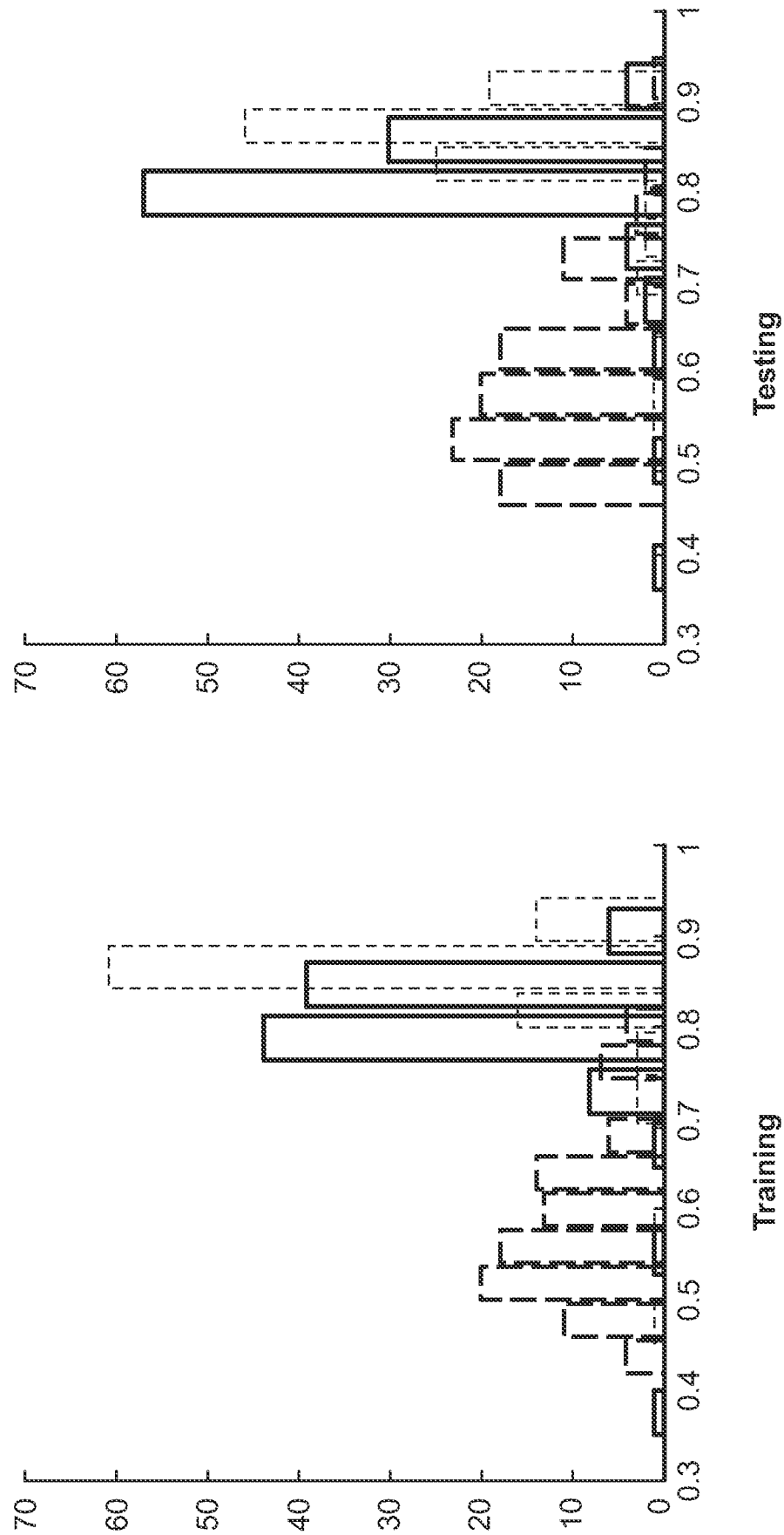
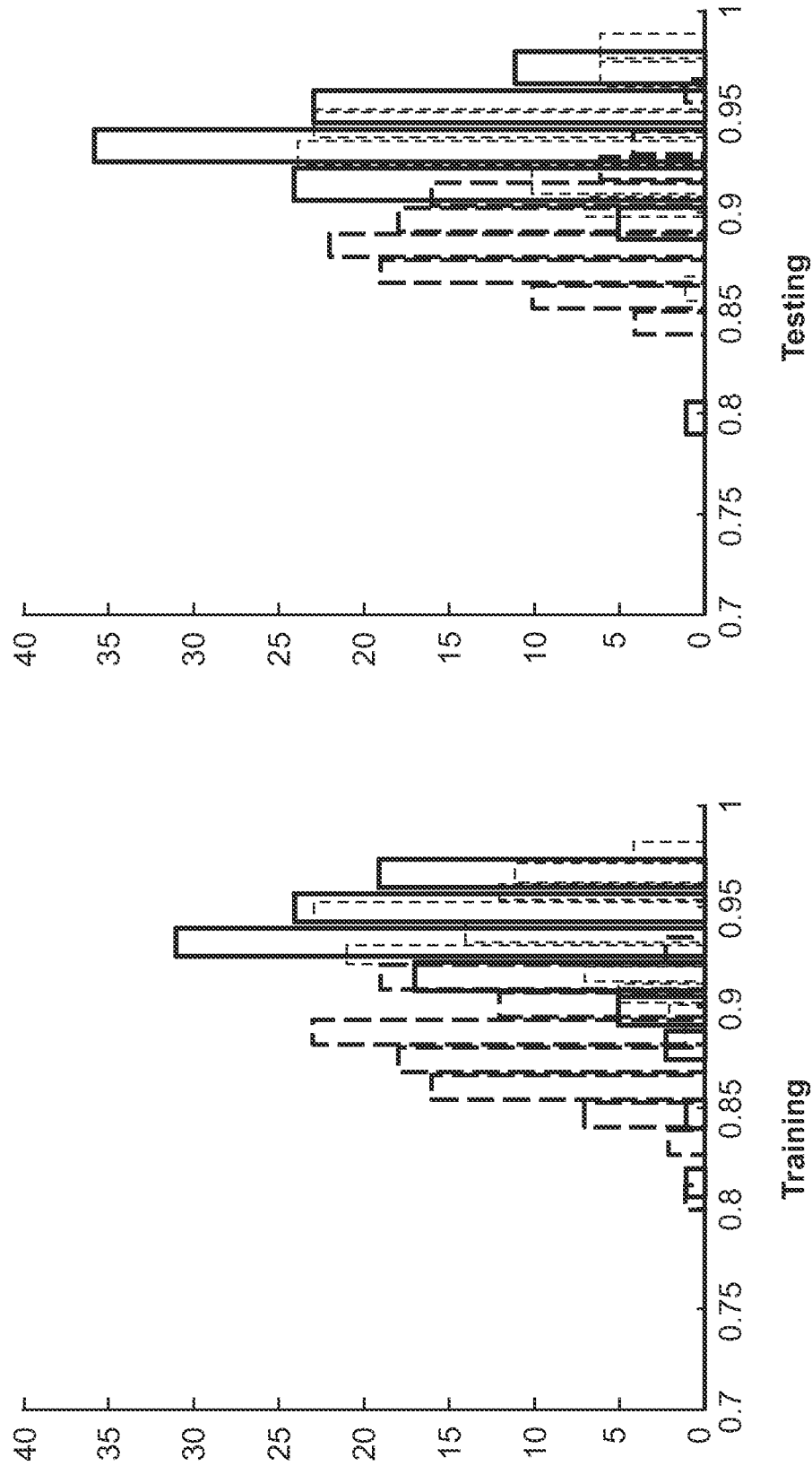


FIG. 14

Nonlinear Models: Distribution of ROC/AUCs among
 1) CA125+Prealb+TRF+HE4 (—), 2) CA125+Prealb+HE4 (---), and 3) OVA1 (---).
 100 cross-validation runs, 50/50 split between training and testing.



Note: There were no noticeable differences in distributions of AUCs between the 3 marker model (—) and 4 marker model (---) since both had reached fairly high values. However, there were differences between the two models comparing specificity at 90% sensitivity, indicating that the addition of the 4th marker may mainly contribute to the shape of the ROC rather than its AUC.

FIG. 15

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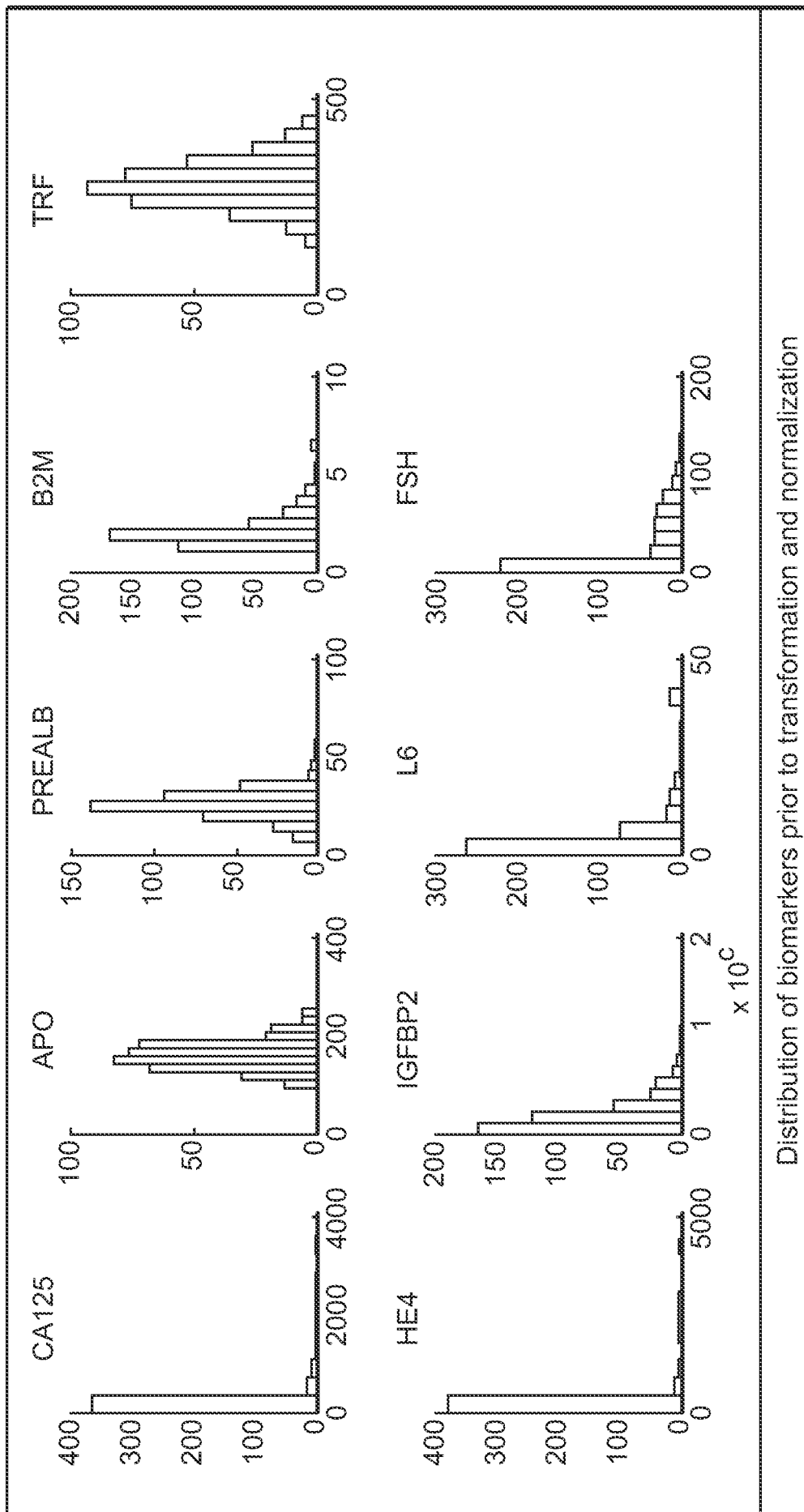


FIG. 16A

Distribution of biomarkers prior to transformation and normalization

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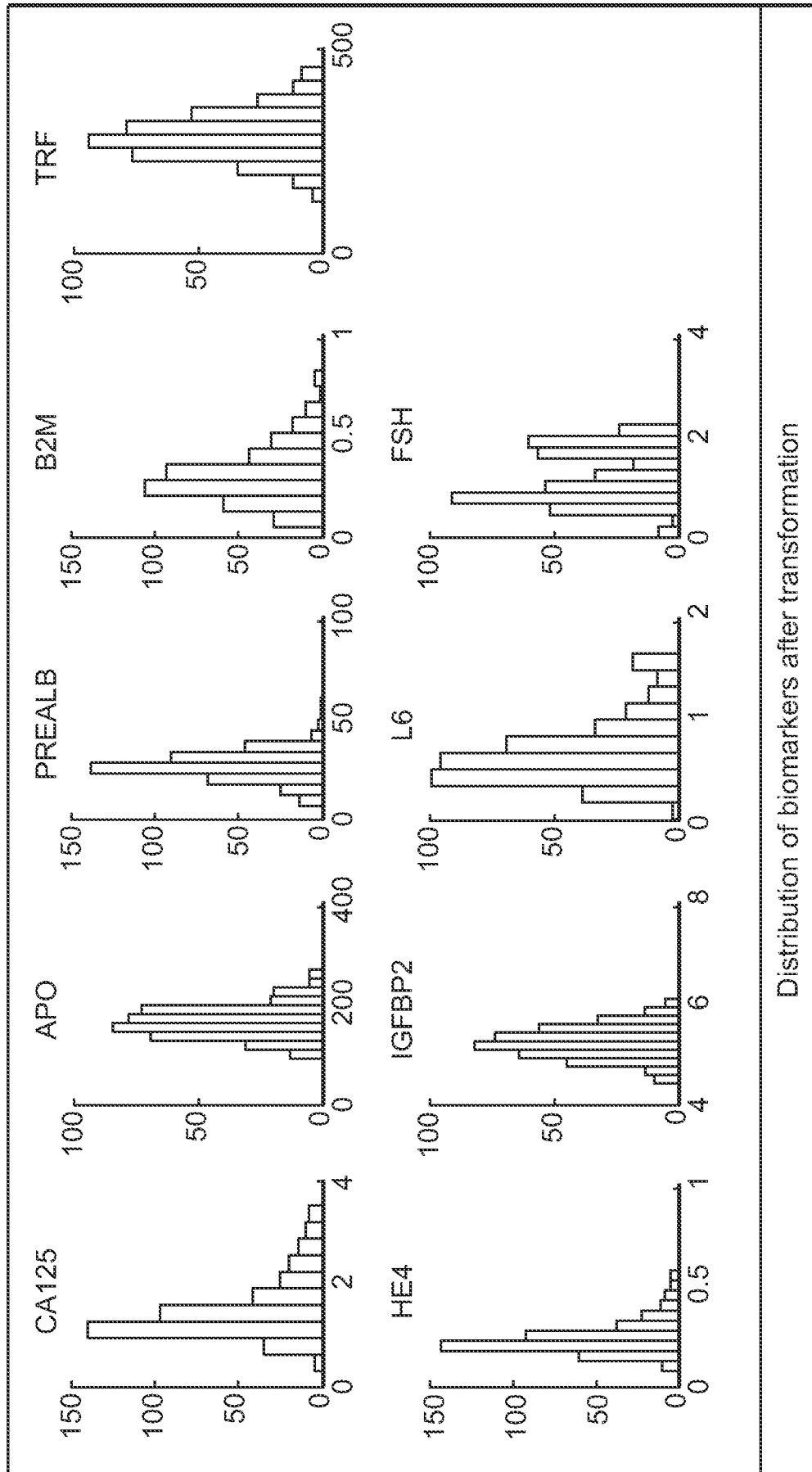


FIG. 16B

Distribution of biomarkers after transformation

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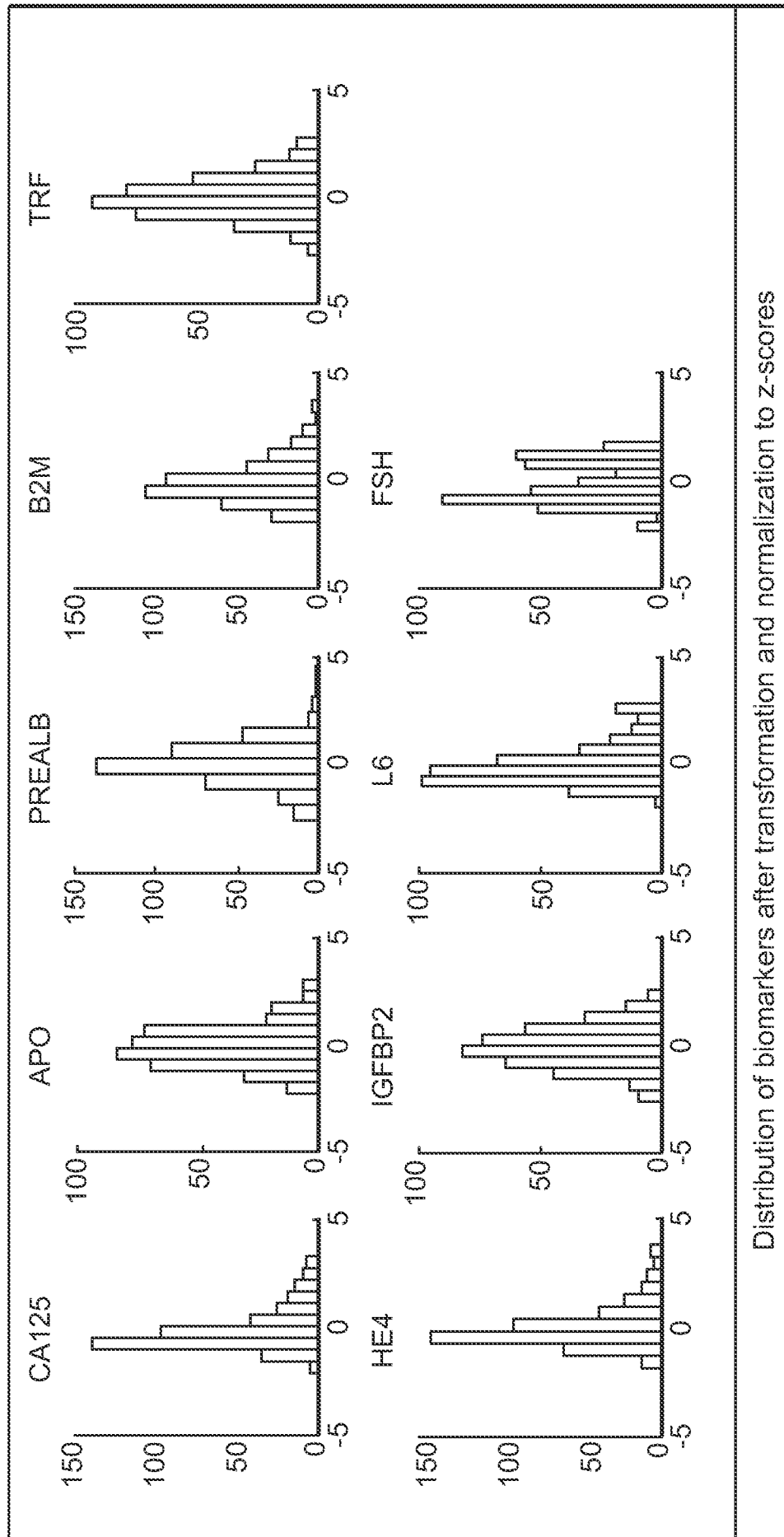
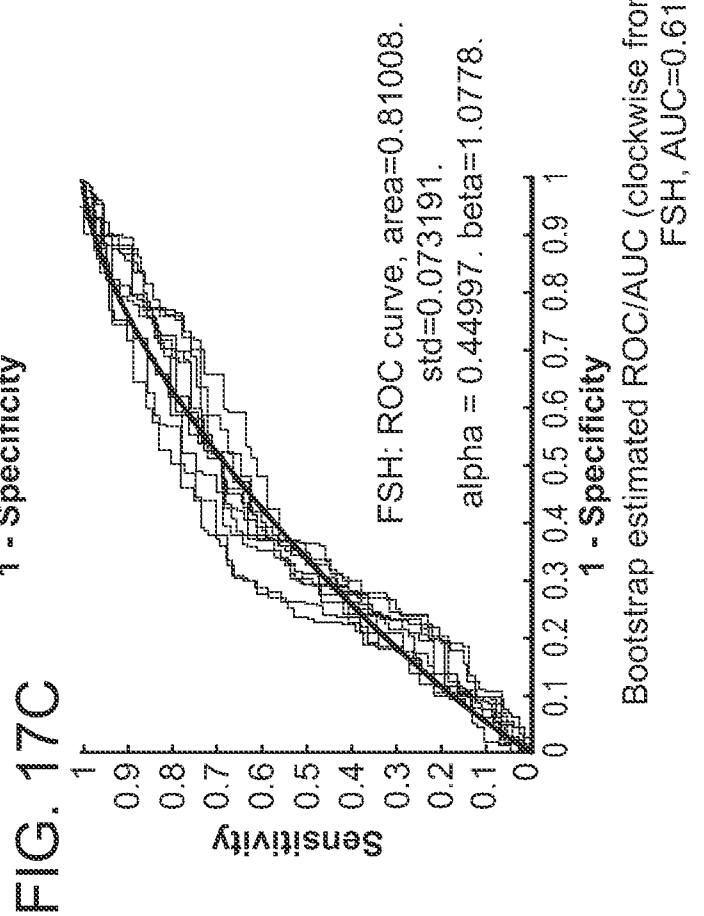
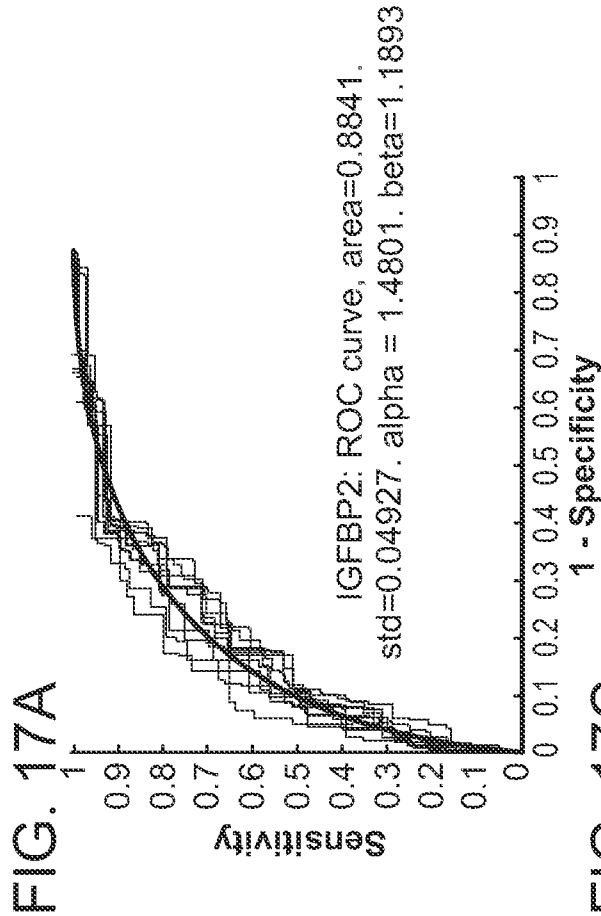
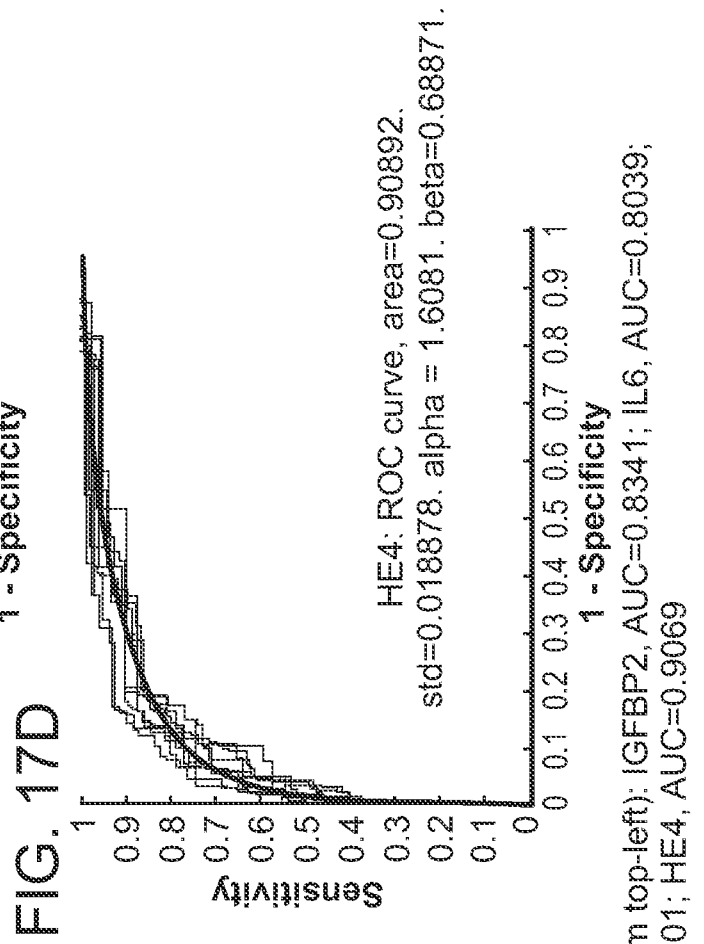
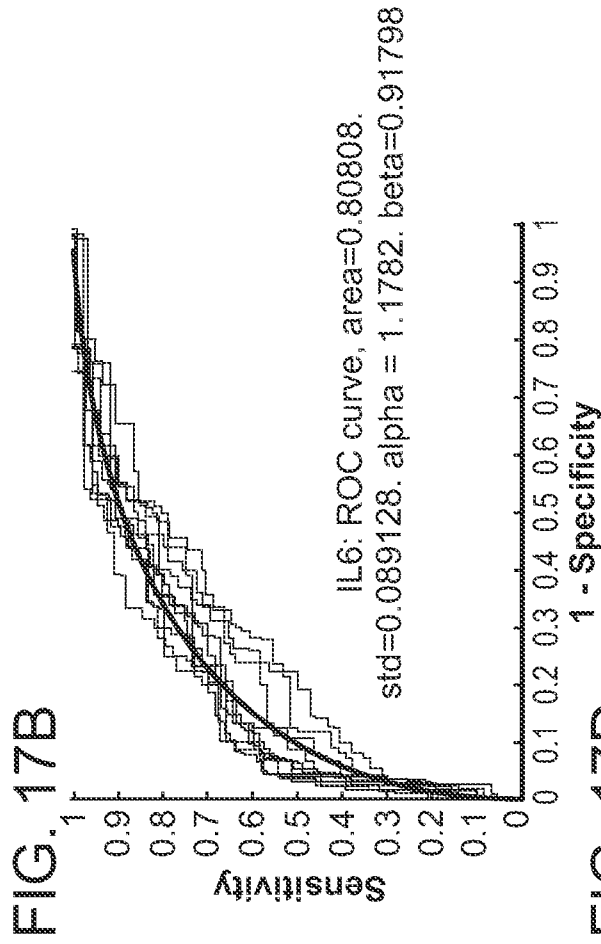


FIG. 16C

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Bootstrap estimated ROC/AUC (clockwise from top-left): IGFBP2, AUC=0.8341; IL6, AUC=0.8039;
FSH, AUC=0.6101; HE4, AUC=0.9069

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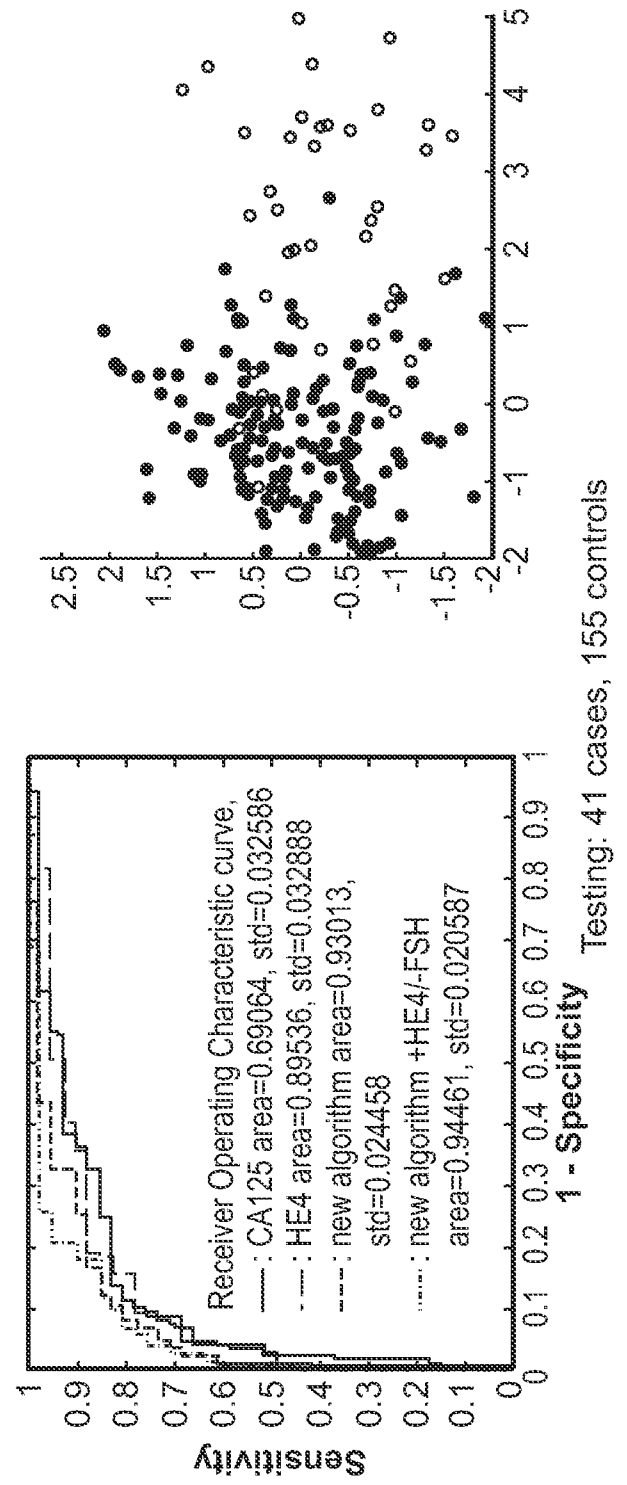
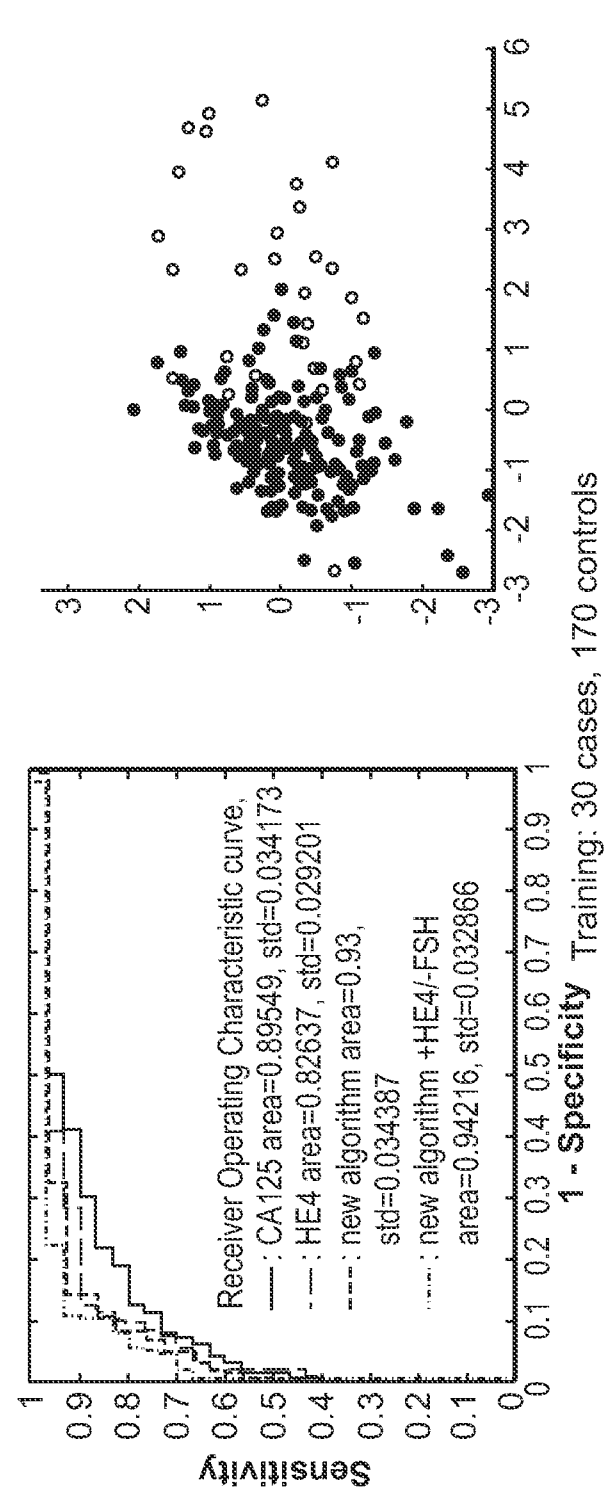


FIG. 19A-1***Insulin-like growth factor binding protein (IGFBP2)***

1 mlprvGPCal plppppllpl lpllllllga sgggggarae vlfrcppctp erlaacgppp
 61 vappaavaav aggarmpcae lvrepgcgcc svcarlegea cgvytprcgq glrcyphpgs
 121 elplqalvmg egtcekrda eygaspeqva dngddhsegg lvenhvdstm nmlggggsag
 181 rkplksgmke lavfrekvte qhrqmgkggk hhlgleepkk lrpptartpc qgelldgvler
 241 istmrlpder gplehlyslh ipncdkhgly nlkqckmsln gqrgeowcwn pntgkllqga
 301 ptirgdpech ifyneqqear gvhtqrmq

Interleukin 6 (IL6)

1 mnsfstsaFg pvaflglill vlpaafpapv ppgedskdva aphrqplts erldkqiryi
 61 ldgisalrke tcnksnmces skealaennl nlpkmaekdg cfqsgfneet clvkiitgll
 121 efevyleylq nrfesseeqa ravqmstkvl iqflqkkakn ldaittpdpt tnaslltklq
 181 aqnqlqdmT thlilrsfke flqsslrar qm

Follicle-stimulating hormone (FSH)

1 mktlqffflf ccwkaiccnS celtnitiai ekeecrfcis inttwcagyc ytrdlvykdp
 61 arpkiqktct fkelvyetvr vpgcahads lytypvatqc hcgkcdsdst dctvrglgps
 121 ycsfgemke

Human Epididymis Protein 4 (HE4)

1 mpacrlgpla aallslslf gftlvsgtga ektgvcpelq adqntqecv sdsecadnlk
 61 ccsagcatfc slpndkegsc pqvninfpql glcrdqcvd sqcpqmkcc rngcgkvscv
 121 tpnf

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FIG. 19B-1

CA 125 (SEQ ID NO:2)

10	20	30	40	50	60
MLKPSGLPGS	SSPTRSLMTG	SRSTKATPEM	DSGLTGATLS	PKTSTGAIVV	TEHTLPFTSP
70	80	90	100	110	120
DKTLASPTSS	VVGRTTQSLG	VMSSALPEST	SRGMTHSEQR	TSPSLSPQVN	GTPSRNYPAT
130	140	150	160	170	180
SMVSGLSSPR	TRTSSTEGNF	TKEASTYTLT	VETTSQGVTE	KYTVPTETST	TEGDSTETPW
190	200	210	220	230	240
DTRYIPVKIT	SPMKTFADST	ASKENAPVSM	TPAETTVTDS	HTPGRNPSF	GTLYSSFLDL
250	260	270	280	290	300
SPKGTNSRG	ETSLELILST	TGYPFSSPEP	GSAGHSRIST	SAPLSSASV	LDNKISETSI
310	320	330	340	350	360
FSGQSLTSPL	SPGVPEARAS	TMPNSAIPFS	MTLSNAETSA	ERVRSTISSL	GTPSISTKQT
370	380	390	400	410	420
AETILTFHAF	AETMDIPSTH	IAKTLASEWL	GSPGTLGGTS	TSALTTTSPS	TTLVSEETNT
430	440	450	460	470	480
HHSTSGKETE	GTLNTSMTPL	ETSAPGEESE	MTATLVPTLG	FTTLDSKIRS	PSQVSSSHPT
490	500	510	520	530	540
RELRTTGSTS	GRQSSSTA AH	GSSDILRATT	SSTSKASSWT	SESTAQQFSE	PQHTQWVETS

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FIG. 19C-1

550	560	570	580	590	600
PSMKTERPPA	STSVAAPIIT	SVPSVVSGET	TLKTSSTKGI	WLEETSADTL	IGESTAGPTT
610	620	630	640	650	660
HQFAVPTGIS	MTGGSSTRGS	QGTTHLLTRA	TASSETSADL	TLATNGVPVS	VSPAUSKTAA
670	680	690	700	710	720
GSSPPGGTKP	SYTMVSSVIP	ETSSLQSSAF	REGTSLGLTP	LNTRHPFSSP	EPDSAGHTKI
730	740	750	760	770	780
STSIPLLSA	SVLEDKVSAT	STFHHKATS	SITGTPEIS	TKTKPSSAVL	SSMTLSNAAT
790	800	810	820	830	840
SPERVRNATS	PLTHPSPSGE	ETAGSVLTLS	TSAETTDSPN	IHPTGTLTSE	SSESPSTLSL
850	860	870	880	890	900
PSVSGVKTFE	SSSTPSTHLF	TSGEETEETS	NPSVSQPEIS	VSRVRTTLAS	TSVPTPVFPT
910	920	930	940	950	960
MDTWPTRSAQ	FSSSHLVSEL	RATSSSTSVTN	STGSALPKIS	HLTGTATMSQ	TNRDTFNDSA
970	980	990	1000	1010	1020
APQSTTWPET	SPRFKTGLPS	ATTTVSTSAT	SLSATVMVSK	FTSPATSSME	ATSIREPSTT
1030	1040	1050	1060	1070	1080
ILTTETTNGP	GSMVAASSTNI	PIGKYITEG	RLDTSHLPIG	TTASSETSMO	FTMAKESVSM

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FIG. 19D-1

1090	1100	1110	1120	1130	1140
SVSPSQSMDA	AGSSTPGRTS	QFVDTFSDDV	YHLTSREITI	PRDGTSSALT	PQMTATHPPS
1150	1160	1170	1180	1190	1200
PDPGSARSTW	LGILSSSPSS	PTPKVTMSST	FSTQRVTISM	IMDTVETSRW	NMPNLPSTTS
1210	1220	1230	1240	1250	1260
LTPSNIPTSG	AIGKSTLVPL	DTPSPATSLE	ASEGGLPTLS	TYPESTNTPS	IHLGAHASSE
1270	1280	1290	1300	1310	1320
SPSTIKLTMA	SVVKPGSYTP	LTFPSIETHI	HVSTARMAYS	SGSSPEMTAP	GETNTGSTWD
1330	1340	1350	1360	1370	1380
PTTYITTTDP	KDTSSAQVST	PHSVRTLRTT	ENHPKTESAT	PAAYSGSPKI	SSSPNLTSPA
1390	1400	1410	1420	1430	1440
TKAWTITDIT	EHSTQLHYTK	LAEKSSGFET	QSAPGPVSVV	IPTSPTIGSS	TLELTSDVPG
1450	1460	1470	1480	1490	1500
EPLVLAPSEQ	TTITLPMATW	LSTSLTEEMA	STDLDISSPS	SPMSTFAIFP	PMSTPSHEL
1510	1520	1530	1540	1550	1560
KSEADTSAIR	NTDSTTLDQH	LGIRSLGRGT	DLTTVPITPL	TTTWTSVIEH	STQAQDTLSA
1570	1580	1590	1600	1610	1620
TMSPTHVTQS	LKDQTSIPAS	ASPSHLTEVY	PELGTQGRSS	SEATTFWKPS	TDTLSREIET

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FIG. 19E-1

1630	1640	1650	1660	1670	1680
GPTNIQSTPP	MDNTTIGSSS	SGVTLGIAHL	PIGTSSPAET	STNMALERRS	STATVSMAGT
1690	1700	1710	1720	1730	1740
MGLLVTSAPG	RSISQSLGRV	SSVLSESTTE	GVTDSKKGSS	PRLNTQGNAT	LSSSLEPSYA
1750	1760	1770	1780	1790	1800
EGSQMSTSI	LTSSPTTPDV	EFIGGSTFWT	KEVTTVMTSD	ISKSSARTES	SSATLMSTAL
1810	1820	1830	1840	1850	1860
GSTENTGKEK	LRTASMDLPS	PTPSMEVTPW	ISLTLSNAPN	TTDSLDSLHG	VHTSSAGTLA
1870	1880	1890	1900	1910	1920
TDRSLNTGVT	RASRLNGSD	TSSKSLSMGN	STHTSMTDTE	KSEVSSSIHP	RPETSAPGAE
1930	1940	1950	1960	1970	1980
TTLTSTPGNR	AISLTLPFSS	IPVEEVISTG	ITSGPDINSA	PMTHSPIITPP	TIVWTSTGTI
1990	2000	2010	2020	2030	2040
EQSTQPLHAV	SSEKVSQVQTQ	STPYVNSVAV	SASPTHENSV	SSGSSSTSSPY	SSASLESLSDS
2050	2060	2070	2080	2090	2100
TISRRNAITS	WLWDLTTSPL	TTTWPSLSLS	EALSSGHSGV	SNPSSTTTEF	PLFSAASTSA
2110	2120	2130	2140	2150	2160
AKQRNPETET	HGPQNTAAST	LNTDASSVTG	LSETPVGASI	SSEVPLPMAI	TSRSDVSGLT
2170	2180	2190	2200	2210	2220
SESTANPSLG	TASSAGTKLT	RTISLPTSES	LVSEFRMNKDP	WTVSIPLGSH	PTTNTETSIP

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FIG. 19F-1

2230	2240	2250	2260	2270	2280
VNSAGPPGLS	TVASDVIDTP	SDGAESIPTV	SFSPSPDTEV	TTISHFEPT	THSFRTISSL
2290	2300	2310	2320	2330	2340
THELTSRVTP	IPGDMSSAM	STKPTGASPS	ITLGERRTIT	SAAPTSPIV	LTASFTEETST
2350	2360	2370	2380	2390	2400
VSLDNETTVK	TSDILDARKT	NELPSDSSSS	SDLINTSIAS	STMDVTKTAS	ISPTSISGMT
2410	2420	2430	2440	2450	2460
ASSSPSLEFS	DRPQVPTSTT	ETNTATSPSV	SSNTYSLDGG	SNVGGTPTSL	PPFTITHPVE
2470	2480	2490	2500	2510	2520
TSSALLAWSR	PVRTFSTMVS	TDTASGENPT	SSNSVVTSVP	APGTWASVGS	TTDLPPAMGFL
2530	2540	2550	2560	2570	2580
KTSPAGEAHS	LLASTIEPAT	AFTPHLSAAV	VTGSSATSEA	SLLTTSESKA	IHSSPQTPTT
2590	2600	2610	2620	2630	2640
PTSGANWETS	ATPESLLVVT	ETSDTTLTSK	ILVTDITLES	TVSTPPSKFP	STGTLGASF
2650	2660	2670	2680	2690	2700
PTLLPDTPAI	PLTATEPTSS	LATSFDSTPL	VTIASDSLGT	VPETTLTMSE	TSNGDALVLK
2710	2720	2730	2740	2750	2760
TVSNPDRSIP	GITIQGVTES	PLHPSSTSPS	KIVAPRNTTY	EGSITVALST	LPAGTTGSLV

FIG. 19G-1

2770	2780	2790	2800	2810	2820
FSQSENSET	TALVDSSAGL	ERASVPLTT	GSQGMASGG	IRSGSTHSTG	TKTFSSLPLT
2830	2840	2850	2860	2870	2880
MNPGEVTAMS	EITTNRLTAT	QSTAPKGIPV	KPTSAESGLL	TPVSASSSPS	KAFASLTTPA
2890	2900	2910	2920	2930	2940
PSTWGIPQST	LTFEFSEVPS	LDTKSASLPT	PGQSLNTIPD	SDASTASSSL	SKSPEKNPRA
2950	2960	2970	2980	2990	3000
RMMTSTKAIS	ASSEQSTGET	ETPEGSASPS	MAGHEPRVPT	SGTGDPRYAS	ESMSYPDPSK
3010	3020	3030	3040	3050	3060
ASSAMTSTSL	ASKLTTLFST	GQAARSGSSS	SPISLSTEKE	TSFLSPTAST	SRKTSLEFLGP
3070	3080	3090	3100	3110	3120
SMARQPNILV	HLQTSALTLS	PTSTLNMSQE	EPPELTSSQT	IAEEEEGTAE	TQTLTFFTPSE
3130	3140	3150	3160	3170	3180
TPTSLLPVSS	PTEPTARRKS	SPETWASSIS	VPAKTSLVET	TDGTLVTTIK	MSSQAAQGNS
3190	3200	3210	3220	3230	3240
TWPAPAEETG	TSPAGTSPGS	PEVSTTLKIM	SSKEPSISPE	IRSTVRNSPW	KTPETTVPME
3250	3260	3270	3280	3290	3300
TTVEPVTLQS	TALGSGSTSI	SHLPTGTTSP	TKSPTENMLA	TERVSLSPSP	PEAWTNLYSG

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FIG. 19H-1

3310	3320	3330	3340	3350	3360
TPGGTRQSLA	TMSSVSLESP	TARSITGTGQ	QSSPELVSKT	TGMEFSMWHG	STGGTTGDTH
3370	3380	3390	3400	3410	3420
VSLSTSSNIL	EDPVTSPNSV	SSLTDKSKHK	TETWVSTTAI	PSTVLNNKIM	AAEQQTSRSV
3430	3440	3450	3460	3470	3480
DEAYSSSTSW	SDQTSGSDIT	LGASPDVTNT	LYITSTAQTT	SLVSLPSGDQ	GITSLTNPSG
3490	3500	3510	3520	3530	3540
GKTSSASSVT	SPSIGLETLR	ANVSAVKSDI	APTAGHLSQT	SSPAEVSILD	VTTAPTGGIS
3550	3560	3570	3580	3590	3600
TTITTMGINS	ISTTTPNPEV	GMSTMDSTPA	TERRTTSTEH	PSTWSSTAAS	DSWTVTDMTS
3610	3620	3630	3640	3650	3660
NLKVARSPGT	ISTMHTTSFL	ASSTELDSMS	TPHGRITVIG	TSLVTPSSDA	SAVKTEITST
3670	3680	3690	3700	3710	3720
ERTLSPSDTT	ASTPISTFSR	VQRMSISVPD	ILSTSWTPSS	TEAEDVPVSM	VSTDHASTKT
3730	3740	3750	3760	3770	3780
DPNTPLSTFL	FDLSLSTLDWD	TGRSLSSATA	TTSAPQGATT	PQELTLETMI	SPATSQLPFS
3790	3800	3810	3820	3830	3840
IGHITSAVTP	AAMARSSGVT	FSRPDPTSKK	AEQSTQLPT	TTSAHPGQVP	RSAAATLTDVI

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FIG. 19I-1

3850	3860	3870	3880	3890	3900
PHTAKTPDAT	FQRQGQTALT	TEARATSDSW	NEKEKSTPSA	PWITEMMNSV	SEDTIKEVTS
3910	3920	3930	3940	3950	3960
SSSVLKDPEY	AGHKLGIWDD	FIPKFGKAAH	MRELPLLSP	QDKEAHPST	NTVETTGWVT
3970	3980	3990	4000	4010	4020
SSEHASHSTI	PAHSASSKLT	SPVVTTSTRE	QAIVSMSTTT	WPESTRARTE	PNSFLTIELR
4030	4040	4050	4060	4070	4080
DVSPYMDTSS	TTQTSIISSP	GSTAITKGPR	TEITSSKRIS	SSFLAQSMRS	SDSPSEAITR
4090	4100	4110	4120	4130	4140
LSNFPAMTES	GGMILAMQTS	PPGATSLSAP	TLDTSATASW	TGTPLATTQR	FTYSEKTTLF
4150	4160	4170	4180	4190	4200
SKGPEDTSQP	SPPSVEETSS	SSSLVPIHAT	TSPSNILLTS	QGHSPSSSTPP	VTSVFLSETS
4210	4220	4230	4240	4250	4260
GLGKTTDMSR	ISLEPGTSLP	PNLSSTAGEA	LSTYEASRDT	KAIHHSADTA	VTNMEATSSE
4270	4280	4290	4300	4310	4320
YSPIPGHTKP	SKATSPLVTS	HIMGDITSST	SVFGSSETTE	IETVSSVNQG	LQERSTSQVA
4330	4340	4350	4360	4370	4380
SSATETSTVI	THVSSGDATT	HVTKTQATFS	SGTSISSPHQ	FITSTNTFTD	VSTNPSTSLI

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FIG. 19J-1

4390	4400	4410	4420	4430	4440
MTESSGVITIT	TQTGPTGAAT	QGPYLLDTST	MPYLTEETPLA	VTPDFMQSEK	TTLISKGPKD
4450	4460	4470	4480	4490	4500
VTWTSPPSVA	ETSYPSSSLTP	FLVTTIPPAT	STLQGQHTSS	PVSATSVLTS	GLVKTTDMLN
4510	4520	4530	4540	4550	4560
TSMEPVTNSP	QNLNNPSNEI	LATLAATTDI	ETIHPSINKA	VTNMGTAASSA	HVLHSTLPVS
4570	4580	4590	4600	4610	4620
SEPSTATSPM	VPASSMGDAL	ASISIPGSET	TDIEGEPTSS	LTAGRKENST	LQEMNSTTES
4630	4640	4650	4660	4670	4680
NIILSNVSVG	AIATEATKMEV	PSFDATFIPT	PAQSTKFPDI	FSVASSRLSN	SPPMTISTHM
4690	4700	4710	4720	4730	4740
TTTQGTGSSGA	TSKIPLALDT	STLETSAAGTP	SVVTEGFAHS	KITTAMNNDV	KDVSQTNPPF
4750	4760	4770	4780	4790	4800
QDEASSPSSQ	APVLVTTLPS	SVAFTPQWHS	TSSPVSMSVV	LTSSLVKTAG	KVDTSLETVT
4810	4820	4830	4840	4850	4860
SSPQSMSNTL	DDISVTSAAAT	TDIETHPSI	NTVVTNVGTT	GSAFESHSTV	SAYPEPSKVT
4870	4880	4890	4900	4910	4920
SPNVTTSTME	DTISRSIPK	SSKTRTEETE	TTSSLTPKLR	ETSIHQEITS	STETSTVPYK

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FIG. 19K-1

4930	4940	4950	4960	4970	4980
ELTGATTEVS	RTDVTSSST	SFPGPDQSTV	SLDISTETNT	RLSTSPIMTE	SAEITITTQT
4990	5000	5010	5020	5030	5040
GPHGATSQDT	FTMDPSNTTP	QAGIHSAMTH	GFSQLDVTTL	MSRIPQDVSW	TSPPSVDKTS
5050	5060	5070	5080	5090	5100
SPSSFLSSPA	MTTPSLISST	LPEDKLSSPM	TSLLTSGLVK	ITDILRTRLE	PVTSSLPNFS
5110	5120	5130	5140	5150	5160
STSDKILATS	KDSKDTKEIF	PSINTEETNV	KANNSGHESH	SPALADSETP	KATTQMVITT
5170	5180	5190	5200	5210	5220
TVGDPAPSTS	MPVHGSSETT	NIKREPTYFL	TPRLRETSTS	QESSEFTDTS	FLLSKVPTGT
5230	5240	5250	5260	5270	5280
ITEVSSSTGVN	SSSKISTPDH	DKSTVPPDTE	TGEIIPRVFTS	SIKTKSAEMT	ITTQASPPES
5290	5300	5310	5320	5330	5340
ASHSTLPLDT	STTLSQGGTH	STVTQGFPPS	EVTTLMGMP	GNVSWMTTTP	VEETSSVSSL
5350	5360	5370	5380	5390	5400
MSSPAMTSPS	PVSSSTSPQSI	PSSPLPVTAL	PTSVLVTTTD	VLGTTSPEVS	TSSPPNLSSI
5410	5420	5430	5440	5450	5460
THERPATYKD	TAHTEAAMHH	STNTAVTNVG	TSGSGHKSQS	SVLADSETSK	ATPLMSTTST

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FIG. 19L-1

5470	5480	5490	5500	5510	5520
LGDTSVSTST	PNISQTNQIQ	TEPTASLSPR	LRESSTSEKT	SSTETNTAF	SYVPTGAIQ
5530	5540	5550	5560	5570	5580
ASRTEISSR	TSISDLDRPT	IAPDISTGMI	TRLFTSPIMT	KSAEMTVTTQ	TTTPGATSQG
5590	5600	5610	5620	5630	5640
ILPWDSTTL	FQGGHSTVS	QGFPHEITT	LRSRTPGDVS	WMTTPPVEET	SSGFSLMSPS
5650	5660	5670	5680	5690	5700
MTSPSPVSST	SPEIPSSPL	PVTALLTSVL	VTTTNVLGTT	SPETVTSSPP	NLSSPTQERL
5710	5720	5730	5740	5750	5760
TTYKDTAHE	AMHASMHTNT	AVANVGTSIS	GHEQSSVPA	DSHTSKATSP	MGITFAMGDT
5770	5780	5790	5800	5810	5820
SVSTSTPAFF	ETRIQTESTS	SLIPGLRDTR	TSEEINTVTE	TSTVLSEVPT	TTTTEVSRTE
5830	5840	5850	5860	5870	5880
VITSSRTTIS	GPDHSMSPY	ISTETITRLS	TFPFVTGSTE	MAITNQTGPI	GTISQATLTL
5890	5900	5910	5920	5930	5940
DTSSTASWEG	THSPVTQRF	HSEETTMSR	STKGVSWQSP	PSVEETSSPS	SPVPLPAITS
5950	5960	5970	5980	5990	6000
HSSLYSAVSG	SSPTSALPVT	SLLTSGRRKT	IDMLDTHSEL	VTSSLPSASS	FSGEILTSEA

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FIG. 19M-1

6010	6020	6030	6040	6050	6060
STNETIHFS	ENTAETNMGT	TNSMHKLHSS	VSIHSQPSGH	TPPKVTGSM	EDAIVSTSTP
6070	6080	6090	6100	6110	6120
GSPETKNVDR	DSTSLTPPEL	KEDSTALVMN	STTESNTVFS	SVSLDAATEV	SRAEVITYYDP
6130	6140	6150	6160	6170	6180
TEMPASAQST	KSPDISPEAS	SSHNSPPPLT	ISTHKTIATQ	TGPSGVTSLG	QLTLDSTIA
6190	6200	6210	6220	6230	6240
TSAGTPSART	QDFVDSETTS	VMNNDLNDVL	KTSPFSAEEA	NSLSSQAPLL	VTTSPSPVTS
6250	6260	6270	6280	6290	6300
TLQEHSTSSL	VSVTSVPTPT	LAKITDMDTN	LEPVTRSPQN	LRNTLATSEA	TTDTHTMHPS
6310	6320	6330	6340	6350	6360
INTAMANVGT	TSSPNEFYFT	VSPDSDPYKA	TSAVVITSTS	GDSIVSTSM	RSSAMKKIES
6370	6380	6390	6400	6410	6420
ETTFSLIFRL	RETSTSQKIG	SSSDTSTVFD	KAFTAAATTEV	SRTSLTSSSR	TSIQGTEKPT
6430	6440	6450	6460	6470	6480
MSPDTSTRSV	TMLSTFAGLT	KSEERTIATQ	TGPHRATSQG	TLTWDTSIIT	SQAGTHSAMT
6490	6500	6510	6520	6530	6540
HGFSQLDLST	LTSRVPEYIS	GTSPPSVEKT	SSSSLLSLP	AITSPSPVPT	TLPESRPSSP

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FIG. 19N-1

6550	6560	6570	6580	6590	6600
VHLSLPTSG	LVKTTDMLAS	VASLPPNLGS	TSHKIPTSE	DIKDTEKMYP	STNIAVTNVG
6610	6620	6630	6640	6650	6660
TTTSEKESYS	SVPAYSEPPK	VTSPMVTSFN	IRDTIVSTSM	PGSSEITRIE	MESTFSVAHG
6670	6680	6690	6700	6710	6720
LKGTSTSQDP	IVSTEKSAVL	HKLTTGATET	SRTEVASSRR	TSIPGPDHST	ESPDISTEVI
6730	6740	6750	6760	6770	6780
PSLPISLGIT	ESSNMTIITR	TGPPPLGSTSQ	GFTTLDTPPT	SSRAGTHSMA	TQEFPHSEMT
6790	6800	6810	6820	6830	6840
TVMNKDPEIL	SWTIPPSIEK	TSFSSSLMPS	PAMTSPPVSS	TLPKTIHTP	SPMTSLLTPS
6850	6860	6870	6880	6890	6900
LVMTTDTLGT	SPEPTTSSPP	NLSSTSHVIL	TTDEDTTAE	AMHPSTSTAA	TNVEITCSGH
6910	6920	6930	6940	6950	6960
GSQSSVLTD	EKTATAPMD	TTSTMGHTTV	STSMVSSET	TKIKRESTYS	LTPGLRETSI
6970	6980	6990	7000	7010	7020
SQNASFSTD	SIVLSEVPTG	TTAEVSRTEV	TSSGRTSIPG	PSQSTVLPEI	STRMTRLFA
7030	7040	7050	7060	7070	7080
SPTMTESAEM	TIPTQTGPSG	STSQDTLLTD	TSTTKSQAKT	HSTLTQRFPH	SEMTTILMSRG

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FIG. 190-1

7090	7100	7110	7120	7130	7140
PGDMSWQSSP	SLENPSSLPS	LLSLPATTSP	PPISSTLPVT	ISSSPLPVT	LLTSSPVTTT
7150	7160	7170	7180	7190	7200
DMLHTSPELV	TSSPPKLSHT	SDERLTTGKD	TTNTEAVHPS	TNTAASNVEI	PSFGHESPSS
7210	7220	7230	7240	7250	7260
ALADSETSKA	TSPMFITSTQ	EDTTVAISTP	HFLETSRIQK	ESISSLSPKL	RETGSSVETS
7270	7280	7290	7300	7310	7320
SAIETSAVLS	EVSIGATTEI	SRTEVTSSSR	TSISGSAEST	MLPEISTTRK	IIKFPTSPIL
7330	7340	7350	7360	7370	7380
AESSEMTIKT	QTSPPGSTSE	STFTLDTSTT	PSLVITHSTM	TQRLPHSEIT	TLVSRGAGDV
7390	7400	7410	7420	7430	7440
PRPSSLPVEE	TSPSSQLSL	SAMISPPSVS	STLPASSHSS	SASVTSPLTP	GQVKTTTEVLD
7450	7460	7470	7480	7490	7500
ASAEPETSSP	PSLSTSVETI	LATSEVTDDT	EKIHPFPNTA	VTKVGTSSSG	HESPSSVLDP
7510	7520	7530	7540	7550	7560
SETTKATSAM	GTISIMGDT	VSTLTPALSN	TRKIQSEPAS	SLTTRLRETS	TSEETSLATE
7570	7580	7590	7600	7610	7620
ANTVLSKVST	GATTEVSRTE	AISFSRTSMS	GPEQSTMSQD	ISIGTIPRIS	ASSVLTESAK

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FIG. 19P-1

7630	7640	7650	7660	7670	7680
MTITTQGPS	ESTLESTLNL	NTATTPSWVE	THSIVIQGF	HPEMTISMGR	GPGGVSWPSP
7690	7700	7710	7720	7730	7740
PFVKETSPPS	SPLSLPAVTS	PHPVSTTFLA	HIPPSPLPVT	SLLTSGPATT	TDILGTSTEP
7750	7760	7770	7780	7790	7800
GTSSSSSLST	TSHERLTTYK	DTAHTAVHP	STNTGGTNVA	TTSSGYKSQS	SVLADSSPMC
7810	7820	7830	7840	7850	7860
TTSTMGDTSV	LTSTPAFLET	RRIQTECLASS	LTPGLRESSG	SEGTSSGTKM	STVLSKVPTG
7870	7880	7890	7900	7910	7920
ATTEISKEDV	TSIPGPAQST	ISPDISTRV	SWFSTSPVMT	ESAEITMNTN	TSPLGATTQG
7930	7940	7950	7960	7970	7980
TSTLATSSST	SLTMTHSTIS	QGFHSQMST	LMRRGPEDVS	WMSPPLLEKT	RPSFSLMSSP
7990	8000	8010	8020	8030	8040
ATTSPSPVSS	TLPESISSSP	LPVTSLLTSG	LAKTTDMLHK	SSEPVTNSPA	NLSSTSVEIL
8050	8060	8070	8080	8090	8100
ATSEVTTDTE	KTHPSSNRTV	TDVGTSSSGH	ESTSEVLADS	QTSKVTSPMV	ITSTMEDTSV
8110	8120	8130	8140	8150	8160
STSTPGFFET	SRIQTEPTSS	LTGLRKTS	SEGTSLATEN	STVLSGVPTG	ATAEVSRTVE

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FIG. 19Q-1

8170	8180	8190	8200	8210	8220
TSSRSISG	FAQLTVSPET	STETITRLPT	SSIMTESAEM	MIKTQTDPPG	STPESTHTVD
8230	8240	8250	8260	8270	8280
ISTTPNWWET	HSTVTQRFH	SEMTTLVRS	PGDMLWPSQS	SVEETSSASS	LLSLPATTSP
8290	8300	8310	8320	8330	8340
SPVSSTLVED	FPSASLPVTS	LLTPGLVITT	DRMGISREPG	TSSTSNLSST	SHERLTILED
8350	8360	8370	8380	8390	8400
TVDTEDMQPS	THTAVTNVRT	SISGHESQSS	VLSDSETPKA	TSPMGTTYTM	GETSVSISTS
8410	8420	8430	8440	8450	8460
DFFETSRIQI	EPTSSLTSGL	RETSSSERIS	SATEGSTVLS	EVPSGATTEV	SRTEVISSRG
8470	8480	8490	8500	8510	8520
TMSGPDQFT	ISPDISTEAI	TRLSTSPIMT	ESAESAITIE	TGSPGATSEG	TLTLDSTTT
8530	8540	8550	8560	8570	8580
FWSGTHSTAS	PGFHSSEMTT	LMSRTPGDVP	WPSLPVVEEA	SSVSSSLSSP	AMTSTSEFFSA
8590	8600	8610	8620	8630	8640
LPESISSSPH	PVTALLTLGP	VKTTDMLRTS	SEPETSSPPN	LSS TSAEILA	TSEVTKDREK
8650	8660	8670	8680	8690	8700
IHPSSNTPVV	NVGTVIYKHL	SPSSVLADLV	TTKPTSPMAT	TSTLGNTSVS	TSTPAFPETM

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FIG. 19R-1

8710	8720	8730	8740	8750	8760
MTQPTSSLTS	GLREISTSQE	TSSATERSAS	LSGMPTGATT	KVSRTEALSL	GRTSTPGPAQ
8770	8780	8790	8800	8810	8820
STISPEISTE	TITRISTPLT	TTGSAEMTIT	PKTGHSGASS	QGTFITLDTSS	RASWPGTHSA
8830	8840	8850	8860	8870	8880
ATHRSPHSGM	TTPMRGPED	VSWPSRPSVE	KTSPPSSLVS	LSAVTSPSPL	YSTPSESSHs
8890	8900	8910	8920	8930	8940
SPLRVTSLFT	PVMKTTDML	DTSLEPVTS	PPSMNITSDE	SLATSKATME	TEAIQLSENT
8950	8960	8970	8980	8990	9000
AVTQMGTISA	RQEFYSSYPG	LPEPSKVTSP	VVTSSTIKDI	VSTIPASSE	ITRIEMESTS
9010	9020	9030	9040	9050	9060
TLTPTPRETS	TSQEIHSATK	PSTVPYKALT	SATIEDSMTQ	VMSSSRGPSP	DQSTMSQDIS
9070	9080	9090	9100	9110	9120
SEVITRLSTS	PIKAESTEMT	ITTQTGSPGA	TSRGTLTLDT	STTFMSGTHS	TASQGFSHSQ
9130	9140	9150	9160	9170	9180
MTALMSRTPG	DVPWLSHPSV	EEASSASFSL	SSPVMTSSSP	VSSTLPDSIH	SSSLPVTSL
9190	9200	9210	9220	9230	9240
TSGLVKTTTEL	LGTSSEPEPETS	SPPNLSSTSA	EILATTEVTT	DTEKLEMTNV	VTSGYTHESP

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FIG. 19S-1

9250	9260	9270	9280	9290	9300
SSVLADSVTT	KATSSMGITY	PTGDTNVLTS	TPAFSDTSRI	QTKSKLSLTP	GLMETSISEE
9310	9320	9330	9340	9350	9360
TSSATEKSTV	LSSVPTGATT	EVSRTAIISS	SRTSIPGPAQ	STMSSDTSME	TITRISTPLT
9370	9380	9390	9400	9410	9420
RKESTDMAIT	PKTGPSGATS	QGTFILDSSS	TASWPGTHSA	TTQRFQSVV	TTPMSRGPED
9430	9440	9450	9460	9470	9480
VSWPPLSVE	KNSPPSSLVS	SSSVTSPSPL	YSTPSGSSHS	SPVPVTSLEF	SIMMKATDML
9490	9500	9510	9520	9530	9540
DASLEPETTS	APNMNITSDE	SLATSKATTE	TEAIIHVFEF	AASHVETISA	TEELYSSSPG
9550	9560	9570	9580	9590	9600
FSEPTKVISP	VVTSSSIRDN	MVSTTMPGSS	GITRIEIESM	SSLTPGLRET	RTSQDITSST
9610	9620	9630	9640	9650	9660
ETSTVLYKMS	SGATPEVSRT	EVMPSRRTSI	PGPAQSTMSL	DISDEVVTRL	STSPIMTESA
9670	9680	9690	9700	9710	9720
EITITQTGY	SLATSQVTLF	LGTSMTFLSG	THSTMSQGLS	HSEMTNLMSR	GPELSWTSP
9730	9740	9750	9760	9770	9780
RFVETTRSSS	SLTSLPLTTS	LSPVSSSTLLD	SSPSSPLPVT	SLILPGLVKT	TEVLDTSSSEF

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FIG. 19T-1

9790	9800	9810	9820	9830	9840
KTSSSPNLSS	TSVEIPATSE	IMDTEKIH	SSNTAVAKVR	TSSSVHESHS	SVLADSETTI
9850	9860	9870	9880	9890	9900
TIPSMGITSA	VDDTTVFSTN	PAFSETRRIP	TEPTFSLTPG	FRETSTSEET	TSITETSAVL
9910	9920	9930	9940	9950	9960
YGVPTSATTE	VSMTEIMSSN	RTHIPDSDQS	TMSPDIIITEV	ITRLSSSSMM	SESTQMTITT
9970	9980	9990	10000	10010	10020
QKSSPGATAQ	STLTATTATTA	PLARTHSTVP	PRFLHSEMTT	LMSRSPENPS	WKSSPFVEKT
10030	10040	10050	10060	10070	10080
SSSSLLSLP	VTTSPSVSST	LPQSIPISSSF	SVTSLLTTPGM	VKTTDTSTEP	GTSLSPLNSG
10090	10100	10110	10120	10130	10140
TSVEILAAASE	VTTDTEKIH	SSSMAVTNVG	TTSSGHELYS	SVSIHSEPSK	ATYPVGPSS
10150	10160	10170	10180	10190	10200
MAETSISTSM	PANFETTGE	AEPFSHLTSG	FRKTNMSLDT	SSVTPTNTPS	SPGSTHLLQS
10210	10220	10230	10240	10250	10260
SKTDFTS	SAK	TSSPDWPPAS	QYTEIPVDII	TPFNASPSIT	ESTGITSEPE
10270	10280	10290	10300	10310	10320
THHLSTDLLP	SAETISTGT	MPSLSEAMTS	FATTGVPRAI	SGSGSPFSRT	ESGPGDATLS

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FIG. 19U-1

10330	10340	10350	10360	10370	10380
TIAESLPST	PVPFSSSTFT	TTDSSSTIPAL	HEITSSSATP	YRVDTSLGTE	SSTTEGR LVM
10390	10400	10410	10420	10430	10440
VSTLDTSSQP	GRTSSSTPILD	TRMTESVELG	TVTSAYQVPS	LSTR LTRTDG	IMEHITKIPN
10450	10460	10470	10480	10490	10500
EAAHRGTIRP	VKGPQTSTSP	ASPKGLHTGG	TKRMETTTTA	LKTTTTALKT	TSRATLTTSV
10510	10520	10530	10540	10550	10560
YTPTLGLTLP	LNASRQMAST	ILTEMMITTP	YVFPDVPETT	SSLATSLGAE	TSTALPR TTP
10570	10580	10590	10600	10610	10620
SVLNRESETT	ASLVSRSGAE	RSPVIQTLDV	SSSEPDTTAS	WVIHPAETIP	TVSKTTPNFF
10630	10640	10650	10660	10670	10680
HSELDTVSST	ATSHGADVSS	AIPTNISPE	LDALTPLVTI	SGTDTSTTFP	TLTKSPHETE
10690	10700	10710	10720	10730	10740
TRTTWLTHPA	ETSSSTIPRTI	PNFHHESDA	TPSIATSPGA	ETSSAIPIMT	VSPGAEDLVT
10750	10760	10770	10780	10790	10800
SQVTSSG TDR	NMTIPTLTLS	PGEPKTIASL	VTHPEAQ TSS	AIPTSTISPA	VSRLVTSMVT
10810	10820	10830	10840	10850	10860
SLAAKTSTN	RALTNSPGE	ATTVSLVTHP	AQTSPTVPWT	TSIFFHKS D	TTPSMTTSHG

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FIG. 19V-1

10870	10880	10890	10900	10910	10920
AESSAVPTP	TVSTEVPGVV	TPLVTSSRAV	ISTTIPILTL	SPGEPETTPS	MATSHGEEAS
10930	10940	10950	10960	10970	10980
SAIPTPTVSP	GVPGVVTSLV	TSSRAVTSTT	IPILTFSLGE	PETTPSMATS	HGTEAGSAVP
10990	11000	11010	11020	11030	11040
TVLPEVPGMV	TSLVASSRAV	TSTTLPTLTL	SPGEPETTPS	MATSHGAEAS	STVPTVSPPEV
11050	11060	11070	11080	11090	11100
PGVVTSLVTS	SSGVNSTSIP	TLILSPGELE	TTPSMATSHG	AEASSAVPTP	TVSPGVSGVV
11110	11120	11130	11140	11150	11160
TPLVTSSRAV	TSTTIPILTL	SSSEPETTPS	MATSHGVEAS	SAVLTVSPEV	PGMVTSLVTS
11170	11180	11190	11200	11210	11220
SRAVTSTTIP	TLTISSEDEPE	TTTSLVTHSE	AKMISAIPTL	AVSPTVQGLV	TSLVTSSGSE
11230	11240	11250	11260	11270	11280
TSAFSNLTVA	SSQPETIDSW	VAHPGTEASS	VVPITLTVSTG	EPFTNISLVT	HPAESSTSLP
11290	11300	11310	11320	11330	11340
RTTSRFSHSE	LDTMPSTVTS	PEAESSSAIS	TTISPGIPGV	LTSLVTSSGR	DISATFTPTVP
11350	11360	11370	11380	11390	11400
ESPHESEATA	SWVTHPAVTS	TTVPRITPNY	SHSEPDTPS	IATSPGAEAT	SDEPTITVSP

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FIG. 19W-1

11410	11420	11430	11440	11450	11460
DVPDMVTSQV	TSSGDTISIT	IPTLTLSSGE	PETTSFITY	SEHTSSAIP	TLPVSPGASK
11470	11480	11490	11500	11510	11520
MLTSLVISSG	TDSTTTFPTL	TETPYEPETT	AIQLIHPAET	NTMVPKTTPK	FSHKSDDTTL
11530	11540	11550	11560	11570	11580
PVAITSPGPE	ASSAVSTTTI	SPDMSDLVTS	LVPSSGTDTS	TTFPTLSETP	YEPETTVTWL
11590	11600	11610	11620	11630	11640
THPAETSTTV	SGTIPNFSHR	GSDTAPSMVT	SPGVDTRSGV	PTTIIPPSIP	GVVTSQVTSS
11650	11660	11670	11680	11690	11700
ATDTSTAIPT	LTPSPGEPET	TASSATHPGT	QTGFTVPIRT	VPSSEPDTMA	SWVTHPPQTS
11710	11720	11730	11740	11750	11760
TPVSRITTSF	SHSSPDATPV	MATSPRTEAS	SAVLTTISPG	APEMVTSQIT	SSGAATSTTV
11770	11780	11790	11800	11810	11820
PTLTHSPGMP	ETTALLSTHP	RTGTSKTFPA	STVFPQVSET	TASLTIRPGA	ETSTALPTQT
11830	11840	11850	11860	11870	11880
TSSLFTLLVT	GTSRVDLSPT	ASPGVSAKTA	PLSTHPGET	STMIPSTLS	LGLLETTGLL
11890	11900	11910	11920	11930	11940
ATSSSAETST	STLTLTVSPA	VSSLSSASIT	TDKPQTVTSW	NTEITSPSVTS	VGPPEFSRTV

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FIG. 19X-1

11950	11960	11970	11980	11990	12000
TGTTMTLIPS	EMTPPKTSH	GEGVSPTTIL	RTTMVEATNL	ATTGSSPTVA	KTTTTENTLA
12010	12020	12030	12040	12050	12060
GSLFTPLTTP	GMSTLASESV	TSRTSYNHR	WISTSSYNR	RYWTPATSTP	VTSTFSPGIS
12070	12080	12090	12100	12110	12120
TSSIPSSATA	TVPFMPFTL	NFTITNLQVE	EDMRHPGSRK	FNATERELQG	LLKPLFRNSS
12130	12140	12150	12160	12170	12180
LEYLYSGCRL	ASLRPEKDSS	AMAVDAICTH	RPDPEDLGLD	RERLYWELSN	LTNGIQELGP
12190	12200	12210	12220	12230	12240
YTLDRNSLYV	NGFTHRSSMP	TTSTPGTSTV	DVGTSCTPSS	SPSPTAAGPL	LMPFTLNFTI
12250	12260	12270	12280	12290	12300
TNLQYEEDMR	RTGSRKFNTM	ESVLQGLLKP	LFKNTSVGPL	YSGCRLTLR	PEKDGAATGV
12310	12320	12330	12340	12350	12360
DAICTHRLDP	KSPGLNREQL	YWELSKLTND	IEELGPYTLD	RNSLYVNGFT	HQSSVSTTST
12370	12380	12390	12400	12410	12420
PGTSTVDLRT	SGTPSSLSSP	TIMAAGPLL	PFTLNFTITN	LQYGEDMGHP	GSRKFNTTER
12430	12440	12450	12460	12470	12480
VLQGLLGPIF	KNTSVGPLY	GCRLTSLRSE	KDGAATGVDA	ICIHHLDPKS	PGLNRERLYW

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FIG. 19Y-1

12490	12500	12510	12520	12530	12540
ELSQLTNGIK	ELGPYTLDNR	SLYVNGFTHR	TSVPTTSTPG	TSTVDLGTSG	TPFSLPSPAT
12550	12560	12570	12580	12590	12600
AGPLLVLFLL	NFTITNLKYE	EDMHRPGSRK	FNTTERVLQT	LLGPMFKNTS	VGLLYSGCRL
12610	12620	12630	12640	12650	12660
TLLRSEKDGA	ATGVDAICTH	RLDPKSPGLD	REQLYWELSQ	LTNGIKELGP	YTLDNRNSLYV
12670	12680	12690	12700	12710	12720
NGFTHWIPVP	TSSTPGTSTV	DLGSGTPSSL	PSPTAAGPLL	VPFTLNFTIT	NLQYEEEDMHH
12730	12740	12750	12760	12770	12780
PGSRKFNTTE	RVLQGLLGPM	FKNTSVGLLY	SGCRLLTLLRS	EKDGAATGVD	AICTHRLDPK
12790	12800	12810	12820	12830	12840
SPGVDRQELY	WELSQLTNGI	KELGPYTLDR	NSLYVNGFTH	QTSAPNTSTP	GTSTVDLGTG
12850	12860	12870	12880	12890	12900
GTPSSLPSPT	SAGPLLVPFT	LNFTITNLQY	EEDMRHPGSR	KFNTTERVLQ	GLLKPLFKST
12910	12920	12930	12940	12950	12960
SVGPLYSGCR	LTLLRSEKDG	AATGVDAICT	HRLLDPKSPGV	DREQLYWELS	QLTNGIKELG
12970	12980	12990	13000	13010	13020
PYTLDNRNSLY	VNGFTHQ TSA	PNTSTPGTST	VDLGTSGTPS	SLPSPTSAGP	LLVPFTLNFT

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FIG. 19Z-1

13030	13040	13050	13060	13070	13080
ITNLQYEEDM	HHPGSRKFNT	TERVLQGLLG	PMFKNTSVGL	LYSGCRLLTLL	RPEKNGAATG
13090	13100	13110	13120	13130	13140
MDAICSHRLD	PKSPGLNREQ	LYWELSQLTH	GIKELGPYTL	DRNSLYVNGF	THRSSVAPTS
13150	13160	13170	13180	13190	13200
TPGTSTVDLG	TSGTPSSLPS	PTTAVPLLVP	FTLNFTITNL	QYGEDMRHPG	SRKFNTTTERV
13210	13220	13230	13240	13250	13260
LQGLLGPLFK	NSSVGPLYSG	CRLISLRSEK	DGAATGVDAI	CTHHLNPQSP	GLDREQLYWQ
13270	13280	13290	13300	13310	13320
LSQMTNGIKE	LGPYTILDRNS	LYVNGFTHRS	SGLTTSTPWT	STVDLGTSGT	PSPVPSPPTA
13330	13340	13350	13360	13370	13380
GPLLVPFTLN	FTITNLQYEE	DMHRPGSRKF	NTTERVLQGL	LSPIFKNSSV	GPLYSGCRLT
13390	13400	13410	13420	13430	13440
SLRPEKDGAA	TGMDAVCLYH	PNPKRPGGLDR	EQLYWELSQL	THNITELGPY	SLDRDSLNVN
13450	13460	13470	13480	13490	13500
GFTHQNSVPT	TSTPGTSTVY	WATTGTPSSF	PGHTEPGPLL	IPFTFNFTIT	NLHYEENMQH
13510	13520	13530	13540	13550	13560
PGSRKFNTTE	RVLQGLLKPL	FKNTSVGPLY	SGCRLTSLRP	EKDGAATGMD	AVCLYHPNPK

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FIG. 19A-2

13570	13580	13590	13600	13610	13620
RPGLDREQLY	WELSQLTHNI	TELGPYSLDR	DSLYVNGFTH	QNSVPTTSTP	GTSTVYWATT
13630	13640	13650	13660	13670	13680
GTPSSFPGHT	EPGPLLIPFT	FNFTITNLHY	EENMQHPGSR	KENTTERVLQ	GLLKPLFKNT
13690	13700	13710	13720	13730	13740
SVGPLYSGCR	LTLRPEKHE	AATGVDTICT	HRVDPIGPGL	DRERLYWELS	QLTNSITTELG
13750	13760	13770	13780	13790	13800
PYTLDRDSLY	VNGFNPRSSV	PTTSTPGTST	VHLATSGTPS	SLPGHTAPVP	LLIPFTLNFT
13810	13820	13830	13840	13850	13860
ITNLHYEENM	QHPGSRKFNT	TERVLQGLLK	PLFKNTSVGP	LYSGCRLTLL	RPEKHEAATG
13870	13880	13890	13900	13910	13920
VDTICTHRVD	PIGPGLXXEX	LYWELSXLTX	XIXELGPYTL	DRXSLYVNGF	THXXSXPTTS
13930	13940	13950	13960	13970	13980
TPGTSTVXXG	TSGTPSSXPX	XTSAGPLLVP	FTLNFTITNL	QYEEDMHHPG	SRKFNTTERV
13990	14000	14010	14020	14030	14040
LQGLLGPMFK	NTSVGLLYSG	CRLTLRPEK	NGAATGMDAI	CSHRLDPKSP	GLDREQLYWE
14050	14060	14070	14080	14090	14100
LSQLTHGIKE	LGPYTLDNRNS	LYVNGFTHRS	SVAPTSTPGT	STVDLGTSGT	PSSLPSPTTA

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FIG. 19B-2

14110	14120	14130	14140	14150	14160
VPLLVPFTLN	FTITNLQYGE	DMRHPGSRKF	NTTERVLQGL	LGPLEKNSSV	GPLYSGCRLI
14170	14180	14190	14200	14210	14220
SLRSEKDGAA	TGVDAICTHH	LNPQSPGLDR	EQLYWQLSQM	TNGIKELGPY	TLLDRNSLYVN
14230	14240	14250	14260	14270	14280
GFTHRSSGLT	TSTPWTSTVD	LGTSGTSPV	PSPTTAGPLL	VPFTLNFTIT	NLQYEEDMHR
14290	14300	14310	14320	14330	14340
PGSRKFNATE	RVLQGLLSPI	FKNSSVGPLY	SGCRLTSLRP	EKDGAATGMD	AVCLYHPNPK
14350	14360	14370	14380	14390	14400
RPGLDREQLY	WELSQLTHNI	TELGPYSLDR	DSLYVNGFTH	QSSMTTTRTP	DTSTMHLATS
14410	14420	14430	14440	14450	14460
RTPASLSGPT	TASPLLVLFT	INCTITNLQY	EEDMRRRTGSR	KFNTMESVLQ	GLLKPLEFKNT
14470	14480	14490	14500	14510	14520
SVGPLYSGCR	LTLRPKKDG	AATGVDAICT	HRLDPKSPGL	NREQLYWELS	KLTNDIEELG
14530	14540	14550	14560	14570	14580
PYTLDRNSLY	VNGFTHQSSV	STTSTPGTST	VDLRTSGTPS	SLSSPTIMXX	XPLLXPFTXN
14590	14600	14610	14620	14630	14640
XTITNLXXXX	XXXXPGSRKF	NTTERVLQGL	LRPLEFKNTSV	SSLYSGCRLT	LLRPEKDGAA

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FIG. 19C-2

14650	14660	14670	14680	14690	14700
TRVDAACTYR	PDPKSPGLDR	EQLYWELS QL	THSITELG PY	TLDRVSLYVN	GFNPRSSVPT
14710	14720	14730	14740	14750	14760
TSTPGTSTVH	LATSGTPSSL	PGHTXXXPLL	XPFTXNX TIT	NLXXXXXXMX	PGSRKFNTTE
14770	14780	14790	14800	14810	14820
RVLQGLLKPL	FRNSSLEYLY	SGCRLASLRP	EKDSSAMAVD	AICTHRPDPE	DLGLDRERLY
14830	14840	14850	14860	14870	14880
WELSNLTNGI	QELGPYTLLDR	NSLYVNGFTH	RSSGLTTSTP	WTSTVDLGTS	GTPSPVPSPT
14890	14900	14910	14920	14930	14940
TAGPLLV PFT	LNFTITNLQY	EEDMHRPGSR	RFNTTERVLQ	GLLTPLFKNT	SVGPLYSGCR
14950	14960	14970	14980	14990	15000
LTLRLRPEKQE	AATGVDTICT	HRVDPIGPGL	DRERLYWELS	QLTNSITELG	PYTLD RDSLY
15010	15020	15030	15040	15050	15060
VNGFNPWSSV	PTTSTPGTST	VHLATSGTPS	SLPGHTAPVP	LLIPFTLNFT	ITDLHYEENM
15070	15080	15090	15100	15110	15120
QHGPSRK FNT	TERVLQGLLK	PLFKSTSVGP	LYSGCRLTLL	RPEKHGAATG	VDAICTLRLD
15130	15140	15150	15160	15170	15180
PTGPGLD RER	LYWELS QL TN	SVTELGPYTL	DRDSLYVNGF	THRSSVPTTS	IPGTS AVHLE

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FIG. 19D-2

15190	15200	15210	15220	15230	15240
TSGTPASLPG	HTAPGPLLVP	FTLNFTITNL	QYEEDMRHPG	SRKFSTTERV	LQGLLKPLFK
15250	15260	15270	15280	15290	15300
NTSVSSLYSG	CRLTLRPEK	DGAATRVDV	CTHRPDPKSP	GLDRERLYWK	LSQLTHGITE
15310	15320	15330	15340	15350	15360
LGPYTLD RHS	LYVNGFTHQS	SMTTTRTPDT	STMHLATSRT	PASLSGPTTA	SPLLVLFTIN
15370	15380	15390	15400	15410	15420
FTITNLR YEE	NMHHPGSRKF	NTTERVLQGL	LRPVFKNTSV	GPLYSGCRLT	TLRPPKDGAA
15430	15440	15450	15460	15470	15480
TKVDAICTYR	PDPKSPGLDR	EQLYWELSQL	THSITELGPY	TQDRDSL YVN	GFTHRSSVPT
15490	15500	15510	15520	15530	15540
TSIPGTS AVH	LETSGTPASL	PGHTAPGPLL	VPFTLNFTIT	NLQYEEDMRH	PGSRKFNTTE
15550	15560	15570	15580	15590	15600
RVLQGLLKPL	FKSTSVGPLY	SGCRLTL LRP	EKRGAATGVD	TICTHRLDPL	NPGLDREQLY
15610	15620	15630	15640	15650	15660
WELSKLTRGI	IELGPYLLDR	GSLYVNGFTH	RTSVPTTSTP	GTSTVDLGTS	GTPFSLPSPA
15670	15680	15690	15700	15710	15720
XXXXPLLXPFT	XXXTITNLXX	XXXXMXXPGSR	KENTTERVLQ	TLLGPMFKNT	SVGLLYSGCR

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FIG. 19E-2

15730	15740	15750	15760	15770	15780
LTLRSEKDG AATGVDAICT	HRLDPKSPGV	DREQLYWELS	QLTNGIKELG	PYTLDRNSLY	
15790	15800	15810	15820	15830	15840
VNGFTHWIPV PTSSTPGTST	VDLGSGTPSS	LPSPTTAGPL	LVPFTLNFTI	TNLKYEEDMH	
15850	15860	15870	15880	15890	15900
CPGSRKFNTT ERVLQSLGPG	MFKNTSVGPL	YSGCRILTLLR	SEKDGAAATGV	DAICTHRLDP	
15910	15920	15930	15940	15950	15960
KSPGVDREQL YWELSQLTNG	IKELGPYTLD	RNSLYVNGFT	HQTSAPNTST	PGTSTVDLGT	
15970	15980	15990	16000	16010	16020
SGTPSSLPSP TXXXPLLPF	TXNXTITNLX	XXXXMXXPGS	RKFNTTEXVL	QGLLXPXFKN	
16030	16040	16050	16060	16070	16080
XSVGXLYSGC RLTXLRXEXX	GAATGXDAIC	XHXXXPKXPG	LXXEXLYWEL	SXLTXIXEL	
16090	16100	16110	16120	16130	16140
GPYTLDRXSL YVNGFTHWIP	VPTSSTPGTS	TVDLGSGTPS	SLPSPTTAGP	LLVPFTLNFT	
16150	16160	16170	16180	16190	16200
ITNLKYEEDM HCPGSRKFNT	TERVLQSLG	PMFKNTSVGP	LYSGCRLTSL	RSEKDGAAATG	
16210	16220	16230	16240	16250	16260
VDAICTHRVD PKSPGVDREQ	LYWELSQLTN	GIKELGPYTL	DRNSLYVNGF	THQTSAPNTS	

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FIG. 19F-2

16270	16280	16290	16300	16310	16320
TPGTSTVXXG	TSGTPSSXPX	XTSAGPLLVP	FTLNFTITNL	QYEEDMHHPG	SRKFNTTTERV
16330	16340	16350	16360	16370	16380
LQGLLGPMFK	NTSVGLLYSG	CRLTLRPEK	NGATTGMDAI	CTHRLDPKSP	GLXXEXLYWE
16390	16400	16410	16420	16430	16440
LSXLTXIXE	LGPYTLDRXS	LYVNGFTHXX	SXPTTSTPGT	STVXXGTSGT	PSSXPPXXTXX
16450	16460	16470	16480	16490	16500
XPLLLXPFTXN	XTITNLXXXX	XXXXPGSRKF	NTTERVLQGL	LKPLFRNSSL	EYLYSGCRLA
16510	16520	16530	16540	16550	16560
SLRPEKDSSA	MAVDAICTHR	PDPEDLGLDR	ERLYWELSNL	TNGIQELGPY	TLDRNSLYVN
16570	16580	16590	16600	16610	16620
GFTHRSMPT	TSTPGTSTVD	VGTSCTPSSS	PSPTTAGPLL	IPFTLNFTIT	NLQYGEDMGH
16630	16640	16650	16660	16670	16680
PGSRKENTTE	RVLQGLLGPI	FKNTSVGPLY	SGCRLTSLRS	EKDGAATGVD	AICIHHLDPK
16690	16700	16710	16720	16730	16740
SPGLNRERLY	WELSQLTNGI	KELGPYTLDL	NSLYVNGFTH	RTSVPTTSTP	GTSTVDLGT
16750	16760	16770	16780	16790	16800
GTPFSLPSPA	TAGPLLVLFT	LNFTITNLKY	EEDMHRPGSR	KFNTTERVLQ	TLLGPMFKNT

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FIG. 19G-2

16810	16820	16830	16840	16850	16860
SVGLLYSGCR	LTLLRSEKDG	AATGVDAICT	HRLDPKSPGL	XXEXLYWELS	XLTXIXELG
16870	16880	16890	16900	16910	16920
PYTLDRXSLY	VNGFTHXXSX	PTSTPGTST	VXXGTSGPS	SXPXXTXXXP	LLXFFTXXXT
16930	16940	16950	16960	16970	16980
ITNLXXXXXM	XXPGSRKFNT	TERVLQGLLR	PVFKNTSVGP	LYSGCRLLTLL	RPKKDGAATK
16990	17000	17010	17020	17030	17040
VDAICTYRPD	PKSPGLDREQ	LYWELSQLTH	SITELGPYTQ	DRDSLTVNGF	THRSSVPTTS
17050	17060	17070	17080	17090	17100
IPGTSAVHLE	TTGTPSSFPG	HTEPGPLLI	FTFNFTITNL	RYEENMQHPG	SRKFNTTTERV
17110	17120	17130	17140	17150	17160
LQGLLTPLFK	NTSVGPLYSG	CRLTLRPEK	QEAATGVDTI	CTHRVDPIGP	GLDRERLYWE
17170	17180	17190	17200	17210	17220
LSQLTNSITE	LGPYTLDRDS	LYVDGFNPWS	SVPTTSTPGT	STVHLATSGT	PSPLPGHTAP
17230	17240	17250	17260	17270	17280
VPLLIPFTLN	FTITDLHYEE	NMQHPGSRKF	NTTERVLQGL	LKPLFKSTSV	GPLYSGCRLT
17290	17300	17310	17320	17330	17340
LLRPEKHGAA	TGVDAICTLR	LDPTGPGGLDR	ERLYWELSQL	TNSITELGPY	TLLDRDSLIVN

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FIG. 19H-2

17350	17360	17370	17380	17390	17400
GENPWSSVPT	TSTPGTSTVH	LATSGTPSSL	PGHTTAGPLL	VPFTLNFTIT	NLKYEEDMHC
17410	17420	17430	17440	17450	17460
PGSRKFNTTE	RVLQSLHGPM	FKNTSVGPLY	SGCRLTLRS	EKDGAATGVD	AICTHRLDPK
17470	17480	17490	17500	17510	17520
SPGLXXEXLY	WELSLTXXI	XELGPYTLDR	XSLYVNGFTH	XXSXPTTSTP	GTSTVXXGTS
17530	17540	17550	17560	17570	17580
GTPSSXPXXT	XXXPLLXPFT	XXXTITNLXX	XXXMXXPGSR	KFNTTEXVLQ	GLLXPXFKNX
17590	17600	17610	17620	17630	17640
SVGXLYSGCR	LTXLRXEKXG	AATGXDAICX	HXXXPKXPGL	XXEXLYWELS	XLTNISITELG
17650	17660	17670	17680	17690	17700
PYTLD RDSLY	VNGFTHRSM	PTTSIPG TSA	VHLETSGTPA	SLPGHTAPGP	LLVPFTLNFT
17710	17720	17730	17740	17750	17760
ITNLQYEEDM	RHPGSRKFNT	TERVLQGLLK	PLFKSTSVGP	LYSGCRLTLL	RPEKRGAATG
17770	17780	17790	17800	17810	17820
VDTICTHRLD	PLNPGLXXEX	LYWELSXLTX	XIXELGPYTL	DRXSLYVNGF	THXXSXPTTS
17830	17840	17850	17860	17870	17880
TPGTSTVXXG	TSGTPSSXPX	XTXXXPLLXP	FTXNXTITNL	XXXXXXMXXPG	SRKFNTTEXV

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FIG. 19I-2

17890	17900	17910	17920	17930	17940
LQGLXPXFK	NXSVGXLYSG	CRLTXLRXEK	XGAATGXDAI	CXHXXPXPKP	GLXXEXLYWE
17950	17960	17970	17980	17990	18000
LSXLTXIXE	LGPYTLDXRS	LYVNGFHPRS	SVPTTSTPGT	STVHLATSGT	PSSLPGHTAP
18010	18020	18030	18040	18050	18060
VPLLIPTLN	FTITNLHYEE	NMQHPGSRKF	NTTERVLQGL	LGPMEKNTSV	GLLYSGCRIT
18070	18080	18090	18100	18110	18120
LLRPEKNGAA	TGMDAICSHR	LDPKSPGLXX	EXLYWELSXL	TXXIXELGPY	TLDRXSLEYVN
18130	18140	18150	18160	18170	18180
GFTHXXSXPT	TSTPGTSTVX	XGTSGTPSSX	PXXTXXXPLL	XPFTXNXTIT	NLXXXXXXMXX
18190	18200	18210	18220	18230	18240
PGSRKENTTE	XVLQGLLXPX	FKNXSVGXLY	SGCRLTXLRX	EKXGAATGXD	AICXHXXPXPK
18250	18260	18270	18280	18290	18300
XPGLXXEXLY	WELSXLTXXI	XELGPYTLDX	XSLYVNGFTH	QNSVPTTSTP	GTSTVYWATT
18310	18320	18330	18340	18350	18360
GTPSSFPGHT	EPGPLLIPT	FNFTITNLHY	EENMQHPGSR	KFNTTERVLQ	GLLTPLFKNT
18370	18380	18390	18400	18410	18420
SVGPLYSGCR	LTLRPEKQE	AATGVDTICT	HRVDPIGPGL	XXEXLYWELS	XLTXIXELG

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FIG. 19J-2

18430	18440	18450	18460	18470	18480
PYTLDRXSLY	VNGFTHXXSX	PTSTPGTST	VXXGTSGTPS	SXPXXTXXXP	LLXPFTXNXT
18490	18500	18510	18520	18530	18540
ITNLXXXXXM	XXPGSRKFNT	TEXVLQGLLX	PXFKNXSVGX	LYSGCRLTXL	RXEKXGAATG
18550	18560	18570	18580	18590	18600
XDAICXHXXX	PKXPGLXXEX	LYWELSLTX	XIXELGPYTL	DRXSLYVNGF	THRSSVPTTS
18610	18620	18630	18640	18650	18660
SPGTSTVHLA	TSGTPSSLPG	HTAPVPLLIP	FTLNFTITNL	HYEENMQHPG	SRKFNTTTERV
18670	18680	18690	18700	18710	18720
LQGLLKPLFK	STSVGPLYSG	CRLTLRPEK	HGAATGVDAI	CTLRLDPTGP	GLXXEXLYWE
18730	18740	18750	18760	18770	18780
LSXLTXXXIXE	LGPYTLDRXS	LYVNGFTHXX	SXPTTSTPGT	STVXXGTSGT	PSSXPXXTXX
18790	18800	18810	18820	18830	18840
XPLLXPFTXN	XTITNLXXXXX	XMXXPGSRKF	NTTEXVLQGL	LXPXFKNXSV	GXLYSGCRLT
18850	18860	18870	18880	18890	18900
XLRXEKGAA	TGXDAICXHX	XXPKXPGLXX	EXLYWELSLX	TXXIXELGPY	TLDRXSLEYVN
18910	18920	18930	18940	18950	18960
GFTHRTSVPT	TSTPGTSTVH	LATSGTPSSL	PGHTAPVPLL	IPFTLNFTIT	NLQYEEDMHR

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FIG. 19K-2

18970	18980	18990	19000	19010	19020
PGSRKFNTE	RVLQGLSPI	EKNSSVGPLY	SGCRLTSLRP	EKDGAATGMD	AVCLYHPNPK
19030	19040	19050	19060	19070	19080
RPGLDREQLY	CELSQLTHNI	TELGPSYSLDR	DSLYVNGFTH	QNSVPTTSTP	GTSTVYWATT
19090	19100	19110	19120	19130	19140
GTPSSFPGHT	XXXPLLPFT	XNXTITNLXX	XXXMXXPGSR	KFNTTEXVLQ	GLLXPXFKNX
19150	19160	19170	19180	19190	19200
SVGXLYSGCR	LTXLRXEKXG	AATGXDAICX	HXXXPKXPGL	XXEXLYWELS	XLTXIXELG
19210	19220	19230	19240	19250	19260
PYTLDRXSLY	VNGETHWSSG	LTTSTPWTST	VDLGTSGTPS	PVPSPPTAGP	LLVPFTLNFT
19270	19280	19290	19300	19310	19320
ITNLQYEEDM	HRPGSRKFNA	TERVLQGLLS	PIFKNTSVGP	LYSGCRLTLL	RPEKQEAATG
19330	19340	19350	19360	19370	19380
VDTICTHRVD	PIGPGLXXEX	LYWELSLTX	XIXELGPYTL	DRXSLYVNGF	THXXSXPTTS
19390	19400	19410	19420	19430	19440
TPGTSTVXXG	TSGTPSSXPX	XTXXXPLLXP	FTXNXITITNL	XXXXXXMXXPG	SRKFNTTEXV
19450	19460	19470	19480	19490	19500
LQGLLXPXFK	NXSVGXLYSG	CRLTXLRXEX	XGAATGXDAI	CXHXXXPKXP	GLXXEXLYWE

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FIG. 19L-2

19510	19520	19530	19540	19550	19560
LSXLTXXIXE	LGPYTLDRXS	LYVNGFTHRS	FGLTTSTPWT	STVDLGTSGT	PSPVPSPTTA
19570	19580	19590	19600	19610	19620
GPLLVPFTLN	FTITNLQYEE	DMHRPGSRKF	NTTERVLQGL	LTPLFRNTSV	SSLYSGCRLT
19630	19640	19650	19660	19670	19680
LLRPEKDGAA	TRVDAVCTHR	PDPKSPGLXX	EXLYWELSXL	TXXIXELGPY	TLDRXSILYVN
19690	19700	19710	19720	19730	19740
GFTHXXSXPT	TSTPGTSTVX	XGTSGETPSSX	PXXTXXXPLL	XPFTXNXTIT	NLXXXXXXMXX
19750	19760	19770	19780	19790	19800
PGSRKENTTE	XVLQGLLXPX	FKNXSVGXLY	SGCRLTXLRX	EKXGAATGXD	AICXHXXPXK
19810	19820	19830	19840	19850	19860
XPGLXXEXLY	WELSXLTXXI	XELGPYTLDL	XSLYVNGFTH	WIPVPTSSTP	GTSTVDLGS
19870	19880	19890	19900	19910	19920
TPSSLPSPTT	AGPLLVPFTL	NFTITNLQYG	EDMGHPGSRK	FNTTERVLQG	LLGPIFKNTS
19930	19940	19950	19960	19970	19980
VGPLYSGCRL	TSLRSEKDKA	ATGVDAICIH	HLDPKSPGLX	XEXLYWELS	LTXIXELGP
19990	20000	20010	20020	20030	20040
YTLDRXSLYV	NGFTHXXSXP	TTSTPGTSTV	XXGTSGETPSS	XPXXTXXXPL	LXPFTXNXTI

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FIG. 19M-2

20050	20060	20070	20080	20090	20100
TNLXXXXMX	XPGSRKFNTT	EXVLQGLLXP	XFKNXSVGXL	YSGCRLTXLR	XEKXGAATGX
20110	20120	20130	20140	20150	20160
DAICXHXXP	KXPGLXXEXL	YWELSLTXX	IXELGPYTLD	RXSLYVNGFT	HQTFAPNTST
20170	20180	20190	20200	20210	20220
PGTSTVDLGT	SGTPSSLPSF	TSAGPLLVPF	TLNFTITNLQ	YEEDMHHPGS	RKFNTTTERVL
20230	20240	20250	20260	20270	20280
QGLLGPMFKN	TSVGLLYSGC	RLTLRPEKN	GAATRVDAVC	THRPDPKSPG	LXXEXLYWEL
20290	20300	20310	20320	20330	20340
SXLTXXIXEL	GPYTLDXXSL	YVNGFTHXXS	XPTSTPGTS	TVXXGTSGTP	SSXPPXXTAPV
20350	20360	20370	20380	20390	20400
PLLIPTLNF	TITNLHYEEN	MQHPGSRKFN	TTERVLQGLL	KPLFKSTSVG	PLYSGCRLTL
20410	20420	20430	20440	20450	20460
LRPEKHGAAT	GVDAICTLRL	DPTGPGLDRE	RLYWELSQLT	NSVTELGPYT	LDRDSLTVNG
20470	20480	20490	20500	20510	20520
FTQRSSVPTT	SIPGTSVAVHL	ETSGTPASLP	GHTAPGPLLV	PFTLNFTITN	LQYEVDMRHP
20530	20540	20550	20560	20570	20580
GSRKFNTER	VLQGLLKPLF	KSTSVGPLY	GCRLTLRPE	KRGAATGVDT	ICTHRLDPLN

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FIG. 19N-2

20590	20600	20610	20620	20630	20640
PGLDREQLYW	ELSKLTRGII	ELGPYLLDRG	SLYVNGFTHR	NFVPITSTPG	TSTVHLGTSE
20650	20660	20670	20680	20690	20700
TPSSLPRIIV	PGPLLVPFTL	NFTITNLQYE	EAMRHPGSRK	FNTTERVLQG	LLRPLFKNTS
20710	20720	20730	20740	20750	20760
IGPLYSSCRL	TLLRPEKDKA	ATRVDAICTH	HPDPQSPGLN	REQLYWELSQ	LTHGITELGP
20770	20780	20790	20800	20810	20820
YTLLDRDSLIV	DGFTHWSPIP	TTSTPGTSIV	NLGTSGIPPS	LPETXXXPL	LXPFTXNXTI
20830	20840	20850	20860	20870	20880
TNLXXXXMX	XPGSRKFNTT	ERVLOGLLKP	LEKSTSVGPL	YSGCRLTLR	PEKDG VATRV
20890	20900	20910	20920	20930	20940
DAICTHRPDP	KIPGLDRQQL	YWELSQLTHS	ITELGPYTLD	RDSLYVNGFT	QRSSVPTTST
20950	20960	20970	20980	20990	21000
PGTFTVQPET	SETPSSLPGP	TATGPVLLPF	TLNFTITNLQ	YEEDMHRPGS	RKFNTTERVL
21010	21020	21030	21040	21050	21060
QGLLMPLFKN	TSVSSLYSGC	RLTLRPEKD	GAATRVDAVC	THRPDPKSPG	LDRERLYWKL
21070	21080	21090	21100	21110	21120
SQLTHGITEL	GPYTLLDRHSL	YVNGFTHQSS	MTTTRTPDTS	TMHLATS RTP	ASLSGPTTAS

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FIG. 190-2

21130	21140	21150	21160	21170	21180
PLLVLFTINF	TITNLRYEEN	MHHPGSRKEN	TTERVLQGLL	RPVFKNTSVG	PLYSGCRLTL
21190	21200	21210	21220	21230	21240
LRPKKDGAAT	KVDAICTYRP	DPKSPGLDRE	QLYWELSQLT	HSITELGPYT	LDRDSLIVNG
21250	21260	21270	21280	21290	21300
FTQRSSVPTT	SIPGTPVDL	GTSCTPVSKP	GPSAASPLL	LFTLNFTITN	LRYEENMQHP
21310	21320	21330	21340	21350	21360
GSRKENTTER	VLQGLLSLF	KSTSVGPLYS	GCRLTLRPE	KDGTATGVDA	ICTHHPPDKS
21370	21380	21390	21400	21410	21420
PRLDREQLYW	ELSCLTHNIT	ELGHYALDND	SLEVNGFTHR	SSVSTSTPG	TPTVYLGASK
21430	21440	21450	21460	21470	21480
TPASIFGPSA	ASHLLILFTL	NFTITNLRYE	ENMWPGRKF	NTTERVLQGL	LRPLFKNTSV
21490	21500	21510	21520	21530	21540
GPLYSGSRLT	LLRPEKDGEA	TGVDAICTHR	PDPTGPGGLDR	EQLYLELSQL	THSITELGPY
21550	21560	21570	21580	21590	21600
TLDRDSLIVN	GFTHRSSVPT	TSTGVVSEEP	FTLNFTINNL	RYMADMGQPG	SLKFNITDNV
21610	21620	21630	21640	21650	21660
MKHLSPLEFQ	RSSLGARYTG	CRVIALRSVK	NGAETRVDLL	CTYLQPLSGP	GLPIKQVFHE

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FIG. 19P-2

21670	21680	21690	21700	21710	21720
LSQQTHGITR	LGPYSLDKDS	LYLNGYNEPG	LDEPPTTPKP	ATFELPLSE	ATTAMGYHLK
21730	21740	21750	21760	21770	21780
TLTLNFTISN	LQYSPDMGKG	SATFNSTEGV	LQHLLRPLFQ	KSSMGPFYLG	CQLISLRPEK
21790	21800	21810	21820	21830	21840
DGAATGVDTT	CTYHPDPVGP	GLDIIQQLYWE	LSQLTHGVTQ	LGFYVLDRDS	LFINGYAPQN
21850	21860	21870	21880	21890	21900
LSIRGEYQIN	FHIVWNLSN	PDPTSSEYIT	LLRDIQDKVT	TLYKGSQLHD	TFRFCLVTNL
21910	21920	21930	21940	21950	21960
TMDSVLVTVK	ALFSSNLDPS	LVEQVFELDKT	LNASEFHWLGS	TYQLVDIHVT	EMESSVYQPT
21970	21980	21990	22000	22010	22020
SSSSTQHFYL	NFTITNLPYS	QDKAQPGTTN	YQRNKRNIED	ALNQLFRNSS	IKSYFSDCCQV
22030	22040	22050	22060	22070	22080
STFRSVPNRH	HTGVDSL CNF	SPLARRVDRV	AIYEEFLRMT	RNGTQLQNFT	LDRSSVLVDG
22090	22100	22110	22120	22130	22140
YSPNRNEPLT	GNSDLPFWAV	ILIGLAGLLG	LITCLICGVL	VTTRRRKKEG	EYNVQQQCPG
22150					
YYQSHLDLED	LQ				

FIG. 19Q-2*Transthyretin*

10	20	30	40	50	60
MASHRLLLC	LAGLVFVSEA	GPTGTGESKC	PLMVKVLDVAV	RGSPAINVAV	HVFRKAADDT
70	80	90	100	110	120
WEPFASGKTS	ESGELHGLTT	EEEFVEGIYK	VEIDTKSYWK	ALGISPFHEH	AEVVFTANDS
130	140				
GPERRYTIAAL	LSPYSYSTTA	VVTNPKE			

Transferrin

10	20	30	40	50	60
MRLAVGALLV	CAVLGLCLAV	PDKTVRWCAV	SEHEATKCQS	FRDHMKSVIP	SDGPSVACVK
70	80	90	100	110	120
KASYLDCIRA	IAANEADAVT	LDAGLVYDAY	LAPNNLKPVV	AEFYGSKEDP	QTFYYAVAVV
130	140	150	160	170	180
KKDSGFQMNQ	LRGKKSCHTG	LGRSAGWNIP	IGLLYCDLPE	PRKPLEKAVA	NEFSGSCAPC
190	200	210	220	230	240
ADGTDFPQLC	QLCPGCGCST	LNQYFGYSGA	EKCLKDGAGD	VAFVKHSTIF	ENLANKADRD
250	260	270	280	290	300
QYELLCLDNT	RKPVDEYKDC	HLAQVPSHTV	VARSIGGKED	LIWELLNQAQ	EHFGKDKSKE

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FIG. 19R-2

310	320	330	340	350	360
FQLFSSPHGK	DLLEFKDSAAG	FLKVPPRMDA	KMYLGYEYVT	AIRNLRGTC	PEAPTDECKP
370	380	390	400	410	420
VKWALSHHE	RLKCDEWSVN	SVGKIECVSA	ETTEDCIAKI	MNGEADAMSL	DGGFVYIAGK
430	440	450	460	470	480
CGLVPVLAEN	YNKSDNCEDT	PGAGYFAVAV	VKKSASDLTW	DNLKGKKSCH	TAVGRTAGWN
490	500	510	520	530	540
IPMGLLYNKI	NHCRFDEFFS	EGCAPGSKKD	SSLCKLCMGS	GLNLCEPNK	EGYYGYTGAF
550	560	570	580	590	600
RCLVEKGDVA	FVKHQTVPPQN	TGGKNPDPWA	KNLNEKDYEL	LCLDGTRKPV	EEYANCHLAR
610	620	630	640	650	660
APNHAVVTRK	DKEACVHKIL	RQQQHLEFGSN	VTDCSGNFCL	FRSETKDLLF	RDDTVCLAKL
670	680	690			
HDRNTYEKYL	GEEYVKAAGN	LRKCSTSSLL	EACTFRRP		

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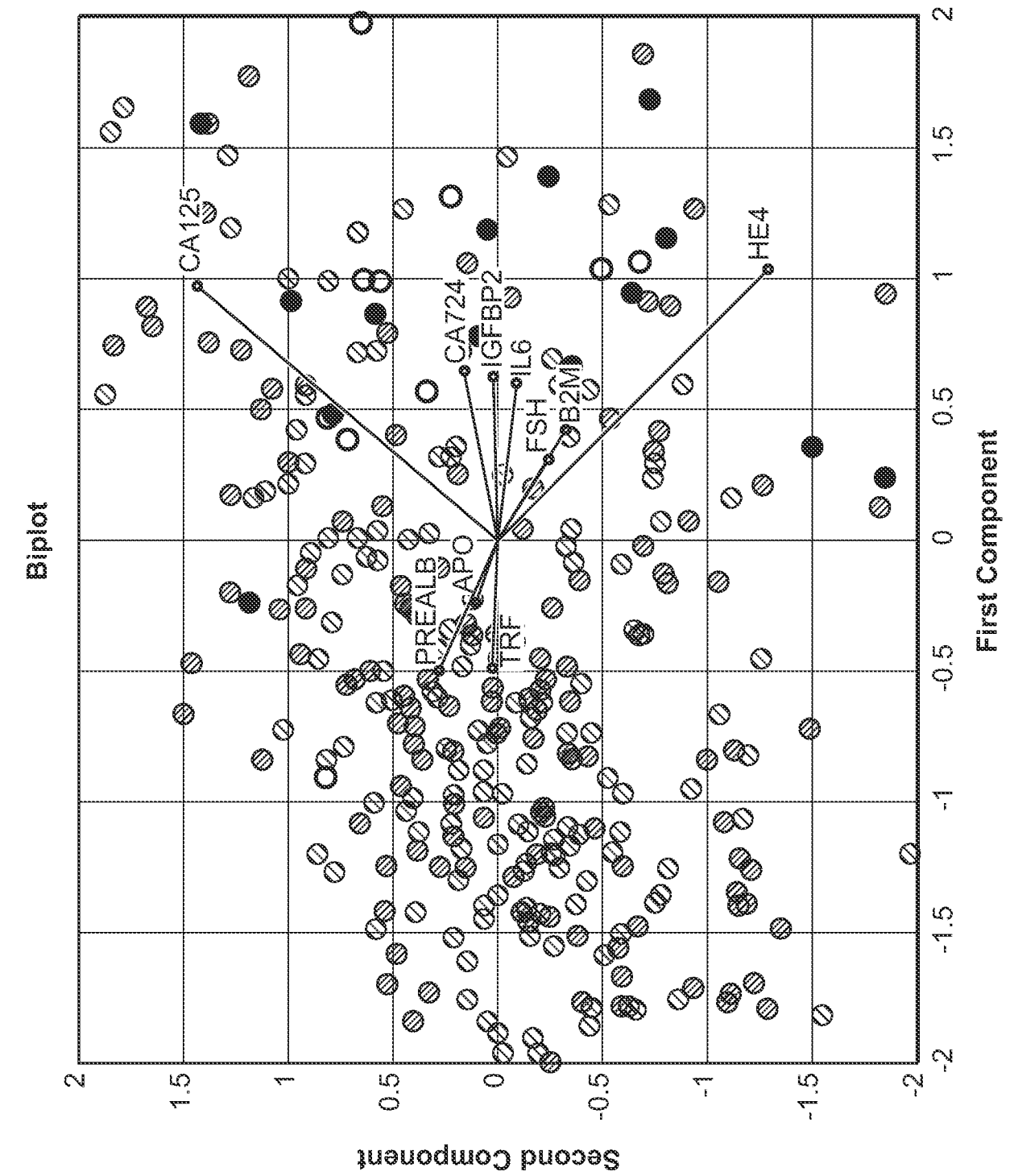
FIG. 19S-2***Apolipoprotein A1***

10	20	30	40	50	60
MKAAVLTIAV	LFLTGSQARH	FWQDEPPQS	PWDRVKDLAT	VYVDVLKDSG	RDYVSQFEGS
70	80	90	100	110	120
ALGKQLNLKL	LDNWDSVTST	FSKLREQLGP	VTQEFWDNLE	KETEGLRQEM	SKDLEEVKAK
130	140	150	160	170	180
VQPYLDDFQK	KWQEEMELYR	QKVEPLRAEL	QEGARQKLHE	LQEKLSPLGE	EMRDRAHAHV
190	200	210	220	230	240
DALRTHLAPY	SDELQRQLAA	RLEALKENG	ARLAEYHAKA	TEHLSTLSEK	AKPALEDLRQ
250	260				
GLLPVLESEK	VSFLSALEEY	TKKLNTQ			

 β -2-microglobulin

10	20	30	40	50	60
MRSVALAVL	ALLSLSGLEA	IQRTPKIQVY	SRHPAENGKS	NFLNCYVSGF	HPSDIEVDLL
70	80	90	100	110	
KNGERIEKVE	HSDLFSKDW	SFYLLYYTEF	TPTEKDEYAC	RVNHVTL SQP	KIVKWDRDM

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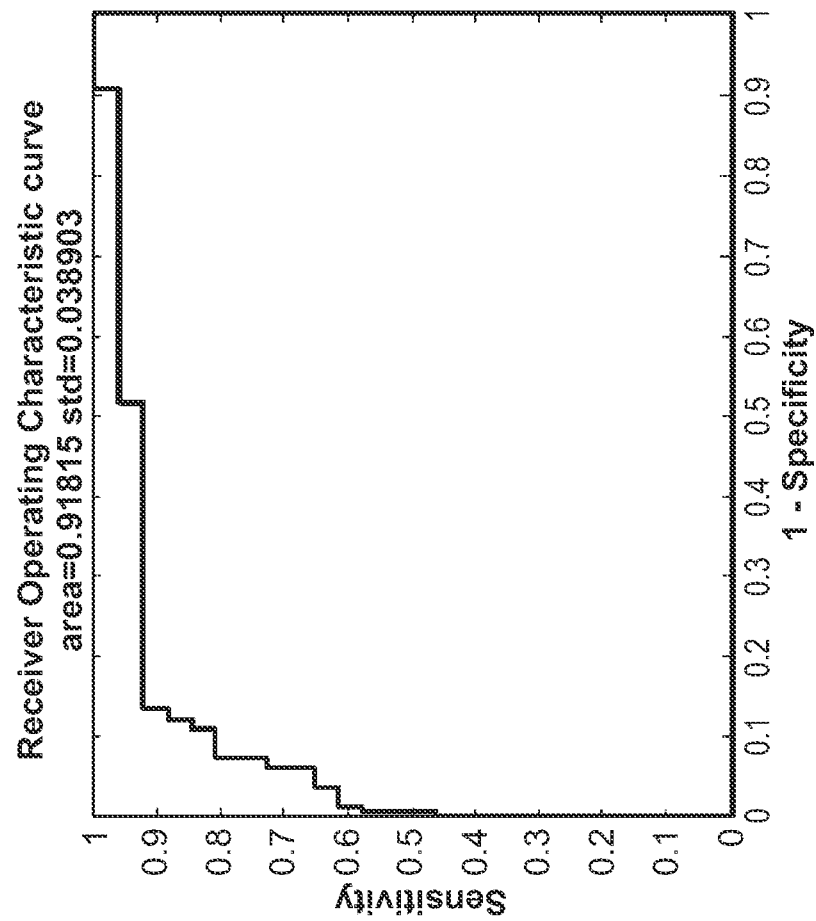


FIG. 21A

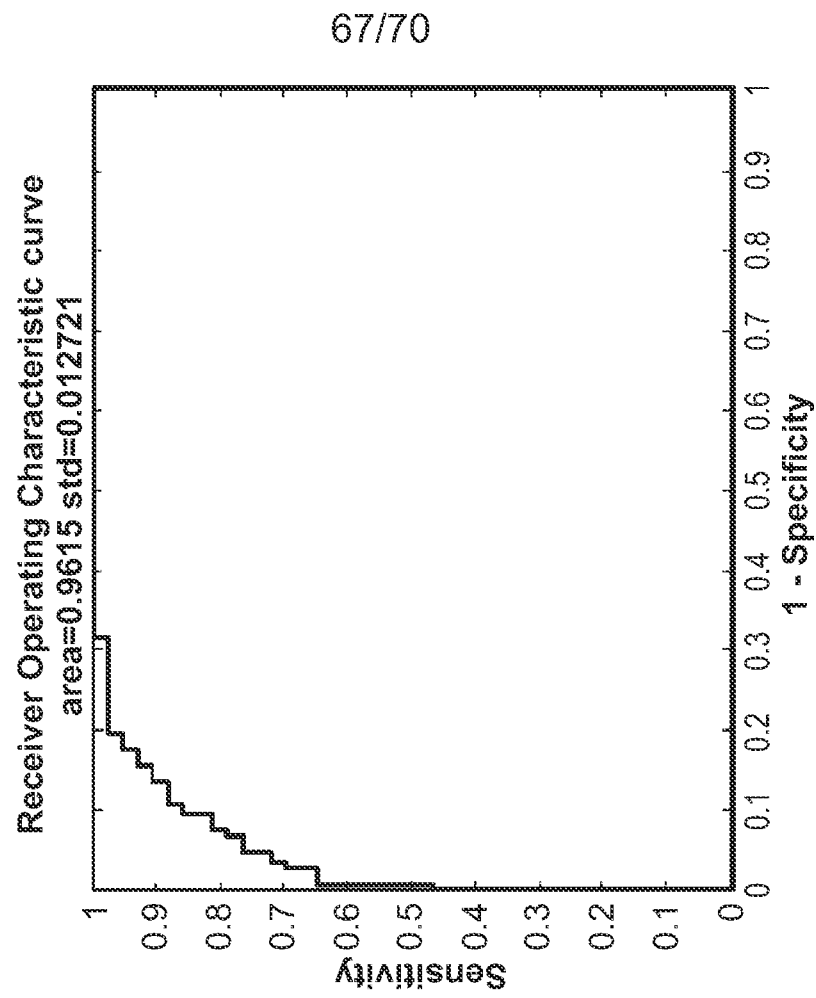


FIG. 21B

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FIG. 23

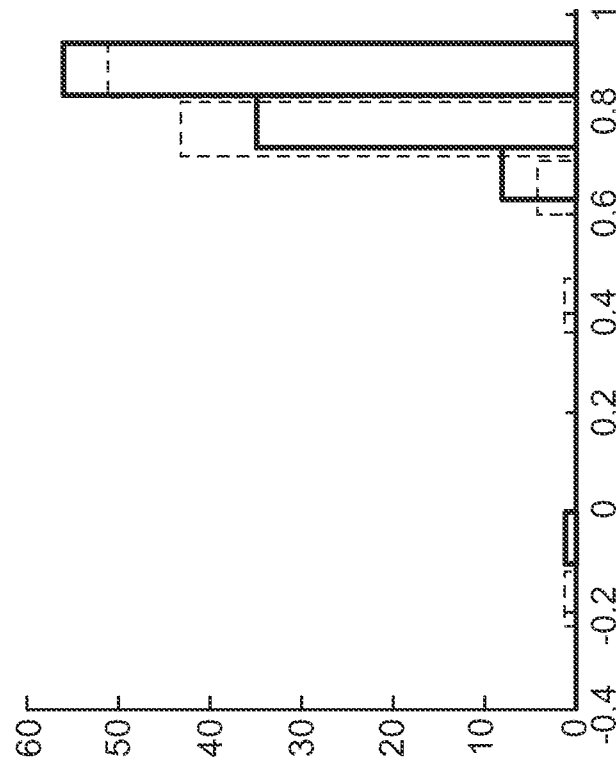


FIG. 22

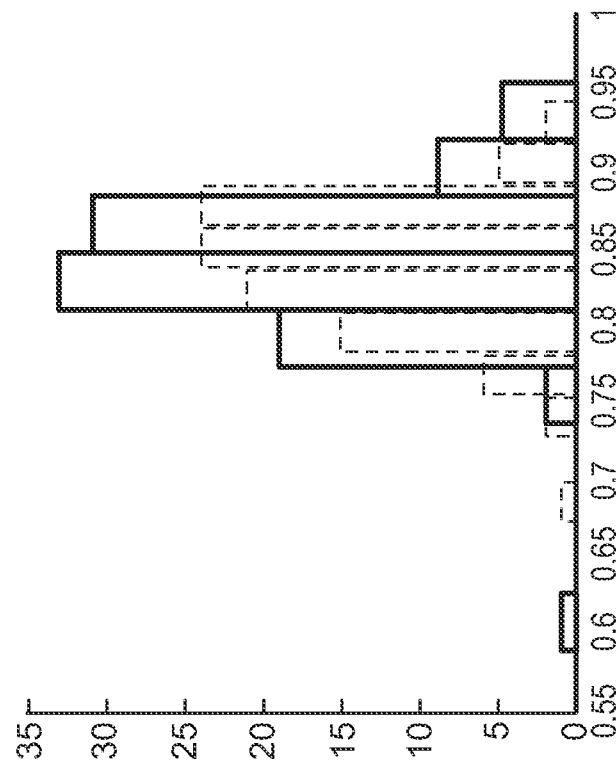


FIG. 25

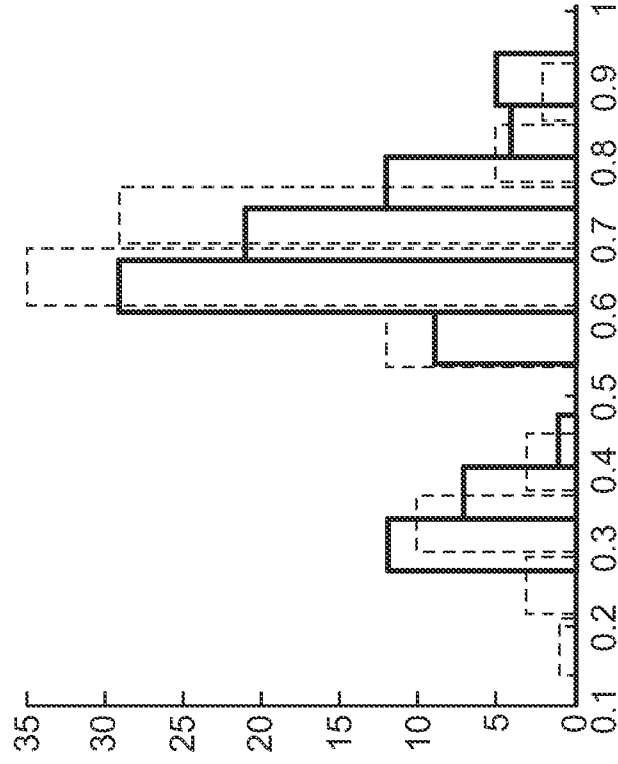
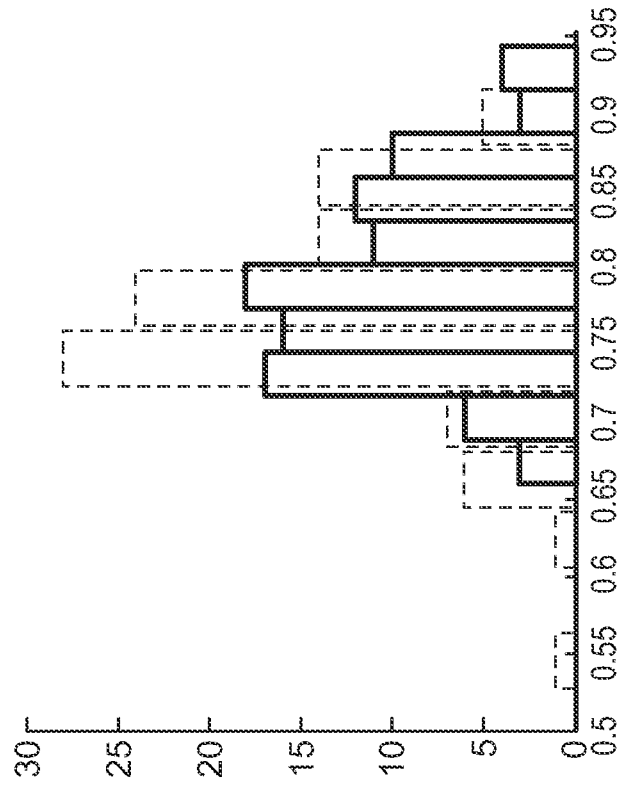
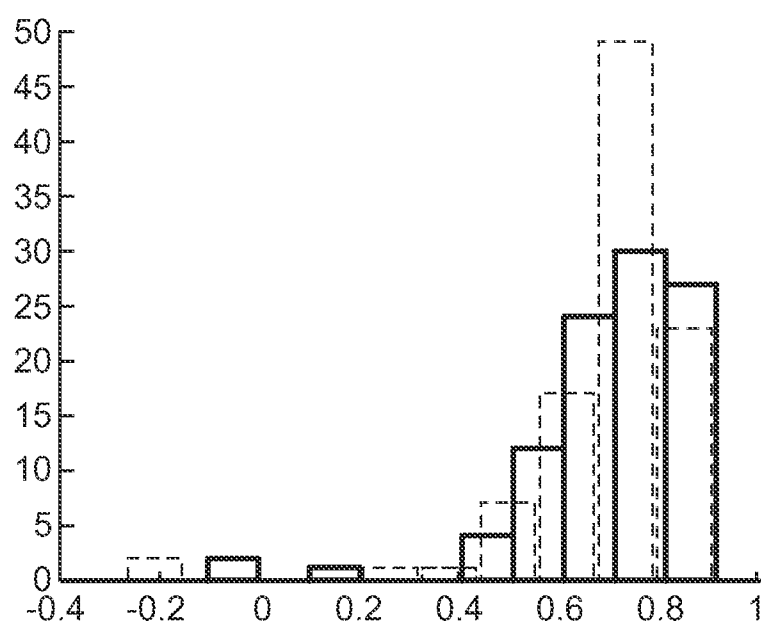


FIG. 24

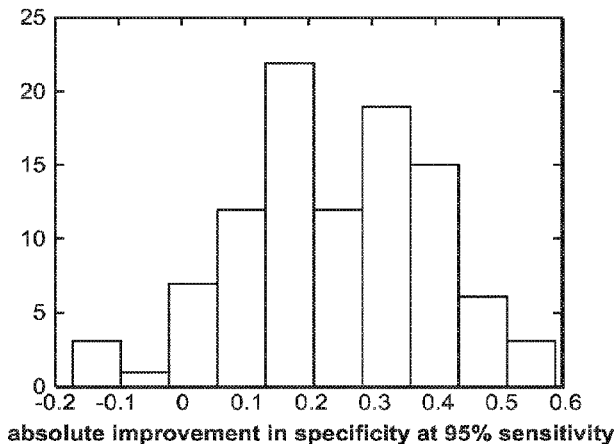


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FIG. 26



Panel: CA125, Prealb, IGFBP2, IL6, CA724



Panel: Transthyretin (TT or prealbumin), Apolipoprotein A-1 (Apo A-1), beta 2-Microglobulin (beta 2M), Transferrin (Tfr) and Cancer Antigen 125 (CA 125 II), IGFBP2, IL6, HE4, and FSH.