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(54) **OVARIAN CANCER BIOMARKERS AND USES THEREOF**

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G06F 17/18	(2006.01)

(52) **U.S. Cl.** **435/7.21**; 436/86; 436/94; 702/19; 702/181

(57) **ABSTRACT**

The present application includes biomarkers, methods, devices, reagents, systems, and kits for the detection and diagnosis of ovarian cancer. In one aspect, the application provides biomarkers that can be used alone or in various combinations to diagnose ovarian cancer or permit the differential diagnosis of a pelvic mass as benign or malignant. In another aspect, methods are provided for diagnosing ovarian cancer in an individual, where the methods include detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on the at least one biomarker value.

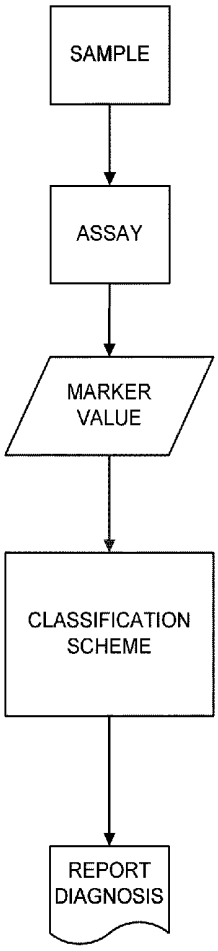


FIG. 1A

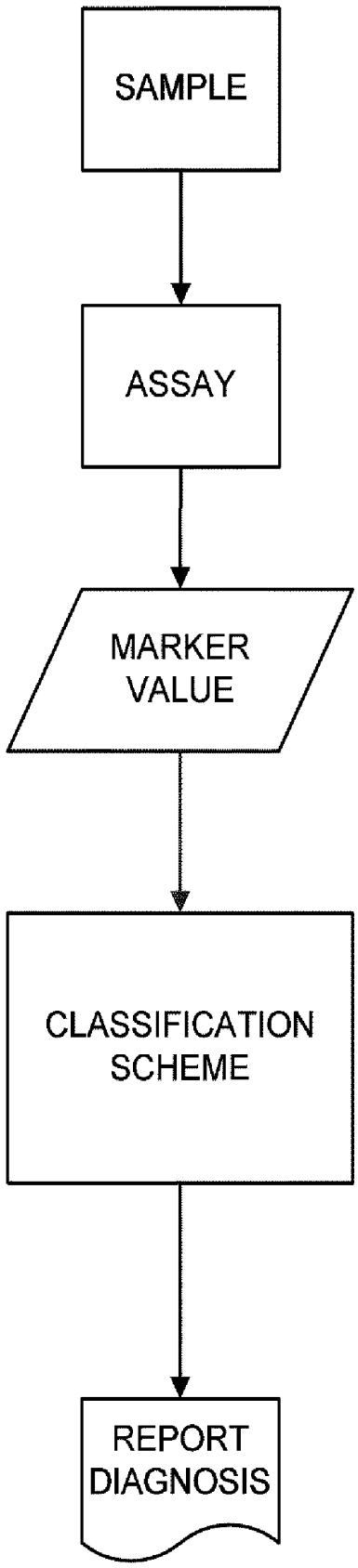

SAMPLE
ASSAY
MARKER VALUE
CLASSIFICATION SCHEME
REPORT DIAGNOSIS

FIG. 1B

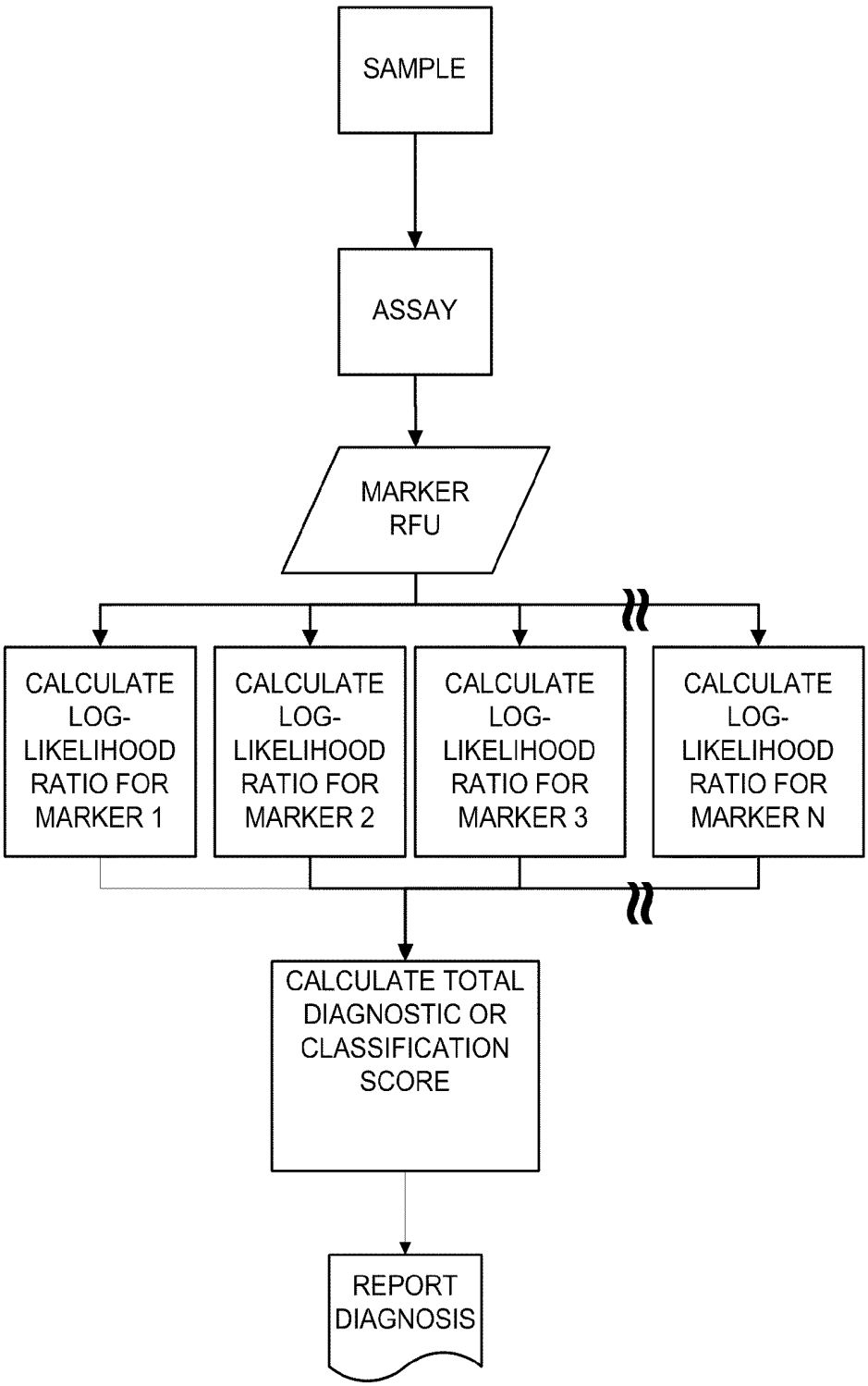

SAMPLE
ASSAY
MARKER RFU
CALCULATE LOG-LIKELIHOOD RATIO FOR MARKER 1
CALCULATE LOG-LIKELIHOOD RATIO FOR MARKER 2
CALCULATE LOG-LIKELIHOOD RATIO FOR MARKER 3
CALCULATE LOG-LIKELIHOOD RATIO FOR MARKER N
CALCULATE TOTAL DIAGNOSTIC OR CLASSIFICATION SCORE
REPORT DIAGNOSIS

FIG. 2

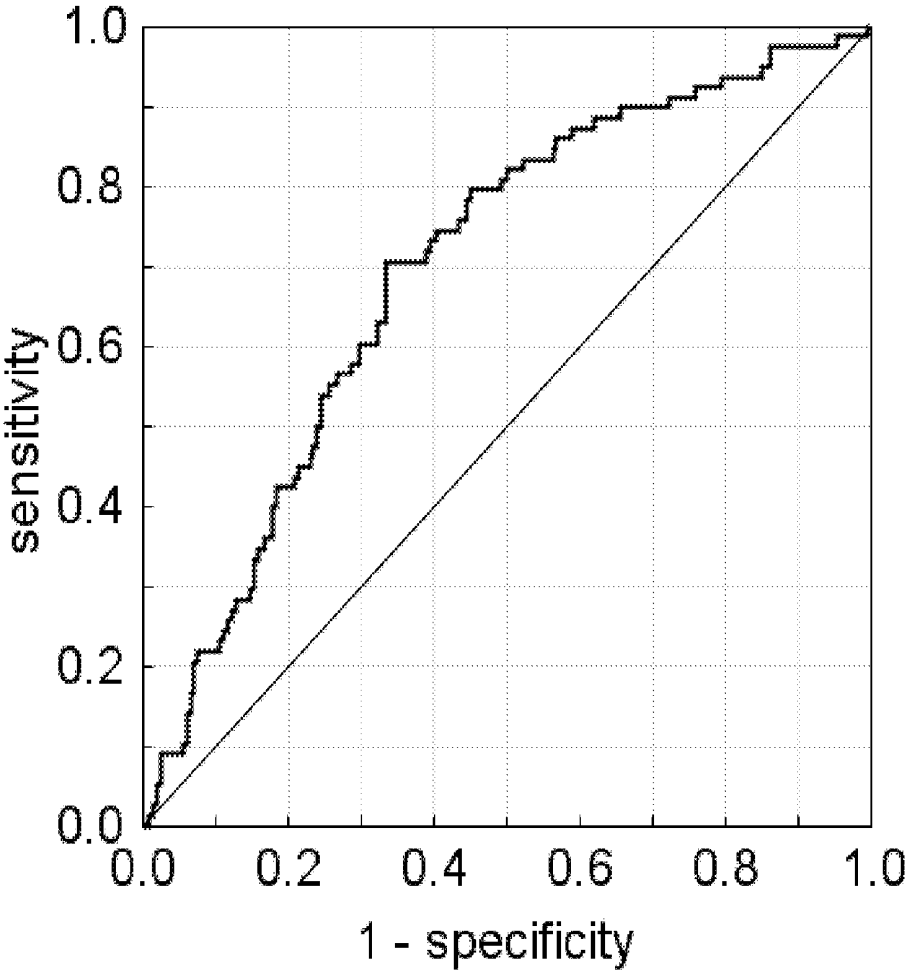


FIG. 3

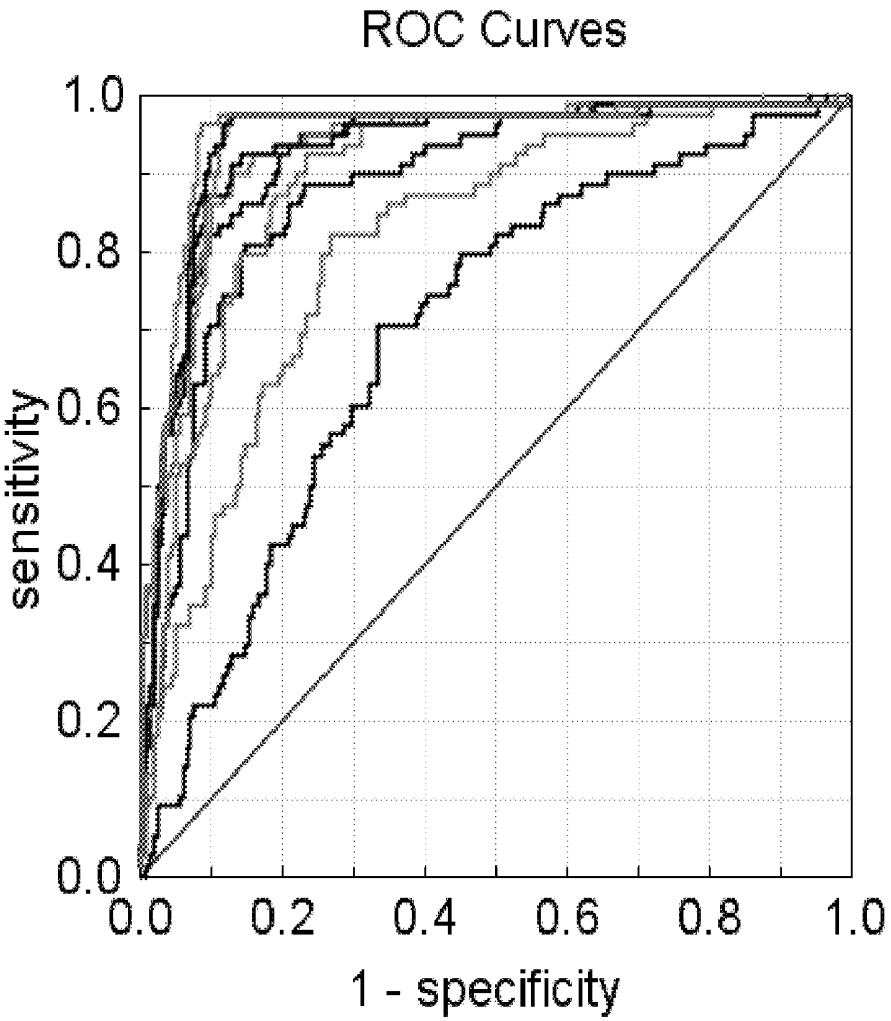


FIG. 4

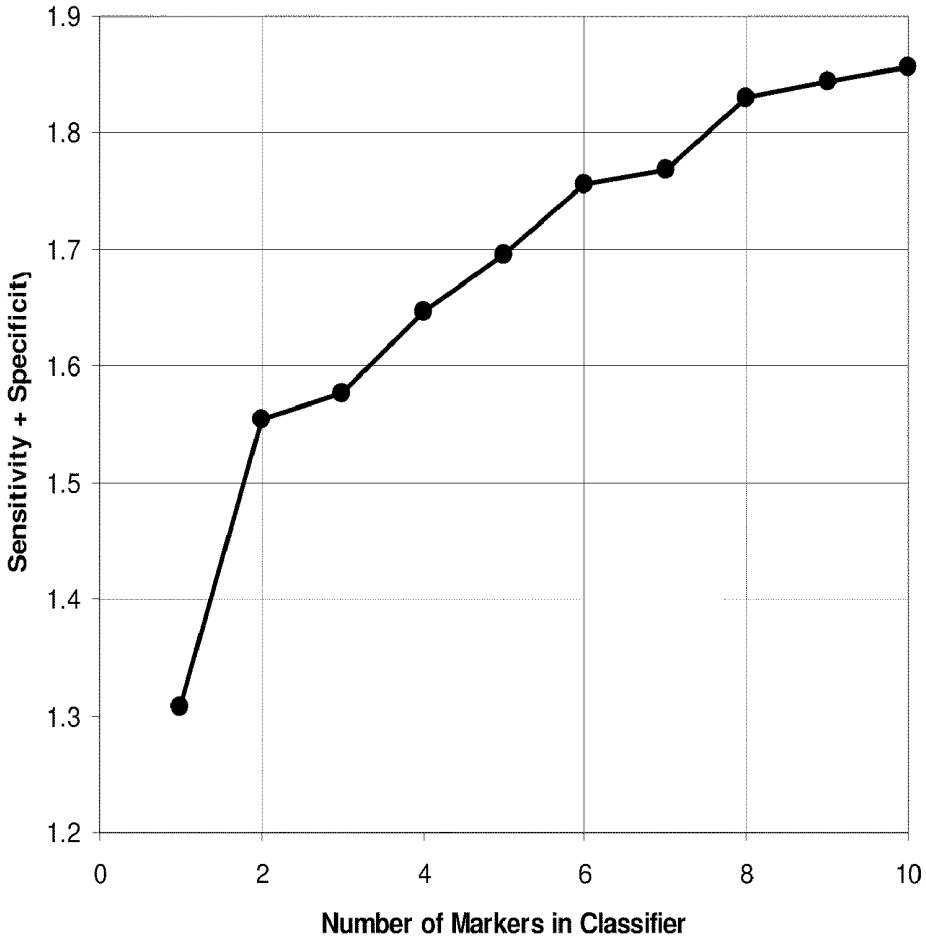

1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
Sensitivity + Specificity
0
2
4
6
8
10
Number of Markers in Classifier

FIG. 5

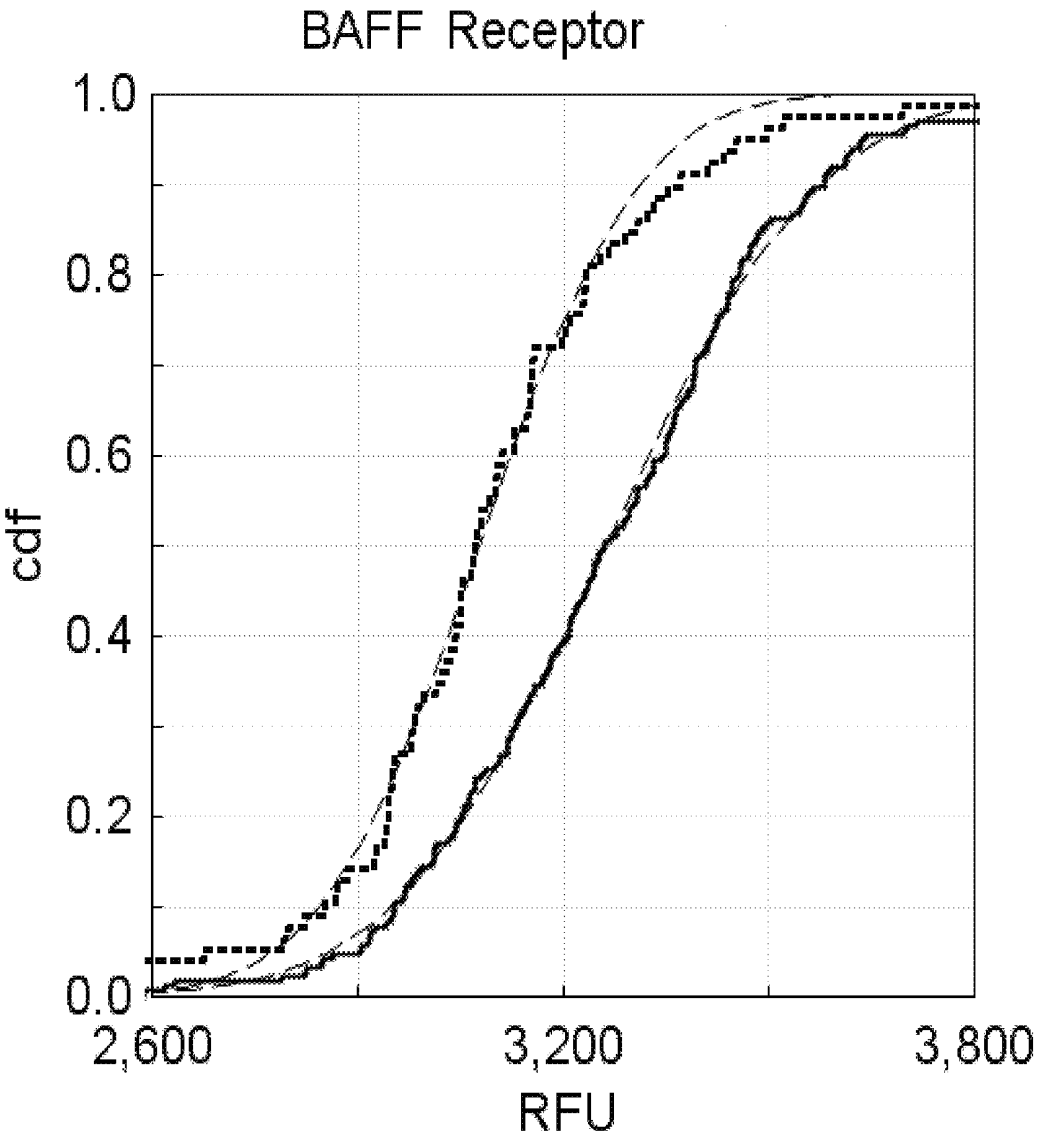


FIG. 6

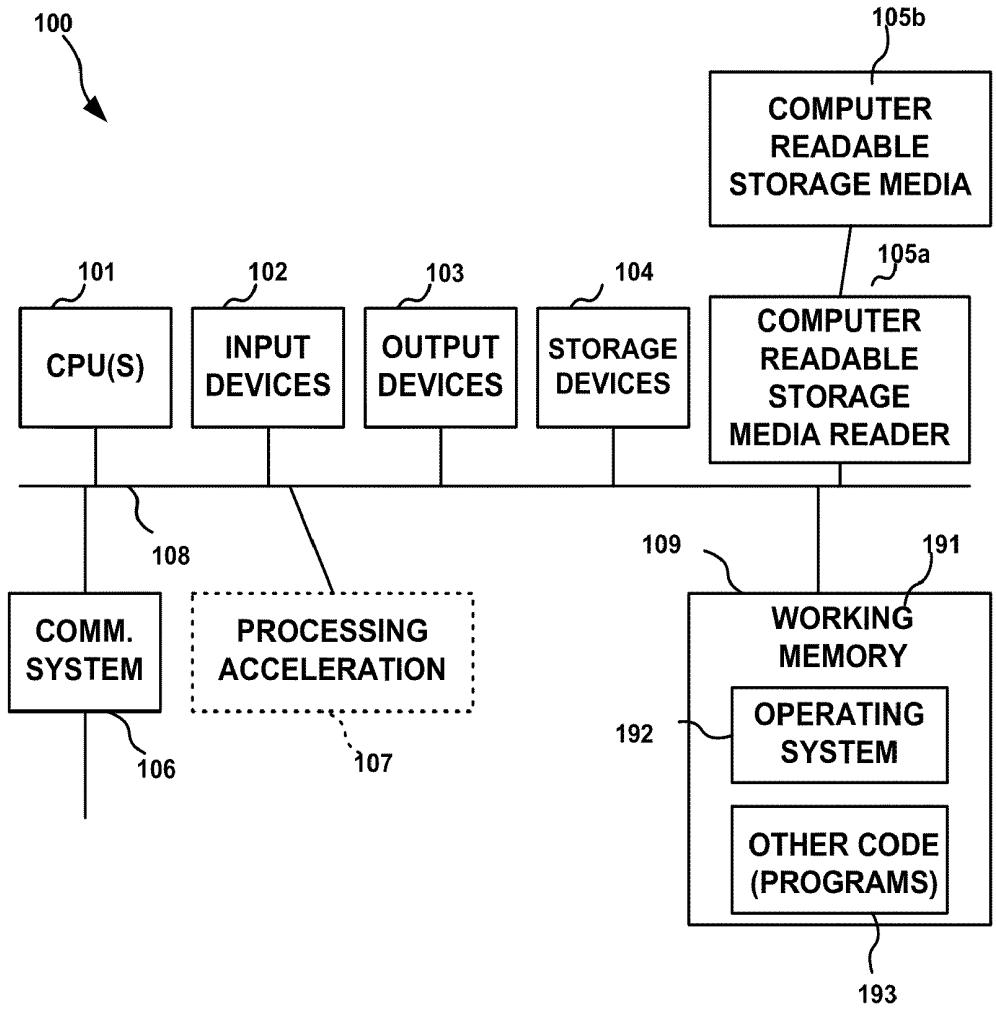

100
105b
COMPUTER READABLE STORAGE MEDIA
105a
101
102
103
104
CPU(S)
INPUT DEVICES
OUTPUT DEVICES
STORAGE DEVICES
COMPUTER READABLE STORAGE MEDIA READER
108
109
191
COMM. SYSTEM
PROCESSING ACCELERATION
WORKING MEMORY
192
OPERATING SYSTEM
OTHER CODE (PROGRAMS)
106
107
193

FIG. 7

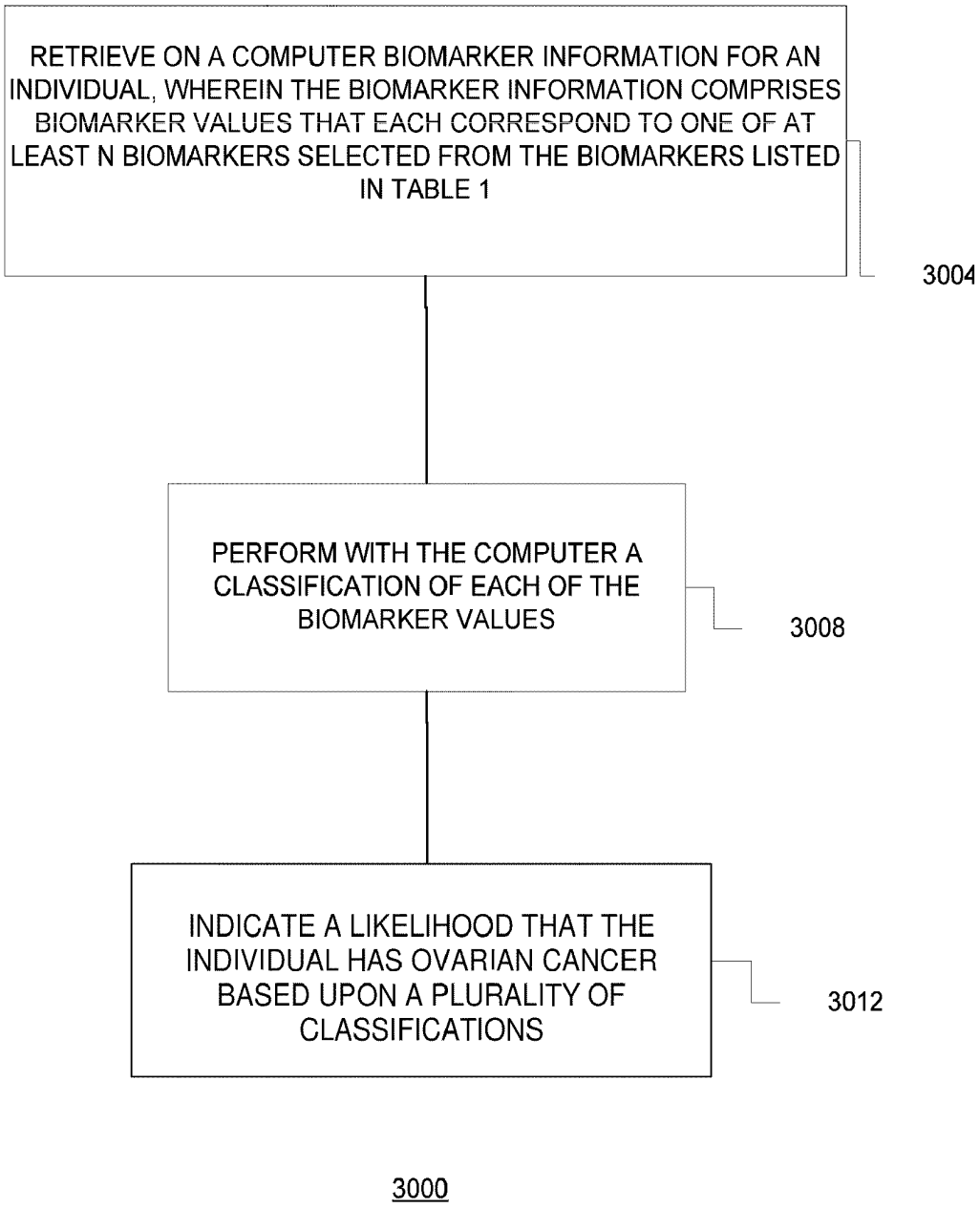

RETRIEVE ON A COMPUTER BIOMARKER INFORMATION FOR AN INDIVIDUAL, WHEREIN THE BIOMARKER INFORMATION COMPRISES BIOMARKER VALUES THAT EACH CORRESPOND TO ONE OF AT LEAST N BIOMARKERS SELECTED FROM THE BIOMARKERS LISTED IN TABLE 1
3004
PERFORM WITH THE COMPUTER A CLASSIFICATION OF EACH OF THE BIOMARKER VALUES
3008
INDICATE A LIKELIHOOD THAT THE INDIVIDUAL HAS OVARIAN CANCER BASED UPON A PLURALITY OF CLASSIFICATIONS
3012
3000

FIG. 8

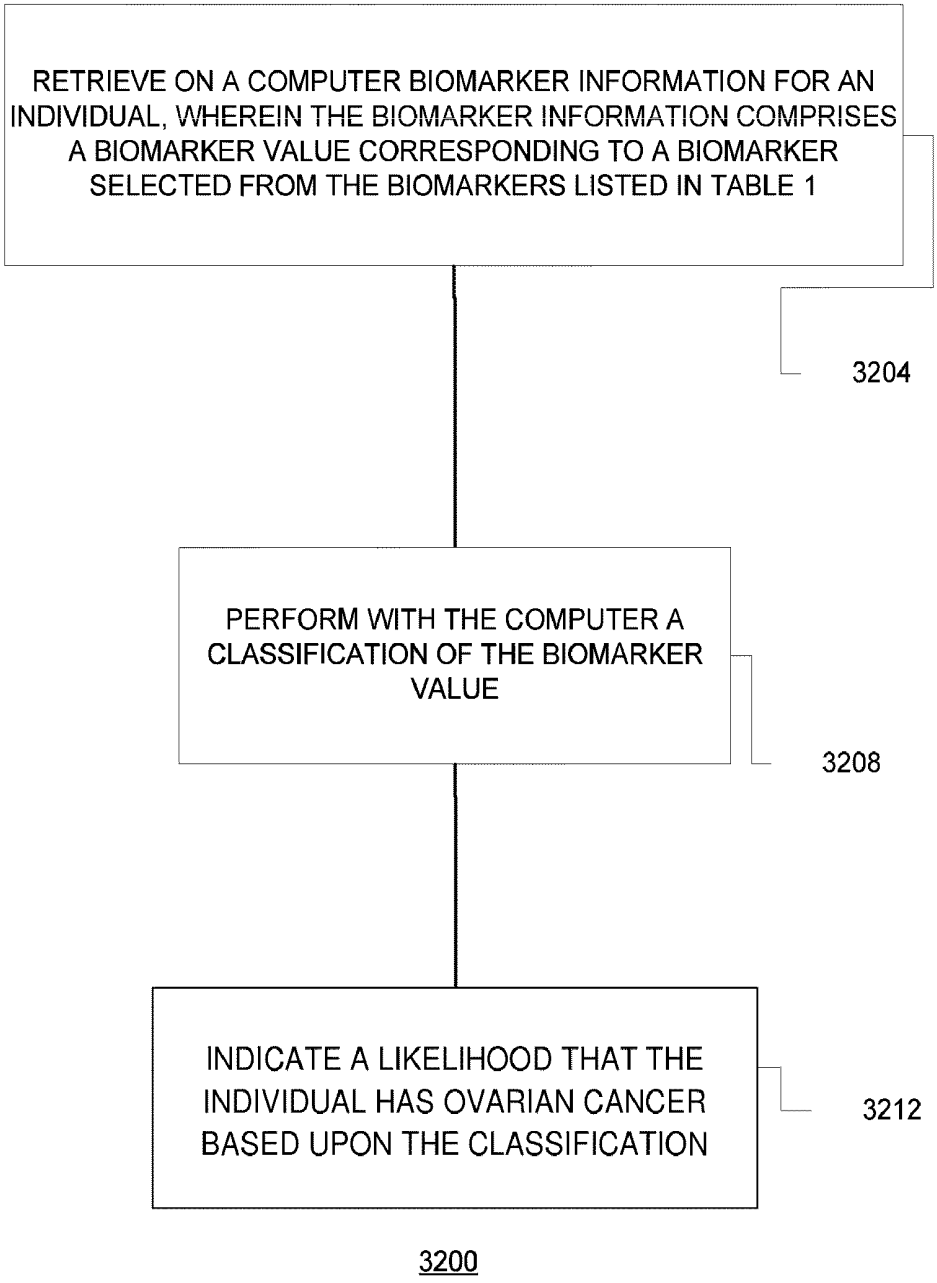
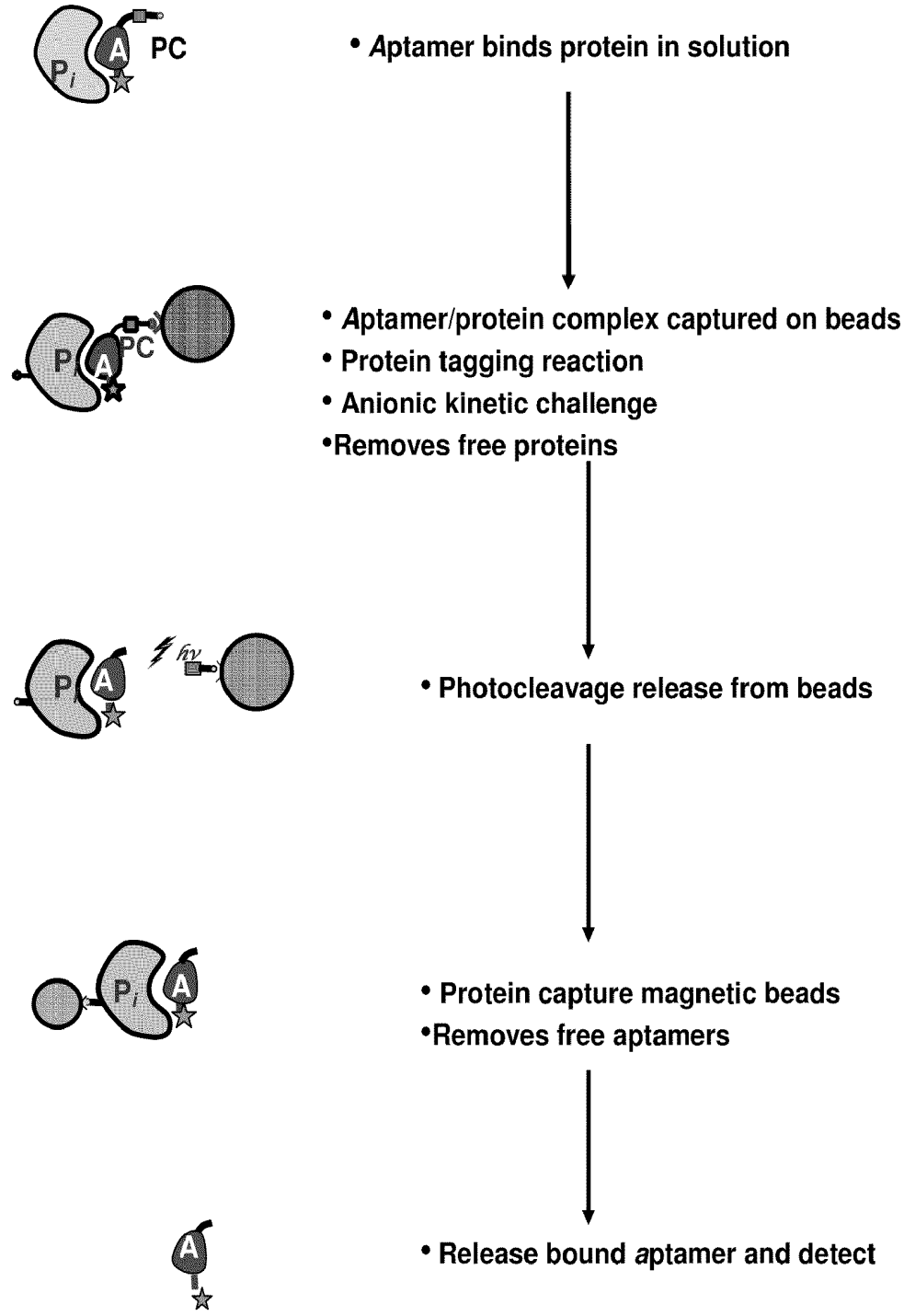

RETRIEVE ON A COMPUTER BIOMARKER INFORMATION FOR AN INDIVIDUAL, WHEREIN THE BIOMARKER INFORMATION COMPRISES A BIOMARKER VALUE CORRESPONDING TO A BIOMARKER SELECTED FROM THE BIOMARKERS LISTED IN TABLE 1
3204
PERFORM WITH THE COMPUTER A CLASSIFICATION OF THE BIOMARKER VALUE
3208
INDICATE A LIKELIHOOD THAT THE INDIVIDUAL HAS OVARIAN CANCER BASED UPON THE CLASSIFICATION
3212
3200

FIG. 9


PC
• Aptamer binds protein in solution
• Aptamer/protein complex captured on beads
• Protein tagging reaction
• Anionic kinetic challenge
•Removes free proteins
hν
• Photocleavage release from beads
• Protein capture magnetic beads
•Removes free aptamers
• Release bound aptamer and detect

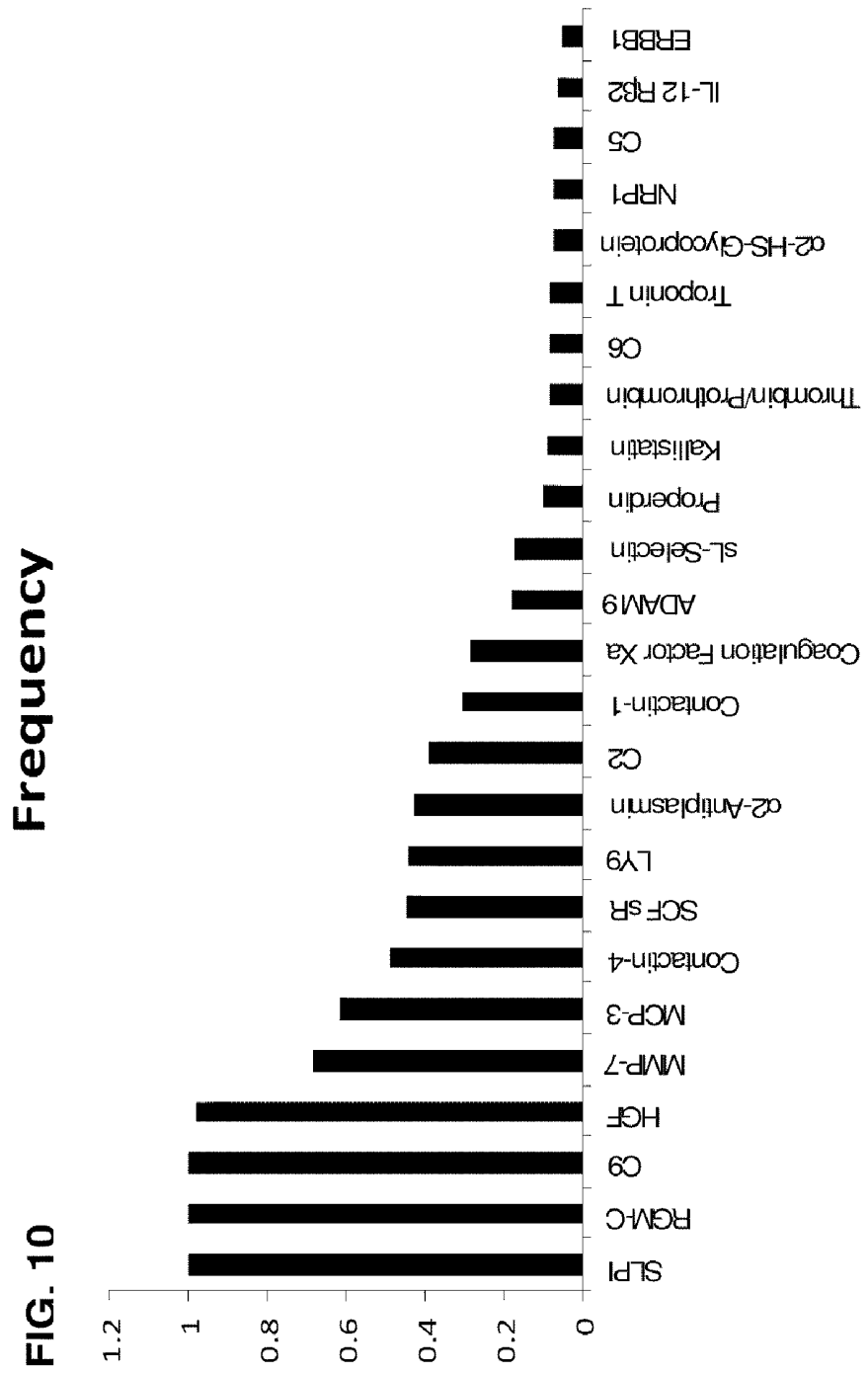

Frequency
1.2
1
0.8
0.6
0.4
0.2
0
SLPI
RGMC
C9
HGF
MMP-7
MCP-3
Contactin-4
SCF sR
LY9
α2-Antiplasmin
C2
Contactin-1
Coagulation Factor Xa
ADAM9
sL-Selectin
Properdin
Kallistatin
Thrombin/Prothrombin
C6
Troponin T
α2-HS-Glycoprotein
NRP1
C5
IL-12 Rβ2
ERBB1

FIG. 10

FIG. 11

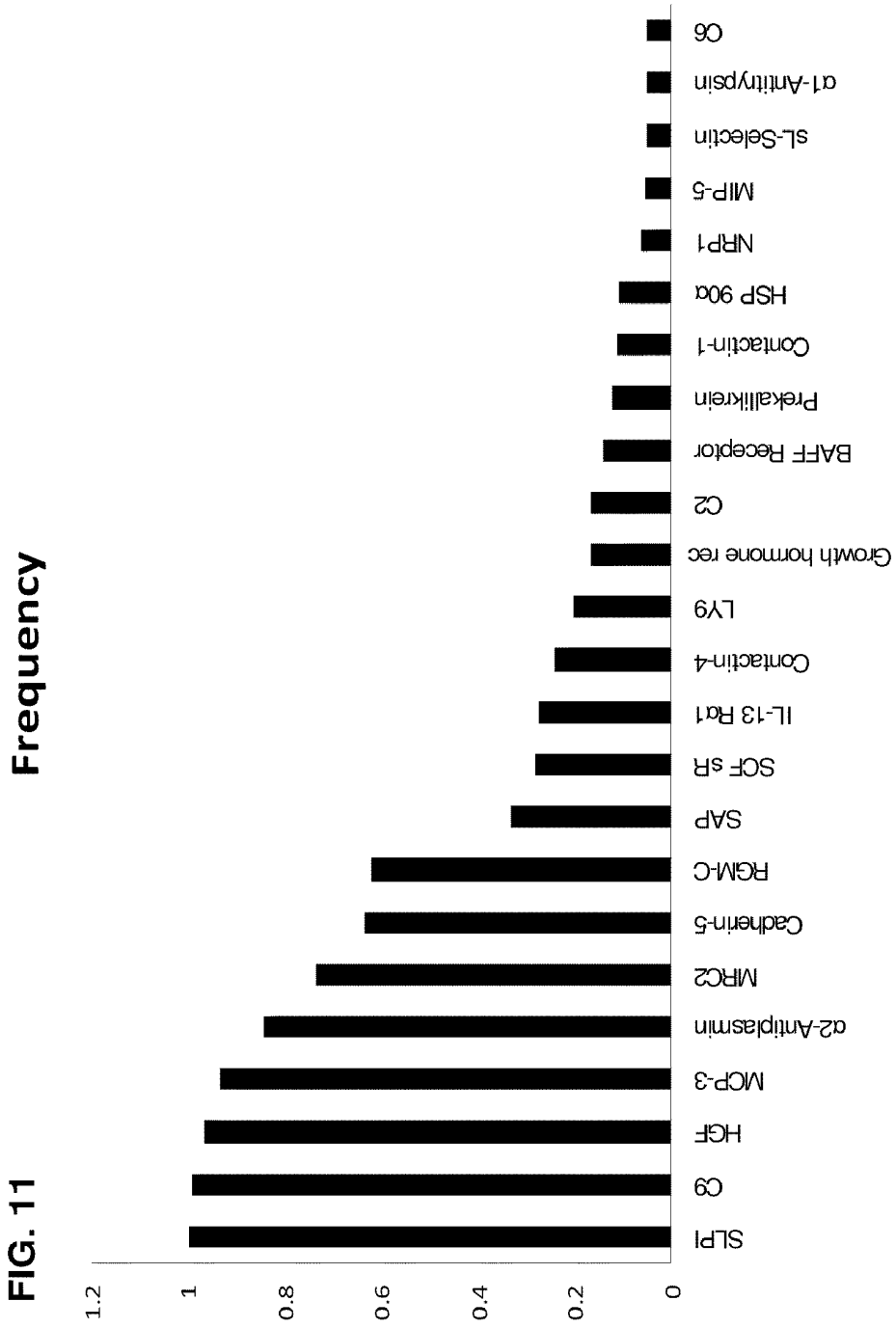

Frequency
1.2
1
0.8
0.6
0.4
0.2
0
SLPI
C9
HGF
MCP-3
α2-Antiplasmin
MRC2
Cadherin-5
RGM-C
SAP
SCF sR
IL-13 Rα1
Contactin-4
LY9
Growth hormone rec
C2
BAFF Receptor
Prekallikrein
Contactin-1
HSP 90α
NRP1
MIP-5
sL-Selectin
α1-Antitrypsin
C6

FIG. 12

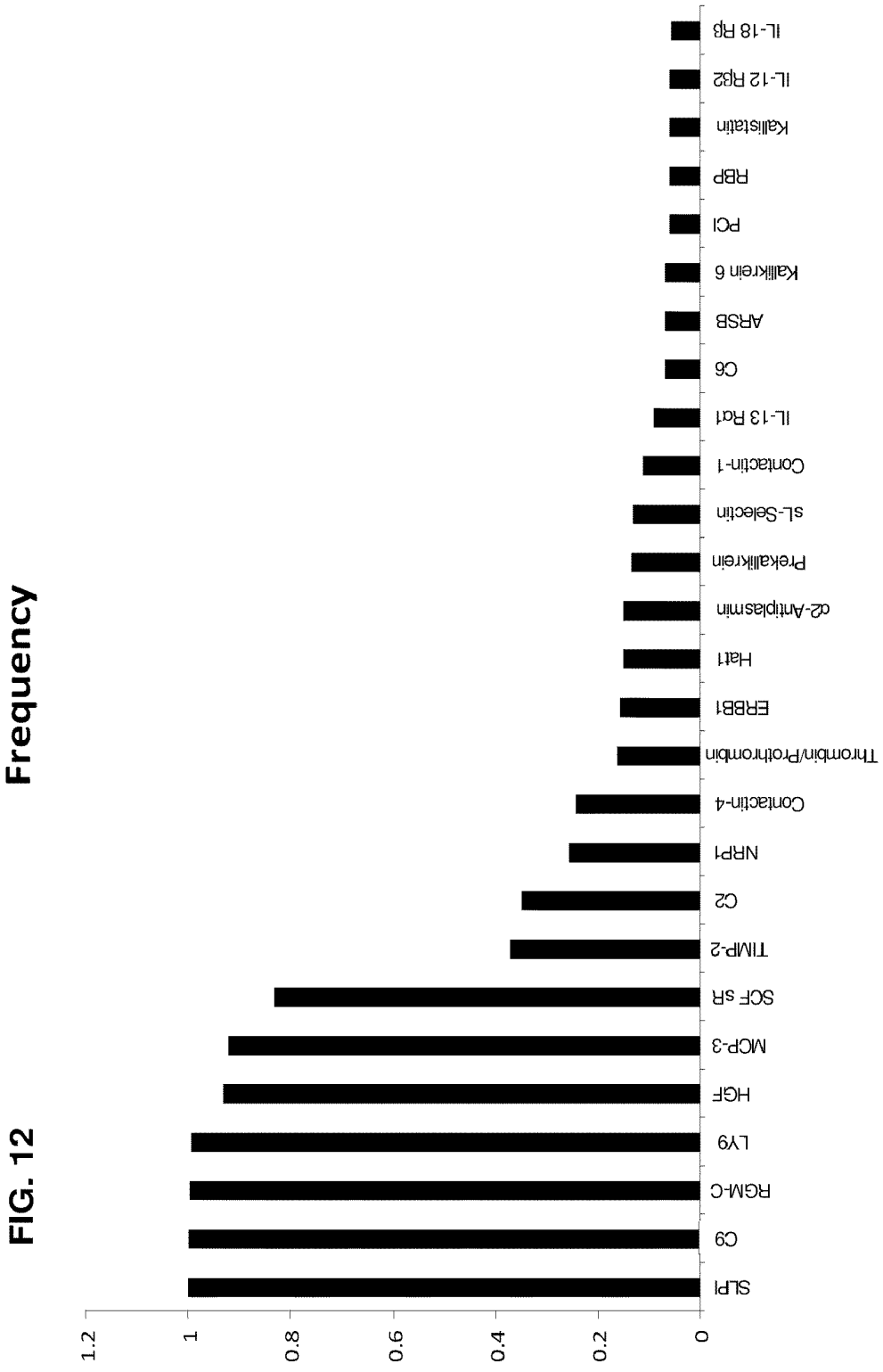

Frequency
1.2
1
0.8
0.6
0.4
0.2
0
SLPI
C9
RGM-C
LY9
HGF
MCP-3
SCF sR
TIMP-2
C2
NRP1
Contactin-4
Thrombin/Prothrombin
ERBB1
Hatt
α2-Antiplasmin
Prekallikrein
sL-Selectin
Contactin-1
IL-13 Rα1
C6
ARSB
Kallikrein 6
PCI
RBP
Kallistatin
IL-12 Rβ2
IL-18 Rβ

FIG. 13

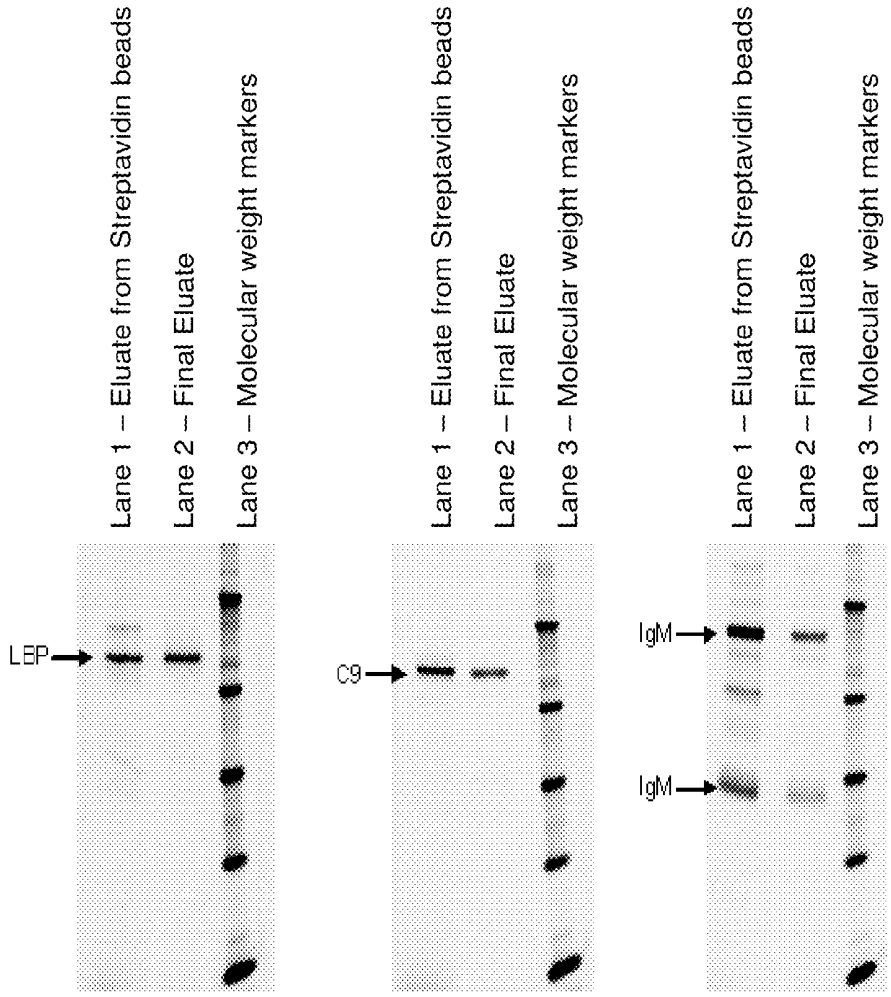

Lane 1 – Eluate from Streptavidin beads
Lane 2 – Final Eluate
Lane 3 – Molecular weight markers
LBP
Lane 1 – Eluate from Streptavidin beads
Lane 2 – Final Eluate
Lane 3 – Molecular weight markers
C9
Lane 1 – Eluate from Streptavidin beads
Lane 2 – Final Eluate
Lane 3 – Molecular weight markers
IgM
IgM

FIG. 14A

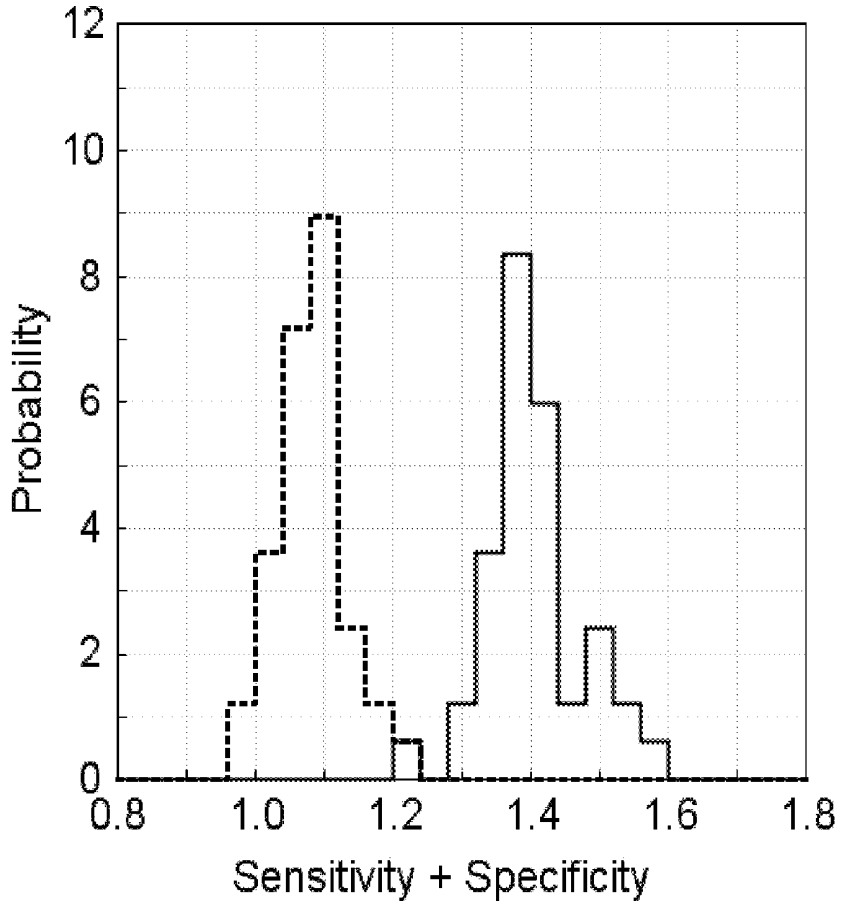

12
10
8
6
4
2
0
Probability
0.8
1.0
1.2
1.4
1.6
1.8
Sensitivity + Specificity

FIG. 14B

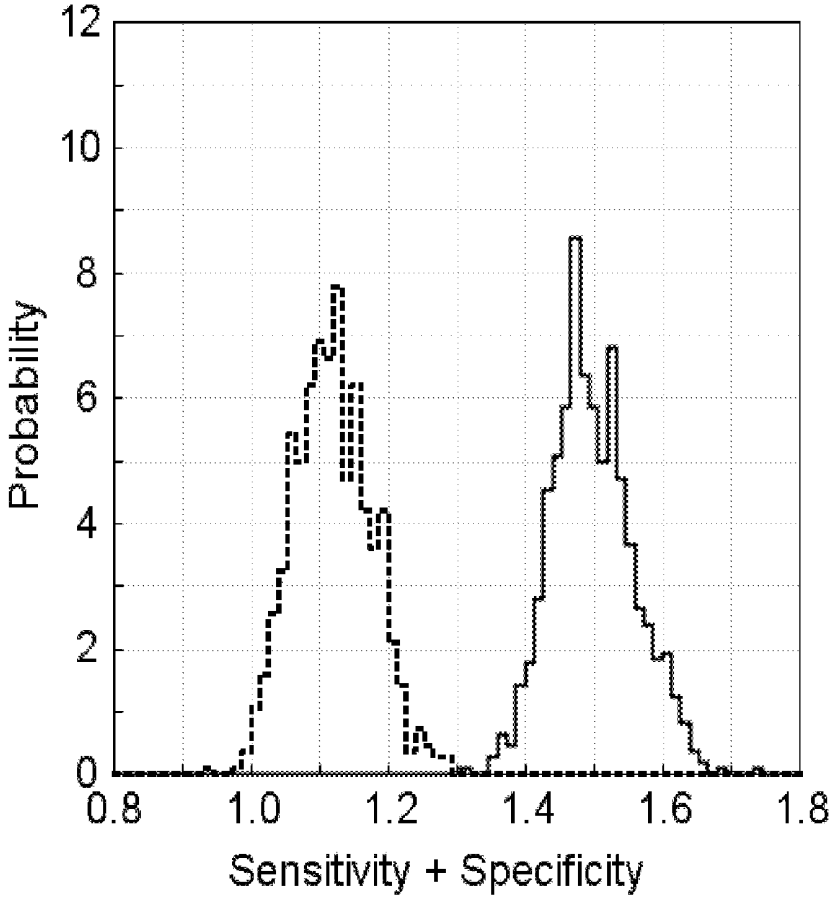

12
10
8
6
4
2
0
Probability
0.8
1.0
1.2
1.4
1.6
1.8
Sensitivity + Specificity

FIG. 14C

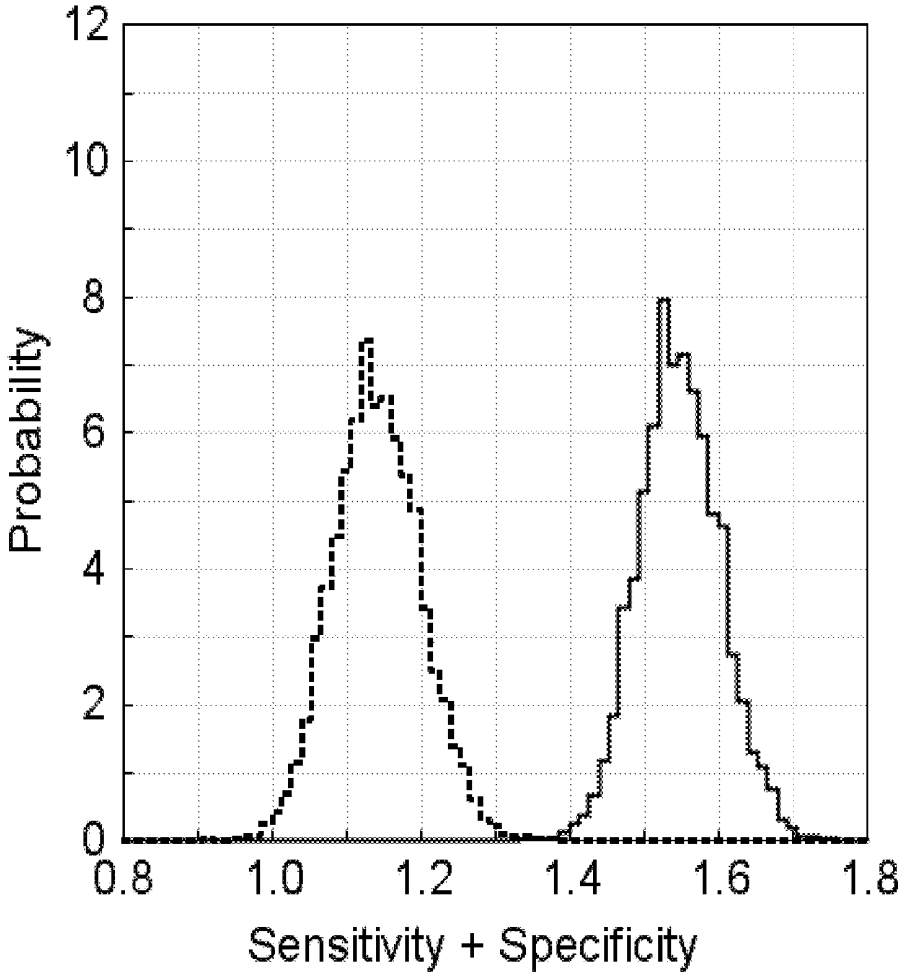

12
10
8
6
4
2
0
Probability
0.8
1.0
1.2
1.4
1.6
1.8
Sensitivity + Specificity

FIG. 15

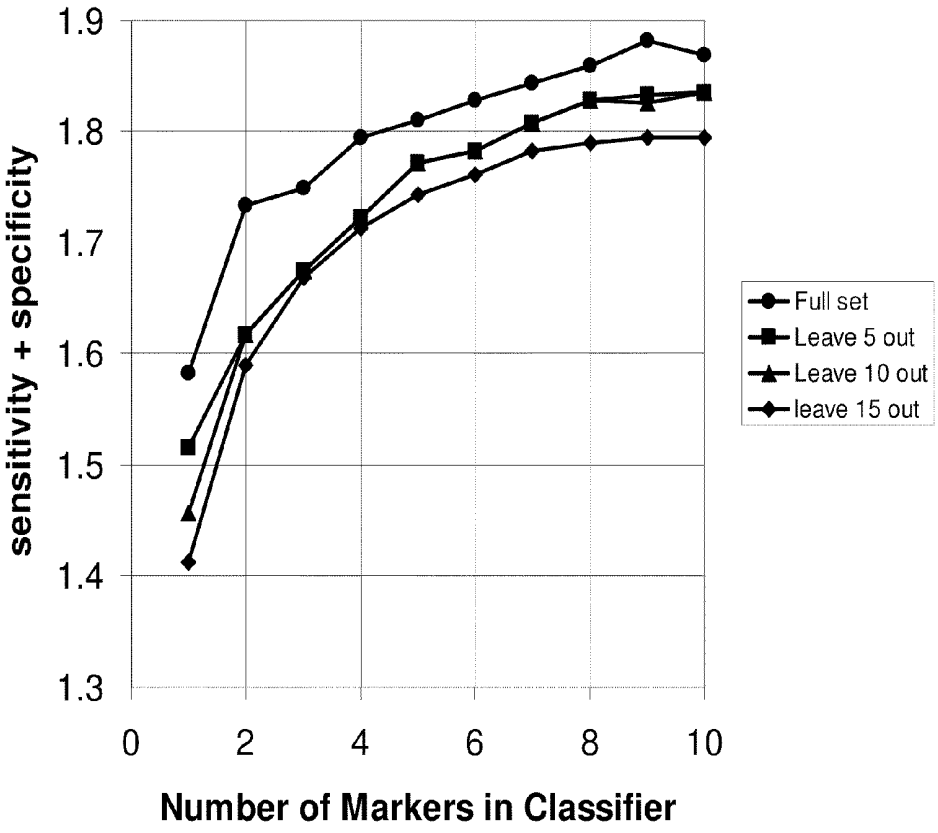

1.9
1.8
1.7
1.6
1.5
1.4
1.3
sensitivity + specificity
0
2
4
6
8
10
Number of Markers in Classifier
Full set
Leave 5 out
Leave 10 out
leave 15 out

FIG. 16A

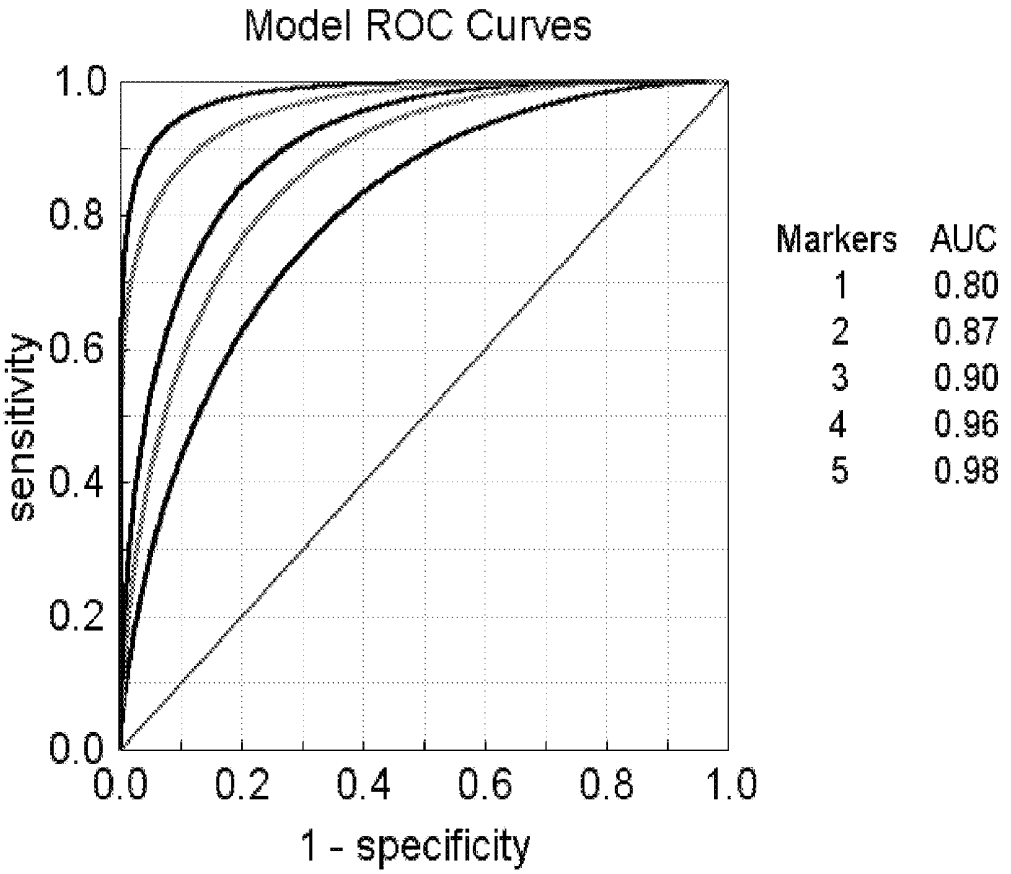
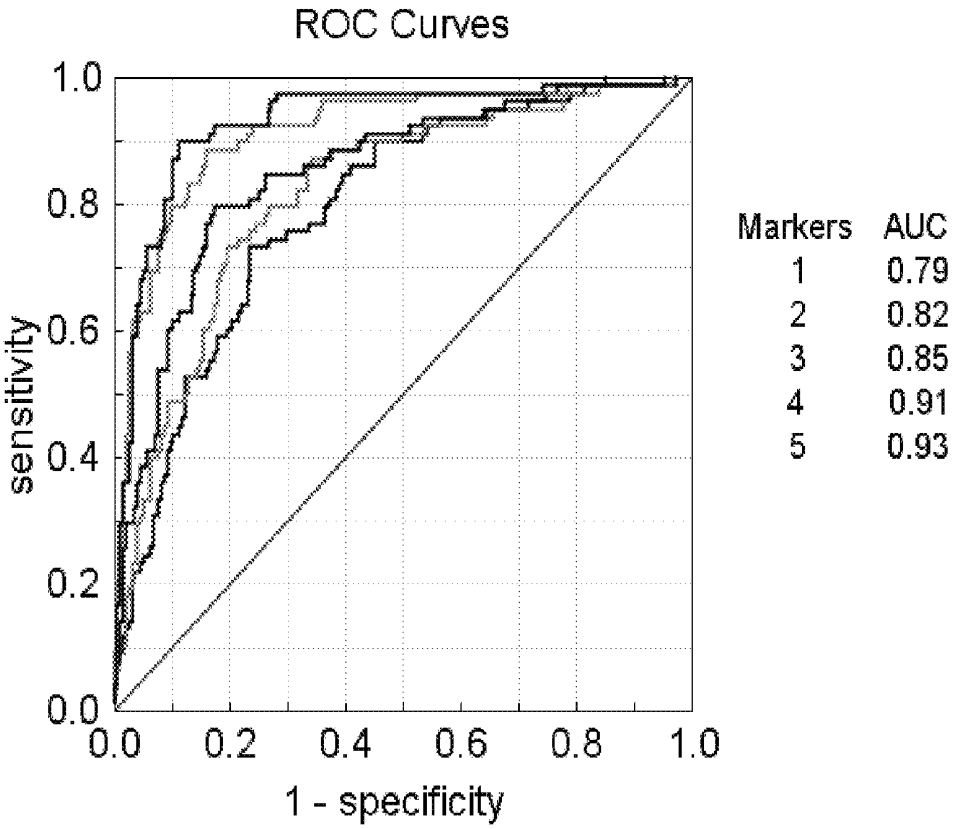

Model ROC Curves
sensitivity
1 - specificity
0.0
0.2
0.4
0.6
0.8
1.0
Markers AUC
1 0.80
2 0.87
3 0.90
4 0.96
5 0.98

FIG. 16B


ROC Curves
sensitivity
1 - specificity
0.0
0.2
0.4
0.6
0.8
1.0
Markers AUC
1 0.79
2 0.82
3 0.85
4 0.91
5 0.93

OVARIAN CANCER BIOMARKERS AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 61/103,149, filed Oct. 6, 2008, entitled "Multiplexed analyses of cancer samples", which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present application relates generally to the detection of biomarkers and the diagnosis of cancer in an individual and, more specifically, to one or more biomarkers, methods, devices, reagents, systems, and kits for diagnosing cancer, more particularly ovarian cancer, in an individual.

BACKGROUND

[0003] The following description provides a summary of information relevant to the present application and is not an admission that any of the information provided or publications referenced herein is prior art to the present application.

[0004] Ovarian cancer is the eighth most common cancer in women and the fifth leading cause of cancer-related deaths in women in the United States. Of all females born in the United States, one of every 70 will develop ovarian cancer and one of every 100 will die from this disease. The American Cancer Society estimates that approximately 21,550 women will be diagnosed with ovarian cancer in 2009 (American Cancer Society. Cancer Facts & Figures 2009. Atlanta: American Cancer Society; 2009). It is estimated that 14,600 women will die from this disease in 2009.

[0005] The survival rate and quality of patient life are improved the earlier ovarian cancer is detected. There is currently no sufficiently accurate screening test proven to be effective in the early detection of ovarian cancer. Thus, a pressing need exists for sensitive and specific methods for detecting ovarian cancer, particularly early-stage ovarian cancer.

[0006] Approximately 7% of the female population is at increased risk for ovarian cancer, based on genetic or family history. The risk for ovarian cancer increases with age. Women who have had breast cancer or who have a family history of breast or ovarian cancer are at increased risk. Inherited mutations in BRCA1 or BRCA2 genes increase risk. Ovarian cancer incidence rates are highest in Western industrialized countries.

[0007] Between 75% and 85% of ovarian cancers are diagnosed at an advanced stage. There is no consistent, reliable, non-invasive test to signal the presence of ovarian cancer. Pelvic examination only occasionally detects ovarian cancer, generally when the disease is advanced. Symptoms are often vague or nonexistent until late stages of the disease. Symptomatic women report frequent (>12 times/month) abdominal pain, bloating, increased girth, difficulty eating or feeling full quickly (Goff et al. Cancer 2007; 109:221). Trans-vaginal ultrasound and serum CA 125 levels have been tested as a screen for ovarian cancer and have not been found satisfactory. A laparotomy is required when ovarian cancer is suspected. The outcome of ovarian cancer patients operated on by a gynecology oncology surgical specialist is improved compared to a general gynecological surgeon, demonstrating that need for differential diagnosis of ovarian cancer from a suspicious pelvic mass prior to surgery. Goff reported on over 10,000 women in nine states undergoing surgery for a suspicious pelvic mass. Among the most important factors for receiving appropriate surgical management were surgeon specialty of gynecologic oncologist and the volume of cases performed by the surgeon annually. There are only 1000 board certified gynecologic oncologists in the United States, mostly in the larger medical centers across the country. Appropriately directing the women who are most likely to benefit from the care of such specialists can be critical for achieving good patient outcomes.

[0008] Currently, cancer antigen 125 (CA-125) is the most widely used serum biomarker for ovarian cancer. Serum concentrations of CA-125 are elevated (>35 U/ml) in 75-80% of patients with advanced-stage disease and this marker is routinely used to follow response to treatment and disease progression in patients from whom CA-125-secreting tumors have been resected. However, because the levels of CA-125 are correlated with tumor volume, only 50% of patients with early-stage disease have elevated levels, indicating that the sensitivity of CA-125 as a screening tool for early stage disease is limited. The utility of CA-125 screening is further limited by the high frequency of false-positive results associated with a variety of benign conditions, including endometriosis, pregnancy, menstruation, pelvic inflammatory disease, peritonitis, pancreatitis, and liver disease.

[0009] Classification of cancers determines appropriate treatment and helps determine the prognosis of the patient. Ovarian cancers are classified according to histology (i.e., "grading") and extent of the disease (i.e., "staging") using recognized grade and stage systems. In grade I, the tumor tissue is well differentiated. In grade II, tumor tissue is moderately well differentiated. In grade III, the tumor tissue is poorly differentiated. Grade III correlates with a less favorable prognosis than either grade I or II. Stage I is generally confined within the capsule surrounding one (stage IA) or both (stage IB) ovaries, although in some stage I (i.e. stage IC) cancers, malignant cells may be detected in ascites, in peritoneal rinse fluid, or on the surface of the ovaries. Stage II involves extension or metastasis of the tumor from one or both ovaries to other pelvic structures. In stage IIA, the tumor extends or has metastasized to the uterus, the fallopian tubes, or both. Stage IIB involves metastasis of the tumor to the pelvis. Stage IIC is stage IIA or IIB with the added requirement that malignant cells may be detected in ascites, in peritoneal rinse fluid, or on the surface of the ovaries. In stage III, the tumor comprises at least one malignant extension to the small bowel or the omentum, has formed extra-pelvic peritoneal implants of microscopic (stage IIIA) or macroscopic (<2 centimeter diameter, stage IIIB; >2 centimeter diameter, stage IIIC) size, or has metastasized to a retroperitoneal or inguinal lymph node (an alternate indicator of stage IIIC). In stage IV, distant (i.e. non-peritoneal) metastases of the tumor can be detected.

[0010] Treatment options include surgery, chemotherapy, and occasionally radiation therapy. Surgery usually involves removal of one or both ovaries, fallopian tubes (salpingoophorectomy), and the uterus (hysterectomy). In younger women with very early stage tumors who wish to have children, only the involved ovary and fallopian tube may be removed. In more advanced disease, surgically removing all abdominal metastases enhances the effect of chemotherapy and helps improve survival. For women with stage III ovarian cancer that has been optimally debulked (removal of as much

of the cancerous tissue as possible), studies have shown that chemotherapy administered both intravenously and directly into the peritoneal cavity improves survival. Studies have found that women who are treated by a gynecologic oncologist have more successful outcomes.

[0011] Relative survival varies by age; women younger than 65 are about twice as likely to survive 5 years (57%) following diagnosis as women 65 and older (29%). Overall, the 1- and 5-year relative survival of ovarian cancer patients is 75% and 46%, respectively. If diagnosed at the localized stage, the 5-year survival rate is 93%; however, only 19% of all cases are detected at this stage, usually fortuitously during another medical procedure. The majority of cases (67%) are diagnosed at distant stage. For women with regional and distant disease, 5-year survival rates are 71% and 31%, respectively; the chance of recurrence in these women is 20-85%. The 10-year relative survival rate for all stages combined is 39%. Therefore, ovarian cancer tends to be diagnosed too late to save women's lives. Detecting recurrence and predicting and monitoring response to therapy is important for making informed decisions on appropriate treatment throughout the care of these patients.

[0012] A blood screening test that can reliably detect early stage ovarian cancer will save thousands of lives each year. Where methods of early diagnosis in cancer exist, the benefits are generally accepted by the medical community. Cancers for which widely utilized screening protocols exist have the highest 5-year survival rates, such as breast cancer (88%) and colon cancer (65%) versus 46% for ovarian cancer. However, fortuitous detection of early stage ovarian cancer is associated with a substantial increase in 5-year survival (>95%). Therefore, early detection could significantly impact long-term survival. This demonstrates the clear need for diagnostic methods that can reliably detect early-stage ovarian cancer.

[0013] Biomarker selection for a specific disease state involves first the identification of markers that have a measurable and statistically significant difference in a disease population compared to a control population for a specific medical application. Biomarkers can include secreted or shed molecules that parallel disease development or progression and readily diffuse into the blood stream from ovarian tissue or from surrounding tissues and circulating cells in response to a tumor. The biomarker or set of biomarkers identified are generally clinically validated or shown to be a reliable indicator for the original intended use for which it was selected. Biomarkers can include small molecules, peptides, proteins, and nucleic acids. Some of the key issues that affect the identification of biomarkers include over-fitting of the available data and bias in the data.

[0014] A variety of methods have been utilized in an attempt to identify biomarkers and diagnose disease. For protein-based markers, these include two-dimensional electrophoresis, mass spectrometry, and immunoassay methods. For nucleic acid markers, these include mRNA expression profiles, microRNA profiles, FISH, serial analysis of gene expression (SAGE), methylation profiles, and large scale gene expression arrays.

[0015] The utility of two-dimensional electrophoresis is limited by low detection sensitivity; issues with protein solubility, charge, and hydrophobicity; gel reproducibility; and the possibility of a single spot representing multiple proteins. For mass spectrometry, depending on the format used, limitations revolve around the sample processing and separation, sensitivity to low abundance proteins, signal to noise considerations, and inability to immediately identify the detected protein. Limitations in immunoassay approaches to biomarker discovery are centered on the inability of antibody-based multiplex assays to measure a large number of analytes. One might simply print an array of high-quality antibodies and, without sandwiches, measure the analytes bound to those antibodies. (This would be the formal equivalent of using a whole genome of nucleic acid sequences to measure by hybridization all DNA or RNA sequences in an organism or a cell. The hybridization experiment works because hybridization can be a stringent test for identity. Even very good antibodies are not stringent enough in selecting their binding partners to work in the context of blood or even cell extracts because the protein ensemble in those matrices have extremely different abundances.) Thus, one must use a different approach with immunoassay-based approaches to biomarker discovery—one would need to use multiplexed ELISA assays (that is, sandwiches) to get sufficient stringency to measure many analytes simultaneously to decide which analytes are indeed biomarkers. Sandwich immunoassays do not scale to high content, and thus biomarker discovery using stringent sandwich immunoassays is not possible using standard array formats. Lastly, antibody reagents are subject to substantial lot variability and reagent instability. The instant platform for protein biomarker discovery overcomes this problem.

[0016] Many of these methods rely on or require some type of sample fractionation prior to the analysis. Thus the sample preparation required to run a sufficiently powered study designed to identify and discover statistically relevant biomarkers in a series of well-defined sample populations is extremely difficult, costly, and time consuming. During fractionation, a wide range of variability can be introduced into the various samples. For example, a potential marker could be unstable to the process, the concentration of the marker could be changed, inappropriate aggregation or disaggregation could occur, and inadvertent sample contamination could occur and thus obscure the subtle changes anticipated in early disease.

[0017] It is widely accepted that biomarker discovery and detection methods using these technologies have serious limitations for the identification of diagnostic biomarkers. These limitations include an inability to detect low-abundance biomarkers, an inability to consistently cover the entire dynamic range of the proteome, irreproducibility in sample processing and fractionation, and overall irreproducibility and lack of robustness of the method. Further, these studies have introduced biases into the data and not adequately addressed the complexity of the sample populations, including appropriate controls, in terms of the distribution and randomization required to identify and validate biomarkers within a target disease population.

[0018] Although efforts aimed at the discovery of new and effective biomarkers have gone on for several decades, the efforts have been largely unsuccessful. Biomarkers for various diseases typically have been identified in academic laboratories, usually through an accidental discovery while doing basic research on some disease process. Based on the discovery and with small amounts of clinical data, papers were published that suggested the identification of a new biomarker. Most of these proposed biomarkers, however, have not been confirmed as real or useful biomarkers; primarily because the small number of clinical samples tested provide only weak statistical proof that an effective biomarker has in

fact been found. That is, the initial identification was not rigorous with respect to the basic elements of statistics. In each of the years 1994 through 2003, a search of the scientific literature shows that thousands of references directed to biomarkers were published. During that same time frame, however, the FDA approved for diagnostic use, at most, three new protein biomarkers a year, and in several years no new protein biomarkers were approved.

[0019] Based on the history of failed biomarker discovery efforts, mathematical theories have been proposed that further promote the general understanding that biomarkers for disease are rare and difficult to find. Biomarker research based on 2D gels or mass spectrometry supports these notions. Very few useful biomarkers have been identified through these approaches. However, it is usually overlooked that 2D gel and mass spectrometry measure proteins that are present in blood at approximately 1 nM concentrations and higher, and that this ensemble of proteins may well be the least likely to change with disease. Other than the instant biomarker discovery platform, proteomic biomarker discovery platforms that are able to accurately measure protein expression levels at much lower concentrations do not exist.

[0020] Much is known about biochemical pathways for complex human biology. Many biochemical pathways culminate in or are started by secreted proteins that work locally within the pathology, for example growth factors are secreted to stimulate the replication of other cells in the pathology, and other factors are secreted to ward off the immune system, and so on. While many of these secreted proteins work in a paracrine fashion, some operate distally in the body. One skilled in the art with a basic understanding of biochemical pathways would understand that many pathology-specific proteins ought to exist in blood at concentrations below (even far below) the detection limits of 2D gels and mass spectrometry. What must precede the identification of this relatively abundant number of disease biomarkers is a proteomic platform that can analyze proteins at concentrations below those detectable by 2D gels or mass spectrometry.

[0021] Accordingly, a need exists for biomarkers, methods, devices, reagents, systems, and kits that enable (a) the differentiation of benign pelvic masses from ovarian cancer; (b) referral to a gynecologic oncology surgeon rather than a general gynecologic surgeon to surgically treat cases of ovarian cancer; (c) the detection of ovarian cancer biomarkers; and (d) the diagnosis of ovarian cancer.

SUMMARY

[0022] The present application includes biomarkers, methods, reagents, devices, systems, and kits for the detection and diagnosis of cancer and more particularly, ovarian cancer. The biomarkers of the present application were identified using a multiplex aptamer-based assay, which is described in detail in Example 1. By using the aptamer-based biomarker identification method described herein, this application describes a surprisingly large number of ovarian cancer biomarkers that are useful for the detection and diagnosis of ovarian cancer. In identifying these biomarkers, over 800 proteins from hundreds of individual samples were measured, some of which were at concentrations in the low femtomolar range. This is about four orders of magnitude lower than biomarker discovery experiments done with 2D gels or mass spectrometry.

[0023] While certain of the described ovarian cancer biomarkers are useful alone for detecting and diagnosing ovarian cancer, methods are described herein for the grouping of multiple subsets of the ovarian cancer biomarkers that are useful as a panel of biomarkers. Once an individual biomarker or subset of biomarkers has been identified, the detection or diagnosis of ovarian cancer in an individual can be accomplished using any assay platform or format that is capable of measuring differences in the levels of the selected biomarker or biomarkers in a biological sample.

[0024] However, it was only by using the aptamer-based biomarker identification method described herein, wherein over 800 separate potential biomarker values were individually screened from a large number of individuals who were postoperatively diagnosed as either having or not having ovarian cancer that it was possible to identify the ovarian cancer biomarkers disclosed herein. This discovery approach is in stark contrast to biomarker discovery using conditioned media or lysed cells as it queries a more patient-relevant system that requires no translation to human pathology.

[0025] Thus, in one aspect of the instant application, one or more biomarkers are provided for use either alone or in various combinations to diagnose ovarian cancer or permit the differential diagnosis of pelvic masses as benign or malignant. Exemplary embodiments include the biomarkers provided in Table 1, which as noted above, were identified using a multiplex aptamer-based assay, as described in Examples 1 and 2. The markers provided in Table 1 are useful in distinguishing benign pelvic masses from ovarian cancer.

[0026] While certain of the described ovarian cancer biomarkers are useful alone for detecting and diagnosing ovarian cancer, methods are also described herein for the grouping of multiple subsets of the ovarian cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least two biomarkers. In other embodiments, N is selected to be any number from 2-42 biomarkers.

[0027] In yet other embodiments, N is selected to be any number from 2-7, 2-10, 2-15, 2-20, 2-25, 2-30, 2-35, 2-40, or 2-42. In other embodiments, N is selected to be any number from 3-7, 3-10, 3-15, 3-20, 3-25, 3-30, 3-35, 3-40, or 3-42. In other embodiments, N is selected to be any number from 4-7, 4-10, 4-15, 4-20, 4-25, 4-30, 4-35, 4-40, or 4-42. In other embodiments, N is selected to be any number from 5-7, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, or 5-42. In other embodiments, N is selected to be any number from 6-10, 6-15, 6-20, 6-25, 6-30, 6-35, 6-40, or 6-42. In other embodiments, N is selected to be any number from 7-10, 7-15, 7-20, 7-25, 7-30, 7-35, 7-40, or 7-42. In other embodiments, N is selected to be any number from 8-10, 8-15, 8-20, 8-25, 8-30, 8-35, 8-40, or 8-42. In other embodiments, N is selected to be any number from 9-15, 9-20, 9-25, 9-30, 9-35, 9-40, or 9-42. In other embodiments, N is selected to be any number from 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, or 10-42. It will be appreciated that N can be selected to encompass similar, but higher order, ranges.

[0028] In another aspect, a method is provided for diagnosing ovarian cancer in an individual, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers provided in Table 1, wherein the individual is classified as having ovarian cancer based on the at least one biomarker value.

[0029] In another aspect, a method is provided for diagnosing ovarian cancer in an individual, the method including

detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the likelihood of the individual having ovarian cancer is determined based on the biomarker values.

[0030] In another aspect, a method is provided for diagnosing ovarian cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer based on the biomarker values, and wherein N=2-10.

[0031] In another aspect, a method is provided for diagnosing ovarian cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the likelihood of the individual having ovarian cancer is determined based on the biomarker values, and wherein N=2-10.

[0032] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on the at least one biomarker value.

[0033] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on the biomarker values, wherein N=2-10.

[0034] In another aspect, a method is provided for diagnosing that an individual does not have ovarian cancer, the method including detecting, in a biological sample from an individual, at least one biomarker value corresponding to at least one biomarker selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as not having ovarian cancer based on the at least one biomarker value.

[0035] In another aspect, a method is provided for diagnosing that an individual does not have ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each corresponding to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as not having ovarian cancer based on the biomarker values, and wherein N=2-10.

[0036] In another aspect, a method is provided for diagnosing ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein a classification of the biomarker values indicates that the individual has ovarian cancer, and wherein N=3-10.

[0037] In another aspect, a method is provided for diagnosing ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein a classification of the biomarker values indicates that the individual has ovarian cancer, and wherein N=3-15.

[0038] In another aspect, a method is provided for diagnosing ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels set forth in Tables 2-14, wherein a classification of the biomarker values indicates that the individual has ovarian cancer.

[0039] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on the biomarker values, and wherein N=3-10.

[0040] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on the biomarker values, and wherein N=3-15.

[0041] In another aspect, a method is provided for diagnosing an absence of ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein a classification of the biomarker values indicates an absence of ovarian cancer in the individual, and wherein N=3-10.

[0042] In another aspect, a method is provided for diagnosing an absence of ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein a classification of the biomarker values indicates an absence of ovarian cancer in the individual, and wherein N=3-15.

[0043] In another aspect, a method is provided for diagnosing an absence of ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of biomarkers selected from the group of panels provided in Tables 2-14, wherein a classification of the biomarker values indicates an absence of ovarian cancer in the individual.

[0044] In another aspect, a method is provided for diagnosing ovarian cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer

based on a classification score that deviates from a predetermined threshold, and wherein N=2-10.

[0045] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on a classification score that deviates from a predetermined threshold, and wherein N=3-10.

[0046] In another aspect, a method is provided for differentiating an individual having a benign pelvic mass from an individual having ovarian cancer, the method including detecting, in a biological sample from an individual, biomarker values that each correspond to a biomarker on a panel of N biomarkers, wherein the biomarkers are selected from the group of biomarkers set forth in Table 1, wherein the individual is classified as having ovarian cancer, or the likelihood of the individual having ovarian cancer is determined, based on a classification score that deviates from a predetermined threshold, wherein N=3-15.

[0047] In another aspect, a method is provided for diagnosing an absence of ovarian cancer in an individual, the method including detecting, in a biological sample from an individual, biomarker values that correspond to one of at least N biomarkers selected from the group of biomarkers set forth in Table 1, wherein said individual is classified as not having ovarian cancer based on a classification score that deviates from a predetermined threshold, and wherein N=2-10.

[0048] In another aspect, a computer-implemented method is provided for indicating a likelihood of ovarian cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers, wherein N is as defined above, selected from the group of biomarkers set forth in Table 1; performing with the computer a classification of each of the biomarker values; and indicating a likelihood that the individual has ovarian cancer based upon a plurality of classifications.

[0049] In another aspect, a computer-implemented method is provided for classifying an individual as either having or not having ovarian cancer. The method comprises: retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers selected from the group of biomarkers provided in Table 1; performing with the computer a classification of each of the biomarker values; and indicating whether the individual has ovarian cancer based upon a plurality of classifications.

[0050] In another aspect, a computer program product is provided for indicating a likelihood of ovarian cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers, wherein N is as defined above, in the biological sample selected from the group of biomarkers set forth in Table 1; and code that executes a classification method that indicates a likelihood that the individual has ovarian cancer as a function of the biomarker values.

[0051] In another aspect, a computer program product is provided for indicating an ovarian cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers in the biological sample selected from the group of biomarkers provided in Table 1; and code that executes a classification method that indicates an ovarian cancer status of the individual as a function of the biomarker values.

[0052] In another aspect, a computer-implemented method is provided for indicating a likelihood of ovarian cancer. The method comprises retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers set forth in Table 1; performing with the computer a classification of the biomarker value; and indicating a likelihood that the individual has ovarian cancer based upon the classification.

[0053] In another aspect, a computer-implemented method is provided for classifying an individual as either having or not having ovarian cancer. The method comprises retrieving, from a computer, biomarker information for an individual, wherein the biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers provided in Table 1; performing with the computer a classification of the biomarker value; and indicating whether the individual has ovarian cancer based upon the classification.

[0054] In still another aspect, a computer program product is provided for indicating a likelihood of ovarian cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers set forth in Table 1; and code that executes a classification method that indicates a likelihood that the individual has ovarian cancer as a function of the biomarker value.

[0055] In still another aspect, a computer program product is provided for indicating an ovarian cancer status of an individual. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers provided in Table 1; and code that executes a classification method that indicates an ovarian cancer status of the individual as a function of the biomarker value.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] FIG. **1**A is a flowchart for an exemplary method for detecting ovarian cancer in a biological sample.

[0057] FIG. **1**B is a flowchart for an exemplary method for detecting ovarian cancer in a biological sample using a naïve Bayes classification method.

[0058] FIG. **2** shows a ROC curve for a single biomarker, BAFF Receptor, using a naïve Bayes classifier for a test that detects ovarian cancer in women with pelvis masses.

[0059] FIG. **3** shows ROC curves for biomarker panels of from one to ten biomarkers using naïve Bayes classifiers for a test that detects ovarian cancer in women with pelvis masses.

[0060] FIG. **4** illustrates the increase in the classification score (specificity+sensitivity) as the number of biomarkers is increased from one to ten using naïve Bayes classification for an ovarian cancer panel.

[0061] FIG. **5** shows the measured biomarker distributions for BAFF Receptor as a cumulative distribution function (cdf) in RFU for the benign control group (solid line) and the ovarian cancer disease group (dotted line) along with their curve fits to a normal cdf (dashed lines) used to train the naïve Bayes classifiers.

[0062] FIG. **6** illustrates an exemplary computer system for use with various computer-implemented methods described herein.

[0063] FIG. **7** is a flowchart for a method of indicating the likelihood that an individual has ovarian cancer in accordance with one embodiment.

[0064] FIG. **8** is a flowchart for a method of indicating the likelihood that an individual has ovarian cancer in accordance with one embodiment.

[0065] FIG. **9** illustrates an exemplary aptamer assay that can be used to detect one or more ovarian cancer biomarkers in a biological sample.

[0066] FIG. **10** shows a histogram of frequencies for which biomarkers were used in building classifiers to distinguish between ovarian cancer and benign pelvic masses from an aggregated set of potential biomarkers.

[0067] FIG. **11** shows a histogram of frequencies for which biomarkers were used in building classifiers to distinguish between ovarian cancer and benign pelvic masses from a site-consistent set of potential biomarkers.

[0068] FIG. **12** shows a histogram of frequencies for which biomarkers were used in building classifiers to distinguish between ovarian cancer and benign pelvic masses from a set of potential biomarkers resulting from a combination of aggregated and site-consistent markers.

[0069] FIG. **13** shows gel images resulting from pull-down experiments that illustrate the specificity of aptamers as capture reagents for the proteins LBP, C9 and IgM. For each gel, lane 1 is the eluate from the Streptavidin-agarose beads, lane 2 is the final eluate, and lane is a MW marker lane (major bands are at 110, 50, 30, 15, and 3.5 kDa from top to bottom).

[0070] FIG. **14**A shows a pair of histograms summarizing all possible single protein naïve Bayes classifier scores (sensitivity+specificity) using the biomarkers set forth in Table 1 (solid) and a set of random non-markers (dotted).

[0071] FIG. **14**B shows a pair of histograms summarizing all possible two-protein protein naïve Bayes classifier scores (sensitivity+specificity) using the biomarkers set forth in Table 1 (solid) and a set of random non-markers (dotted).

[0072] FIG. **14**C shows a pair of histograms summarizing all possible three-protein naïve Bayes classifier scores (sensitivity+specificity) using the biomarkers set forth in Table 1 (solid) and a set of non-random markers (dotted).

[0073] FIG. **15** shows the sensitivity+specificity score for naïve Bayes classifiers using from 2-10 markers selected from the full panel (●) and the scores obtained by dropping the best 5 (■), 10 (▲) and 15 (♦) markers during classifier generation.

[0074] FIG. **16**A shows a set of ROC curves modeled from the data in Table 18 for panels of from one to five markers.

[0075] FIG. **16**B shows a set of ROC curves computed from the training data for panels of from one to five markers as in FIG. **16**A.

DETAILED DESCRIPTION

[0076] Reference will now be made in detail to representative embodiments of the invention. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that the invention is not intended to be limited to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents that may be included within the scope of the present invention as defined by the claims.

[0077] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in and are within the scope of the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[0078] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[0079] All publications, published patent documents, and patent applications cited in this application are indicative of the level of skill in the art(s) to which the application pertains. All publications, published patent documents, and patent applications cited herein are hereby incorporated by reference to the same extent as though each individual publication, published patent document, or patent application was specifically and individually indicated as being incorporated by reference.

[0080] As used in this application, including the appended claims, the singular forms "a," "an," and "the" include plural references, unless the content clearly dictates otherwise, and are used interchangeably with "at least one" and "one or more." Thus, reference to "an aptamer" includes mixtures of aptamers, reference to "a probe" includes mixtures of probes, and the like.

[0081] As used herein, the term "about" represents an insignificant modification or variation of the numerical value such that the basic function of the item to which the numerical value relates is unchanged.

[0082] As used herein, the terms "comprises," "comprising," "includes," "including," "contains," "containing," and any variations thereof, are intended to cover a non-exclusive inclusion, such that a process, method, product-by-process, or composition of matter that comprises, includes, or contains an element or list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, product-by-process, or composition of matter.

[0083] The present application includes biomarkers, methods, devices, reagents, systems, and kits for the detection and diagnosis of ovarian cancer.

[0084] In one aspect, one or more biomarkers are provided for use either alone or in various combinations to diagnose ovarian cancer, permit the differential diagnosis of pelvic masses as benign or malignant, monitor ovarian cancer recurrence, or address other clinical indications. As described in

detail below, exemplary embodiments include the biomarkers provided in Table 1, which were identified using a multiplex aptamer-based assay, as described generally in Example 1 and more specifically in Example 2.

[0085] Table 1 sets forth the findings obtained from analyzing blood samples from 142 individuals diagnosed with ovarian cancer and blood samples from 195 individuals diagnosed with a benign pelvic mass. The benign pelvic mass group was designed to match the population with which an ovarian cancer diagnostic test can have significant benefit. (These cases and controls were obtained from two clinical sites). The potential biomarkers were measured in individual samples rather than pooling the disease and control blood; this allowed a better understanding of the individual and group variations in the phenotypes associated with the presence and absence of disease (in this case ovarian cancer). Since over 800 protein measurements were made on each sample, and 337 samples from both the disease and the control populations were individually measured, Table 1 resulted from an analysis of an uncommonly large set of data. The measurements were analyzed using the methods described in the section, "Classification of Biomarkers and Calculation of Disease Scores" herein.

[0086] Table 1 lists the biomarkers found to be useful in distinguishing samples obtained from individuals with ovarian cancer from "control" samples obtained from individuals with benign pelvic masses. Using a multiplex aptamer assay, forty-two biomarkers were discovered that distinguished samples obtained from individuals with ovarian cancer from samples obtained from people who had benign pelvic masses (see Table 1).

[0087] While certain of the described ovarian cancer biomarkers are useful alone for detecting and diagnosing ovarian cancer, methods are also described herein for the grouping of multiple subsets of the ovarian cancer biomarkers, where each grouping or subset selection is useful as a panel of three or more biomarkers, interchangeably referred to herein as a "biomarker panel" and a panel. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least two biomarkers. In other embodiments, N is selected from 2-42 biomarkers.

[0088] In yet other embodiments, N is selected to be any number from 2-7, 2-10, 2-15, 2-20, 2-25, 2-30, 2-35, 2-40, or 2-42. In other embodiments, N is selected to be any number from 3-7, 3-10, 3-15, 3-20, 3-25, 3-30, 3-35, 3-40, or 3-42. In other embodiments, N is selected to be any number from 4-7, 4-10, 4-15, 4-20, 4-25, 4-30, 4-35, 4-40, or 4-42. In other embodiments, N is selected to be any number from 5-7, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, or 5-42. In other embodiments, N is selected to be any number from 6-10, 6-15, 6-20, 6-25, 6-30, 6-35, 6-40, or 6-42. In other embodiments, N is selected to be any number from 7-10, 7-15, 7-20, 7-25, 7-30, 7-35, 7-40, or 7-42. In other embodiments, N is selected to be any number from 8-10, 8-15, 8-20, 8-25, 8-30, 8-35, 8-40, or 8-42. In other embodiments, N is selected to be any number from 9-15, 9-20, 9-25, 9-30, 9-35, 9-40, or 9-42. In other embodiments, N is selected to be any number from 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, or 10-42. It will be appreciated that N can be selected to encompass similar, but higher order, ranges.

[0089] In one embodiment, the number of biomarkers useful for a biomarker subset or panel is based on the sensitivity and specificity value for the particular combination of biomarker values. The terms "sensitivity" and "specificity" are used herein with respect to the ability to correctly classify an individual, based on one or more biomarker values detected in their biological sample, as having ovarian cancer or not having ovarian cancer. "Sensitivity" indicates the performance of the biomarker(s) with respect to correctly classifying individuals that have ovarian cancer. "Specificity" indicates the performance of the biomarker(s) with respect to correctly classifying individuals who do not have ovarian cancer. For example, 85% specificity and 90% sensitivity for a panel of markers used to test a set of control samples and ovarian cancer samples indicates that 85% of the control samples were correctly classified as control samples by the panel, and 90% of the ovarian cancer samples were correctly classified as ovarian cancer samples by the panel. The desired or preferred minimum value can be determined as described in Example 3. Representative panels are set forth in Tables 2-14, which set forth a series of 100 different panels of 3-15 biomarkers, which have the indicated levels of specificity and sensitivity for each panel. The total number of occurrences of each marker in each of these panels is indicated at the bottom of each Table.

[0090] In one aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to at least one of the biomarkers SLPI, C9, HGF and RGM-C and at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In a further aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarkers SLPI, C9, HGF and RGM-C and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13. In a further aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker SLPI and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In a further aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker C9 and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In a further aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker HGF and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In a further aspect, ovarian cancer is detected or diagnosed in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker RGM-C and one of at least N additional biomarkers selected from the list of biomarkers in Table 1, wherein N equals 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

[0091] The ovarian cancer biomarkers identified herein represent a relatively large number of choices for subsets or panels of biomarkers that can be used to effectively detect or diagnose ovarian cancer. Selection of the desired number of

such biomarkers depends on the specific combination of biomarkers chosen. It is important to remember that panels of biomarkers for detecting or diagnosing ovarian cancer may also include biomarkers not found in Table 1, and that the inclusion of additional biomarkers not found in Table 1 may reduce the number of biomarkers in the particular subset or panel that is selected from Table 1. The number of biomarkers from Table 1 used in a subset or panel may also be reduced if additional biomedical information is used in conjunction with the biomarker values to establish acceptable sensitivity and specificity values for a given assay.

[0092] Another factor that can affect the number of biomarkers to be used in a subset or panel of biomarkers is the procedures used to obtain biological samples from individuals who are being evaluated for ovarian cancer. In a carefully controlled sample procurement environment, the number of biomarkers necessary to meet desired sensitivity and specificity values will be lower than in a situation where there can be more variation in sample collection, handling and storage. In developing the list of biomarkers set forth in Table 1, two sample collection sites were utilized to collect data for classifier training.

[0093] One aspect of the instant application can be described generally with reference to FIGS. **1**A and B. A biological sample is obtained from an individual or individuals of interest. The biological sample is then assayed to detect the presence of one or more (N) biomarkers of interest and to determine a biomarker value for each of said N biomarkers (referred to in FIG. **1**B as marker RFU (relative fluorescence units)). Once a biomarker has been detected and a biomarker value assigned each marker is scored or classified as described in detail herein. The marker scores are then combined to provide a total diagnostic score, which indicates the likelihood that the individual from whom the sample was obtained has ovarian cancer.

[0094] "Biological sample", "sample", and "test sample" are used interchangeably herein to refer to any material, biological fluid, tissue, or cell obtained or otherwise derived from an individual. This includes blood (including whole blood, leukocytes, peripheral blood mononuclear cells, buffy coat, plasma, and serum), sputum, tears, mucus, nasal washes, nasal aspirate, breath, urine, semen, saliva, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, synovial fluid, joint aspirate, ascites, cells, a cellular extract, and cerebrospinal fluid. This also includes experimentally separated fractions of all of the preceding. For example, a blood sample can be fractionated into serum or into fractions containing particular types of blood cells, such as red blood cells or white blood cells (leukocytes). If desired, a sample can be a combination of samples from an individual, such as a combination of a tissue and fluid sample. The term "biological sample" also includes materials containing homogenized solid material, such as from a stool sample, a tissue sample, or a tissue biopsy, for example. The term "biological sample" also includes materials derived from a tissue culture or a cell culture. Any suitable methods for obtaining a biological sample can be employed; exemplary methods include, e.g., phlebotomy, swab (e.g., buccal swab), and a fine needle aspirate biopsy procedure. Samples can also be collected, e.g., by micro dissection (e.g., laser capture micro dissection (LCM) or laser micro dissection (LMD)), bladder wash, smear (e.g., a PAP smear), or ductal lavage. A "biological sample" obtained or derived from an individual includes any such sample that has been processed in any suitable manner after being obtained from the individual.

[0095] Further, it should be realized that a biological sample can be derived by taking biological samples from a number of individuals and pooling them or pooling an aliquot of each individual's biological sample. The pooled sample can be treated as a sample from a single individual and if the presence of cancer is established in the pooled sample, then each individual biological sample can be re-tested to determine which individuals have ovarian cancer.

[0096] For purposes of this specification, the phrase "data attributed to a biological sample from an individual" is intended to mean that the data in some form derived from, or were generated using, the biological sample of the individual. The data may have been reformatted, revised, or mathematically altered to some degree after having been generated, such as by conversion from units in one measurement system to units in another measurement system; but, the data are understood to have been derived from, or were generated using, the biological sample.

[0097] "Target", "target molecule", and "analyte" are used interchangeably herein to refer to any molecule of interest that may be present in a biological sample.

[0098] A "molecule of interest" includes any minor variation of a particular molecule, such as, in the case of a protein, for example, minor variations in amino acid sequence, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component, which does not substantially alter the identity of the molecule. A "target molecule", "target", or "analyte" is a set of copies of one type or species of molecule or multi-molecular structure. "Target molecules", "targets", and "analytes" refer to more than one such set of molecules. Exemplary target molecules include proteins, polypeptides, nucleic acids, carbohydrates, lipids, polysaccharides, glycoproteins, hormones, receptors, antigens, antibodies, affybodies, antibody mimics, viruses, pathogens, toxic substances, substrates, metabolites, transition state analogs, cofactors, inhibitors, drugs, dyes, nutrients, growth factors, cells, tissues, and any fragment or portion of any of the foregoing.

[0099] As used herein, "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. Polypeptides can be single chains or associated chains. Also included within the definition are preproteins and intact mature proteins; peptides or polypeptides derived from a mature protein; fragments of a protein; splice variants; recombinant forms of a protein; protein variants with amino acid modifications, deletions, or substitutions; digests; and post-translational modifications, such as glycosylation, acetylation, phosphorylation, and the like.

[0100] As used herein, "thrombin" refers to thrombin, prothrombin, or both thrombin and prothrombin.

[0101] As used herein, "marker" and "biomarker" are used interchangeably to refer to a target molecule that indicates or is a sign of a normal or abnormal process in an individual or of a disease or other condition in an individual. More specifically, a "marker" or "biomarker" is an anatomic, physiologic, biochemical, or molecular parameter associated with the presence of a specific physiological state or process, whether normal or abnormal, and, if abnormal, whether chronic or acute. Biomarkers are detectable and measurable by a variety of methods including laboratory assays and medical imaging. When a biomarker is a protein, it is also possible to use the expression of the corresponding gene as a surrogate measure of the amount or presence or absence of the corresponding protein biomarker in a biological sample or methylation state of the gene encoding the biomarker or proteins that control expression of the biomarker.

[0102] As used herein, "biomarker value", "value", "biomarker level", and "level" are used interchangeably to refer to a measurement that is made using any analytical method for detecting the biomarker in a biological sample and that indicates the presence, absence, absolute amount or concentration, relative amount or concentration, titer, a level, an expression level, a ratio of measured levels, or the like, of, for, or corresponding to the biomarker in the biological sample. The exact nature of the "value" or "level" depends on the specific design and components of the particular analytical method employed to detect the biomarker.

[0103] When a biomarker indicates or is a sign of an abnormal process or a disease or other condition in an individual, that biomarker is generally described as being either over-expressed or under-expressed as compared to an expression level or value of the biomarker that indicates or is a sign of a normal process or an absence of a disease or other condition in an individual. "Up-regulation", "up-regulated", "over-expression", "over-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

[0104] "Down-regulation", "down-regulated", "under-expression", "under-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

[0105] Further, a biomarker that is either over-expressed or under-expressed can also be referred to as being "differentially expressed" or as having a "differential level" or "differential value" as compared to a "normal" expression level or value of the biomarker that indicates or is a sign of a normal process or an absence of a disease or other condition in an individual. Thus, "differential expression" of a biomarker can also be referred to as a variation from a "normal" expression level of the biomarker.

[0106] The term "differential gene expression" and "differential expression" are used interchangeably to refer to a gene (or its corresponding protein expression product) whose expression is activated to a higher or lower level in a subject suffering from a specific disease, relative to its expression in a normal or control subject. The terms also include genes (or the corresponding protein expression products) whose expression is activated to a higher or lower level at different stages of the same disease. It is also understood that a differentially expressed gene may be either activated or inhibited at the nucleic acid level or protein level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a variety of changes including mRNA levels, surface expression, secretion or other partitioning of a polypeptide. Differential gene expression may include a comparison of expression between two or more genes or their gene products; or a comparison of the ratios of the expression between two or more genes or their gene products; or even a comparison of two differently processed products of the same gene, which differ between normal subjects and subjects suffering from a disease; or between various stages of the same disease. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a gene or its expression products among, for example, normal and diseased cells, or among cells which have undergone different disease events or disease stages.

[0107] As used herein, "individual" refers to a test subject or patient. The individual can be a mammal or a non-mammal. In various embodiments, the individual is a mammal. A mammalian individual can be a human or non-human. In various embodiments, the individual is a human. A healthy or normal individual is an individual in which the disease or condition of interest (including, for example, ovarian diseases, ovarian-associated diseases, or other ovarian conditions) is not detectable by conventional diagnostic methods.

[0108] "Diagnose", "diagnosing", "diagnosis", and variations thereof refer to the detection, determination, or recognition of a health status or condition of an individual on the basis of one or more signs, symptoms, data, or other information pertaining to that individual. The health status of an individual can be diagnosed as healthy/normal (i.e., a diagnosis of the absence of a disease or condition) or diagnosed as ill/abnormal (i.e., a diagnosis of the presence, or an assessment of the characteristics, of a disease or condition). The terms "diagnose", "diagnosing", "diagnosis", etc., encompass, with respect to a particular disease or condition, the initial detection of the disease; the characterization or classification of the disease; the detection of the progression, remission, or recurrence of the disease; and the detection of disease response after the administration of a treatment or therapy to the individual. The diagnosis of ovarian cancer includes distinguishing individuals who have cancer from individuals who do not. It further includes distinguishing benign pelvic masses from ovarian cancer.

[0109] "Prognose", "prognosing", "prognosis", and variations thereof refer to the prediction of a future course of a disease or condition in an individual who has the disease or condition (e.g., predicting patient survival), and such terms encompass the evaluation of disease response after the administration of a treatment or therapy to the individual.

[0110] "Evaluate", "evaluating", "evaluation", and variations thereof encompass both "diagnose" and "prognose" and also encompass determinations or predictions about the

future course of a disease or condition in an individual who does not have the disease as well as determinations or predictions regarding the likelihood that a disease or condition will recur in an individual who apparently has been cured of the disease. The term "evaluate" also encompasses assessing an individual's response to a therapy, such as, for example, predicting whether an individual is likely to respond favorably to a therapeutic agent or is unlikely to respond to a therapeutic agent (or will experience toxic or other undesirable side effects, for example), selecting a therapeutic agent for administration to an individual, or monitoring or determining an individual's response to a therapy that has been administered to the individual. Thus, "evaluating" ovarian cancer can include, for example, any of the following: prognosing the future course of ovarian cancer in an individual; predicting the recurrence of ovarian cancer in an individual who apparently has been cured of ovarian cancer; or determining or predicting an individual's response to an ovarian cancer treatment or selecting an ovarian cancer treatment to administer to an individual based upon a determination of the biomarker values derived from the individual's biological sample.

[0111] Any of the following examples may be referred to as either "diagnosing" or "evaluating" ovarian cancer: initially detecting the presence or absence of ovarian cancer; determining a specific stage, type or sub-type, or other classification or characteristic of ovarian cancer; determining whether a pelvic mass is benign or malignant; or detecting or monitoring ovarian cancer progression (e.g., monitoring ovarian tumor growth or metastatic spread), remission, or recurrence.

[0112] As used herein, "additional biomedical information" refers to one or more evaluations of an individual, other than using any of the biomarkers described herein, that are associated with ovarian cancer risk. "Additional biomedical information" includes any of the following: physical descriptors of an individual; physical descriptors of a pelvic mass observed by MRI, abdominal ultrasound, or CT imaging; the height and/or weight of an individual; change in weight; the ethnicity of an individual; occupational history; family history of ovarian cancer (or other cancer); the presence of a genetic marker(s) correlating with a higher risk of ovarian cancer in the individual or a family member; the presence of a pelvic mass; size of mass; location of mass; morphology of mass and associated pelvic region (e.g., as observed through imaging); clinical symptoms such as bloating, abdominal pain, or sudden weight gain or loss; and the like. Additional biomedical information can be obtained from an individual using routine techniques known in the art, such as from the individual themselves by use of a routine patient questionnaire or health history questionnaire, etc., or from a medical practitioner, etc. Alternately, additional biomedical information can be obtained from routine imaging techniques, including abdominal or transvaginal ultrasound, MRI, CT imaging, and PET-CT. Testing of biomarker levels in combination with an evaluation of any additional biomedical information, including other laboratory tests (e.g., CA-125 testing), may, for example, improve sensitivity, specificity, and/or AUC for detecting ovarian cancer (or other ovarian cancer-related uses) as compared to biomarker testing alone or evaluating any particular item of additional biomedical information alone (e.g., ultrasound imaging alone).

[0113] The term "area under the curve" or "AUC" refers to the area under the curve of a receiver operating characteristic (ROC) curve, both of which are well known in the art. AUC measures are useful for comparing the accuracy of a classifier across the complete data range. Classifiers with a greater AUC have a greater capacity to classify unknowns correctly between two groups of interest (e.g., ovarian cancer samples and normal or control samples). ROC curves are useful for plotting the performance of a particular feature (e.g., any of the biomarkers described herein and/or any item of additional biomedical information) in distinguishing between two populations (e.g., cases having ovarian cancer and controls without ovarian cancer). Typically, the feature data across the entire population (e.g., the cases and controls) are sorted in ascending order based on the value of a single feature. Then, for each value for that feature, the true positive and false positive rates for the data are calculated. The true positive rate is determined by counting the number of cases above the value for that feature and then dividing by the total number of cases. The false positive rate is determined by counting the number of controls above the value for that feature and then dividing by the total number of controls. Although this definition refers to scenarios in which a feature is elevated in cases compared to controls, this definition also applies to scenarios in which a feature is lower in cases compared to the controls (in such a scenario, samples below the value for that feature would be counted). ROC curves can be generated for a single feature as well as for other single outputs, for example, a combination of two or more features can be mathematically combined (e.g., added, subtracted, multiplied, etc.) to provide a single sum value, and this single sum value can be plotted in a ROC curve. Additionally, any combination of multiple features, in which the combination derives a single output value, can be plotted in a ROC curve. These combinations of features may comprise a test. The ROC curve is the plot of the true positive rate (sensitivity) of a test against the false positive rate (1-specificity) of the test.

[0114] As used herein, "detecting" or "determining" with respect to a biomarker value includes the use of both the instrument required to observe and record a signal corresponding to a biomarker value and the material/s required to generate that signal. In various embodiments, the biomarker value is detected using any suitable method, including fluorescence, chemiluminescence, surface plasmon resonance, surface acoustic waves, mass spectrometry, infrared spectroscopy, Raman spectroscopy, atomic force microscopy, scanning tunneling microscopy, electrochemical detection methods, nuclear magnetic resonance, quantum dots, and the like.

[0115] "Solid support" refers herein to any substrate having a surface to which molecules may be attached, directly or indirectly, through either covalent or non-covalent bonds. A "solid support" can have a variety of physical formats, which can include, for example, a membrane; a chip (e.g., a protein chip); a slide (e.g., a glass slide or coverslip); a column; a hollow, solid, semi-solid, pore- or cavity-containing particle, such as, for example, a bead; a gel; a fiber, including a fiber optic material; a matrix; and a sample receptacle. Exemplary sample receptacles include sample wells, tubes, capillaries, vials, and any other vessel, groove or indentation capable of holding a sample. A sample receptacle can be contained on a multi-sample platform, such as a microtiter plate, slide, microfluidics device, and the like. A support can be composed of a natural or synthetic material, an organic or inorganic material. The composition of the solid support on which capture reagents are attached generally depends on the method of attachment (e.g., covalent attachment). Other exemplary receptacles include microdroplets and microfluidic controlled or bulk oil/aqueous emulsions within which

assays and related manipulations can occur. Suitable solid supports include, for example, plastics, resins, polysaccharides, silica or silica-based materials, functionalized glass, modified silicon, carbon, metals, inorganic glasses, membranes, nylon, natural fibers (such as, for example, silk, wool and cotton), polymers, and the like. The material composing the solid support can include reactive groups such as, for example, carboxy, amino, or hydroxyl groups, which are used for attachment of the capture reagents. Polymeric solid supports can include, e.g., polystyrene, polyethylene glycol tetraphthalate, polyvinyl acetate, polyvinyl chloride, polyvinyl pyrrolidone, polyacrylonitrile, polymethyl methacrylate, polytetrafluoroethylene, butyl rubber, styrenebutadiene rubber, natural rubber, polyethylene, polypropylene, (poly)tetrafluoroethylene, (poly)vinylidenefluoride, polycarbonate, and polymethylpentene. Suitable solid support particles that can be used include, e.g., encoded particles, such as Luminex®-type encoded particles, magnetic particles, and glass particles.

Exemplary Uses of Biomarkers

[0116] In various exemplary embodiments, methods are provided for diagnosing ovarian cancer in an individual by detecting one or more biomarker values corresponding to one or more biomarkers that are present in the circulation of an individual, such as in serum or plasma, by any number of analytical methods, including any of the analytical methods described herein. These biomarkers are, for example, differentially expressed in individuals with ovarian cancer as compared to individuals without ovarian cancer. Detection of the differential expression of a biomarker in an individual can be used, for example, to permit the early diagnosis of ovarian cancer, to distinguish between a benign pelvic mass and ovarian cancer (such as, for example, a mass observed on an abdominal ultrasound or computed tomography (CT) scan), to monitor ovarian cancer recurrence, or for other clinical indications.

[0117] Any of the biomarkers described herein may be used in a variety of clinical indications for ovarian cancer, including any of the following: detection of ovarian cancer (such as in a high-risk individual or population); characterizing ovarian cancer (e.g., determining ovarian cancer type, sub-type, or stage), such as by determining whether a pelvic mass is benign or malignant; determining ovarian cancer prognosis; monitoring ovarian cancer progression or remission; monitoring for ovarian cancer recurrence; monitoring metastasis; treatment selection (e.g., pre- or post-operative chemotherapy selection); monitoring response to a therapeutic agent or other treatment; combining biomarker testing with additional biomedical information, such as CA-125 level, the presence of a genetic marker(s) indicating a higher risk for ovarian cancer, etc., or with mass size, morphology, presence of ascites, etc. (such as to provide an assay with increased diagnostic performance compared to CA-125 testing or other biomarker testing alone); facilitating the diagnosis of a pelvic mass as malignant or benign; facilitating clinical decision making once a pelvic mass is observed through imaging; and facilitating decisions regarding clinical follow-up (e.g., whether to refer an individual to a surgical specialist, such as a gynecologic oncology surgeon). Biomarker testing may improve positive predictive value (PPV) over CA-125 testing and imaging alone. Furthermore, the described biomarkers may also be useful in permitting certain of these uses before indications of ovarian cancer are detected by imaging modalities or other clinical correlates, or before symptoms appear.

[0118] As an example of the manner in which any of the biomarkers described herein can be used to diagnose ovarian cancer, differential expression of one or more of the described biomarkers in an individual who is not known to have ovarian cancer may indicate that the individual has ovarian cancer, thereby enabling detection of ovarian cancer at an early stage of the disease when treatment is most effective, perhaps before the ovarian cancer is detected by other means or before symptoms appear. Increased differential expression from "normal" (since some biomarkers may be down-regulated with disease) of one or more of the biomarkers during the course of ovarian cancer may be indicative of ovarian cancer progression, e.g., an ovarian tumor is growing and/or metastasizing (and thus indicate a poor prognosis), whereas a decrease in the degree to which one or more of the biomarkers is differentially expressed (i.e., in subsequent biomarker tests, the expression level in the individual is moving toward or approaching a "normal" expression level) may be indicative of ovarian cancer remission, e.g., an ovarian tumor is shrinking (and thus indicate a good or better prognosis). Similarly, an increase in the degree to which one or more of the biomarkers is differentially expressed (i.e., in subsequent biomarker tests, the expression level in the individual is moving further away from a "normal" expression level) during the course of ovarian cancer treatment may indicate that the ovarian cancer is progressing and therefore indicate that the treatment is ineffective, whereas a decrease in differential expression of one or more of the biomarkers during the course of ovarian cancer treatment may be indicative of ovarian cancer remission and therefore indicate that the treatment is working successfully. Additionally, an increase or decrease in the differential expression of one or more of the biomarkers after an individual has apparently been cured of ovarian cancer may be indicative of ovarian cancer recurrence. In a situation such as this, for example, the individual can be restarted on therapy (or the therapeutic regimen modified such as to increase dosage amount and/or frequency, if the individual has maintained therapy) at an earlier stage than if the recurrence of ovarian cancer was not detected until later. Furthermore, a differential expression level of one or more of the biomarkers in an individual may be predictive of the individual's response to a particular therapeutic agent. In monitoring for ovarian cancer recurrence or progression, changes in the biomarker expression levels may indicate the need for repeat imaging, such as to determine ovarian cancer activity or to determine the need for changes in treatment.

[0119] Detection of any of the biomarkers described herein may be particularly useful following, or in conjunction with, ovarian cancer treatment, such as to evaluate the success of the treatment or to monitor ovarian cancer remission, recurrence, and/or progression (including metastasis) following treatment. Ovarian cancer treatment may include, for example, administration of a therapeutic agent to the individual, performance of surgery (e.g., surgical resection of at least a portion of a pelvic mass), administration of radiation therapy, or any other type of ovarian cancer treatment used in the art, and any combination of these treatments. For example, any of the biomarkers may be detected at least once after treatment or may be detected multiple times after treatment (such as at periodic intervals), or may be detected both before and after treatment. Differential expression levels of any of the biomarkers in an individual over time may be

indicative of ovarian cancer progression, remission, or recurrence, examples of which include any of the following: an increase or decrease in the expression level of the biomarkers after treatment compared with the expression level of the biomarker before treatment; an increase or decrease in the expression level of the biomarker at a later time point after treatment compared with the expression level of the biomarker at an earlier time point after treatment; and a differential expression level of the biomarker at a single time point after treatment compared with normal levels of the biomarker.

[0120] As a specific example, the biomarker levels for any of the biomarkers described herein can be determined in pre-surgery and post-surgery (e.g., 2-8 weeks after surgery) serum or plasma samples. An increase in the biomarker expression level(s) in the post-surgery sample compared with the pre-surgery sample can indicate progression of ovarian cancer (e.g., unsuccessful surgery), whereas a decrease in the biomarker expression level(s) in the post-surgery sample compared with the pre-surgery sample can indicate regression of ovarian cancer (e.g., the surgery successfully removed the ovarian tumor). Similar analyses of the biomarker levels can be carried out before and after other forms of treatment, such as before and after radiation therapy or administration of a therapeutic agent or cancer vaccine.

[0121] In addition to testing biomarker levels as a standalone diagnostic test, biomarker levels can also be done in conjunction with determination of SNPs or other genetic lesions or variability that are indicative of increased risk of susceptibility of disease. (See, e.g., Amos et al., Nature Genetics 40, 616-622 (2009)).

[0122] In addition to testing biomarker levels as a standalone diagnostic test, biomarker levels can also be done in conjunction with relevant symptoms or abdominal ultrasound and CT imaging.

[0123] Detection of any of the biomarkers described herein may be useful after a pelvic mass has been observed through imaging to aid in the diagnosis of ovarian cancer and guide appropriate clinical care of the individual, including care by an appropriate surgical specialist.

[0124] In addition to testing biomarker levels in conjunction with relevant symptoms or abdominal ultrasound or CT imaging, information regarding the biomarkers can also be evaluated in conjunction with other types of data, particularly data that indicates an individual's risk for ovarian cancer (e.g., patient clinical history, symptoms, family history of cancer, risk factors such as the presence of a genetic marker(s), and/or status of other biomarkers, etc.). These various data can be assessed by automated methods, such as a computer program/software, which can be embodied in a computer or other apparatus/device.

[0125] Any of the described biomarkers may also be used in imaging tests. For example, an imaging agent can be coupled to any of the described biomarkers, which can be used to aid in ovarian cancer diagnosis, to monitor disease progression/remission or metastasis, to monitor for disease recurrence, or to monitor response to therapy, among other uses.

Detection and Determination of Biomarkers and Biomarker Values

[0126] A biomarker value for the biomarkers described herein can be detected using any of a variety of known analytical methods. In one embodiment, a biomarker value is detected using a capture reagent. As used herein, a "capture agent" or "capture reagent" refers to a molecule that is capable of binding specifically to a biomarker. In various embodiments, the capture reagent can be exposed to the biomarker in solution or can be exposed to the biomarker while the capture reagent is immobilized on a solid support. In other embodiments, the capture reagent contains a feature that is reactive with a secondary feature on a solid support. In these embodiments, the capture reagent can be exposed to the biomarker in solution, and then the feature on the capture reagent can be used in conjunction with the secondary feature on the solid support to immobilize the biomarker on the solid support. The capture reagent is selected based on the type of analysis to be conducted. Capture reagents include but are not limited to aptamers, antibodies, adnectins, ankyrins, other antibody mimetics and other protein scaffolds, autoantibodies, chimeras, small molecules, an $F(ab')_2$ fragment, a single chain antibody fragment, an Fv fragment, a single chain Fv fragment, a nucleic acid, a lectin, a ligand-binding receptor, affybodies, nanobodies, imprinted polymers, avimers, peptidomimetics, a hormone receptor, a cytokine receptor, and synthetic receptors, and modifications and fragments of these.

[0127] In some embodiments, a biomarker value is detected using a biomarker/capture reagent complex.

[0128] In other embodiments, the biomarker value is derived from the biomarker/capture reagent complex and is detected indirectly, such as, for example, as a result of a reaction that is subsequent to the biomarker/capture reagent interaction, but is dependent on the formation of the biomarker/capture reagent complex.

[0129] In some embodiments, the biomarker value is detected directly from the biomarker in a biological sample.

[0130] In one embodiment, the biomarkers are detected using a multiplexed format that allows for the simultaneous detection of two or more biomarkers in a biological sample. In one embodiment of the multiplexed format, capture reagents are immobilized, directly or indirectly, covalently or non-covalently, in discrete locations on a solid support. In another embodiment, a multiplexed format uses discrete solid supports where each solid support has a unique capture reagent associated with that solid support, such as, for example quantum dots. In another embodiment, an individual device is used for the detection of each one of multiple biomarkers to be detected in a biological sample. Individual devices can be configured to permit each biomarker in the biological sample to be processed simultaneously. For example, a microtiter plate can be used such that each well in the plate is used to uniquely analyze one of multiple biomarkers to be detected in a biological sample.

[0131] In one or more of the foregoing embodiments, a fluorescent tag can be used to label a component of the biomarker/capture complex to enable the detection of the biomarker value. In various embodiments, the fluorescent label can be conjugated to a capture reagent specific to any of the biomarkers described herein using known techniques, and the fluorescent label can then be used to detect the corresponding biomarker value. Suitable fluorescent labels include rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, allophycocyanin, PBXL-3, Qdot 605, Lissamine, phycoerythrin, Texas Red, and other such compounds.

[0132] In one embodiment, the fluorescent label is a fluorescent dye molecule. In some embodiments, the fluorescent dye molecule includes at least one substituted indolium ring system in which the substituent on the 3-carbon of the indo-

lium ring contains a chemically reactive group or a conjugated substance. In some embodiments, the dye molecule includes an AlexFluor molecule, such as, for example, AlexaFluor 488, AlexaFluor 532, AlexaFluor 647, AlexaFluor 680, or AlexaFluor 700. In other embodiments, the dye molecule includes a first type and a second type of dye molecule, such as, e.g., two different AlexaFluor molecules. In other embodiments, the dye molecule includes a first type and a second type of dye molecule, and the two dye molecules have different emission spectra.

[0133] Fluorescence can be measured with a variety of instrumentation compatible with a wide range of assay formats. For example, spectrofluorimeters have been designed to analyze microtiter plates, microscope slides, printed arrays, cuvettes, etc. See Principles of Fluorescence Spectroscopy, by J. R. Lakowicz, Springer Science+Business Media, Inc., 2004. See Bioluminescence & Chemiluminescence: Progress & Current Applications; Philip E. Stanley and Larry J. Kricka editors, World Scientific Publishing Company, January 2002.

[0134] In one or more of the foregoing embodiments, a chemiluminescence tag can optionally be used to label a component of the biomarker/capture complex to enable the detection of a biomarker value. Suitable chemiluminescent materials include any of oxalyl chloride, Rodamin 6G, $Ru(bipy)_3^{2+}$, TMAE (tetrakis(dimethylamino)ethylene), Pyrogallol (1,2,3-trihydroxibenzene), Lucigenin, peroxyoxalates, Aryl oxalates, Acridinium esters, dioxetanes, and others.

[0135] In yet other embodiments, the detection method includes an enzyme/substrate combination that generates a detectable signal that corresponds to the biomarker value. Generally, the enzyme catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques, including spectrophotometry, fluorescence, and chemiluminescence. Suitable enzymes include, for example, luciferases, luciferin, malate dehydrogenase, urease, horseradish peroxidase (HRPO), alkaline phosphatase, beta-galactosidase, glucoamylase, lysozyme, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, uricase, xanthine oxidase, lactoperoxidase, microperoxidase, and the like.

[0136] In yet other embodiments, the detection method can be a combination of fluorescence, chemiluminescence, radionuclide or enzyme/substrate combinations that generate a measurable signal. Multimodal signaling could have unique and advantageous characteristics in biomarker assay formats.

[0137] More specifically, the biomarker values for the biomarkers described herein can be detected using known analytical methods including, singleplex aptamer assays, multiplexed aptamer assays, singleplex or multiplexed immunoassays, mRNA expression profiling, miRNA expression profiling, mass spectrometric analysis, histological/cytological methods, etc. as detailed below.

Determination of Biomarker Values using Aptamer-Based Assays

[0138] Assays directed to the detection and quantification of physiologically significant molecules in biological samples and other samples are important tools in scientific research and in the health care field. One class of such assays involves the use of a microarray that includes one or more aptamers immobilized on a solid support. The aptamers are each capable of binding to a target molecule in a highly specific manner and with very high affinity. See, e.g., U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands"; see also, e.g., U.S. Pat. No. 6,242,246, U.S. Pat. No. 6,458,543, and U.S. Pat. No. 6,503,715, each of which is entitled "Nucleic Acid Ligand Diagnostic Biochip". Once the microarray is contacted with a sample, the aptamers bind to their respective target molecules present in the sample and thereby enable a determination of a biomarker value corresponding to a biomarker.

[0139] As used herein, an "aptamer" refers to a nucleic acid that has a specific binding affinity for a target molecule. It is recognized that affinity interactions are a matter of degree; however, in this context, the "specific binding affinity" of an aptamer for its target means that the aptamer binds to its target generally with a much higher degree of affinity than it binds to other components in a test sample. An "aptamer" is a set of copies of one type or species of nucleic acid molecule that has a particular nucleotide sequence. An aptamer can include any suitable number of nucleotides, including any number of chemically modified nucleotides. "Aptamers" refers to more than one such set of molecules. Different aptamers can have either the same or different numbers of nucleotides. Aptamers can be DNA or RNA or chemically modified nucleic acids and can be single stranded, double stranded, or contain double stranded regions, and can include higher ordered structures. An aptamer can also be a photoaptamer, where a photoreactive or chemically reactive functional group is included in the aptamer to allow it to be covalently linked to its corresponding target. Any of the aptamer methods disclosed herein can include the use of two or more aptamers that specifically bind the same target molecule. As further described below, an aptamer may include a tag. If an aptamer includes a tag, all copies of the aptamer need not have the same tag. Moreover, if different aptamers each include a tag, these different aptamers can have either the same tag or a different tag.

[0140] An aptamer can be identified using any known method, including the SELEX process. Once identified, an aptamer can be prepared or synthesized in accordance with any known method, including chemical synthetic methods and enzymatic synthetic methods.

[0141] The terms "SELEX" and "SELEX process" are used interchangeably herein to refer generally to a combination of (1) the selection of aptamers that interact with a target molecule in a desirable manner, for example binding with high affinity to a protein, with (2) the amplification of those selected nucleic acids. The SELEX process can be used to identify aptamers with high affinity to a specific target or biomarker.

[0142] SELEX generally includes preparing a candidate mixture of nucleic acids, binding of the candidate mixture to the desired target molecule to form an affinity complex, separating the affinity complexes from the unbound candidate nucleic acids, separating and isolating the nucleic acid from the affinity complex, purifying the nucleic acid, and identifying a specific aptamer sequence. The process may include multiple rounds to further refine the affinity of the selected aptamer. The process can include amplification steps at one or more points in the process. See, e.g., U.S. Pat. No. 5,475,096, entitled "Nucleic Acid Ligands". The SELEX process can be used to generate an aptamer that covalently binds its target as well as an aptamer that non-covalently binds its target. See, e.g., U.S. Pat. No. 5,705,337 entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Chemi-SELEX."

[0143] The SELEX process can be used to identify high-affinity aptamers containing modified nucleotides that confer improved characteristics on the aptamer, such as, for example, improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX process-identified aptamers containing modified nucleotides are described in U.S. Pat. No. 5,660,985, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides", which describes oligonucleotides containing nucleotide derivatives chemically modified at the 5'- and 2'-positions of pyrimidines. U.S. Pat. No. 5,580,737, see supra, describes highly specific aptamers containing one or more nucleotides modified with 2'-amino (2'-NH2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). See also, U.S. Patent Application Publication 20090098549, entitled "SELEX and PHOTOSELEX", which describes nucleic acid libraries having expanded physical and chemical properties and their use in SELEX and photoSELEX.

[0144] SELEX can also be used to identify aptamers that have desirable off-rate characteristics. See U.S. Patent Application Publication 20090004667, entitled "Method for Generating Aptamers with Improved Off-Rates", which describes improved SELEX methods for generating aptamers that can bind to target molecules. Methods for producing aptamers and photoaptamers having slower rates of dissociation from their respective target molecules are described. The methods involve contacting the candidate mixture with the target molecule, allowing the formation of nucleic acid-target complexes to occur, and performing a slow off-rate enrichment process wherein nucleic acid-target complexes with fast dissociation rates will dissociate and not reform, while complexes with slow dissociation rates will remain intact. Additionally, the methods include the use of modified nucleotides in the production of candidate nucleic acid mixtures to generate aptamers with improved off-rate performance.

[0145] A variation of this assay employs aptamers that include photoreactive functional groups that enable the aptamers to covalently bind or "photocrosslink" their target molecules. See, e.g., U.S. Pat. No. 6,544,776 entitled "Nucleic Acid Ligand Diagnostic Biochip". These photoreactive aptamers are also referred to as photoaptamers. See, e.g., U.S. Pat. No. 5,763,177, U.S. Pat. No. 6,001,577, and U.S. Pat. No. 6,291,184, each of which is entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Photoselection of Nucleic Acid Ligands and Solution SELEX"; see also, e.g., U.S. Pat. No. 6,458,539, entitled "Photoselection of Nucleic Acid Ligands". After the microarray is contacted with the sample and the photoaptamers have had an opportunity to bind to their target molecules, the photoaptamers are photoactivated, and the solid support is washed to remove any non-specifically bound molecules. Harsh wash conditions may be used, since target molecules that are bound to the photoaptamers are generally not removed, due to the covalent bonds created by the photoactivated functional group(s) on the photoaptamers. In this manner, the assay enables the detection of a biomarker value corresponding to a biomarker in the test sample.

[0146] In both of these assay formats, the aptamers are immobilized on the solid support prior to being contacted with the sample. Under certain circumstances, however, immobilization of the aptamers prior to contact with the sample may not provide an optimal assay. For example, pre-immobilization of the aptamers may result in inefficient mixing of the aptamers with the target molecules on the surface of the solid support, perhaps leading to lengthy reaction times and, therefore, extended incubation periods to permit efficient binding of the aptamers to their target molecules. Further, when photoaptamers are employed in the assay and depending upon the material utilized as a solid support, the solid support may tend to scatter or absorb the light used to effect the formation of covalent bonds between the photoaptamers and their target molecules. Moreover, depending upon the method employed, detection of target molecules bound to their aptamers can be subject to imprecision, since the surface of the solid support may also be exposed to and affected by any labeling agents that are used. Finally, immobilization of the aptamers on the solid support generally involves an aptamer-preparation step (i.e., the immobilization) prior to exposure of the aptamers to the sample, and this preparation step may affect the activity or functionality of the aptamers.

[0147] Aptamer assays that permit an aptamer to capture its target in solution and then employ separation steps that are designed to remove specific components of the aptamer-target mixture prior to detection have also been described (see U.S. Patent Application Publication 20090042206, entitled "Multiplexed Analyses of Test Samples"). The described aptamer assay methods enable the detection and quantification of a non-nucleic acid target (e.g., a protein target) in a test sample by detecting and quantifying a nucleic acid (i.e., an aptamer). The described methods create a nucleic acid surrogate (i.e., the aptamer) for detecting and quantifying a non-nucleic acid target, thus allowing the wide variety of nucleic acid technologies, including amplification, to be applied to a broader range of desired targets, including protein targets.

[0148] Aptamers can be constructed to facilitate the separation of the assay components from an aptamer biomarker complex (or photoaptamer biomarker covalent complex) and permit isolation of the aptamer for detection and/or quantification. In one embodiment, these constructs can include a cleavable or releasable element within the aptamer sequence. In other embodiments, additional functionality can be introduced into the aptamer, for example, a labeled or detectable component, a spacer component, or a specific binding tag or immobilization element. For example, the aptamer can include a tag connected to the aptamer via a cleavable moiety, a label, a spacer component separating the label, and the cleavable moiety. In one embodiment, a cleavable element is a photocleavable linker. The photocleavable linker can be attached to a biotin moiety and a spacer section, can include an NHS group for derivatization of amines, and can be used to introduce a biotin group to an aptamer, thereby allowing for the release of the aptamer later in an assay method.

[0149] Homogenous assays, done with all assay components in solution, do not require separation of sample and reagents prior to the detection of signal. These methods are rapid and easy to use. These methods generate signal based on a molecular capture or binding reagent that reacts with its specific target. For ovarian cancer, the molecular capture reagents would be an aptamer or an antibody or the like and the specific target would be an ovarian cancer biomarker of Table 1.

[0150] In one embodiment, a method for signal generation takes advantage of anisotropy signal change due to the interaction of a fluorophore-labeled capture reagent with its specific biomarker target. When the labeled capture reacts with its target, the increased molecular weight causes the rotational motion of the fluorophore attached to the complex to

become much slower changing the anisotropy value. By monitoring the anisotropy change, binding events may be used to quantitatively measure the biomarkers in solutions. Other methods include fluorescence polarization assays, molecular beacon methods, time resolved fluorescence quenching, chemiluminescence, fluorescence resonance energy transfer, and the like.

[0151] An exemplary solution-based aptamer assay that can be used to detect a biomarker value corresponding to a biomarker in a biological sample includes the following: (a) preparing a mixture by contacting the biological sample with an aptamer that includes a first tag and has a specific affinity for the biomarker, wherein an aptamer affinity complex is formed when the biomarker is present in the sample; (b) exposing the mixture to a first solid support including a first capture element, and allowing the first tag to associate with the first capture element; (c) removing any components of the mixture not associated with the first solid support; (d) attaching a second tag to the biomarker component of the aptamer affinity complex; (e) releasing the aptamer affinity complex from the first solid support; (f) exposing the released aptamer affinity complex to a second solid support that includes a second capture element and allowing the second tag to associate with the second capture element; (g) removing any non-complexed aptamer from the mixture by partitioning the non-complexed aptamer from the aptamer affinity complex; (h) eluting the aptamer from the solid support; and (i) detecting the biomarker by detecting the aptamer component of the aptamer affinity complex.

Determination of Biomarker Values Using Immunoassays

[0152] Immunoassay methods are based on the reaction of an antibody to its corresponding target or analyte and can detect the analyte in a sample depending on the specific assay format. To improve specificity and sensitivity of an assay method based on immuno-reactivity, monoclonal antibodies are often used because of their specific epitope recognition. Polyclonal antibodies have also been successfully used in various immunoassays because of their increased affinity for the target as compared to monoclonal antibodies. Immunoassays have been designed for use with a wide range of biological sample matrices. Immunoassay formats have been designed to provide qualitative, semi-quantitative, and quantitative results.

[0153] Quantitative results are generated through the use of a standard curve created with known concentrations of the specific analyte to be detected. The response or signal from an unknown sample is plotted onto the standard curve, and a quantity or value corresponding to the target in the unknown sample is established.

[0154] Numerous immunoassay formats have been designed. ELISA or EIA can be quantitative for the detection of an analyte. This method relies on attachment of a label to either the analyte or the antibody and the label component includes, either directly or indirectly, an enzyme. ELISA tests may be formatted for direct, indirect, competitive, or sandwich detection of the analyte. Other methods rely on labels such as, for example, radioisotopes (I^{125}) or fluorescence. Additional techniques include, for example, agglutination, nephelometry, turbidimetry, Western blot, immunoprecipitation, immunocytochemistry, immunohistochemistry, flow cytometry, Luminex assay, and others (see ImmunoAssay: A Practical Guide, edited by Brian Law, published by Taylor & Francis, Ltd., 2005 edition).

[0155] Exemplary assay formats include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, fluorescent, chemiluminescence, and fluorescence resonance energy transfer (FRET) or time resolved-FRET (TR-FRET) immunoassays. Examples of procedures for detecting biomarkers include biomarker immunoprecipitation followed by quantitative methods that allow size and peptide level discrimination, such as gel electrophoresis, capillary electrophoresis, planar electrochromatography, and the like.

[0156] Methods of detecting and/or quantifying a detectable label or signal generating material depend on the nature of the label. The products of reactions catalyzed by appropriate enzymes (where the detectable label is an enzyme; see above) can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors suitable for detecting such detectable labels include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

[0157] Any of the methods for detection can be performed in any format that allows for any suitable preparation, processing, and analysis of the reactions. This can be, for example, in multi-well assay plates (e.g., 96 wells or 384 wells) or using any suitable array or microarray. Stock solutions for various agents can be made manually or robotically, and all subsequent pipetting, diluting, mixing, distribution, washing, incubating, sample readout, data collection and analysis can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting a detectable label.

Determination of Biomarker Values Using Gene Expression Profiling

[0158] Measuring mRNA in a biological sample may be used as a surrogate for detection of the level of the corresponding protein in the biological sample. Thus, any of the biomarkers or biomarker panels described herein can also be detected by detecting the appropriate RNA.

[0159] mRNA expression levels are measured by reverse transcription quantitative polymerase chain reaction (RT-PCR followed with qPCR). RT-PCR is used to create a cDNA from the mRNA. The cDNA may be used in a qPCR assay to produce fluorescence as the DNA amplification process progresses. By comparison to a standard curve, qPCR can produce an absolute measurement such as number of copies of mRNA per cell. Northern blots, microarrays, Invader assays, and RT-PCR combined with capillary electrophoresis have all been used to measure expression levels of mRNA in a sample. See Gene Expression Profiling: Methods and Protocols, Richard A. Shimkets, editor, Humana Press, 2004.

[0160] miRNA molecules are small RNAs that are non-coding but may regulate gene expression. Any of the methods suited to the measurement of mRNA expression levels can also be used for the corresponding miRNA. Recently many laboratories have investigated the use of miRNAs as biomarkers for disease. Many diseases involve wide-spread transcriptional regulation, and it is not surprising that miRNAs might find a role as biomarkers. The connection between miRNA concentrations and disease is often even less clear than the connections between protein levels and disease, yet the value of miRNA biomarkers might be substantial. Of course, as with any RNA expressed differentially during disease, the problems facing the development of an in vitro diagnostic product will include the requirement that the miR-

NAs survive in the diseased cell and are easily extracted for analysis, or that the miRNAs are released into blood or other matrices where they must survive long enough to be measured. Protein biomarkers have similar requirements, although many potential protein biomarkers are secreted intentionally at the site of pathology and function, during disease, in a paracrine fashion. Many potential protein biomarkers are designed to function outside the cells within which those proteins are synthesized.

Detection of Biomarkers Using In Vivo Molecular Imaging Technologies

[0161] Any of the described biomarkers (see Table 1) may also be used in molecular imaging tests. For example, an imaging agent can be coupled to any of the described biomarkers, which can be used to aid in ovarian cancer diagnosis, to monitor disease progression/remission or metastasis, to monitor for disease recurrence, or to monitor response to therapy, among other uses.

[0162] In vivo imaging technologies provide non-invasive methods for determining the state of a particular disease in the body of an individual. For example, entire portions of the body, or even the entire body, may be viewed as a three dimensional image, thereby providing valuable information concerning morphology and structures in the body. Such technologies may be combined with the detection of the biomarkers described herein to provide information concerning the cancer status, in particular the ovarian cancer status, of an individual.

[0163] The use of in vivo molecular imaging technologies is expanding due to various advances in technology. These advances include the development of new contrast agents or labels, such as radiolabels and/or fluorescent labels, which can provide strong signals within the body; and the development of powerful new imaging technology, which can detect and analyze these signals from outside the body, with sufficient sensitivity and accuracy to provide useful information. The contrast agent can be visualized in an appropriate imaging system, thereby providing an image of the portion or portions of the body in which the contrast agent is located. The contrast agent may be bound to or associated with a capture reagent, such as an aptamer or an antibody, for example, and/or with a peptide or protein, or an oligonucleotide (for example, for the detection of gene expression), or a complex containing any of these with one or more macromolecules and/or other particulate forms.

[0164] The contrast agent may also feature a radioactive atom that is useful in imaging. Suitable radioactive atoms include technetium-99m or iodine-123 for scintigraphic studies. Other readily detectable moieties include, for example, spin labels for magnetic resonance imaging (MRI) such as, for example, iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Such labels are well known in the art and could easily be selected by one of ordinary skill in the art.

[0165] Standard imaging techniques include but are not limited to magnetic resonance imaging, contrast-enhanced abdominal or transvaginal ultrasound, computed tomography (CT) scanning, positron emission tomography (PET), single photon emission computed tomography (SPECT), and the like. For diagnostic in vivo imaging, the type of detection instrument available is a major factor in selecting a given contrast agent, such as a given radionuclide and the particular biomarker that it is used to target (protein, mRNA, and the like). The radionuclide chosen typically has a type of decay that is detectable by a given type of instrument. Also, when selecting a radionuclide for in vivo diagnosis, its half-life should be long enough to enable detection at the time of maximum uptake by the target tissue but short enough that deleterious radiation of the host is minimized.

[0166] Exemplary imaging techniques include but are not limited to PET and SPECT, which are imaging techniques in which a radionuclide is synthetically or locally administered to an individual. The subsequent uptake of the radiotracer is measured over time and used to obtain information about the targeted tissue and the biomarker. Because of the high-energy (gamma-ray) emissions of the specific isotopes employed and the sensitivity and sophistication of the instruments used to detect them, the two-dimensional distribution of radioactivity may be inferred from outside of the body.

[0167] Commonly used positron-emitting nuclides in PET include, for example, carbon-11, nitrogen-13, oxygen-15, and fluorine-18. Isotopes that decay by electron capture and/or gamma-emission are used in SPECT and include, for example iodine-123 and technetium-99m. An exemplary method for labeling amino acids with technetium-99m is the reduction of pertechnetate ion in the presence of a chelating precursor to form the labile technetium-99m-precursor complex, which, in turn, reacts with the metal binding group of a bifunctionally modified chemotactic peptide to form a technetium-99m-chemotactic peptide conjugate.

[0168] Antibodies are frequently used for such in vivo imaging diagnostic methods. The preparation and use of antibodies for in vivo diagnosis is well known in the art. Labeled antibodies which specifically bind any of the biomarkers in Table 1 can be injected into an individual suspected of having a certain type of cancer (e.g., ovarian cancer), detectable according to the particular biomarker used, for the purpose of diagnosing or evaluating the disease status of the individual. The label used will be selected in accordance with the imaging modality to be used, as previously described. Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

[0169] Similarly, aptamers may be used for such in vivo imaging diagnostic methods. For example, an aptamer that was used to identify a particular biomarker described in Table 1 (and therefore binds specifically to that particular biomarker) may be appropriately labeled and injected into an individual suspected of having ovarian cancer, detectable according to the particular biomarker, for the purpose of diagnosing or evaluating the ovarian cancer status of the individual. The label used will be selected in accordance with the imaging modality to be used, as previously described. Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue. Aptamer-directed imaging agents could have unique and advantageous characteristics relating to tissue penetration, tissue distribution, kinetics, elimination, potency, and selectivity as compared to other imaging agents.

[0170] Such techniques may also optionally be performed with labeled oligonucleotides, for example, for detection of gene expression through imaging with antisense oligonucleotides. These methods are used for in situ hybridization, for example, with fluorescent molecules or radionuclides as the

label. Other methods for detection of gene expression include, for example, detection of the activity of a reporter gene.

[0171] Another general type of imaging technology is optical imaging, in which fluorescent signals within the subject are detected by an optical device that is external to the subject. These signals may be due to actual fluorescence and/or to bioluminescence. Improvements in the sensitivity of optical detection devices have increased the usefulness of optical imaging for in vivo diagnostic assays.

[0172] The use of in vivo molecular biomarker imaging is increasing, including for clinical trials, for example, to more rapidly measure clinical efficacy in trials for new cancer therapies and/or to avoid prolonged treatment with a placebo for those diseases, such as multiple sclerosis, in which such prolonged treatment may be considered to be ethically questionable.

[0173] For a review of other techniques, see N. Blow, Nature Methods, 6, 465-469, 2009.

Determination of Biomarker Values Using Histology or Cytology Methods

[0174] For evaluation of ovarian cancer, a variety of tissue samples may be used in histological or cytological methods. Sample selection depends on the primary tumor location and sites of metastases. For example, fine needle aspirates, cutting needles, and core biopsies can be used for histology. Ascites can be used for cyotology. While cytological analysis is still used in the diagnosis of ovarian cancer, histological methods are known to provide better sensitivity for the detection of cancer. Any of the biomarkers identified herein that were shown to be up-regulated (see Table 15) in the individuals with ovarian cancer can be used to stain a histological specimen as an indication of disease.

[0175] In one embodiment, one or more capture reagents specific to the corresponding biomarker is used in a cytological evaluation of an ovarian cell sample and may include one or more of the following: collecting a cell sample, fixing the cell sample, dehydrating, clearing, immobilizing the cell sample on a microscope slide, permeabilizing the cell sample, treating for analyte retrieval, staining, destaining, washing, blocking, and reacting with one or more capture reagent/s in a buffered solution. In another embodiment, the cell sample is produced from a cell block.

[0176] In another embodiment, one or more capture reagents specific to the corresponding biomarker is used in a histological evaluation of an ovarian tissue sample and may include one or more of the following: collecting a tissue specimen, fixing the tissue sample, dehydrating, clearing, immobilizing the tissue sample on a microscope slide, permeabilizing the tissue sample, treating for analyte retrieval, staining, destaining, washing, blocking, rehydrating, and reacting with capture reagent/s in a buffered solution. In another embodiment, fixing and dehydrating are replaced with freezing.

[0177] In another embodiment, the one or more aptamers specific to the corresponding biomarker is reacted with the histological or cytological sample and can serve as the nucleic acid target in a nucleic acid amplification method. Suitable nucleic acid amplification methods include, for example, PCR, q-beta replicase, rolling circle amplification, strand displacement, helicase dependent amplification, loop mediated isothermal amplification, ligase chain reaction, and restriction and circularization aided rolling circle amplification.

[0178] In one embodiment, the one or more capture reagent/s specific to the corresponding biomarkers for use in the histological or cytological evaluation are mixed in a buffered solution that can include any of the following: blocking materials, competitors, detergents, stabilizers, carrier nucleic acid, polyanionic materials, etc.

[0179] A "cytology protocol" generally includes sample collection, sample fixation, sample immobilization, and staining. "Cell preparation" can include several processing steps after sample collection, including the use of one or more slow off-rate aptamers for the staining of the prepared cells.

[0180] Sample collection can include directly placing the sample in an untreated transport container, placing the sample in a transport container containing some type of media, or placing the sample directly onto a slide (immobilization) without any treatment or fixation.

[0181] Sample immobilization can be improved by applying a portion of the collected specimen to a glass slide that is treated with polylysine, gelatin, or a silane. Slides can be prepared by smearing a thin and even layer of cells across the slide. Care is generally taken to minimize mechanical distortion and drying artifacts. Liquid specimens can be processed in a cell block method. Or, alternatively, liquid specimens can be mixed 1:1 with the fixative solution for about 10 minutes at room temperature.

[0182] Cell blocks can be prepared from residual effusions, sputum, urine sediments, gastrointestinal fluids, cell scraping, ascites, or fine needle aspirates. Cells are concentrated or packed by centrifugation or membrane filtration. A number of methods for cell block preparation have been developed. Representative procedures include the fixed sediment, bacterial agar, or membrane filtration methods. In the fixed sediment method, the cell sediment is mixed with a fixative like Bouins, picric acid, or buffered formalin and then the mixture is centrifuged to pellet the fixed cells. The supernatant is removed, drying the cell pellet as completely as possible. The pellet is collected and wrapped in lens paper and then placed in a tissue cassette. The tissue cassette is placed in a jar with additional fixative and processed as a tissue sample. Agar method is very similar but the pellet is removed and dried on paper towel and then cut in half. The cut side is placed in a drop of melted agar on a glass slide and then the pellet is covered with agar making sure that no bubbles form in the agar. The agar is allowed to harden and then any excess agar is trimmed away. This is placed in a tissue cassette and the tissue process completed. Alternatively, the pellet may be directly suspended in 2% liquid agar at 65° C. and the sample centrifuged. The agar cell pellet is allowed to solidify for an hour at 4° C. The solid agar may be removed from the centrifuge tube and sliced in half. The agar is wrapped in filter paper and then the tissue cassette. Processing from this point forward is as described above. Centrifugation can be replaced in any these procedures with membrane filtration. Any of these processes may be used to generate a "cell block sample".

[0183] Cell blocks can be prepared using specialized resin including Lowicryl resins, LR White, LR Gold, Unicryl, and MonoStep. These resins have low viscosity and can be polymerized at low temperatures and with ultra violet (UV) light. The embedding process relies on progressively cooling the sample during dehydration, transferring the sample to the resin, and polymerizing a block at the final low temperature at the appropriate UV wavelength.

[0184] Cell block sections can be stained with hematoxylin-eosin for cytomorphological examination while additional sections are used for examination for specific markers.

[0185] Whether the process is cytologoical or histological, the sample may be fixed prior to additional processing to prevent sample degradation. This process is called "fixation" and describes a wide range of materials and procedures that may be used interchangeably. The sample fixation protocol and reagents are best selected empirically based on the targets to be detected and the specific cell/tissue type to be analyzed. Sample fixation relies on reagents such as ethanol, polyethylene glycol, methanol, formalin, or isopropanol. The samples should be fixed as soon after collection and affixation to the slide as possible. However, the fixative selected can introduce structural changes into various molecular targets making their subsequent detection more difficult. The fixation and immobilization processes and their sequence can modify the appearance of the cell and these changes must be anticipated and recognized by the cytotechnologist. Fixatives can cause shrinkage of certain cell types and cause the cytoplasm to appear granular or reticular. Many fixatives function by crosslinking cellular components. This can damage or modify specific epitopes, generate new epitopes, cause molecular associations, and reduce membrane permeability. Formalin fixation is one of the most common cytological and histological approaches. Formalin forms methyl bridges between neighboring proteins or within proteins. Precipitation or coagulation is also used for fixation and ethanol is frequently used in this type of fixation. A combination of crosslinking and precipitation can also be used for fixation. A strong fixation process is best at preserving morphological information while a weaker fixation process is best for the preservation of molecular targets.

[0186] A representative fixative is 50% absolute ethanol, 2 mM polyethylene glycol (PEG), 1.85% formaldehyde. Variations on this formulation include ethanol (50% to 95%), methanol (20%-50%), and formalin (formaldehyde) only. Another common fixative is 2% PEG 1500, 50% ethanol, and 3% methanol. Slides are place in the fixative for about 10 to 15 minutes at room temperature and then removed and allowed to dry. Once slides are fixed they can be rinsed with a buffered solution like PBS.

[0187] A wide range of dyes can be used to differentially highlight and contrast or "stain" cellular, sub-cellular, and tissue features or morphological structures. Hematoylin is used to stain nuclei a blue or black color. Orange G-6 and Eosin Azure both stain the cell's cytoplasm. Orange G stains keratin and glycogen containing cells yellow. Eosin Y is used to stain nucleoli, cilia, red blood cells, and superficial epithelial squamous cells. Romanowsky stains are used for air dried slides and are useful in enhancing pleomorphism and distinguishing extracellular from intracytoplasmic material.

[0188] The staining process can include a treatment to increase the permeability of the cells to the stain. Treatment of the cells with a detergent can be used to increase permeability. To increase cell and tissue permeability, fixed samples can be further treated with solvents, saponins, or non-ionic detergents. Enzymatic digestion can also improve the accessibility of specific targets in a tissue sample.

[0189] After staining, the sample is dehydrated using a succession of alcohol rinses with increasing alcohol concentration. The final wash is done with xylene or a xylene substitute, such as a citrus terpene, that has a refractive index close to that of the coverslip to be applied to the slide. This final step is referred to as clearing. Once the sample is dehydrated and cleared, a mounting medium is applied. The mounting medium is selected to have a refractive index close to the glass and is capable of bonding the coverslip to the slide. It will also inhibit the additional drying, shrinking, or fading of the cell sample.

[0190] Regardless of the stains or processing used, the final evaluation of the ovarian cytological specimen is made by some type of microscopy to permit a visual inspection of the morphology and a determination of the marker's presence or absence. Exemplary microscopic methods include bright-field, phase contrast, fluorescence, and differential interference contrast.

[0191] If secondary tests are required on the sample after examination, the coverslip may be removed and the slide destained. Destaining involves using the original solvent systems used in staining the slide originally without the added dye and in a reverse order to the original staining procedure. Destaining may also be completed by soaking the slide in an acid alcohol until the cells are colorless. Once colorless the slides are rinsed well in a water bath and the second staining procedure applied.

[0192] In addition, specific molecular differentiation may be possible in conjunction with the cellular morphological analysis through the use of specific molecular reagents such as antibodies or nucleic acid probes or aptamers. This improves the accuracy of diagnostic cytology. Micro-dissection can be used to isolate a subset of cells for additional evaluation, in particular, for genetic evaluation of abnormal chromosomes, gene expression, or mutations.

[0193] Preparation of a tissue sample for histological evaluation involves fixation, dehydration, infiltration, embedding, and sectioning. The fixation reagents used in histology are very similar or identical to those used in cytology and have the same issues of preserving morphological features at the expense of molecular ones such as individual proteins. Time can be saved if the tissue sample is not fixed and dehydrated but instead is frozen and then sectioned while frozen. This is a more gentle processing procedure and can preserve more individual markers. However, freezing is not acceptable for long term storage of a tissue sample as subcellular information is lost due to the introduction of ice crystals. Ice in the frozen tissue sample also prevents the sectioning process from producing a very thin slice and thus some microscopic resolution and imaging of subcellular structures can be lost. In addition to formalin fixation, osmium tetroxide is used to fix and stain phospholipids (membranes).

[0194] Dehydration of tissues is accomplished with successive washes of increasing alcohol concentration. Clearing employs a material that is miscible with alcohol and the embedding material and involves a stepwise process starting at 50:50 alcohol:clearing reagent and then 100% clearing agent (xylene or xylene substitute). Infiltration involves incubating the tissue with a liquid form of the embedding agent (warm wax, nitrocellulose solution) first at 50:50 embedding agent: clearing agent and the 100% embedding agent. Embedding is completed by placing the tissue in a mold or cassette and filling with melted embedding agent such as wax, agar, or gelatin. The embedding agent is allowed to harden. The hardened tissue sample may then be sliced into thin section for staining and subsequent examination.

[0195] Prior to staining, the tissue section is dewaxed and rehydrated. Xylene is used to dewax the section, one or more changes of xylene may be used, and the tissue is rehydrated by

successive washes in alcohol of decreasing concentration. Prior to dewax, the tissue section may be heat immobilized to a glass slide at about 80° C. for about 20 minutes.

[0196] Laser capture micro-dissection allows the isolation of a subset of cells for further analysis from a tissue section.

[0197] As in cytology, to enhance the visualization of the microscopic features, the tissue section or slice can be stained with a variety of stains. A large menu of commercially available stains can be used to enhance or identify specific features.

[0198] To further increase the interaction of molecular reagents with cytological or histological samples, a number of techniques for "analyte retrieval" have been developed. The first such technique uses high temperature heating of a fixed sample. This method is also referred to as heat-induced epitope retrieval or HIER. A variety of heating techniques have been used, including steam heating, microwaving, autoclaving, water baths, and pressure cooking or a combination of these methods of heating. Analyte retrieval solutions include, for example, water, citrate, and normal saline buffers. The key to analyte retrieval is the time at high temperature but lower temperatures for longer times have also been successfully used. Another key to analyte retrieval is the pH of the heating solution. Low pH has been found to provide the best immunostaining but also gives rise to backgrounds that frequently require the use of a second tissue section as a negative control. The most consistent benefit (increased immunostaining without increase in background) is generally obtained with a high pH solution regardless of the buffer composition. The analyte retrieval process for a specific target is empirically optimized for the target using heat, time, pH, and buffer composition as variables for process optimization. Using the microwave analyte retrieval method allows for sequential staining of different targets with antibody reagents. But the time required to achieve antibody and enzyme complexes between staining steps has also been shown to degrade cell membrane analytes. Microwave heating methods have improved in situ hybridization methods as well.

[0199] To initiate the analyte retrieval process, the section is first dewaxed and hydrated. The slide is then placed in 10 mM sodium citrate buffer pH 6.0 in a dish or jar. A representative procedure uses an 1100 W microwave and microwaves the slide at 100% power for 2 minutes followed by microwaving the slides using 20% power for 18 minutes after checking to be sure the slide remains covered in liquid. The slide is then allowed to cool in the uncovered container and then rinsed with distilled water. HIER may be used in combination with an enzymatic digestion to improve the reactivity of the target to immunochemical reagents.

[0200] One such enzymatic digestion protocol uses proteinase K. A 20 μg/ml concentration of proteinase K is prepared in 50 mM Tris Base, 1 mM EDTA, 0.5% Triton X-100, pH 8.0 buffer. The process first involves dewaxing sections in 2 changes of xylene, 5 minutes each. Then the sample is hydrated in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each, and then rinsed in distilled water. Sections are covered with Proteinase K working solution and incubated 10-20 minutes at 37° C. in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation). The sections are cooled at room temperature for 10 minutes and then rinsed in PBS Tween 20 for 2×2 min. If desired, sections can be blocked to eliminate potential interference from endogenous compounds and enzymes. The section is then incubated with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight at 4° C. The section is then rinsed with PBS Tween 20 for 2×2 min. Additional blocking can be performed, if required for the specific application, followed by additional rinsing with PBS Tween 20 for 3×2 min and then finally the immunostaining protocol completed.

[0201] A simple treatment with 1% SDS at room temperature has also been demonstrated to improve immunohistochemical staining. Analyte retrieval methods have been applied to slide mounted sections as well as free floating sections. Another treatment option is to place the slide in a jar containing citric acid and 0.1 Nonident P40 at pH 6.0 and heating to 95° C. The slide is then washed with a buffer solution like PBS.

[0202] For immunological staining of tissues it may be useful to block non-specific association of the antibody with tissue proteins by soaking the section in a protein solution like serum or non-fat dry milk.

[0203] Blocking reactions may include the need to do any of the following, either alone or in combination: reduce the level of endogenous biotin; eliminate endogenous charge effects; inactivate endogenous nucleases; and inactivate endogenous enzymes like peroxidase and alkaline phosphatase. Endogenous nucleases may be inactivated by degradation with proteinase K, by heat treatment, use of a chelating agent such as EDTA or EGTA, the introduction of carrier DNA or RNA, treatment with a chaotrope such as urea, thiourea, guanidine hydrochloride, guanidine thiocyanate, lithium perchlorate, etc, or diethyl pyrocarbonate. Alkaline phosphatase may be inactivated by treated with 0.1N HCl for 5 minutes at room temperature or treatment with 1 mM levamisole. Peroxidase activity may be eliminated by treatment with 0.03% hydrogen peroxide. Endogenous biotin may be blocked by soaking the slide or section in an avidin (streptavidin, neutravidin may be substituted) solution for at least 15 minutes at room temperature. The slide or section is then washed for at least 10 minutes in buffer. This may be repeated at least three times. Then the slide or section is soaked in a biotin solution for 10 minutes. This may be repeated at least three times with a fresh biotin solution each time. The buffer wash procedure is repeated. Blocking protocols should be minimized to prevent damaging either the cell or tissue structure or the target or targets of interest but one or more of these protocols could be combined to "block" a slide or section prior to reaction with one or more slow off-rate aptamers. See Basic Medical Histology: the Biology of Cells, Tissues and Organs, authored by Richard G. Kessel, Oxford University Press, 1998.

Determination of Biomarker Values Using Mass Spectrometry Methods

[0204] A variety of configurations of mass spectrometers can be used to detect biomarker values. Several types of mass spectrometers are available or can be produced with various configurations. In general, a mass spectrometer has the following major components: a sample inlet, an ion source, a mass analyzer, a detector, a vacuum system, and instrument-control system, and a data system. Difference in the sample inlet, ion source, and mass analyzer generally define the type of instrument and its capabilities. For example, an inlet can be a capillary-column liquid chromatography source or can be a direct probe or stage such as used in matrix-assisted laser desorption. Common ion sources are, for example, electro-

spray, including nanospray and microspray or matrix-assisted laser desorption. Common mass analyzers include a quadrupole mass filter, ion trap mass analyzer and time-of-flight mass analyzer. Additional mass spectrometry methods are well known in the art (see Burlingame et al. Anal. Chem. 70:647 R-716R (1998); Kinter and Sherman, New York (2000)).

[0205] Protein biomarkers and biomarker values can be detected and measured by any of the following: electrospray ionization mass spectrometry (ESI-MS), ESI-MS/MS, ESI-MS/(MS)n, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), desorption/ionization on silicon (DIOS), secondary ion mass spectrometry (SIMS), quadrupole time-of-flight (Q-TOF), tandem time-of-flight (TOF/TOF) technology, called ultraflex III TOF/TOF, atmospheric pressure chemical ionization mass spectrometry (APCI-MS), APCI-MS/MS, APCI-$(MS)^N$, atmospheric pressure photoionization mass spectrometry (APPI-MS), APPI-MS/MS, and APPI-$(MS)^N$, quadrupole mass spectrometry, Fourier transform mass spectrometry (FTMS), quantitative mass spectrometry, and ion trap mass spectrometry.

[0206] Sample preparation strategies are used to label and enrich samples before mass spectroscopic characterization of protein biomarkers and determination biomarker values. Labeling methods include but are not limited to isobaric tag for relative and absolute quantitation (iTRAQ) and stable isotope labeling with amino acids in cell culture (SILAC). Capture reagents used to selectively enrich samples for candidate biomarker proteins prior to mass spectroscopic analysis include but are not limited to aptamers, antibodies, nucleic acid probes, chimeras, small molecules, an $F(ab')_2$ fragment, a single chain antibody fragment, an Fv fragment, a single chain Fv fragment, a nucleic acid, a lectin, a ligand-binding receptor, affybodies, nanobodies, ankyrins, domain antibodies, alternative antibody scaffolds (e.g. diabodies etc) imprinted polymers, avimers, peptidomimetics, peptoids, peptide nucleic acids, threose nucleic acid, a hormone receptor, a cytokine receptor, and synthetic receptors, and modifications and fragments of these.

[0207] The foregoing assays enable the detection of biomarker values that are useful in methods for diagnosing ovarian cancer, where the methods comprise detecting, in a biological sample from an individual, at least N biomarker values that each correspond to a biomarker selected from the group consisting of the biomarkers provided in Table 1, wherein a classification, as described in detail below, using the biomarker values indicates whether the individual has ovarian cancer. While certain of the described ovarian cancer biomarkers are useful alone for detecting and diagnosing ovarian cancer, methods are also described herein for the grouping of multiple subsets of the ovarian cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. In other embodiments, N is selected to be any number from 2-42 biomarkers. It will be appreciated that N can be selected to be any number from any of the above described ranges, as well as similar, but higher order, ranges. In accordance with any of the methods described herein, biomarker values can be detected and classified individually or they can be detected and classified collectively, as for example in a multiplex assay format.

[0208] In another aspect, methods are provided for detecting an absence of ovarian cancer, the methods comprising detecting, in a biological sample from an individual, at least N biomarker values that each correspond to a biomarker selected from the group consisting of the biomarkers provided in Table 1, wherein a classification, as described in detail below, of the biomarker values indicates an absence of ovarian cancer in the individual. While certain of the described ovarian cancer biomarkers are useful alone for detecting and diagnosing the absence of ovarian cancer, methods are also described herein for the grouping of multiple subsets of the ovarian cancer biomarkers that are each useful as a panel of three or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. In other embodiments, N is selected to be any number from 2-42 biomarkers. It will be appreciated that N can be selected to be any number from any of the above described ranges, as well as similar, but higher order, ranges. In accordance with any of the methods described herein, biomarker values can be detected and classified individually or they can be detected and classified collectively, as for example in a multiplex assay format.

Classification of Biomarkers and Calculation of Disease Scores

[0209] A biomarker "signature" for a given diagnostic test contains a set of markers, each marker having different levels in the populations of interest. Different levels, in this context, may refer to different means of the marker levels for the individuals in two or more groups, or different variances in the two or more groups, or a combination of both. For the simplest form of a diagnostic test, these markers can be used to assign an unknown sample from an individual into one of two groups, either diseased or not diseased. The assignment of a sample into one of two or more groups is known as classification, and the procedure used to accomplish this assignment is known as a classifier or a classification method. Classification methods may also be referred to as scoring methods. There are many classification methods that can be used to construct a diagnostic classifier from a set of biomarker values. In general, classification methods are most easily performed using supervised learning techniques where a data set is collected using samples obtained from individuals within two (or more, for multiple classification states) distinct groups one wishes to distinguish. Since the class (group or population) to which each sample belongs is known in advance for each sample, the classification method can be trained to give the desired classification response. It is also possible to use unsupervised learning techniques to produce a diagnostic classifier.

[0210] Common approaches for developing diagnostic classifiers include decision trees; bagging+boosting+forests; rule inference based learning; Parzen Windows; linear models; logistic; neural network methods; unsupervised clustering; K-means; hierarchical ascending/descending; semi-supervised learning; prototype methods; nearest neighbor; kernel density estimation; support vector machines; hidden Markov models; Boltzmann Learning; and classifiers may be combined either simply or in ways which minimize particular objective functions. For a review, see, e.g., Pattern Classification, R. O. Duda, et al., editors, John Wiley & Sons, 2nd edition, 2001; see also, The Elements of Statistical Learning—Data Mining, Inference, and Prediction, T. Hastie, et al.,

editors, Springer Science+Business Media, LLC, 2nd edition, 2009; each of which is incorporated by reference in its entirety.

[0211] To produce a classifier using supervised learning techniques, a set of samples called training data are obtained. In the context of diagnostic tests, training data includes samples from the distinct groups (classes) to which unknown samples will later be assigned. For example, samples collected from individuals in a control population and individuals in a particular disease population can constitute training data to develop a classifier that can classify unknown samples (or, more particularly, the individuals from whom the samples were obtained) as either having the disease or being free from the disease. The development of the classifier from the training data is known as training the classifier. Specific details on classifier training depend on the nature of the supervised learning technique. For purposes of illustration, an example of training a naïve Bayesian classifier will be described below (see, e.g., Pattern Classification, R. O. Duda, et al., editors, John Wiley & Sons, 2nd edition, 2001; see also, The Elements of Statistical Learning—Data Mining, Inference, and Prediction, T. Hastie, et al., editors, Springer Science+Business Media, LLC, 2nd edition, 2009).

[0212] Since typically there are many more potential biomarker values than samples in a training set, care must be used to avoid over-fitting. Over-fitting occurs when a statistical model describes random error or noise instead of the underlying relationship. Over-fitting can be avoided in a variety of way, including, for example, by limiting the number of markers used in developing the classifier, by assuming that the marker responses are independent of one another, by limiting the complexity of the underlying statistical model employed, and by ensuring that the underlying statistical model conforms to the data.

[0213] An illustrative example of the development of a diagnostic test using a set of biomarkers includes the application of a naïve Bayes classifier, a simple probabilistic classifier based on Bayes theorem with strict independent treatment of the biomarkers. Each biomarker is described by a class-dependent probability density function (pdf) for the measured RFU values or log RFU (relative fluorescence units) values in each class. The joint pdfs for the set of markers in one class is assumed to be the product of the individual class-dependent pdfs for each biomarker. Training a naïve Bayes classifier in this context amounts to assigning parameters ("parameterization") to characterize the class dependent pdfs. Any underlying model for the class-dependent pdfs may be used, but the model should generally conform to the data observed in the training set.

[0214] Specifically, the class-dependent probability of measuring a value x_i for biomarker i in the disease class is written as $p(x_i\backslash d)$ and the overall naïve Bayes probability of observing n markers with values $\underset{\sim}{x}=(x_1, x_2, \ldots x_n)$ is written as

$$p(\underset{\sim}{x}\,|\,d)=\prod_{i=1}^{n} p(x_i\,|\,d)$$

where the individual x_is are the measured biomarker levels in RFU or log RFU. The classification assignment for an unknown is facilitated by calculating the probability of being diseased $p(d\backslash\underset{\sim}{x})$ having measured $\underset{\sim}{x}$ compared to the probability of being disease free (control) $p(c\backslash\underset{\sim}{x})$ for the same measured values. The ratio of these probabilities is computed from the class-dependent pdfs by application of Bayes theorem, i.e.,

$$\frac{p(c\,|\,\underset{\sim}{x})}{p(d\,|\,\underset{\sim}{x})}=\frac{p(\underset{\sim}{x}\,|\,c)(1-P(d))}{p(\underset{\sim}{x}\,|\,d)P(d)}$$

where P(d) is the prevalence of the disease in the population appropriate to the test. Taking the logarithm of both sides of this ratio and substituting the naïve Bayes class-dependent probabilities from above gives

$$\ln\frac{p(c\,|\,\underset{\sim}{x})}{p(d\,|\,\underset{\sim}{x})}=\sum_{i=1}^{n}\ln\frac{p(x_i\,|\,c)}{p(x_i\,|\,d)}+\ln\frac{(1-P(d))}{P(d)}.$$

This form is known as the log likelihood ratio and simply states that the log likelihood of being free of the particular disease versus having the disease and is primarily composed of the sum of individual log likelihood ratios of the n individual biomarkers. In its simplest form, an unknown sample (or, more particularly, the individual from whom the sample was obtained) is classified as being free of the disease if the above ratio is greater than zero and having the disease if the ratio is less than zero.

[0215] In one exemplary embodiment, the class-dependent biomarker pdfs $p(x_i\backslash c)$ and $p(x_i\backslash d)$ are assumed to be normal or log-normal distributions in the measured RFU values x_i, i.e.

$$p(x_i\,|\,c)=\frac{1}{\sqrt{2\pi}\,\sigma_{c,i}}e^{-\frac{(x_i-\mu_{c,i})^2}{2\sigma_{c,i}^2}}$$

with a similar expression for $p(x_i\backslash d)$ with $\mu_{d,i}$ and $\sigma_{d,i}^2$. Parameterization of the model requires estimation of two parameters for each class-dependent pdf, a mean μ and a variance σ^2, from the training data. This may be accomplished in a number of ways, including, for example, by maximum likelihood estimates, by least-squares, and by any other methods known to one skilled in the art. Substituting the normal distributions for $p(x_i\backslash c)$ and $p(x_i\backslash d)$ into the log-likelihood ratio defined above gives the following expression:

$$\ln\frac{p(c\,|\,\underset{\sim}{x})}{p(d\,|\,\underset{\sim}{x})}=\sum_{i=1}^{n}\ln\frac{\sigma_{d,i}}{\sigma_{c,i}}-\frac{1}{2}\sum_{i=1}^{n}\left[\left(\frac{x_i-\mu_{c,i}}{\sigma_{c,i}}\right)^2-\left(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}}\right)^2\right]+\ln\frac{(1-P(d))}{P(d)}.$$

Once a set of μs and σ^2s have been defined for each pdf in each class from the training data and the disease prevalence in the population is specified, the Bayes classifier is fully determined and may be used to classify unknown samples with measured values $\underset{\sim}{x}$.

[0216] The performance of the naïve Bayes classifier is dependent upon the number and quality of the biomarkers used to construct and train the classifier. A single biomarker

will perform in accordance with its KS-distance (Kolmogorov-Smirnov), as defined in Example 3, below. If a classifier performance metric is defined as the sum of the sensitivity (fraction of true positives, f_{TP}) and specificity (one minus the fraction of false positives, $1-f_{FP}$), a perfect classifier will have a score of two and a random classifier, on average, will have a score of one. Using the definition of the KS-distance, that value x* which maximizes the difference in the cdf functions can be found by solving

$$\frac{\partial KS}{\partial x} = \frac{\partial (cdf_c(x) - cdf_d(x))}{\partial x} = 0$$

for x which leads to p(x*\c)=p(x*\d), i.e, the KS distance occurs where the class-dependent pdfs cross. Substituting this value of x* into the expression for the KS-distance yields the following definition for KS

$$\begin{aligned} KS &= cdf_c(x^*) - cdf_d(x^*) \\ &= \int_{-\infty}^{x^*} p(x \mid c)\,dx - \int_{-\infty}^{x^*} p(x \mid d)\,dx \\ &= 1 - \int_{x^*}^{-\infty} p(x \mid c)\,dx - \int_{-\infty}^{x^*} p(x \mid d)\,dx \\ &= 1 - f_{FP} - f_{FN}, \end{aligned}$$

the KS distance is one minus the total fraction of errors using a test with a cut-off at x*, essentially a single analyte Bayesian classifier. Since we define a score of sensitivity+specificity=$2-f_{FP}-f_{FN}$, combining the above definition of the KS-distance we see that sensitivity+specificity=1+KS. We select biomarkers with a statistic that is inherently suited for building naïve Bayes classifiers.

[0217] The addition of subsequent markers with good KS distances (>0.3, for example) will, in general, improve the classification performance if the subsequently added markers are independent of the first marker. Using the sensitivity plus specificity as a classifier score, it is straightforward to generate many high scoring classifiers with a variation of a greedy algorithm. (A greedy algorithm is any algorithm that follows the problem solving metaheuristic of making the locally optimal choice at each stage with the hope of finding the global optimum.)

[0218] The algorithm approach used here is described in detail in Example 4. Briefly, all single analyte classifiers are generated from a table of potential biomarkers and added to a list. Next, all possible additions of a second analyte to each of the stored single analyte classifiers is then performed, saving a predetermined number of the best scoring pairs, say, for example, a thousand, on a new list. All possible three-marker classifiers are explored using this new list of the best two-marker classifiers, again saving the best thousand of these. This process continues until the score either plateaus or begins to deteriorate as additional markers are added. Those high scoring classifiers that remain after convergence can be evaluated for the desired performance for an intended use. For example, in one diagnostic application, classifiers with a high sensitivity and modest specificity may be more desirable than modest sensitivity and high specificity. In another diagnostic application, classifiers with a high specificity and a modest sensitivity may be more desirable. The desired level of performance is generally selected based upon a trade-off that must be made between the number of false positives and false negatives that can each be tolerated for the particular diagnostic application. Such trade-offs generally depend on the medical consequences of an error, either false positive or false negative.

[0219] Various other techniques are known in the art and may be employed to generate many potential classifiers from a list of biomarkers using a naïve Bayes classifier. In one embodiment, what is referred to as a genetic algorithm can be used to combine different markers using the fitness score as defined above. Genetic algorithms are particularly well suited to exploring a large diverse population of potential classifiers. In another embodiment, so-called ant colony optimization can be used to generate sets of classifiers. Other strategies that are known in the art can also be employed, including, for example, other evolutionary strategies as well as simulated annealing and other stochastic search methods. Metaheuristic methods, such as, for example, harmony search may also be employed.

[0220] Exemplary embodiments use any number of the ovarian cancer biomarkers listed in Table 1 in various combinations to produce diagnostic tests for detecting ovarian cancer (see Example 2 for a detailed description of how these biomarkers were identified). In one embodiment, a method for diagnosing ovarian cancer uses a naïve Bayes classification method in conjunction with any number of the ovarian cancer biomarkers listed in Table 1. In an illustrative example (see Example 3), the simplest test for detecting ovarian cancer from a population of women with pelvic masses can be constructed using a single biomarker, for example, BAFF Receptor which is down-regulated in ovarian cancer with a KS-distance of 0.39 (1+KS=1.39). Using the parameters $\mu_{c,i}$, $\sigma_{c,i}$, $\mu_{d,i}$ and $\sigma_{d,i}$ for BAFF Receptor from Table 16 and the equation for the log-likelihood described above, a diagnostic test with a sensitivity of 0.74 and specificity of 0.56 (sensitivity+specificity=1.31) can be produced, see Table 17. The ROC curve for this test is displayed in FIG. **2** and has an AUC of 0.70.

[0221] Addition of biomarker RGM-C, for example, with a KS-distance of 0.43, significantly improves the classifier performance to a sensitivity of 82% and specificity of 0.73% (sensitivity+specificity=1.51) and an AUC=0.81. Note that the score for a classifier constructed of two biomarkers is not a simple sum of the KS-distances; KS-distances are not additive when combining biomarkers, and it takes many more weak markers to achieve the same level of performance as a strong marker. Adding a third marker, HGF, for example, boosts the classifier performance to 83% sensitivity and 74% specificity and AUC=0.84. Adding additional biomarkers, such as, for example, SLPI, C9, α2-Antiplasmin, SAP, MMP-7, MCP-3, and HSP90α, produces a series of ovarian cancer tests summarized in Table 17 and displayed as a series of ROC curves in FIG. **3**. The score of the classifiers as a function of the number of analytes used in classifier construction is shown in FIG. **4**. This exemplary ten-marker classifier has a sensitivity of 97% and a specificity of 88% with an AUC of 0.94.

[0222] The markers listed in Table 1 can be combined in many ways to produce classifiers for diagnosing ovarian cancer. In some embodiments, panels of biomarkers are comprised of different numbers of analytes depending on a specific diagnostic performance criterion that is selected. For

example, certain combinations of biomarkers will produce tests that are more sensitive (or more specific) than other combinations.

[0223] Once a panel is defined to include a particular set of biomarkers from Table 1 and a classifier is constructed from a set of training data, the definition of the diagnostic test is complete. In one embodiment, the procedure used to classify an unknown sample is outlined in FIG. **1**A. In another embodiment the procedure used to classify an unknown sample is outlined in FIG. **1**B. The biological sample is appropriately diluted and then run in one or more assays to produce the relevant quantitative biomarker levels used for classification. The measured biomarker levels are used as input for the classification method that outputs a classification and an optional score for the sample that reflects the confidence of the class assignment.

[0224] Table 1 identifies 42 biomarkers that are useful for diagnosing ovarian cancer. This is a surprisingly larger number than expected when compared to what is typically found during biomarker discovery efforts and may be attributable to the scale of the described study, which encompassed over 800 proteins measured in hundreds of individual samples, in some cases at concentrations in the low femtomolar range. Presumably, the large number of discovered biomarkers reflects the diverse biochemical pathways implicated in both tumor biology and the body's response to the tumor's presence; each pathway and process involves many proteins. The results show that no single protein of a small group of proteins is uniquely informative about such complex processes; rather, that multiple proteins are involved in relevant processes, such as apoptosis or extracellular matrix repair, for example.

[0225] Given the numerous biomarkers identified during the described study, one would expect to be able to derive large numbers of high-performing classifiers that can be used in various diagnostic methods. To test this notion, tens of thousands of classifiers were evaluated using the biomarkers in Table 1. As described in Example 4, many subsets of the biomarkers presented in Table 1 can be combined to generate useful classifiers. By way of example, descriptions are provided for classifiers containing 1, 2, and 3 biomarkers for the diagnosis of ovarian cancer, particularly, the diagnosis of ovarian cancer in individuals who have a pelvic mass that is detectable by CT. As described in Example 4, all classifiers that were built using the biomarkers in Table 1 perform distinctly better than classifiers that were built using "non-markers".

[0226] The performance of ten-marker classifiers obtained by excluding the "best" individual markers from the ten-marker aggregation was tested. As described in Example 4, Part 3, classifiers constructed without the "best" markers in Table 1 performed well. Many subsets of the biomarkers listed in Table 1 performed close to optimally, even after removing the top 15 of the markers listed in the Table. This implies that the performance characteristics of any particular classifier are likely not due to some small core group of biomarkers and that the disease process likely impacts numerous biochemical pathways, which alters the expression level of many proteins.

[0227] The results from Example 4 suggest certain possible conclusions: First, the identification of a large number of biomarkers enables their aggregation into a vast number of classifiers that offer similarly high performance. Second, classifiers can be constructed such that particular biomarkers may be substituted for other biomarkers in a manner that reflects the redundancies that undoubtedly pervade the complexities of the underlying disease processes. That is to say, the information about the disease contributed by any individual biomarker identified in Table 1 overlaps with the information contributed by other biomarkers, such that it may be that no particular biomarker or small group of biomarkers in Table 1 must be included in any classifier.

[0228] Exemplary embodiments use naïve Bayes classifiers constructed from the data in Table 18 to classify an unknown sample. The procedure is outlined in FIGS. **1**A and B. In one embodiment, the biological sample is optionally diluted and run in a multiplexed aptamer assay. The data from the assay are normalized and calibrated as outlined in Example 3, and the resulting biomarker levels are used as input to a Bayes classification scheme. The log-likelihood ratio is computed for each measured biomarker individually and then summed to produce a final classification score, which is also referred to as a diagnostic score. The resulting assignment as well as the overall classification score can be reported. Optionally, the individual log-likelihood risk factors computed for each biomarker level can be reported as well. The details of the classification score calculation are presented in Example 3.

Kits

[0229] Any combination of the biomarkers of Table 1 (as well as additional biomedical information) can be detected using a suitable kit, such as for use in performing the methods disclosed herein. Furthermore, any kit can contain one or more detectable labels as described herein, such as a fluorescent moiety, etc.

[0230] In one embodiment, a kit includes (a) one or more capture reagents (such as, for example, at least one aptamer or antibody) for detecting one or more biomarkers in a biological sample, wherein the biomarkers include any of the biomarkers set forth in Table 1, and optionally (b) one or more software or computer program products for classifying the individual from whom the biological sample was obtained as either having or not having ovarian cancer or for determining the likelihood that the individual has ovarian cancer, as further described herein. Alternatively, rather than one or more computer program products, one or more instructions for manually performing the above steps by a human can be provided.

[0231] The combination of a solid support with a corresponding capture reagent and a signal generating material is referred to herein as a "detection device" or "kit". The kit can also include instructions for using the devices and reagents, handling the sample, and analyzing the data. Further the kit may be used with a computer system or software to analyze and report the result of the analysis of the biological sample.

[0232] The kits can also contain one or more reagents (e.g., solubilization buffers, detergents, washes, or buffers) for processing a biological sample. Any of the kits described herein can also include, e.g., buffers, blocking agents, mass spectrometry matrix materials, antibody capture agents, positive control samples, negative control samples, software and information such as protocols, guidance and reference data.

[0233] In one aspect, the invention provides kits for the analysis of ovarian cancer status. The kits include PCR primers for one or more biomarkers selected from Table 1. The kit may further include instructions for use and correlation of the biomarkers with ovarian cancer. The kit may also include any of the following, either alone or in combination: a DNA array

containing the complement of one or more of the biomarkers selected from Table 1, reagents, and enzymes for amplifying or isolating sample DNA. The kits may include reagents for real-time PCR, such as, for example, TaqMan probes and/or primers, and enzymes.

[0234] For example, a kit can comprise (a) reagents comprising at least capture reagent for quantifying one or more biomarkers in a test sample, wherein said biomarkers comprise the biomarkers set forth in Table 1, or any other biomarkers or biomarkers panels described herein, and optionally (b) one or more algorithms or computer programs for performing the steps of comparing the amount of each biomarker quantified in the test sample to one or more predetermined cutoffs and assigning a score for each biomarker quantified based on said comparison, combining the assigned scores for each biomarker quantified to obtain a total score, comparing the total score with a predetermined score, and using said comparison to determine whether an individual has ovarian cancer. Alternatively, rather than one or more algorithms or computer programs, one or more instructions for manually performing the above steps by a human can be provided.

Computer Methods and Software

[0235] Once a biomarker or biomarker panel is selected, a method for diagnosing an individual can comprise the following: 1) collect or otherwise obtain a biological sample; 2) perform an analytical method to detect and measure the biomarker or biomarkers in the panel in the biological sample; 3) perform any data normalization or standardization required for the method used to collect biomarker values; 4) calculate the marker score; 5) combine the marker scores to obtain a total diagnostic score; and 6) report the individual's diagnostic score. In this approach, the diagnostic score may be a single number determined from the sum of all the marker calculations that is compared to a preset threshold value that is an indication of the presence or absence of disease. Or the diagnostic score may be a series of bars that each represent a biomarker value and the pattern of the responses may be compared to a pre-set pattern for determination of the presence or absence of disease.

[0236] At least some embodiments of the methods described herein can be implemented with the use of a computer. An example of a computer system **100** is shown in FIG. **6**. With reference to FIG. **6**, system **100** is shown comprised of hardware elements that are electrically coupled via bus **108**, including a processor **101**, input device **102**, output device **103**, storage device **104**, computer-readable storage media reader **105***a*, communications system **106** processing acceleration (e.g., DSP or special-purpose processors) **107** and memory **109**. Computer-readable storage media reader **105***a* is further coupled to computer-readable storage media **105***b*, the combination comprehensively representing remote, local, fixed and/or removable storage devices plus storage media, memory, etc. for temporarily and/or more permanently containing computer-readable information, which can include storage device **104**, memory **109** and/or any other such accessible system **100** resource. System **100** also comprises software elements (shown as being currently located within working memory **191**) including an operating system **192** and other code **193**, such as programs, data and the like.

[0237] With respect to FIG. **6**, system **100** has extensive flexibility and configurability. Thus, for example, a single architecture might be utilized to implement one or more servers that can be further configured in accordance with currently desirable protocols, protocol variations, extensions, etc. However, it will be apparent to those skilled in the art that embodiments may well be utilized in accordance with more specific application requirements. For example, one or more system elements might be implemented as sub-elements within a system **100** component (e.g., within communications system **106**). Customized hardware might also be utilized and/or particular elements might be implemented in hardware, software or both. Further, while connection to other computing devices such as network input/output devices (not shown) may be employed, it is to be understood that wired, wireless, modem, and/or other connection or connections to other computing devices might also be utilized.

[0238] In one aspect, the system can comprise a database containing features of biomarkers characteristic of ovarian cancer. The biomarker data (or biomarker information) can be utilized as an input to the computer for use as part of a computer implemented method. The biomarker data can include the data as described herein.

[0239] In one aspect, the system further comprises one or more devices for providing input data to the one or more processors.

[0240] The system further comprises a memory for storing a data set of ranked data elements.

[0241] In another aspect, the device for providing input data comprises a detector for detecting the characteristic of the data element, e.g., such as a mass spectrometer or gene chip reader.

[0242] The system additionally may comprise a database management system. User requests or queries can be formatted in an appropriate language understood by the database management system that processes the query to extract the relevant information from the database of training sets.

[0243] The system may be connectable to a network to which a network server and one or more clients are connected. The network may be a local area network (LAN) or a wide area network (WAN), as is known in the art. Preferably, the server includes the hardware necessary for running computer program products (e.g., software) to access database data for processing user requests.

[0244] The system may include an operating system (e.g., UNIX or Linux) for executing instructions from a database management system. In one aspect, the operating system can operate on a global communications network, such as the internet, and utilize a global communications network server to connect to such a network.

[0245] The system may include one or more devices that comprise a graphical display interface comprising interface elements such as buttons, pull down menus, scroll bars, fields for entering text, and the like as are routinely found in graphical user interfaces known in the art. Requests entered on a user interface can be transmitted to an application program in the system for formatting to search for relevant information in one or more of the system databases. Requests or queries entered by a user may be constructed in any suitable database language.

[0246] The graphical user interface may be generated by a graphical user interface code as part of the operating system and can be used to input data and/or to display inputted data. The result of processed data can be displayed in the interface, printed on a printer in communication with the system, saved in a memory device, and/or transmitted over the network or can be provided in the form of the computer readable medium.

[0247] The system can be in communication with an input device for providing data regarding data elements to the system (e.g., expression values). In one aspect, the input device can include a gene expression profiling system including, e.g., a mass spectrometer, gene chip or array reader, and the like.

[0248] The methods and apparatus for analyzing ovarian cancer biomarker information according to various embodiments may be implemented in any suitable manner, for example, using a computer program operating on a computer system. A conventional computer system comprising a processor and a random access memory, such as a remotely-accessible application server, network server, personal computer or workstation may be used. Additional computer system components may include memory devices or information storage systems, such as a mass storage system and a user interface, for example a conventional monitor, keyboard and tracking device. The computer system may be a stand-alone system or part of a network of computers including a server and one or more databases.

[0249] The ovarian cancer biomarker analysis system can provide functions and operations to complete data analysis, such as data gathering, processing, analysis, reporting and/or diagnosis. For example, in one embodiment, the computer system can execute the computer program that may receive, store, search, analyze, and report information relating to the ovarian cancer biomarkers. The computer program may comprise multiple modules performing various functions or operations, such as a processing module for processing raw data and generating supplemental data and an analysis module for analyzing raw data and supplemental data to generate an ovarian cancer status and/or diagnosis. Diagnosing ovarian cancer status may comprise generating or collecting any other information, including additional biomedical information, regarding the condition of the individual relative to the disease, identifying whether further tests may be desirable, or otherwise evaluating the health status of the individual.

[0250] Referring now to FIG. **7**, an example of a method of utilizing a computer in accordance with principles of a disclosed embodiment can be seen. In FIG. **7**, a flowchart **3000** is shown. In block **3004**, biomarker information can be retrieved for an individual. The biomarker information can be retrieved from a computer database, for example, after testing of the individual's biological sample is performed. The biomarker information can comprise biomarker values that each correspond to one of at least N biomarkers selected from a group consisting of the biomarkers provided in Table 1, wherein N=2-42. In block **3008**, a computer can be utilized to classify each of the biomarker values. And, in block **3012**, a determination can be made as to the likelihood that an individual has ovarian cancer based upon a plurality of classifications. The indication can be output to a display or other indicating device so that it is viewable by a person. Thus, for example, it can be displayed on a display screen of a computer or other output device.

[0251] Referring now to FIG. **8**, an alternative method of utilizing a computer in accordance with another embodiment can be illustrated via flowchart **3200**. In block **3204**, a computer can be utilized to retrieve biomarker information for an individual. The biomarker information comprises a biomarker value corresponding to a biomarker selected from the group of biomarkers provided in Table 1. In block **3208**, a classification of the biomarker value can be performed with the computer. And, in block **3212**, an indication can be made as to the likelihood that the individual has ovarian cancer based upon the classification. The indication can be output to a display or other indicating device so that it is viewable by a person. Thus, for example, it can be displayed on a display screen of a computer or other output device.

[0252] Some embodiments described herein can be implemented so as to include a computer program product. A computer program product may include a computer readable medium having computer readable program code embodied in the medium for causing an application program to execute on a computer with a database.

[0253] As used herein, a "computer program product" refers to an organized set of instructions in the form of natural or programming language statements that are contained on a physical media of any nature (e.g., written, electronic, magnetic, optical or otherwise) and that may be used with a computer or other automated data processing system. Such programming language statements, when executed by a computer or data processing system, cause the computer or data processing system to act in accordance with the particular content of the statements. Computer program products include without limitation: programs in source and object code and/or test or data libraries embedded in a computer readable medium. Furthermore, the computer program product that enables a computer system or data processing equipment device to act in pre-selected ways may be provided in a number of forms, including, but not limited to, original source code, assembly code, object code, machine language, encrypted or compressed versions of the foregoing and any and all equivalents.

[0254] In one aspect, a computer program product is provided for indicating a likelihood of ovarian cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers in the biological sample selected from the group of biomarkers provided in Table 1, wherein N=2-42; and code that executes a classification method that indicates an ovarian disease status of the individual as a function of the biomarker values.

[0255] In still another aspect, a computer program product is provided for indicating a likelihood of ovarian cancer. The computer program product includes a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising: code that retrieves data attributed to a biological sample from an individual, wherein the data comprises a biomarker value corresponding to a biomarker in the biological sample selected from the group of biomarkers provided in Table 1; and code that executes a classification method that indicates an ovarian disease status of the individual as a function of the biomarker value.

[0256] While various embodiments have been described as methods or apparatuses, it should be understood that embodiments can be implemented through code coupled with a computer, e.g., code resident on a computer or accessible by the computer. For example, software and databases could be utilized to implement many of the methods discussed above. Thus, in addition to embodiments accomplished by hardware, it is also noted that these embodiments can be accomplished through the use of an article of manufacture comprised of a computer usable medium having a computer readable pro-

gram code embodied therein, which causes the enablement of the functions disclosed in this description. Therefore, it is desired that embodiments also be considered protected by this patent in their program code means as well. Furthermore, the embodiments may be embodied as code stored in a computer-readable memory of virtually any kind including, without limitation, RAM, ROM, magnetic media, optical media, or magneto-optical media. Even more generally, the embodiments could be implemented in software, or in hardware, or any combination thereof including, but not limited to, software running on a general purpose processor, microcode, PLAs, or ASICs.

[0257] It is also envisioned that embodiments could be accomplished as computer signals embodied in a carrier wave, as well as signals (e.g., electrical and optical) propagated through a transmission medium. Thus, the various types of information discussed above could be formatted in a structure, such as a data structure, and transmitted as an electrical signal through a transmission medium or stored on a computer readable medium.

[0258] It is also noted that many of the structures, materials, and acts recited herein can be recited as means for performing a function or step for performing a function. Therefore, it should be understood that such language is entitled to cover all such structures, materials, or acts disclosed within this specification and their equivalents, including the matter incorporated by reference.

EXAMPLES

[0259] The following examples are provided for illustrative purposes only and are not intended to limit the scope of the application as defined by the appended claims. All examples described herein were carried out using standard techniques, which are well known and routine to those of skill in the art. Routine molecular biology techniques described in the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (2001).

Example 1

Multiplexed Aptamer Analysis of Samples for Ovarian Cancer Biomarker Selection

[0260] This example describes the multiplex aptamer assay used to analyze the samples and controls for the identification of the biomarkers set forth in Table 1 (see FIG. **9**). In this case, the multiplexed analysis utilized 811 aptamers, each unique to a specific target.

[0261] In this method, pipette tips were changed for each solution addition.

[0262] Also, unless otherwise indicated, most solution transfers and wash additions used the 96-well head of a Beckman Biomek Fx^P. Method steps manually pipetted used a twelve channel P200 Pipetteman (Rainin Instruments, LLC, Oakland, Calif.), unless otherwise indicated. A custom buffer referred to as SB17 was prepared in-house, comprising 40 mM HEPES, 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA at pH7.5. All steps were performed at room temperature unless otherwise indicated.

[0263] 1. Preparation of Aptamer Stock Solution

[0264] For aptamers without a photo-cleavable biotin linker, custom stock aptamer solutions for 10%, 1% and 0.03% plasma were prepared at 8× concentration in 1×SB17, 0.05% Tween-20 with appropriate photo-cleavable, biotinylated primers, where the resultant primer concentration was 3 times the relevant aptamer concentration. The primers hybridized to all or part of the corresponding aptamer.

[0265] Each of the 3, 8× aptamer solutions were diluted separately 1:4 into 1×SB17, 0.05% Tween-20 (1500 μL of 8× stock into 4500 μL of 1×SB17, 0.05% Tween-20) to achieve a 2× concentration. Each diluted aptamer master mix was then split, 1500 μL each, into 4, 2 mL screw cap tubes and brought to 95° C. for 5 minutes, followed by a 37° C. incubation for 15 minutes. After incubation, the 4, 2 mL tubes corresponding to a particular aptamer master mix were combined into a reagent trough, and 55 μL of a 2× aptamer mix (for all three mixes) was manually pipetted into a 96-well Hybaid plate and the plate foil sealed. The final result was 3, 96-well, foil-sealed Hybaid plates. The individual aptamer concentration was 0.5 nM.

[0266] 2. Assay Sample Preparation

[0267] Frozen aliquots of 100% plasma, stored at −80° C., were placed in 25° C. water bath for 10 minutes. Thawed samples were placed on ice, gently vortexed (set on 4) for 8 seconds and then replaced on ice.

[0268] A 20% sample solution was prepared by transferring 16 μL of sample using a 50 μL 8-channel spanning pipettor into 96-well Hybaid plates, each well containing 64 μL of the appropriate sample diluent at 4° C. (0.8×SB17, 0.05% Tween-20, 2 μM Z-block_2, 0.6 mM $MgCl_2$ for plasma). This plate was stored on ice until the next sample dilution steps were initiated.

[0269] To commence sample and aptamer equilibration, the 20% sample plate was briefly centrifuged and placed on the Beckman FX where it was mixed by pipetting up and down with the 96-well pipettor. A 2% sample was then prepared by diluting 10 μL of the 20% sample into 90 μL of 1×SB17, 0.05% Tween-20. Next, dilution of 6 μL of the resultant 2% sample into 194 μL of 1×SB17, 0.05% Tween-20 made a 0.06% sample plate. Dilutions were done on the Beckman Biomek Fx^P. After each transfer, the solutions were mixed by pipetting up and down. The 3 sample dilution plates were then transferred to their respective aptamer solutions by adding 55 μL of the sample to 55 μL of the appropriate 2× aptamer mix. The sample and aptamer solutions were mixed on the robot by pipetting up and down.

[0270] 3. Sample Equilibration Binding

[0271] The sample/aptamer plates were foil sealed and placed into a 37° C. incubator for 3.5 hours before proceeding to the Catch 1 step.

[0272] 4. Preparation of Catch 2 Bead Plate

[0273] An 11 mL aliquot of MyOne (Invitrogen Corp., Carlsbad, Calif.) Streptavidin C1 beads was washed 2 times with equal volumes of 20 mM NaOH (5 minute incubation for each wash), 3 times with equal volumes of 1×SB17, 0.05% Tween-20 and resuspended in 11 mL 1×SB17, 0.05% Tween-20. Using a 12-span multichannel pipettor, 50 μL of this solution was manually pipetted into each well of a 96-well Hybaid plate. The plate was then covered with foil and stored at 4° C. for use in the assay.

[0274] 5. Preparation of Catch 1 Bead Plates

[0275] Three 0.45 μm Millipore HV plates (Durapore membrane, Cat# MAHVN4550) were equilibrated with 100 μL of 1×SB17, 0.05% Tween-20 for at least 10 minutes. The equilibration buffer was then filtered through the plate and 133.3 μL of a 7.5% Streptavidin-agarose bead slurry (in 1×SB17, 0.05% Tween-20) was added into each well. To keep

the streptavidin-agarose beads suspended while transferring them into the filter plate, the bead solution was manually mixed with a 200 μL, 12-channel pipettor, 15 times. After the beads were distributed across the 3 filter plates, a vacuum was applied to remove the bead supernatant. Finally, the beads were washed in the filter plates with 200 μL 1×SB17, 0.05% Tween-20 and then resuspended in 200 μL 1×SB17, 0.05% Tween-20. The bottoms of the filter plates were blotted and the plates stored for use in the assay.

[0276] 6. Loading the Cytomat

[0277] The cytomat was loaded with all tips, plates, all reagents in troughs (except NHS-biotin reagent which was prepared fresh right before addition to the plates), 3 prepared catch 1 filter plates and 1 prepared MyOne plate.

[0278] 7. Catch 1

[0279] After a 3.5 hour equilibration time, the sample/aptamer plates were removed from the incubator, centrifuged for about 1 minute, foil removed, and placed on the deck of the Beckman Biomek Fx^P. The Beckman Biomek Fx^P program was initiated. All subsequent steps in Catch 1 were performed by the Beckman Biomek Fx^P robot unless otherwise noted. Within the program, the vacuum was applied to the Catch 1 filter plates to remove the bead supernatant. One hundred microlitres of each of the 10%, 1% and 0.03% equilibration binding reactions were added to their respective Catch 1 filtration plates, and each plate was mixed using an on-deck orbital shaker at 800 rpm for 10 minutes.

[0280] Unbound solution was removed via vacuum filtration. The catch 1 beads were washed with 190 μL of 100 μM biotin in 1×SB17, 0.05% Tween-20 followed by 190 μL of 1×SB17, 0.05% Tween-20 by dispensing the solution and immediately drawing a vacuum to filter the solution through the plate.

[0281] Next, 190 μL 1×SB17, 0.05% Tween-20 was added to the Catch 1 plates. Plates were blotted to remove droplets using an on-deck blot station and then incubated with orbital shakers at 800 rpm for 10 minutes at 25° C.

[0282] The robot removed this wash via vacuum filtration and blotted the bottom of the filter plate to remove droplets using the on-deck blot station.

[0283] 8. Tagging

[0284] A NHS-PEO4-biotin aliquot was thawed at 37° C. for 6 minutes and then diluted 1:100 with tagging buffer (SB17 at pH=7.25 0.05% Tween-20). The NHS-PEO4-biotin reagent was dissolved at 100 mM concentration in anhydrous DMSO and had been stored frozen at −20° C. Upon a robot prompt, the diluted NHS-PEO4-biotin reagent was manually added to an on-deck trough and the robot program was manually re-initiated to dispense 100 μL of the NHS-PEO4-biotin into each well of each Catch 1 filter plate. This solution was allowed to incubate with Catch 1 beads shaking at 800 rpm for 5 minutes on the obital shakers.

[0285] 9. Kinetic Challenge and Photo-Cleavage

[0286] The tagging reaction was quenched by the addition of 150 μL of 20 mM glycine in 1×SB17, 0.05% Tween-20 to the Catch 1 plates while still containing the NHS tag. The plates were then incubated for 1 minute on orbital shakers at 800 rpm. The NHS-tag/glycine solution was removed via vacuum filtration. Next, 190 μL 20 mM glycine (1×SB17, 0.05% Tween-20) was added to each plate and incubated for 1 minute on orbital shakers at 800 rpm before removal by vacuum filtration.

[0287] 190 μL of 1×SB17, 0.05% Tween-20 was added to each plate and removed by vacuum filtration.

[0288] The wells of the Catch 1 plates were subsequently washed three times by adding 190 μL 1×SB17, 0.05% Tween-20, placing the plates on orbital shakers for 1 minute at 800 rpm followed by vacuum filtration. After the last wash the plates were placed on top of a 1 mL deep-well plate and removed from the deck. The Catch 1 plates were centrifuged at 1000 rpm for 1 minute to remove as much extraneous volume from the agarose beads before elution as possible.

[0289] The plates were placed back onto the Beckman Biomek Fx^P and 85 μL of 10 mM $DxSO_4$ in 1×SB17, 0.05% Tween-20 was added to each well of the filter plates.

[0290] The filter plates were removed from the deck, placed onto a Variomag Thermoshaker (Thermo Fisher Scientific, Inc., Waltham, Mass.) under the BlackRay (Ted Pella, Inc., Redding, Calif.) light sources, and irradiated for 10 minutes while shaking at 800 rpm.

[0291] The photocleaved solutions were sequentially eluted from each Catch 1 plate into a common deep well plate by first placing the 10% Catch 1 filter plate on top of a 1 mL deep-well plate and centrifuging at 1000 rpm for 1 minute. The 1% and 0.03% catch 1 plates were then sequentially centrifuged into the same deep well plate.

[0292] 10. Catch 2 Bead Capture

[0293] The 1 mL deep well block containing the combined eluates of catch 1 was placed on the deck of the Beckman Biomek Fx^P for catch 2.

[0294] The robot transferred all of the photo-cleaved eluate from the 1 mL deep-well plate onto the Hybaid plate containing the previously prepared catch 2 MyOne magnetic beads (after removal of the MyOne buffer via magnetic separation).

[0295] The solution was incubated while shaking at 1350 rpm for 5 minutes at 25° C. on a Variomag Thermoshaker (Thermo Fisher Scientific, Inc., Waltham, Mass.).

[0296] The robot transferred the plate to the on deck magnetic separator station. The plate was incubated on the magnet for 90 seconds before removal and discarding of the supernatant.

[0297] 11. 37° C. 30% Glycerol Washes

[0298] The catch 2 plate was moved to the on-deck thermal shaker and 75 μL of 1×SB17, 0.05% Tween-20 was transferred to each well. The plate was mixed for 1 minute at 1350 rpm and 37° C. to resuspend and warm the beads. To each well of the catch 2 plate, 75 μL of 60% glycerol at 37° C. was transferred and the plate continued to mix for another minute at 1350 rpm and 37° C. The robot transferred the plate to the 37° C. magnetic separator where it was incubated on the magnet for 2 minutes and then the robot removed and discarded the supernatant. These washes were repeated two more times.

[0299] After removal of the third 30% glycerol wash from the catch 2 beads, 150 μL of 1×SB17, 0.05% Tween-20 was added to each well and incubated at 37° C., shaking at 1350 rpm for 1 minute, before removal by magnetic separation on the 37° C. magnet.

[0300] The catch 2 beads were washed a final time using 150 μL 1×SB19, 0.05% Tween-20 with incubation for 1 minute while shaking at 1350 rpm, prior to magnetic separation.

[0301] 12. Catch 2 Bead Elution and Neutralization

[0302] The aptamers were eluted from catch 2 beads by adding 105 μL of 100 mM CAPSO with 1 M NaCl, 0.05% Tween-20 to each well. The beads were incubated with this solution with shaking at 1300 rpm for 5 minutes.

[0303] The catch 2 plate was then placed onto the magnetic separator for 90 seconds prior to transferring 90 μL of the eluate to a new 96-well plate containing 10 μL of 500 mM HCl, 500 mM HEPES, 0.05% Tween-20 in each well. After transfer, the solution was mixed robotically by pipetting 90 μL up and down five times.

[0304] 13. Hybridization

[0305] The Beckman Biomek Fx^P transferred 20 μL of the neutralized catch 2 eluate to a fresh Hybaid plate, and 5 μL of 10× Agilent Block, containing a 10× spike of hybridization controls, was added to each well. Next, 25 μL of 2× Agilent Hybridization buffer was manually pipetted to the each well of the plate containing the neutralized samples and blocking buffer and the solution was mixed by manually pipetting 25 μL up and down 15 times slowly to avoid extensive bubble formation. The plate was spun at 1000 rpm for 1 minute.

[0306] A gasket slide was placed into an Agilent hybridization chamber and 40 μL of each of the samples containing hybridization and blocking solution was manually pipetted into each gasket. An 8-channel variable spanning pipettor was used in a manner intended to minimize bubble formation. Custom Agilent microarray slides (Agilent Technologies, Inc., Santa Clara, Calif.), with their Number Barcode facing up, were then slowly lowered onto the gasket slides (see Agilent manual for Detailed Description).

[0307] The top of the hybridization chambers were placed onto the slide/backing sandwich and clamping brackets slid over the whole assembly. These assemblies were tightly clamped by turning the screws securely.

[0308] Each slide/backing slide sandwich was visually inspected to assure the solution bubble could move freely within the sample. If the bubble did not move freely the hybridization chamber assembly was gently tapped to disengage bubbles lodged near the gasket.

[0309] The assembled hybridization chambers were incubated in an Agilent hybridization oven for 19 hours at 60° C. rotating at 20 rpm.

[0310] 14. Post Hybridization Washing

[0311] Approximately 400 mL Agilent Wash Buffer 1 was placed into each of two separate glass staining dishes. One of the staining dishes was placed on a magnetic stir plate and a slide rack and stir bar were placed into the buffer.

[0312] A staining dish for Agilent Wash 2 was prepared by placing a stir bar into an empty glass staining dish.

[0313] A fourth glass staining dish was set aside for the final acetonitrile wash.

[0314] Each of six hybridization chambers was disassembled. One-by-one, the slide/backing sandwich was removed from its hybridization chamber and submerged into the staining dish containing Wash 1. The slide/backing sandwich was pried apart using a pair of tweezers, while still submerging the microarray slide. The slide was quickly transferred into the slide rack in the Wash 1 staining dish on the magnetic stir plate.

[0315] The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0316] When one minute was remaining for Wash 1, Wash Buffer 2 pre-warmed to 37° C. in an incubator was added to the second prepared staining dish. The slide rack was quickly transferred to Wash Buffer 2 and any excess buffer on the bottom of the rack was removed by scraping it on the top of the stain dish. The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0317] The slide rack was slowly pulled out of Wash 2, taking approximately 15 seconds to remove the slides from the solution.

[0318] With one minute remaining in Wash 2 acetonitrile (ACN) was added to the fourth staining dish. The slide rack was transferred to the acetonitrile stain dish. The slide rack was gently raised and lowered 5 times. The magnetic stirrer was turned on at a low setting and the slides incubated for 5 minutes.

[0319] The slide rack was slowly pulled out of the ACN stain dish and placed on an absorbent towel. The bottom edges of the slides were quickly dried and the slide was placed into a clean slide box.

[0320] 15. Microarray Imaging

[0321] The microarray slides were placed into Agilent scanner slide holders and loaded into the Agilent Microarray scanner according to the manufacturer's instructions.

[0322] The slides were imaged in the Cy3-channel at 5 μm resolution at the 100% PMT setting and the XRD option enabled at 0.05. The resulting tiff images were processed using Agilent feature extraction software version 10.5.

Example 2

Biomarker Identification

[0323] The identification of potential ovarian cancer biomarkers was performed for diagnosis of ovarian cancer in women with pelvic masses. Enrollment criteria for this study were women scheduled for laparotomy or pelvic surgery for suspicion of ovarian cancer. The primary criteria for exclusion were women suffering from chronic infectious (e.g. hepatitis B, Hepatitis C or HIV), autoimmune, or inflammatory conditions or women being treated for malignancy (other than basal or squamous cell carcinomas of the skin) within the last two years. Plasma samples were collected from two different clinical sites and included 142 cases and 195 benign controls. Table 19 summarizes the site sample information. The multiplexed aptamer affinity assay was used to measure and report the RFU value for 811 analytes in each of these 337 samples. Since the plasma samples were obtained from two independent sites under similar protocols, an examination of site differences prior to the analysis for biomarkers discovery was performed. Each of the two populations, benign pelvic mass and ovarian cancer, was separately compared between sites by generating within-site, class-dependent cumulative distribution functions (cdfs) for each of the 811 analytes. The KS-test was then applied to each analyte between both site pairs within a common class to identify those analytes that differed not by class but rather by site. In both site comparisons among the two classes, statistically significant site-dependent differences were observed.

[0324] Such site-dependent effects tend to obscure the ability to identify specific control-disease differences. In order to minimize such effects and identify key disease dependent biomarkers, three distinct strategies were employed for biomarker discovery, namely (1) aggregated class-dependent cdfs across sites, (2) comparison of within-site class-dependent cdfs, and (3) blending methods (1) with (2). Details of these three methodologies and their results follow.

[0325] These three sets of potential biomarkers can be used to build classifiers that assign samples to either a control or disease group. In fact, many such classifiers were produced

from these sets of biomarkers and the frequency with which any biomarker was used in good scoring classifiers determined. Those biomarkers that occurred most frequently among the top scoring classifiers were the most useful for creating a diagnostic test. In this example, Bayesian classifiers were used to explore the classification space but many other supervised learning techniques may be employed for this purpose. The scoring fitness of any individual classifier was gauged by summing the sensitivity and specificity of the classifier at the Bayesian surface assuming a disease prevalence of 0.5. This scoring metric varies from zero to two, with two being an error-free classifier. The details of constructing a Bayesian classifier from biomarker population measurements are described in Example 3.

[0326] By aggregating the class-dependent samples across all sites in method (1), those analyte measurements that showed large site-to-site variation, on average, failed to exhibit class-dependent differences due to the large site-to-site differences. Such analytes were automatically removed from further analysis. However, those analytes that did show class-dependent differences across the sites are robust biomarkers that were relatively insensitive to sample collection and sample handling variability. KS-distances were computed for all analytes using the class-dependent cdfs aggregated across all sites. Using a KS-distance threshold of 0.4, fifty-nine potential biomarkers for diagnosing malignant ovarian cancer from benign pelvic masses were identified.

[0327] Using the fifty-nine potential biomarkers identified above, a total of 1966 10-analyte classifiers were found with a score of 1.75 or better (>87.5% sensitivity and >87.5% specificity, on average) for diagnosing ovarian cancer from a control group with benign pelvic masses using measurements from both sites. From this set of classifiers, a total of twenty-five biomarkers were found to be present in 5.0% or more of the high scoring classifiers. Table 20 provides a list of these potential biomarkers and FIG. **10** is a frequency plot for the identified biomarkers. This completed the biomarker identification using method (1).

[0328] Method (2) focused on consistency of potential biomarker changes between the control and case groups among the individual sites. The class-dependent cdfs were constructed for all analytes within each site separately and from these cdfs the KS-distances were computed to identify potential biomarkers. Sixty-three analytes were found to have a KS-distance greater than 0.4 in all the sites. Using these Sixty-three analytes to build potential 10-analyte Bayesian classifiers, there were 2031 classifiers that had a score of 1.75 or better. Twenty-four analytes occurred with a frequency greater than 5% among these classifiers and are presented in Table 21 and shown in FIG. **11**.

[0329] Finally, by combining the criteria for potential biomarker selection described for method (1) and (2) above, a set of potential biomarkers were produced by requiring an analyte to have a KS distance of 0.4 or better in the aggregated set as well as the two site comparisons. Forty-five analytes satisfy these requirements and are referred to as a blended set of potential biomarkers. For a classification score of 1.75 or better, a total of 1563 Bayesian classifiers were built from this set of potential biomarkers and twenty-seven biomarkers were identified from this set of classifiers using a frequency cut-off of 5%. These analytes are displayed in Table 22 and FIG. **12** is a frequency plot for the identified biomarkers.

[0330] A final list of biomarkers is obtained by combining the three sets of biomarkers identified above with frequencies greater than 5% in high scoring classifiers, Tables 20-22. From these sets of twenty-five, twenty-four, and twenty-seven biomarkers, forty-two unique biomarkers were identified and are shown in Table 1. Table 15 includes a dissociation constant for the aptamer used to identify the biomarker, the limit of quantification for the marker in the multiplex aptamer assay, and whether the marker was up-regulated or down-regulated in the disease population relative to the control population.

Example 3

Naïve Bayesian Classification for Ovarian Cancer

[0331] From the list of biomarkers identified as useful for discriminating between benign pelvic masses and ovarian malignancies, a panel of ten biomarkers was selected and a naïve Bayes classifier was constructed, see Table 18. The class-dependent probability density functions (pdfs), $p(x_i \backslash c)$ and $p(x_i \backslash d)$, where x_i is the measured RFU value for biomarker i, and c and d refer to the control and disease populations, were modeled as normal distribution functions characterized by a mean μ and variance σ^2. The parameters for pdfs of the ten biomarkers are listed in Table 18 and an example of the raw data along with the model fit to a normal cdf is shown in FIG. **5** for biomarker BAFF Receptor. The underlying assumption appears to fit the data quite well as evidenced by FIG. **5**.

[0332] The naïve Bayes classification for such a model is given by the following equation, where P(d) is the prevalence of the disease in the population

$$\ln\frac{p(c\mid \underset{\sim}{x})}{p(d\mid \underset{\sim}{x})}=\sum_{i=1}^{n}\left(\ln\frac{\sigma_{d,i}}{\sigma_{c,i}}-\frac{1}{2}\left[\left(\frac{x_i-\mu_{c,i}}{\sigma_{c,i}}\right)^2-\left(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}}\right)^2\right]\right)+\ln\frac{(1-P(d))}{P(d)}$$

appropriate to the test and n=10 here. Each of the terms in the summation is a log-likelihood ratio for an individual marker and the total log-likelihood ratio of a sample $\underset{\sim}{x}$ being free from the disease of interest versus having the disease (i.e. in this case, ovarian cancer) is simply the sum of these individual terms plus a term that accounts for the prevalence of the disease. For simplicity, we assume P(d)=0.5 so that

$$\ln\frac{(1-P(d))}{P(d)}=0.$$

[0333] Given an unknown sample measurement in RFU for each of the ten biomarkers of $\underset{\sim}{x}$=(701, 34158, 182792, 19531, 170310, 896, 3207, 22545, 733, 12535), the calculation of the classification is detailed in Table 23. The individual components comprising the log likelihood ratio for control versus disease class are tabulated and can be computed from the parameters in Table 18 and the values of $\underset{\sim}{x}$. The sum of the individual log likelihood ratios is 1.965, or a likelihood of being free from the disease versus having the disease of 7:1, where likelihood=$e^{1.965}$==7.14. Four of the ten biomarker values have likelihoods more consistent with the disease group (log likelihood <0) while the remaining six biomarkers favor the control group, the largest by a factor of 3.5:1. Multiplying the likelihoods together gives the same result as that shown above; an aggregate likelihood of 7:1 that the unknown sample is free from the disease. In fact, this sample came from the control population in the training set.

Example 4

Greedy Algorithm for Selecting Biomarker Panels for Classifiers

Part 1

[0334] This example describes the selection of biomarkers from Table 1 to form panels that can be used as classifiers in any of the methods described herein. Subsets of the biomarkers in Table 1 were selected to construct classifiers with good performance. This method was also used to determine which potential markers were included as biomarkers in Example 2.

[0335] The measure of classifier performance used here is the sum of the sensitivity and specificity; a performance of 1.0 is the baseline expectation for a random (coin toss) classifier, a classifier worse than random would score between 0.0 and 1.0, a classifier with better than random performance would score between 1.0 and 2.0. A perfect classifier with no errors would have a sensitivity of 1.0 and a specificity of 1.0, therefore a performance of 2.0 (1.0+1.0). One can apply other common measures of performance such as area under the ROC curve, the F-measure, or the product of sensitivity and specificity. Specifically one might want to treat sensitivity and specificity with differing weight, in order to select those classifiers that perform with higher specificity at the expense of some sensitivity, or to select those classifiers which perform with higher sensitivity at the expense of some specificity. Since the method described here only involves a measure of "performance", any weighting scheme which results in a single performance measure can be used. Different applications will have different benefits for true positive and true negative findings, and will have different costs associated with false positive findings from false negative findings. For example, screening and the differential diagnosis of benign pelvic masses will not in general have the same optimal trade-off between specificity and sensitivity. The different demands of the two tests will in general require setting different weighting to positive and negative misclassifications, which will be reflected in the performance measure. Changing the performance measure will in general change the exact subset of markers selected from Table 1 for a given set of data.

[0336] For the Bayesian approach to the discrimination of ovarian cancer samples from control samples described in Example 3, the classifier was completely parameterized by the distributions of biomarkers in the disease and non-disease training samples, and the list of biomarkers was chosen from Table 1; that is to say, the subset of markers chosen for inclusion determined a classifier in a one-to-one manner given a set of training data.

[0337] The greedy method employed here was used to search for the optimal subset of markers from Table 1. For small numbers of markers or classifiers with relatively few markers, every possible subset of markers was enumerated and evaluated in terms of the performance of the classifier constructed with that particular set of markers (see Example 4, Part 2). (This approach is well known in the field of statistics as "best subset selection"; see, e.g., Hastie et al, supra). However, for the classifiers described herein, the number of combinations of multiple markers can be very large, and it was not feasible to evaluate every possible set of 10 markers, for example, from the list of 42 markers (Table 1) (i.e., 1,471,442,973 combinations). Because of the impracticality of searching through every subset of markers, the single optimal subset may not be found; however, by using this approach, many excellent subsets were found, and, in many cases, any of these subsets may represent an optimal one.

[0338] Instead of evaluating every possible set of markers, a "greedy" forward stepwise approach may be followed (see, e.g., Dabney A R, Storey J D (2007) Optimality Driven Nearest Centroid Classification from Genomic Data. PLoS ONE 2(10): e1002. doi:10.1371/journal.pone.0001002). Using this method, a classifier is started with the best single marker (based on KS-distance for the individual markers) and is grown at each step by trying, in turn, each member of a marker list that is not currently a member of the set of markers in the classifier. The one marker that scores the best in combination with the existing classifier is added to the classifier. This is repeated until no further improvement in performance is achieved. Unfortunately, this approach may miss valuable combinations of markers for which some of the individual markers are not all chosen before the process stops.

[0339] The greedy procedure used here was an elaboration of the preceding forward stepwise approach, in that, to broaden the search, rather than keeping just a single candidate classifier (marker subset) at each step, a list of candidate classifiers was kept. The list was seeded with every single marker subset (using every marker in the table on its own). The list was expanded in steps by deriving new classifiers (marker subsets) from the ones currently on the list and adding them to the list. Each marker subset currently on the list was extended by adding any marker from Table 1 not already part of that classifier, and which would not, on its addition to the subset, duplicate an existing subset (these are termed "permissible markers"). Every existing marker subset was extended by every permissible marker from the list. Clearly, such a process would eventually generate every possible subset, and the list would run out of space. Therefore, all the generated classifiers were kept only while the list was less than some predetermined size (often enough to hold all three marker subsets). Once the list reached the predetermined size limit, it became elitist; that is, only those classifiers which showed a certain level of performance were kept on the list, and the others fell off the end of the list and were lost. This was achieved by keeping the list sorted in order of classifier performance; new classifiers which were at least as good as the worst classifier currently on the list were inserted, forcing the expulsion of the current bottom underachiever. One further implementation detail is that the list was completely replaced on each generational step; therefore, every classifier on the list had the same number of markers, and at each step the number of markers per classifier grew by one.

[0340] Since this method produced a list of candidate classifiers using different combinations of markers, one may ask if the classifiers can be combined in order to avoid errors that might be made by the best single classifier, or by minority groups of the best classifiers. Such "ensemble" and "committee of experts" methods are well known in the fields of statistical and machine learning and include, for example, "Averaging", "Voting", "Stacking", "Bagging" and "Boosting" (see, e.g., Hastie et al., supra). These combinations of simple classifiers provide a method for reducing the variance in the classifications due to noise in any particular set of markers by including several different classifiers and therefore information from a larger set of the markers from the biomarker table, effectively averaging between the classifiers. An example of the usefulness of this approach is that it can prevent outliers in a single marker from adversely affecting the classification of a single sample. The requirement to measure a larger number of signals may be impractical in conventional "one marker at a time" antibody assays but has no downside for a fully multiplexed aptamer assay. Techniques such as these benefit from a more extensive table of biomarkers and use the multiple sources of information concerning the disease processes to provide a more robust classification.

Part 2

[0341] The biomarkers selected in Table 1 gave rise to classifiers that perform better than classifiers built with "non-markers" (i.e., proteins having signals that did not meet the criteria for inclusion in Table 1 (as described in Example 2)).

[0342] For classifiers containing only one, two, and three markers, all possible classifiers obtained using the biomarkers in Table 1 were enumerated and examined for the distribution of performance compared to classifiers built from a similar table of randomly selected non-markers signals.

[0343] In FIG. **14**, the sum of the sensitivity and specificity was used as the measure of performance; a performance of 1.0 is the baseline expectation for a random (coin toss) classifier. The histogram of classifier performance was compared with the histogram of performance from a similar exhaustive enumeration of classifiers built from a "non-marker" table of 42 non-marker analytes; the 42 analytes were randomly chosen from 387 aptamer measurements that did not demonstrate differential signaling between control and disease populations (KS-distance<0.2).

[0344] FIG. **14** shows histograms of the performance of all possible one, two, and three-marker classifiers built from the biomarker parameters in Table 18 for biomarkers that can discriminate between benign pelvic masses and ovarian cancer and compares these classifiers with all possible one, two, and three-marker classifiers built using the 42 "non-marker" aptamer RFU signals. FIG. **14**A shows the histograms of single marker classifier performance, FIG. **14**B shows the histogram of two-marker classifier performance, and FIG. **14**C shows the histogram of three-marker classifier performance.

[0345] In FIG. **14**, the solid lines represent the histograms of the classifier performance of all one, two, and three-marker classifiers using the biomarker data for benign pelvic masses and ovarian cancer in Table 18. The dotted lines are the histograms of the classifier performance of all one, two, and three-marker classifiers using the data for benign pelvic masses and ovarian cancer but using the set of random non-marker signals.

[0346] The classifiers built from the markers listed in Table 1 form a distinct histogram, well separated from the classifiers built with signals from the "non-markers" for all one-marker, two-marker, and three-marker comparisons. The performance and AUC score of the classifiers built from the biomarkers in Table 1 also increase at a higher rate as markers are added than do the classifiers built from the non-markers. The separation of performance increases between the marker and non-marker classifiers as the number of markers per classifier increases. All classifiers built using the biomarkers listed in Table 1 perform distinctly better than classifiers built using the "non-markers".

Part 3

[0347] The distributions of classifier performance show that there are many possible multiple-marker classifiers that can be derived from the set of analytes in Table 1. Although some biomarkers are better than others on their own, as evidenced by the distribution of classifier scores and AUCs for single analytes, it was desirable to determine whether such biomarkers are required to construct high performing classifiers. To make this determination, the behavior of classifier performance was examined by leaving out some number of the best biomarkers. FIG. **15** compares the performance of classifiers built with the full list of biomarkers in Table 1 with the performance of classifiers built with subsets of biomarkers from Table 1 that excluded top-ranked markers.

[0348] FIG. **15** demonstrates that classifiers constructed without the best markers perform well, implying that the performance of the classifiers was not due to some small core group of markers and that the changes in the underlying processes associated with disease are reflected in the activities of many proteins. Many subsets of the biomarkers in Table 1 performed close to optimally, even after removing the top 15 of the 42 markers from Table 1. After dropping the 15 top-ranked markers (ranked by KS-distance) from Table 1, the classifier performance increased with the number of markers selected from the table to reach almost 1.80 (sensitivity+specificity), close to the performance of the optimal classifier score of 1.87 selected from the full list of biomarkers.

[0349] Finally, FIG. **16** shows how the ROC performance of typical classifiers constructed from the list of parameters in Table 18 according to Example 3. A five analyte classifier was constructed with TIMP-2, MCP-3, Cadherin-5, SLPI, and C9. FIG. **16**A shows the performance of the model, assuming independence of these markers, as in Example 3, and FIG. **16**B shows the empirical ROC curves generated from the study data set used to define the parameters in Table 18. It can be seen that the performance for a given number of selected markers was qualitatively in agreement, and that quantitative agreement was generally quite good, as evidenced by the AUCs, although the model calculation tends to overestimate classifier performance. This is consistent with the notion that the information contributed by any particular biomarker concerning the disease processes is redundant with the information contributed by other biomarkers provided in Table 1 while the model calculation assumes complete independence. FIG. **16** thus demonstrates that Table 1 in combination with the methods described in Example 3 enable the construction and evaluation of a great many classifiers useful for the discrimination of ovarian cancer from benign pelvic masses.

Example 5

Aptamer Specificity Demonstration in a Pull-Down Assay

[0350] The final readout on the multiplex assay is based on the amount of aptamer recovered after the successive capture steps in the assay. The multiplex assay is based on the premise that the amount of aptamer recovered at the end of the assay is proportional to the amount of protein in the original complex mixture (e.g., plasma). In order to demonstrate that this signal is indeed derived from the intended analyte rather than from non-specifically bound proteins in plasma, we developed a gel-based pull-down assay in plasma. This assay can be used to visually demonstrate that a desired protein is in fact pulled out from plasma after equilibration with an aptamer as well as to demonstrate that aptamers bound to their intended protein targets can survive as a complex through the kinetic challenge steps in the assay. In the experiments described in this example, recovery of protein at the end of this pull-down assay requires that the protein remain non-covalently bound to the aptamer for nearly two hours after equilibration. Importantly, in this example we also provide evidence that non-specifically bound proteins dissociate during these steps and do not contribute significantly to the final signal. It should be

noted that the pull-down procedure described in this example includes all of the key steps in the multiplex assay described above.

[0351] A. Plasma Pull-Down Assay

[0352] Plasma samples were prepared by diluting 50 μL EDTA-plasma to 100 μL in SB18 with 0.05% Tween-20 (SB18T) and 2 μM Z-Block. The plasma solution was equilibrated with 10 pmoles of a PBDC-aptamer in a final volume of 150 μL for 2 hours at 37° C. After equilibration, complexes and unbound aptamer were captured with 133 μL of a 7.5% Streptavidin-agarose bead slurry by incubating with shaking for 5 minutes at RT in a Durapore filter plate. The samples bound to beads were washed with biotin and with buffer under vacuum as described in Example 1. After washing, bound proteins were labeled with 0.5 mM NHS-S-S-biotin, 0.25 mM NHS-Alexa647 in the biotin diluent for 5 minutes with shaking at RT. This staining step allows biotinylation for capture of protein on streptavidin beads as well as highly sensitive staining for detection on a gel. The samples were washed with glycine and with buffer as described in Example 1. Aptamers were released from the beads by photocleavage using a Black Ray light source for 10 minutes with shaking at RT. At this point, the biotinylated proteins were captured on 0.5 mg MyOne Streptavidin beads by shaking for 5 minutes at RT. This step will capture proteins bound to aptamers as well as proteins that may have dissociated from aptamers since the initial equilibration. The beads were washed as described in Example 1. Proteins were eluted from the MyOne Streptavidin beads by incubating with 50 mM DTT in SB17T for 25 minutes at 37° C. with shaking. The eluate was then transferred to MyOne beads coated with a sequence complimentary to the 3' fixed region of the aptamer and incubated for 25 minutes at 37° C. with shaking. This step captures all of the remaining aptamer. The beads were washed 2× with 100 μL SB17T for 1 minute and 1× with 100 μL SB19T for 1 minute. Aptamer was eluted from these final beads by incubating with 45 μL 20 mM NaOH for 2 minutes with shaking to disrupt the hybridized strands. 40 μL of this eluate was neutralized with 10 μL 80 mM HCl containing 0.05% Tween-20. Aliquots representing 5% of the eluate from the first set of beads (representing all plasma proteins bound to the aptamer) and 20% of the eluate from the final set of beads (representing all plasma proteins remaining bound at the end of our clinical assay) were run on a NuPAGE 4-12% Bis-Tris gel (Invitrogen) under reducing and denaturing conditions. Gels were imaged on an Alpha Innotech FluorChem Q scanner in the Cy5 channel to image the proteins.

[0353] B. Pull-down gels for aptamers were selected against LBP (~1×10^{-7} M in plasma, polypeptide MW ~60 kDa), C9 (~1×10^{-6} M in plasma, polypeptide MW ~60 kDa), and IgM (~9×10^{-6} M in plasma, MW ~70 kDa and 23 kDa), respectively. (See FIG. **13**).

[0354] For each gel, lane 1 is the eluate from the Streptavidin-agarose beads, lane 2 is the final eluate, and lane 3 is a MW marker lane (major bands are at 110, 50, 30, 15, and 3.5 kDa from top to bottom). It is evident from these gels that there is a small amount non-specific binding of plasma proteins in the initial equilibration, but only the target remains after performing the capture steps of the assay. It is clear that the single aptamer reagent is sufficient to capture its intended analyte with no up-front depletion or fractionation of the plasma. The amount of remaining aptamer after these steps is then proportional to the amount of the analyte in the initial sample.

[0355] The foregoing embodiments and examples are intended only as examples. No particular embodiment, example, or element of a particular embodiment or example is to be construed as a critical, required, or essential element or feature of any of the claims. Further, no element described herein is required for the practice of the appended claims unless expressly described as "essential" or "critical." Various alterations, modifications, substitutions, and other variations can be made to the disclosed embodiments without departing from the scope of the present application, which is defined by the appended claims. The specification, including the figures and examples, is to be regarded in an illustrative manner, rather than a restrictive one, and all such modifications and substitutions are intended to be included within the scope of the application. Accordingly, the scope of the application should be determined by the appended claims and their legal equivalents, rather than by the examples given above. For example, steps recited in any of the method or process claims may be executed in any feasible order and are not limited to an order presented in any of the embodiments, the examples, or the claims. Further, in any of the aforementioned methods, one or more biomarkers of Table 1 can be specifically excluded either as an individual biomarker or as a biomarker from any panel.

TABLE 1

Biomarkers for Ovarian Cancer

Biomarker Designation	Alternate Protein Names	Gene Designation
α1-Antitrypsin	Alpha-1-antitrypsin API Alpha-1 protease inhibitor alpha 1 antitrypsin alpha1-protease inhibitor Serpin A1 AAT	SERPINA1
α2-Antiplasmin	alpha-2-plasmin inhibitor	SERPINF2
α2-HS-Glycoprotein	fetuin fetuin A alpha-2-HS glycoprotein AHSG Alpha2-Heremans Schmid glycoprotein Ba-alpha-2-glycoprotein Alpha-2-Z-globulin	AHSG
ADAM 9	Disintegrin and metalloproteinase domain-containing protein 9 Metalloprotease/disintegrin/cysteine-rich protein 9 Myeloma cell metalloproteinase Meltrin-gamma Cellular disintegrin-related protein	ADAM9
ARSB	Arylsulfatase B G4S N-acetylgalactosamine-4-sulfatase ASB G4S	ARSB
BAFF Receptor	B cell-activating factor receptor BLyS receptor 3 Tumor necrosis factor receptor superfamily member 13C TNFRSF13C CD268 antigen	TNFRSF13C
C2	Complement C2 C3/C5 convertase	C2
C5	Complement Factor C5 Complement C5 C3 and PZP-like alpha-2-macroglobulin	C5

TABLE 2

100 Panels of 3 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers			Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	ADAM 9	α1-Antitrypsin	α2-Antiplαsmin	0.846	0.851	1.697	0.866
2	ARSB	SLPI	C9	0.846	0.856	1.703	0.913
3	BAFF Receptor	SLPI	C9	0.833	0.862	1.695	0.916
4	C2	LY9	SLPI	0.808	0.923	1.731	0.916
5	C5	Troponin T	C9	0.897	0.800	1.697	0.885
6	C6	ERBB1	SLPI	0.808	0.887	1.695	0.902
7	Cadherin-5	C9	SLPI	0.859	0.887	1.746	0.929
8	Coagulation Factor Xa	LY9	SLPI	0.821	0.882	1.703	0.911
9	Contactin-4	LY9	SLPI	0.833	0.872	1.705	0.906
10	Growth hormone receptor	SLPI	C9	0.859	0.856	1.715	0.916
11	HGF	Troponin T	C9	0.897	0.795	1.692	0.886
12	HSP 90α	LY9	SLPI	0.846	0.882	1.728	0.896
13	Hat1	SLPI	C9	0.846	0.867	1.713	0.914
14	IL-12 Rβ2	C9	SLPI	0.833	0.872	1.705	0.916
15	IL-13 Rα1	SLPI	C9	0.846	0.856	1.703	0.920
16	IL-18 Rβ	SLPI	C9	0.846	0.856	1.703	0.925
17	Kallikrein 6	SLPI	C9	0.821	0.851	1.672	0.921
18	LY9	Kallistatin	SLPI	0.795	0.897	1.692	0.912
19	MCP-3	SLPI	C9	0.833	0.882	1.715	0.924
20	MIP-5	C9	SLPI	0.821	0.846	1.667	0.919
21	MRC2	MMP-7	C9	0.859	0.846	1.705	0.898
22	SAP	NRP1	SLPI	0.821	0.887	1.708	0.917
23	LY9	PCI	SLPI	0.833	0.867	1.700	0.902
24	C2	Prekallikrein	SLPI	0.808	0.892	1.700	0.911
25	Properdin	LY9	SLPI	0.846	0.877	1.723	0.905
26	LY9	RBP	SLPI	0.782	0.903	1.685	0.897
27	SAP	RGM-C	SLPI	0.872	0.877	1.749	0.923
28	SCF sR	C9	SLPI	0.846	0.856	1.703	0.915
29	TIMP-2	C9	SLPI	0.885	0.856	1.741	0.926
30	MCP-3	Thrombin/ Prothrombin	C9	0.833	0.826	1.659	0.875
31	α2-HS-Glycoprotein	α2-Antiplαsmin	SLPI	0.808	0.872	1.679	0.887
32	Contactin-1	LY9	SLPI	0.808	0.882	1.690	0.909
33	sL-Selectin	C9	SLPI	0.821	0.872	1.692	0.929
34	C2	ADAM 9	SLPI	0.795	0.897	1.692	0.879
35	Cadherin-5	ARSB	α1-Antitrypsin	0.769	0.897	1.667	0.867
36	BAFF Receptor	C6	SLPI	0.782	0.897	1.679	0.876
37	C5	RGM-C	SLPI	0.833	0.862	1.695	0.906
38	Coagulation Factor Xa	SLPI	C9	0.846	0.846	1.692	0.923
39	SAP	Contactin-4	SLPI	0.821	0.867	1.687	0.891
40	ERBB1	C9	SLPI	0.846	0.846	1.692	0.920
41	SAP	Growth hormone receptor	SLPI	0.808	0.892	1.700	0.917
42	HGF	MCP-3	C9	0.872	0.815	1.687	0.872
43	HSP 90α	SLPI	C9	0.859	0.862	1.721	0.927
44	SAP	Hat1	SLPI	0.808	0.903	1.710	0.902
45	IL-12 Rβ2	Prekallikrein	SLPI	0.821	0.856	1.677	0.889
46	IL-13 Rα1	RGM-C	C9	0.872	0.805	1.677	0.886
47	IL-18 Rβ	LY9	C9	0.859	0.826	1.685	0.870
48	Kallikrein 6	LY9	SLPI	0.795	0.872	1.667	0.896
49	Cadherin-5	Kallistatin	SLPI	0.769	0.903	1.672	0.910
50	MIP-5	RGM-C	C9	0.885	0.774	1.659	0.893
51	RGM-C	MMP-7	C9	0.885	0.815	1.700	0.908
52	MRC2	C9	SLPI	0.859	0.862	1.721	0.911
53	NRP1	LY9	SLPI	0.821	0.877	1.697	0.908
54	PCI	C9	SLPI	0.821	0.856	1.677	0.917
55	Cadherin-5	Properdin	SLPI	0.782	0.908	1.690	0.907
56	RBP	SLPI	C9	0.833	0.851	1.685	0.910
57	SCF sR	α1-Antitrypsin	SLPI	0.808	0.872	1.679	0.885
58	TIMP-2	α2-Antiplαsmin	SLPI	0.821	0.882	1.703	0.900
59	NRP1	Thrombin/ Prothrombin	C9	0.846	0.805	1.651	0.873
60	SCF sR	α2-HS-Glycoprotein	SLPI	0.795	0.872	1.667	0.879
61	Contactin-1	NRP1	SLPI	0.782	0.897	1.679	0.906
62	RGM-C	sL-Selectin	C9	0.872	0.805	1.677	0.901
63	Cadherin-5	ADAM 9	α1-Antitrypsin	0.795	0.892	1.687	0.862

TABLE 2-continued

64	Properdin	ARSB	SLPI	0.769	0.892	1.662	0.889
65	BAFF Receptor	α2-Antiplasmin	SLPI	0.782	0.887	1.669	0.880
66	C5	Properdin	SLPI	0.808	0.882	1.690	0.898
67	C6	RGM-C	SLPI	0.821	0.872	1.692	0.908
68	SAP	Coagulation Factor Xa	SLPI	0.808	0.872	1.679	0.907
69	Contactin-4	Coagulation Factor Xa	MMP-7	0.808	0.867	1.674	0.868
70	C2	ERBB1	SLPI	0.795	0.892	1.687	0.904
71	Cadherin-5	Growth hormone receptor	α1-Antitrypsin	0.821	0.872	1.692	0.876
72	HGF	SLPI	C9	0.872	0.815	1.687	0.916
73	HSP 90α	C2	SLPI	0.808	0.872	1.679	0.900
74	Hat1	LY9	SLPI	0.808	0.877	1.685	0.903
75	IL-12 Rβ2	α2-Antiplasmin	SLPI	0.808	0.867	1.674	0.883
76	IL-13 Rα1	LY9	SLPI	0.795	0.877	1.672	0.900
77	IL-18 Rβ	Prekallikrein	C9	0.859	0.826	1.685	0.890
78	Kallikrein 6	SCF sR	C9	0.846	0.821	1.667	0.882
79	C2	Kallistatin	SLPI	0.782	0.887	1.669	0.903
80	MIP-5	Cadherin-5	SLPI	0.782	0.867	1.649	0.885
81	MRC2	Hat1	SLPI	0.782	0.897	1.679	0.889
82	PCI	α2-Antiplasmin	SLPI	0.795	0.867	1.662	0.891
83	SAP	RBP	SLPI	0.782	0.892	1.674	0.895
84	Cadherin-5	TIMP-2	SLPI	0.808	0.877	1.685	0.907
85	SCF sR	Thrombin/ Prothrombin	C9	0.859	0.790	1.649	0.865
86	Troponin T	SLPI	C9	0.833	0.851	1.685	0.923
87	α2-HS-Glycoprotein	C9	SLPI	0.808	0.851	1.659	0.915
88	Cadherin-5	Contactin-1	SLPI	0.808	0.867	1.674	0.897
89	Cadherin-5	sL-Selectin	SLPI	0.795	0.882	1.677	0.901
90	ADAM 9	SLPI	α2-Antiplasmin	0.782	0.892	1.674	0.883
91	ARSB	ADAM 9	α2-Antiplasmin	0.808	0.851	1.659	0.836
92	BAFF Receptor	α1-Antitrypsin	SLPI	0.769	0.897	1.667	0.889
93	C5	C9	SLPI	0.833	0.856	1.690	0.920
94	C6	LY9	SLPI	0.782	0.908	1.690	0.908
95	C5	Contactin-4	SLPI	0.808	0.862	1.669	0.883
96	ERBB1	α1-Antitrypsin	SLPI	0.808	0.877	1.685	0.893
97	C5	Growth hormone receptor	C9	0.872	0.810	1.682	0.881
98	HGF	Hat1	C9	0.872	0.810	1.682	0.871
99	HSP 90α	IL-18 Rβ	C9	0.859	0.815	1.674	0.885
100	IL-12 Rβ2	α1-Antitrypsin	SLPI	0.795	0.877	1.672	0.887

Marker	Count	Marker	Count
SLPI	77	Contactin-4	4
C9	41	Coagulation Factor Xa	4
LY9	15	C6	4
Cadherin-5	10	BAFF Receptor	4
α2-Antiplasmin	8	ARSB	4
α1-Antitrypsin	8	sL-Selectin	3
SAP	7	Contactin-1	3
RGM-C	7	α2-HS-Glycoprotein	3
C5	6	Troponin T	3
C2	6	Thrombin/ Prothrombin	3
SCF sR	5	TIMP-2	3
Hat1	5	RBP	3
ADAM 9	5	Prekallikrein	3
Properdin	4	PCI	3
NRP1	4	MRC2	3
IL-18 Rβ	4	MMP-7	3
IL-12 Rβ2	4	MIP-5	3
HSP 90α	4	MCP-3	3
HGF	4	Kallistatin	3
Growth hormone receptor	4	Kallikrein 6	3
ERBB1	4	IL-13 Rα1	3

TABLE 3

100 Panels of 4 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers				Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	LY9	ADAM 9	C9	SLPI	0.872	0.867	1.738	0.910
2	ARSB	LY9	C9	SLPI	0.872	0.877	1.749	0.920
3	BAFF Receptor	MCP-3	SLPI	C9	0.885	0.862	1.746	0.923
4	Cadherin-5	C2	SLPI	LY9	0.859	0.918	1.777	0.923
5	C5	C2	SLPI	LY9	0.846	0.897	1.744	0.907
6	C6	LY9	C9	SLPI	0.885	0.867	1.751	0.923
7	Coagulation Factor Xa	LY9	C9	SLPI	0.897	0.862	1.759	0.930
8	Hat1	LY9	Contactin-4	SLPI	0.872	0.897	1.769	0.910
9	IL-13 Rα1	LY9	ERBB1	SLPI	0.872	0.877	1.749	0.906
10	Cadherin-5	SAP	Growth hormone receptor	SLPI	0.885	0.892	1.777	0.924
11	HGF	MRC2	C9	SLPI	0.910	0.856	1.767	0.911
12	HSP 90α	LY9	C9	SLPI	0.897	0.897	1.795	0.924
13	Cadherin-5	IL-12 Rβ2	C9	SLPI	0.846	0.892	1.738	0.923
14	IL-18 Rβ	SLPI	RGM-C	C9	0.897	0.862	1.759	0.930
15	Cadherin-5	LY9	Kallikrein 6	SLPI	0.885	0.887	1.772	0.915
16	MMP-7	α2-Antiplαsmin	Kallistatin	SLPI	0.859	0.882	1.741	0.921
17	MIP-5	LY9	C9	SLPI	0.872	0.877	1.749	0.925
18	NRP1	LY9	Cadherin-5	SLPI	0.859	0.908	1.767	0.924
19	LY9	PCI	C9	SLPI	0.872	0.867	1.738	0.917
20	LY9	Prekallikrein	C9	SLPI	0.897	0.856	1.754	0.925
21	SAP	Properdin	RGM-C	SLPI	0.859	0.903	1.762	0.931
22	LY9	RBP	C9	SLPI	0.897	0.862	1.759	0.917
23	SCF sR	LY9	C9	SLPI	0.885	0.867	1.751	0.923
24	MCP-3	TIMP-2	C9	SLPI	0.897	0.862	1.759	0.920
25	MMP-7	Thrombin/ Prothrombin	SLPI	C9	0.885	0.841	1.726	0.925
26	LY9	Troponin T	C9	SLPI	0.872	0.872	1.744	0.924
27	α1-Antitrypsin	C9	LY9	SLPI	0.885	0.862	1.746	0.919
28	Cadherin-5	α2-HS-Glycoprotein	SLPI	sL-Selectin	0.821	0.897	1.718	0.900
29	Contactin-1	LY9	C9	SLPI	0.885	0.882	1.767	0.927
30	Properdin	ADAM 9	C9	SLPI	0.872	0.862	1.733	0.907
31	Cadherin-5	ARSB	C9	SLPI	0.872	0.862	1.733	0.922
32	BAFF Receptor	LY9	C9	SLPI	0.885	0.856	1.741	0.915
33	Properdin	MCP-3	C5	SLPI	0.833	0.908	1.741	0.909
34	C6	C2	SLPI	LY9	0.833	0.918	1.751	0.922
35	SAP	C9	Coagulation Factor Xa	SLPI	0.885	0.867	1.751	0.929
36	Contactin-4	LY9	MCP-3	SLPI	0.859	0.892	1.751	0.914
37	LY9	ERBB1	C9	SLPI	0.872	0.872	1.744	0.923
38	Cadherin-5	Growth hormone receptor	C9	SLPI	0.872	0.877	1.749	0.926
39	HGF	RGM-C	α2-Antiplαsmin	C9	0.936	0.821	1.756	0.909
40	HSP 90α	Cadherin-5	C9	SLPI	0.859	0.892	1.751	0.928
41	Hat1	LY9	C9	SLPI	0.885	0.877	1.762	0.926
42	IL-12 Rβ2	C2	SLPI	LY9	0.833	0.903	1.736	0.907
43	IL-13 Rα1	SLPI	Cadherin-5	C9	0.885	0.882	1.767	0.928
44	MRC2	LY9	IL-18 Rβ	SLPI	0.833	0.908	1.741	0.913
45	Kallikrein 6	LY9	C9	SLPI	0.897	0.867	1.764	0.921
46	BAFF Receptor	LY9	Kallistatin	SLPI	0.833	0.903	1.736	0.900
47	MIP-5	SCF sR	SLPI	C9	0.872	0.862	1.733	0.914
48	NRP1	LY9	C9	SLPI	0.885	0.877	1.762	0.927
49	SAP	PCI	RGM-C	SLPI	0.872	0.862	1.733	0.916
50	BAFF Receptor	HGF	SLPI	Prekallikrein	0.897	0.841	1.738	0.893
51	RGM-C	RBP	MMP-7	C9	0.897	0.841	1.738	0.905
52	Cadherin-5	TIMP-2	C9	SLPI	0.872	0.882	1.754	0.931
53	C2	Thrombin/ Prothrombin	Growth hormone receptor	SLPI	0.859	0.862	1.721	0.904

TABLE 3-continued

54	RGM-C	Troponin T	C9	α1-Antitrypsin	0.872	0.867	1.738	0.908
55	sL-Selectin	α2-HS-Glycoprotein	C9	SLPI	0.833	0.882	1.715	0.920
56	Contactin-1	C2	SLPI	Cadherin-5	0.846	0.903	1.749	0.908
57	Cadherin-5	ADAM 9	C9	SLPI	0.833	0.897	1.731	0.916
58	Cadherin-5	Properdin	ARSB	SLPI	0.821	0.908	1.728	0.909
59	C5	LY9	α1-Antitrypsin	SLPI	0.859	0.882	1.741	0.909
60	RGM-C	LY9	C6	SLPI	0.859	0.887	1.746	0.920
61	NRP1	LY9	Coagulation Factor Xa	SLPI	0.872	0.872	1.744	0.915
62	RGM-C	Contactin-4	MCP-3	SLPI	0.846	0.897	1.744	0.919
63	MCP-3	LY9	ERBB1	SLPI	0.859	0.877	1.736	0.906
64	HSP 90α	MCP-3	C9	SLPI	0.897	0.851	1.749	0.922
65	Hat1	LY9	C2	SLPI	0.859	0.897	1.756	0.917
66	MRC2	IL-12 Rβ2	Properdin	SLPI	0.833	0.897	1.731	0.885
67	Cadherin-5	LY9	IL-13 Rα1	SLPI	0.872	0.887	1.759	0.917
68	IL-18 Rβ	SLPI	Cadherin-5	C9	0.859	0.882	1.741	0.933
69	Kallikrein 6	LY9	SCF sR	SLPI	0.859	0.887	1.746	0.898
70	Cadherin-5	LY9	Kallistatin	SLPI	0.833	0.903	1.736	0.921
71	MIP-5	Hat1	SLPI	C9	0.859	0.872	1.731	0.907
72	Cadherin-5	LY9	PCI	SLPI	0.846	0.887	1.733	0.909
73	Prekallikrein	α1-Antitrypsin	LY9	SLPI	0.846	0.887	1.733	0.911
74	SCF sR	RBP	SLPI	C9	0.872	0.856	1.728	0.908
75	RGM-C	TIMP-2	C9	SLPI	0.885	0.867	1.751	0.931
76	C2	LY9	Thrombin/ Prothrombin	SLPI	0.846	0.867	1.713	0.922
77	SAP	α1-Antitrypsin	Troponin T	SLPI	0.833	0.903	1.736	0.917
78	HGF	α2-Antiplαsmin	C9	SLPI	0.910	0.841	1.751	0.922
79	Cadherin-5	α2-HS-Glycoprotein	SLPI	LY9	0.833	0.882	1.715	0.908
80	Contactin-1	LY9	Growth hormone receptor	SLPI	0.859	0.887	1.746	0.914
81	sL-Selectin	LY9	C9	SLPI	0.885	0.867	1.751	0.926
82	Cadherin-5	Prekallikrein	ADAM 9	SLPI	0.846	0.882	1.728	0.897
83	Cadherin-5	ARSB	SLPI	LY9	0.846	0.882	1.728	0.907
84	Hat1	LY9	C5	SLPI	0.859	0.877	1.736	0.909
85	C6	MRC2	Hat1	SLPI	0.833	0.908	1.741	0.893
86	Cadherin-5	Coagulation Factor Xa	C9	SLPI	0.872	0.872	1.744	0.929
87	HSP 90α	Contactin-4	SLPI	LY9	0.872	0.872	1.744	0.902
88	Cadherin-5	ERBB1	C9	SLPI	0.846	0.887	1.733	0.926
89	Properdin	IL-12 Rβ2	MCP-3	SLPI	0.821	0.908	1.728	0.898
90	IL-13 Rα1	LY9	C9	SLPI	0.872	0.867	1.738	0.921
91	Cadherin-5	LY9	IL-18 Rβ	SLPI	0.846	0.882	1.728	0.918
92	RGM-C	Kallikrein 6	SLPI	C9	0.872	0.862	1.733	0.926
93	HSP 90α	LY9	Kallistatin	SLPI	0.833	0.903	1.736	0.911
94	MIP-5	RGM-C	SLPI	C9	0.872	0.856	1.728	0.930
95	MMP-7	SLPI	C9	LY9	0.897	0.877	1.774	0.935
96	Cadherin-5	NRP1	C9	SLPI	0.885	0.877	1.762	0.931
97	Coagulation Factor Xa	LY9	PCI	SLPI	0.833	0.892	1.726	0.909

TABLE 3-continued

98	Growth hormone receptor	RBP	C9	SLPI	0.859	0.867	1.726	0.907
99	Properdin	TIMP-2	C9	SLPI	0.872	0.872	1.744	0.927
100	Cadherin-5	Thrombin/ Prothrombin	Kallistatin	SLPI	0.821	0.892	1.713	0.908

Marker	Count	Marker	Count
SLPI	97	NRP1	4
C9	53	MRC2	4
LY9	51	MMP-7	4
Cadherin-5	26	MIP-5	4
RGM-C	11	Kallikrein 6	4
MCP-3	8	IL-18 Rβ	4
C2	8	IL-13 Rα1	4
Properdin	7	IL-12 Rβ2	4
Hat1	6	HGF	4
α1-Antitrypsin	5	ERBB1	4
SAP	5	Contactin-4	4
Kallistatin	5	C6	4
HSP 90α	5	C5	4
Growth hormone receptor	5	BAFF Receptor	4
Coagulation Factor Xa	5	ARSB	4
Thrombin/ Prothrombin	4	ADAM 9	4
TIMP-2	4	sL-Selectin	3
SCF sR	4	Contactin-1	3
RBP	4	α2-HS-Glycoprotein	3
Prekallikrein	4	α2-Antiplαsmin	3
PCI	4	Troponin T	3

TABLE 4

100 Panels of 5 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers					Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	SCF sR	C9	SLPI	MCP-3	ADAM 9	0.897	0.882	1.779	0.916
2	IL-18 Rβ	C9	SLPI	Cadherin-5	ARSB	0.885	0.882	1.767	0.924
3	BAFF Receptor	SLPI	C9	LY9	MMP-7	0.885	0.877	1.762	0.924
4	C6	SLPI	LY9	RGM-C	C2	0.885	0.913	1.797	0.931
5	C5	SLPI	LY9	α1-Antitrypsin	RGM-C	0.885	0.892	1.777	0.919
6	SAP	Coagulation Factor Xa	SLPI	LY9	NRP1	0.897	0.892	1.790	0.932
7	Cadherin-5	SLPI	LY9	IL-13 Rα1	Contactin-4	0.910	0.887	1.797	0.919
8	Cadherin-5	C9	MCP-3	SLPI	ERBB1	0.859	0.908	1.767	0.928
9	Growth hormone receptor	SLPI	C9	LY9	Contactin-4	0.910	0.882	1.792	0.923
10	HGF	SLPI	C9	MMP-7	Cadherin-5	0.949	0.862	1.810	0.938
11	SLPI	NRP1	LY9	SAP	HSP 90α	0.923	0.887	1.810	0.923
12	Hat1	SLPI	C9	RGM-C	C2	0.910	0.877	1.787	0.925
13	SLPI	C9	Properdin	TIMP-2	IL-12 Rβ2	0.885	0.872	1.756	0.922
14	SLPI	NRP1	LY9	SAP	Kallikrein 6	0.910	0.887	1.797	0.918
15	LY9	α1-Antitrypsin	SLPI	Growth hormone receptor	Kallistatin	0.885	0.887	1.772	0.909
16	SLPI	NRP1	LY9	SAP	MIP-5	0.885	0.908	1.792	0.923
17	HGF	SLPI	C9	MMP-7	MRC2	0.923	0.862	1.785	0.932
18	RGM-C	SLPI	Cadherin-5	C9	PCI	0.897	0.877	1.774	0.926
19	LY9	C9	SLPI	Prekallikrein	MMP-7	0.923	0.862	1.785	0.933
20	RBP	C9	SLPI	LY9	RGM-C	0.897	0.877	1.774	0.923
21	RGM-C	SLPI	LY9	C9	Thrombin/ Prothrombin	0.910	0.862	1.772	0.930
22	Troponin T	C9	SLPI	LY9	NRP1	0.910	0.867	1.777	0.924
23	HGF	SLPI	C9	α2-Antiplαsmin	HSP 90α	0.949	0.851	1.800	0.924
24	HSP 90α	C9	SLPI	LY9	α2-HS-Glycoprotein	0.885	0.882	1.767	0.920
25	SLPI	NRP1	Cadherin-5	LY9	Contactin-1	0.885	0.913	1.797	0.928
26	Cadherin-5	C9	SLPI	MMP-7	sL-Selectin	0.885	0.892	1.777	0.939

TABLE 4-continued

27	RGM-C	C9	MCP-3	SLPI	ADAM 9	0.897	0.872	1.769	0.923
28	ARSB	SLPI	C9	LY9	C2	0.885	0.882	1.767	0.923
29	SCF sR	C9	SLPI	MCP-3	BAFF Receptor	0.885	0.877	1.762	0.924
30	HGF	SLPI	C9	α2-Antiplαsmin	C5	0.923	0.851	1.774	0.921
31	C6	SLPI	LY9	C9	Cadherin-5	0.897	0.882	1.779	0.928
32	LY9	SLPI	MMP-7	C2	Coagulation Factor Xa	0.885	0.897	1.782	0.942
33	ERBB1	SLPI	LY9	C9	IL-13 Rα1	0.897	0.867	1.764	0.919
34	Hat1	SLPI	LY9	C9	Contactin-4	0.885	0.897	1.782	0.922
35	Growth hormone receptor	SLPI	SAP	α1-Antitrypsin	IL-12 Rβ2	0.872	0.882	1.754	0.904
36	IL-18 Rβ	C9	SLPI	Cadherin-5	RGM-C	0.885	0.882	1.767	0.936
37	Cadherin-5	C9	SLPI	MMP-7	Kallikrein 6	0.897	0.887	1.785	0.940
38	Growth hormone receptor	SLPI	C9	LY9	Kallistatin	0.897	0.872	1.769	0.922
39	LY9	C9	SLPI	MIP-5	HSP 90α	0.897	0.877	1.774	0.923
40	MRC2	C9	SLPI	LY9	NRP1	0.897	0.887	1.785	0.926
41	LY9	C9	SLPI	PCI	Cadherin-5	0.885	0.887	1.772	0.923
42	SLPI	Contactin-4	LY9	MCP-3	Prekallikrein	0.872	0.903	1.774	0.916
43	SAP	SLPI	RGM-C	Properdin	Growth hormone receptor	0.897	0.882	1.779	0.926
44	RBP	C9	SLPI	LY9	MMP-7	0.897	0.872	1.769	0.927
45	LY9	SLPI	TIMP-2	C9	Kallikrein 6	0.910	0.872	1.782	0.919
46	Troponin T	C9	SLPI	LY9	RGM-C	0.897	0.872	1.769	0.931
47	Growth hormone receptor	SLPI	C9	LY9	Contactin-1	0.897	0.892	1.790	0.925
48	RGM-C	C9	MMP-7	SLPI	sL-Selectin	0.897	0.877	1.774	0.940
49	Growth hormone receptor	SLPI	SAP	α1-Antitrypsin	ADAM 9	0.872	0.892	1.764	0.899
50	C2	SLPI	LY9	C9	ARSB	0.885	0.882	1.767	0.923
51	SAP	SLPI	RGM-C	MCP-3	BAFF Receptor	0.885	0.877	1.762	0.924
52	SLPI	NRP1	LY9	C9	C5	0.897	0.877	1.774	0.924
53	IL-13 Rα1	C9	SLPI	Cadherin-5	C6	0.885	0.892	1.777	0.925
54	Coagulation Factor Xa	SLPI	C9	Cadherin-5	MMP-7	0.885	0.892	1.777	0.945
55	Cadherin-5	C9	SLPI	MMP-7	ERBB1	0.872	0.892	1.764	0.933
56	Hat1	SLPI	LY9	C2	SAP	0.872	0.908	1.779	0.922
57	SLPI	NRP1	LY9	C9	IL-12 Rβ2	0.872	0.882	1.754	0.919
58	IL-18 Rβ	C9	SLPI	RGM-C	Cadherin-5	0.885	0.882	1.767	0.936
59	Growth hormone receptor	SLPI	C9	Cadherin-5	Kallistatin	0.885	0.882	1.767	0.927
60	RGM-C	C9	MMP-7	MRC2	MIP-5	0.923	0.846	1.769	0.926
61	Cadherin-5	SLPI	LY9	C9	PCI	0.885	0.887	1.772	0.923
62	C2	SLPI	LY9	C9	Prekallikrein	0.897	0.877	1.774	0.931
63	SAP	SLPI	RGM-C	Properdin	MCP-3	0.859	0.918	1.777	0.932
64	LY9	SLPI	MMP-7	C9	RBP	0.897	0.872	1.769	0.927
65	SCF sR	C9	SLPI	MCP-3	Cadherin-5	0.885	0.897	1.782	0.930
66	LY9	SLPI	TIMP-2	C9	C2	0.897	0.877	1.774	0.928
67	RGM-C	SLPI	LY9	C9	Troponin T	0.897	0.872	1.769	0.931
68	α2-Antiplαsmin	C9	SLPI	LY9	HGF	0.936	0.856	1.792	0.925
69	MCP-3	SLPI	C9	Contactin-1	Cadherin-5	0.872	0.908	1.779	0.930
70	sL-Selectin	C9	SLPI	LY9	HSP 90α	0.885	0.882	1.767	0.923
71	Cadherin-5	SLPI	LY9	C9	ADAM 9	0.872	0.892	1.764	0.917
72	LY9	α1-Antitrypsin	SLPI	Cadherin-5	ARSB	0.846	0.913	1.759	0.913
73	BAFF Receptor	SLPI	C9	LY9	MIP-5	0.897	0.862	1.759	0.915
74	RGM-C	C9	MCP-3	SLPI	C5	0.897	0.877	1.774	0.928
75	C6	SLPI	LY9	RGM-C	Cadherin-5	0.897	0.877	1.774	0.925
76	Coagulation Factor Xa	SLPI	C9	LY9	MMP-7	0.897	0.877	1.774	0.938
77	IL-13 Rα1	C9	SLPI	Cadherin-5	ERBB1	0.872	0.892	1.764	0.926
78	MCP-3	SLPI	C9	Contactin-1	Hat1	0.885	0.892	1.777	0.917
79	SAP	Coagulation Factor Xa	SLPI	LY9	IL-12 Rβ2	0.859	0.892	1.751	0.918
80	IL-18 Rβ	C9	SLPI	RGM-C	LY9	0.910	0.856	1.767	0.928
81	LY9	C9	SLPI	Kallikrein 6	Cadherin-5	0.897	0.877	1.774	0.928
82	Cadherin-5	SLPI	LY9	C9	Kallistatin	0.885	0.882	1.767	0.930
83	Growth hormone receptor	SLPI	C9	LY9	MRC2	0.885	0.897	1.782	0.925
84	LY9	C9	SLPI	PCI	Contactin-1	0.885	0.882	1.767	0.918

TABLE 4-continued

85	LY9	C9	SLPI	Prekallikrein	RGM-C	0.923	0.851	1.774	0.929
86	HSP 90α	C9	SLPI	LY9	Properdin	0.897	0.877	1.774	0.926
87	RBP	C9	SLPI	LY9	NRP1	0.885	0.877	1.762	0.916
88	SCF sR	C9	SLPI	LY9	C2	0.897	0.882	1.779	0.926
89	TIMP-2	SLPI	Cadherin-5	C9	MCP-3	0.885	0.887	1.772	0.927
90	SAP	SLPI	RGM-C	Properdin	Troponin T	0.859	0.908	1.767	0.933
91	α2-Antiplαsmin	C9	SLPI	Cadherin-5	HGF	0.936	0.851	1.787	0.926
92	HSP 90α	C9	SLPI	LY9	sL-Selectin	0.885	0.882	1.767	0.923
93	SAP	SLPI	RGM-C	Properdin	ADAM 9	0.859	0.903	1.762	0.920
94	SCF sR	C9	SLPI	MCP-3	ARSB	0.872	0.887	1.759	0.918
95	LY9	C9	SLPI	MIP-5	BAFF Receptor	0.897	0.862	1.759	0.915
96	SCF sR	C9	SLPI	MCP-3	C5	0.897	0.867	1.764	0.922
97	SAP	SLPI	RGM-C	MCP-3	C6	0.872	0.903	1.774	0.926
98	SLPI	Contactin-4	LY9	HSP 90α	NRP1	0.885	0.892	1.777	0.916
99	ERBB1	SLPI	LY9	C9	Cadherin-5	0.885	0.877	1.762	0.927
100	Hat1	SLPI	Cadherin-5	α1-Antitrypsin	MCP-3	0.872	0.903	1.774	0.902

Marker	Count	Marker	Count
SLPI	99	Coagulation Factor Xa	5
C9	75	C6	5
LY9	60	C5	5
Cadherin-5	29	BAFF Receptor	5
RGM-C	23	ARSB	5
MCP-3	16	ADAM 9	5
SAP	14	sL-Selectin	4
MMP-7	14	α2-Antiplαsmin	4
NRP1	11	Troponin T	4
Growth hormone receptor	9	TIMP-2	4
C2	9	RBP	4
HSP 90α	8	Prekallikrein	4
α1-Antitrypsin	6	PCI	4
SCF sR	6	MRC2	4
Properdin	6	Kallistatin	4
HGF	6	Kallikrein 6	4
Contactin-1	5	IL-18 Rβ	4
MIP-5	5	IL-13 Rα1	4
Hat1	5	IL-12 Rβ2	4
ERBB1	5	α2-HS-Glycoprotein	1
Contactin-4	5	Thrombin/Prothrombin	1

TABLE 5

100 Panels of 6 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers					Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	SCF sR	C9	ADAM9	SLPI	SAP	MCP-3	0.923	0.872	1.795	0.923
2	SCF sR	C9	Cadherin-5	SLPI	ARSB	MCP-3	0.897	0.892	1.790	0.923
3	LY9	C9	MMP-7	SLPI	BAFF Receptor	Prekallikrein	0.923	0.867	1.790	0.922
4	LY9	SLPI	Coagulation Factor Xa	MMP-7	Cadherin-5	C2	0.910	0.918	1.828	0.943
5	C5	SLPI	RGM-C	LY9	Troponin T	α1-Antitrypsin	0.897	0.903	1.800	0.921
6	Cadherin-5	SLPI	C9	LY9	C6	IL-13 Rα1	0.923	0.887	1.810	0.926
7	SLPI	Contactin-4	Prekallikrein	LY9	Cadherin-5	MCP-3	0.885	0.923	1.808	0.921
8	Cadherin-5	SLPI	C9	LY9	ERBB1	IL-13 Rα1	0.910	0.897	1.808	0.924
9	Cadherin-5	C9	C2	SLPI	Growth hormone receptor	MMP-7	0.923	0.887	1.810	0.941

TABLE 5-continued

10	HGF	SLPI MRC2	C9 α2-Antiplαsmin	MMP-7	0.962	0.856	1.818	0.940
11	HGF	SLPI MRC2	C9 HSP 90α	MMP-7	0.949	0.856	1.805	0.934
12	HGF	SLPI MRC2	C9 Hat1	MMP-7	0.936	0.862	1.797	0.927
13	SLPI	Contactin-4 Prekallikrein	LY9 IL-12 Rβ2	MCP-3	0.859	0.923	1.782	0.910
14	MRC2	C9 NRP1	SLPI IL-18 Rβ	LY9	0.910	0.887	1.797	0.925
15	Growth hormone receptor	SLPI Contactin-4	C9 Kallikrein 6	LY9	0.923	0.882	1.805	0.916
16	RGM-C	C9 LY9	MMP-7 Kallistatin	SLPI	0.910	0.882	1.792	0.942
17	SLPI	NRP1 MIP-5	LY9 Cadherin-5	SAP	0.897	0.897	1.795	0.932
18	C6	SLPI Cadherin-5	LY9 PCI	C9	0.897	0.882	1.779	0.921
19	HGF	SLPI MRC2	C9 Properdin	MMP-7	0.923	0.877	1.800	0.936
20	RGM-C	C9 SAP	MMP-7 RBP	SLPI	0.936	0.862	1.797	0.940
21	HSP 90α	C9 IL-13 Rα1	SLPI TIMP-2	LY9	0.910	0.877	1.787	0.919
22	RGM-C	SLPI Thrombin/ Prothrombin	LY9 NRP1	C9	0.897	0.877	1.774	0.932
23	RGM-C	C9 SAP	MMP-7 α2-HS-Glycoprotein	SLPI	0.923	0.856	1.779	0.941
24	RGM-C	SLPI NRP1	LY9 Contactin-1	SAP	0.910	0.903	1.813	0.932
25	Cadherin-5	C9 sL-Selectin	SLPI Growth hormone receptor	MMP-7	0.910	0.897	1.808	0.938
26	RGM-C	SLPI α1-Antitrypsin	LY9 ADAM 9	SAP	0.885	0.908	1.792	0.910
27	RGM-C	SLPI α1-Antitrypsin	LY9 ARSB	SAP	0.885	0.897	1.782	0.917
28	RGM-C	SLPI α1-Antitrypsin	LY9 BAFF Receptor	SAP	0.885	0.897	1.782	0.913
29	RGM-C	SLPI NRP1	LY9 C5	SAP	0.923	0.877	1.800	0.928
30	Coagulation Factor Xa	SLPI MMP-7	C9 RGM-C	Cadherin-5	0.923	0.892	1.815	0.949
31	Coagulation Factor Xa	SLPI MMP-7	C9 ERBB1	Cadherin-5	0.910	0.892	1.803	0.937
32	SLPI	NRP1 C2	Cadherin-5 Hat1	LY9	0.885	0.908	1.792	0.930
33	Growth hormone receptor	SLPI LY9	SAP IL-12 Rβ2	α1-Antitrypsin	0.885	0.897	1.782	0.910
34	HGF	SLPI MRC2	C9 IL-18 Rβ	MMP-7	0.949	0.846	1.795	0.931
35	RGM-C	C9 SAP	MMP-7 Kallikrein 6	SLPI	0.936	0.867	1.803	0.941
36	Growth hormone receptor	SLPI Contactin-1	C9 Kallistatin	LY9	0.885	0.903	1.787	0.923
37	RGM-C	SLPI NRP1	LY9 MIP-5	SAP	0.910	0.877	1.787	0.930
38	RGM-C	SLPI HSP 90α	LY9 PCI	C9	0.897	0.877	1.774	0.921
39	SAP	SLPI MCP-3	RGM-C Cadherin-5	Properdin	0.885	0.913	1.797	0.935
40	HGF	SLPI MRC2	C9 RBP	MMP-7	0.936	0.856	1.792	0.930
41	RGM-C	C9 SAP	MMP-7 TIMP-2	SLPI	0.923	0.862	1.785	0.942
42	RGM-C	C9 MRC2	MCP-3 Thrombin/ Prothrombin	SLPI	0.885	0.887	1.772	0.928
43	HGF	SLPI MRC2	C9 Troponin T	MMP-7	0.949	0.846	1.795	0.936
44	α2-Antiplαsmin	C9 HGF	SLPI MMP-7	Cadherin-5	0.949	0.862	1.810	0.943
45	HGF	SLPI MRC2	C9 α2-HS- Glycoprotein	MMP-7	0.923	0.856	1.779	0.934

TABLE 5-continued

46	Cadherin-5	C9 sL-Selectin	SLPI HGF	MMP-7	0.936	0.867	1.803	0.941
47	SAP	SLPI MCP-3	RGM-C ADAM 9	Properdin	0.885	0.903	1.787	0.926
48	Coagulation Factor Xa	SLPI MMP-7	C9 ARSB	LY9	0.897	0.882	1.779	0.932
49	LY9	SLPI Coagulation Factor Xa	MMP-7 BAFF Receptor	C2	0.872	0.908	1.779	0.926
50	SLPI	NRP1 C5	LY9 HSP 90α	C9	0.923	0.872	1.795	0.924
51	Growth hormone receptor	SLPI SAP	C2 C6	LY9	0.885	0.918	1.803	0.933
52	Cadherin-5	C9 SAP	SLPI ERBB1	MMP-7	0.910	0.887	1.797	0.939
53	Hat1	SLPI Contactin-4	LY9 NRP1	C9	0.897	0.892	1.790	0.925
54	SLPI	Contactin-4 NRP1	LY9 IL-12 Rβ2	HSP 90α	0.872	0.908	1.779	0.912
55	SCF sR	C9 Cadherin-5	SLPI IL-18 Rβ	MCP-3	0.885	0.897	1.782	0.928
56	SLPI	NRP1 Kallikrein 6	LY9 Cadherin-5	SAP	0.910	0.892	1.803	0.928
57	Growth hormone receptor	SLPI C2	C9 Kallistatin	LY9	0.885	0.892	1.777	0.927
58	SLPI	NRP1 MIP-5	LY9 RGM-C	SAP	0.910	0.877	1.787	0.930
59	C6	SLPI Cadherin-5	LY9 PCI	RGM-C	0.885	0.887	1.772	0.920
60	RBP	C9 RGM-C	SLPI NRP1	LY9	0.910	0.877	1.787	0.923
61	Growth hormone receptor	SLPI LY9	SAP TIMP-2	α1-Antitrypsin	0.885	0.897	1.782	0.915
62	HGF	SLPI MRC2	C9 Thrombin/ Prothrombin	MMP-7	0.936	0.836	1.772	0.934
63	Growth hormone receptor	SLPI Cadherin-5	SAP Troponin T	α1-Antitrypsin	0.872	0.913	1.785	0.921
64	α2-Antiplαsmin	C9 C2	SLPI Cadherin-5	LY9	0.910	0.897	1.808	0.938
65	Growth hormone receptor	SLPI MRC2	C9 α2-HS-Glycoprotein	LY9	0.885	0.892	1.777	0.920
66	Growth hormone receptor	SLPI C2	C9 Contactin-1	LY9	0.910	0.897	1.808	0.929
67	HGF	SLPI MRC2	C9 sL-Selectin	MMP-7	0.936	0.867	1.803	0.938
68	Growth hormone receptor	SLPI Cadherin-5	SAP ADAM 9	α1-Antitrypsin	0.872	0.913	1.785	0.904
69	SCF sR	C9 ADAM 9	SLPI ARSB	MCP-3	0.897	0.882	1.779	0.911
70	Cadherin-5	C9 MRC2	MCP-3 BAFF Receptor	SLPI	0.872	0.903	1.774	0.923
71	HGF	SLPI C5	C9 Cadherin-5	α2-Antiplαsmin	0.936	0.856	1.792	0.927
72	Cadherin-5	C9 C2	SLPI ERBB1	MMP-7	0.897	0.897	1.795	0.939
73	Cadherin-5	SLPI C2	LY9 Hat1	IL-13 Rα1	0.897	0.892	1.790	0.922
74	Cadherin-5	C9 SAP	SLPI IL-12 Rβ2	MMP-7	0.897	0.882	1.779	0.939
75	SLPI	NRP1 C2	LY9 IL-18 Rβ	SAP	0.885	0.897	1.782	0.932
76	Cadherin-5	C9 Kallikrein 6	SLPI HSP 90α	MMP-7	0.923	0.872	1.795	0.935
77	SLPI	NRP1 LY9	Cadherin-5 Kallistatin	C9	0.885	0.887	1.772	0.928
78	SLPI	NRP1 LY9	Cadherin-5 MIP-5	C9	0.897	0.887	1.785	0.931
79	Growth hormone receptor	SLPI Contactin-1	C9 PCI	LY9	0.885	0.887	1.772	0.918
80	LY9	C9 RGM-C	SLPI IL-13 Rα1	Prekallikrein	0.949	0.851	1.800	0.923
81	RGM-C	SLPI MMP-7	LY9 Properdin	SAP	0.910	0.882	1.792	0.939

TABLE 5-continued

82	Cadherin-5	C9 LY9	SLPI RBP	MMP-7	0.897	0.887	1.785	0.933
83	C5	SLPI RGM-C	LY9 TIMP-2	α1-Antitrypsin	0.897	0.882	1.779	0.915
84	RGM-C	SLPI Thrombin/ Prothrombin	LY9 MCP-3	C9	0.897	0.872	1.769	0.926
85	SLPI	Contactin-4 Prekallikrein	LY9 Troponin T	MCP-3	0.885	0.897	1.782	0.911
86	HSP 90α	C9 LY9	SLPI α2-HS- Glycoprotein	Cadherin-5	0.885	0.887	1.772	0.922
87	RGM-C	C9 sL-Selectin	MMP-7 LY9	SLPI	0.910	0.887	1.797	0.941
88	Growth hormone receptor	SLPI Cadherin-5	SAP ARSB	α1-Antitrypsin	0.872	0.903	1.774	0.912
89	Growth hormone receptor	SLPI LY9	SAP BAFF Receptor	α1-Antitrypsin	0.885	0.887	1.772	0.907
90	Growth hormone receptor	SLPI Cadherin-5	SAP C6	LY9	0.897	0.903	1.800	0.929
91	RGM-C	SLPI NRP1	LY9 ERBB1	SAP	0.897	0.892	1.790	0.927
92	Hat1	SLPI SAP	LY9 Kallikrein 6	C2	0.885	0.897	1.782	0.913
93	SLPI	NRP1 C5	LY9 IL-12 Rβ2	C9	0.897	0.877	1.774	0.917
94	SLPI	NRP1 LY9	Cadherin-5 IL-18 Rβ	C9	0.897	0.877	1.774	0.930
95	Cadherin-5	SLPI C9	LY9 Kallistatin	IL-13 Rα1	0.897	0.872	1.769	0.926
96	Growth hormone receptor	SLPI MRC2	C9 MIP-5	LY9	0.897	0.887	1.785	0.927
97	RGM-C	SLPI PCI	Cadherin-5 LY9	C9	0.897	0.872	1.769	0.927
98	SAP	SLPI MCP-3	RGM-C Contactin-1	Properdin	0.859	0.928	1.787	0.932
99	RBP	C9 RGM-C	SLPI HGF	LY9	0.923	0.856	1.779	0.925
100	SCF sR	C9 Cadherin-5	SLPI IL-13 Rα1	MCP-3	0.897	0.903	1.800	0.926

Marker	Count	Marker	Count
SLPI	100	Properdin	5
C9	65	Prekallikrein	5
LY9	62	PCI	5
Cadherin-5	38	MIP-5	5
MMP-7	32	Kallistatin	5
SAP	31	Kallikrein 6	5
RGM-C	30	IL-18 Rβ	5
NRP1	19	IL-12 Rβ2	5
Growth hormone receptor	17	Hat1	5
MRC2	15	ERBB1	5
MCP-3	14	Coagulation Factor Xa	5
HGF	14	C6	5
C2	12	BAFF Receptor	5
α1-Antitrypsin	11	ARSB	5
IL-13 Rα1	7	ADAM 9	5
HSP 90α	7	sL-Selectin	4
Contactin-4	6	α2-HS-Glycoprotein	4
C5	6	α2-Antiplαsmin	4
Contactin-1	5	Troponin T	4
SCF sR	5	Thrombin/ Prothrombin	4
RBP	5	TIMP-2	4

TABLE 6

100 Panels of 7 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers				Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	SAP	SLPI	RGM-C	MCP-3	0.897	0.923	1.821	0.919
		α1-Antitrypsin	Cadherin-5	ADAM 9				
2	Cadherin-5	C9	SLPI	MMP-7	0.923	0.882	1.805	0.940
		LY9	RGM-C	ARSB				
3	HGF	SLPI	C9	MMP-7	0.936	0.887	1.823	0.928
		MRC2	Properdin	BAFF Receptor				
4	α2-Antiplasmin	C9	SLPI	Cadherin-5	0.949	0.882	1.831	0.946
		HGF	C2	MMP-7				
5	LY9	C9	SLPI	Prekallikrein	0.936	0.872	1.808	0.932
		MMP-7	HSP 90α	C5				
6	α2-Antiplasmin	C9	SLPI	Cadherin-5	0.936	0.887	1.823	0.945
		HGF	MMP-7	C6				
7	SLPI	NRP1	LY9	SAP	0.923	0.908	1.831	0.934
		MMP-7	Coagulation Factor Xa	MRC2				
8	HGF	SLPI	C9	α2-Antiplasmin	0.962	0.867	1.828	0.942
		SAP	MMP-7	Contactin-4				
9	HSP 90α	C9	SLPI	LY9	0.949	0.862	1.810	0.925
		HGF	C2	ERBB1				
10	HGF	SLPI	C9	α2-Antiplasmin	0.962	0.862	1.823	0.939
		SAP	MMP-7	Growth hormone receptor				
11	HGF	SLPI	C9	MMP-7	0.949	0.867	1.815	0.932
		MRC2	Hat1	LY9				
12	HGF	SLPI	C9	MMP-7	0.936	0.867	1.803	0.939
		MRC2	α2-Antiplasmin	IL-12 Rβ2				
13	SLPI	NRP1	Cadherin-5	C9	0.923	0.892	1.815	0.925
		LY9	Contactin-1	IL-13 Rα1				
14	HGF	SLPI	C9	MMP-7	0.949	0.856	1.805	0.937
		MRC2	Coagulation Factor Xa	IL-18 Rβ				
15	Cadherin-5	C9	SLPI	MMP-7	0.936	0.882	1.818	0.940
		Kallikrein 6	HSP 90α	RGM-C				
16	α2-Antiplasmin	C9	SLPI	Cadherin-5	0.936	0.872	1.808	0.946
		HGF	MMP-7	Kallistatin				
17	RGM-C	C9	MMP-7	SLPI	0.923	0.887	1.810	0.941
		sL-Selectin	LY9	MIP-5				
18	Cadherin-5	C9	SLPI	MMP-7	0.936	0.862	1.797	0.949
		SAP	RGM-C	PCI				
19	MRC2	C9	SLPI	LY9	0.923	0.897	1.821	0.925
		NRP1	MMP-7	RBP				
20	HGF	SLPI	C9	MMP-7	0.949	0.877	1.826	0.935
		MRC2	MCP-3	SCF sR				
21	HGF	SLPI	C9	MMP-7	0.949	0.867	1.815	0.942
		MRC2	α2-Antiplasmin	TIMP-2				
22	HGF	SLPI	C9	MMP-7	0.949	0.851	1.800	0.941
		MRC2	α2-Antiplasmin	Thrombin/ Prothrombin				
23	HGF	SLPI	C9	MMP-7	0.949	0.872	1.821	0.941
		MRC2	Troponin T	α2-Antiplasmin				
24	Cadherin-5	C9	SLPI	MMP-7	0.910	0.887	1.797	0.946
		C2	RGM-C	α2-HS-Glycoprotein				
25	LY9	C9	SLPI	Prekallikrein	0.923	0.892	1.815	0.927
		MMP-7	SAP	ADAM 9				
26	Growth hormone receptor	SLPI	C9	LY9	0.910	0.887	1.797	0.911
		Contactin-4	Kallikrein 6	ARSB				
27	HGF	SLPI	C9	α2-Antiplasmin	0.962	0.856	1.818	0.931
		SAP	MMP-7	BAFF Receptor				
28	LY9	C9	SLPI	Prekallikrein	0.923	0.877	1.800	0.926
		RGM-C	MCP-3	C5				
29	SLPI	NRP1	Cadherin-5	C9	0.923	0.887	1.810	0.940
		LY9	MMP-7	C6				
30	Cadherin-5	C9	SLPI	MMP-7	0.910	0.897	1.808	0.939
		SAP	ERBB1	Growth hormone receptor				
31	HGF	SLPI	C9	MMP-7	0.949	0.862	1.810	0.933
		MRC2	Hat1	SAP				
32	α2-Antiplasmin	C9	SLPI	Cadherin-5	0.936	0.862	1.797	0.941
		HGF	MMP-7	IL-12 Rβ2				

TABLE 6-continued

33	Cadherin-5	C9 C2	SLPI RGM-C	MMP-7 IL-13 Rα1	0.936	0.877	1.813	0.947
34	HGF	SLPI MRC2	C9 IL-18 Rβ	MMP-7 RGM-C	0.949	0.856	1.805	0.941
35	RGM-C	C9 SAP	MMP-7 LY9	SLPI Kallistatin	0.936	0.862	1.797	0.944
36	RGM-C	C9 SAP	MMP-7 MRC2	SLPI MIP-5	0.923	0.882	1.805	0.946
37	Coagulation Factor Xa	SLPI MMP-7	C9 RGM-C	Cadherin-5 PCI	0.910	0.887	1.797	0.945
38	HGF	SLPI MRC2	C9 Properdin	MMP-7 MCP-3	0.949	0.882	1.831	0.932
39	Cadherin-5	C9 LY9	SLPI RGM-C	MMP-7 RBP	0.923	0.892	1.815	0.940
40	HGF	SLPI Cadherin-5	C9 SCF sR	MMP-7 MCP-3	0.936	0.887	1.823	0.937
41	RGM-C	C9 SAP	MMP-7 MRC2	SLPI TIMP-2	0.936	0.867	1.803	0.942
42	SLPI	NRP1 RGM-C	LY9 MRC2	C9 Thrombin/ Prothrombin	0.910	0.887	1.797	0.933
43	HGF	SLPI MRC2	C9 Troponin T	MMP-7 RGM-C	0.962	0.856	1.818	0.944
44	Growth hormone receptor	SLPI Cadherin-5	SAP LY9	α1-Antitrypsin HGF	0.936	0.872	1.808	0.921
45	Cadherin-5	C9 SAP	SLPI RGM-C	MMP-7 α2-HS-Glycoprotein	0.923	0.872	1.795	0.949
46	Cadherin-5	C9 SAP	SLPI HGF	MMP-7 Contactin-1	0.962	0.862	1.823	0.945
47	HGF	SLPI MRC2	C9 sL-Selectin	MMP-7 α2-Antiplαsmin	0.962	0.867	1.828	0.942
48	Cadherin-5	C9 LY9	SLPI Prekallikrein	MMP-7 ADAM 9	0.910	0.897	1.808	0.927
49	Growth hormone receptor	SLPI Cadherin-5	SAP LY9	α1-Antitrypsin ARSB	0.885	0.908	1.792	0.916
50	α2-Antiplαsmin	C9 HGF	SLPI MMP-7	Cadherin-5 BAFF Receptor	0.949	0.867	1.815	0.932
51	C5	SLPI RGM-C	LY9 Troponin T	α1-Antitrypsin Growth hormone receptor	0.910	0.887	1.797	0.916
52	LY9	SLPI Coagulation Factor Xa	MMP-7 Cadherin-5	C2 C6	0.897	0.913	1.810	0.942
53	RGM-C	C9 SAP	MMP-7 HGF	SLPI Contactin-4	0.962	0.856	1.818	0.946
54	Cadherin-5	C9 C2	SLPI ERBB1	MMP-7 HSP 90α	0.923	0.882	1.805	0.938
55	HGF	SLPI MRC2	C9 Hat1	MMP-7 α2-Antiplαsmin	0.923	0.882	1.805	0.934
56	LY9	SLPI Coagulation Factor Xa	MMP-7 Cadherin-5	C2 IL-12 Rβ2	0.885	0.913	1.797	0.938
57	HGF	SLPI MRC2	C9 HSP 90α	MMP-7 IL-13 Rα1	0.962	0.851	1.813	0.936
58	HGF	SLPI MRC2	C9 IL-18 Rβ	MMP-7 LY9	0.936	0.867	1.803	0.932
59	HGF	SLPI MRC2	C9 Coagulation Factor Xa	MMP-7 Kallikrein 6	0.949	0.867	1.815	0.937
60	Cadherin-5	C9 Kallikrein 6	SLPI HSP 90α	MMP-7 Kallistatin	0.910	0.887	1.797	0.936
61	RGM-C	C9 LY9	MMP-7 HGF	SLPI MIP-5	0.962	0.841	1.803	0.939
62	RGM-C	C9 SAP	MMP-7 LY9	SLPI PCI	0.923	0.862	1.785	0.940
63	HGF	SLPI MRC2	C9 Properdin	MMP-7 RGM-C	0.949	0.877	1.826	0.945
64	C2	SLPI RGM-C	LY9 MMP-7	C9 RBP	0.923	0.892	1.815	0.943
65	RGM-C	C9 LY9	MMP-7 HGF	SLPI SCF sR	0.949	0.867	1.815	0.945
66	Growth hormone receptor	SLPI Cadherin-5	SAP C6	LY9 TIMP-2	0.897	0.897	1.795	0.927

TABLE 6-continued

67	Contactin-1	SLPI MMP-7	LY9 SAP	Growth hormone receptor Thrombin/ Prothrombin	0.910	0.887	1.797	0.931
68	Cadherin-5	C9 LY9	SLPI RGM-C	MMP-7 α2-HS-Glycoprotein	0.923	0.872	1.795	0.944
69	Cadherin-5	C9 sL-Selectin	SLPI HGF	MMP-7 MRC2	0.936	0.887	1.823	0.943
70	RGM-C	C9 MRC2	MCP-3 α2-Antiplαsmin	SLPI ADAM 9	0.897	0.908	1.805	0.928
71	Cadherin-5	C9 LY9	SLPI Prekallikrein	MMP-7 ARSB	0.897	0.892	1.790	0.932
72	HGF	SLPI MRC2	C9 MCP-3	MMP-7 BAFF Receptor	0.936	0.877	1.813	0.930
73	C5	SLPI RGM-C	LY9 Troponin T	α1-Antitrypsin C2	0.897	0.897	1.795	0.919
74	LY9	SLPI Coagulation Factor Xa	MMP-7 Cadherin-5	C2 Contactin-4	0.897	0.918	1.815	0.937
75	HGF	SLPI MRC2	C9 Properdin	MMP-7 ERBB1	0.923	0.882	1.805	0.935
76	HGF	SLPI MRC2	C9 α2-Antiplαsmin	MMP-7 Hat1	0.923	0.882	1.805	0.934
77	Growth hormone receptor	SLPI Cadherin-5	SAP LY9	α1-Antitrypsin IL-12 Rβ2	0.897	0.897	1.795	0.913
78	HGF	SLPI MRC2	C9 LY9	MMP-7 IL-13 Rα1	0.949	0.862	1.810	0.932
79	HGF	SLPI MRC2	C9 LY9	MMP-7 IL-18 Rβ	0.936	0.867	1.803	0.932
80	SLPI	NRP1 LY9	Cadherin-5 MMP-7	C9 Kallistatin	0.910	0.887	1.797	0.940
81	Cadherin-5	C9 LY9	SLPI Prekallikrein	MMP-7 MIP-5	0.923	0.877	1.800	0.939
82	α2-Antiplαsmin	C9 HGF	SLPI MMP-7	Cadherin-5 PCI	0.923	0.862	1.785	0.941
83	Cadherin-5	C9 sL-Selectin	SLPI Growth hormone	MMP-7 RBP receptor	0.923	0.892	1.815	0.931
84	SCF sR	C9 Cadherin-5	SLPI HGF	MCP-3 SAP	0.936	0.877	1.813	0.933
85	C2	SLPI RGM-C	LY9 MMP-7	C9 TIMP-2	0.923	0.872	1.795	0.943
86	α2-Antiplαsmin	C9 HGF	SLPI MMP-7	Cadherin-5 Thrombin/ Prothrombin	0.936	0.856	1.792	0.943
87	HGF	SLPI Cadherin-5	C9 SCF sR	MMP-7 α2-HS-Glycoprotein	0.923	0.867	1.790	0.942
88	RGM-C	C9 SAP	MMP-7 HGF	SLPI Contactin-1	0.962	0.856	1.818	0.948
89	C2	SLPI RGM-C	LY9 MMP-7	C9 ADAM 9	0.923	0.877	1.800	0.934
90	Cadherin-5	C9 SAP	SLPI NRP1	MMP-7 ARSB	0.897	0.892	1.790	0.940
91	RGM-C	C9 SAP	MMP-7 HGF	SLPI BAFF Receptor	0.949	0.862	1.810	0.936
92	C5	SLPI RGM-C	LY9 Troponin T	α1-Antitrypsin MCP-3	0.897	0.897	1.795	0.913
93	Growth hormone receptor	SLPI SAP	C2 C6	LY9 IL-13 Rα1	0.910	0.897	1.808	0.931
94	RGM-C	C9 LY9	MMP-7 HGF	SLPI Contactin-4	0.949	0.862	1.810	0.942
95	Cadherin-5	C9 SAP	SLPI ERBB1	MMP-7 HGF	0.949	0.856	1.805	0.943
96	HGF	SLPI MRC2	C9 Hat1	MMP-7 SCF sR	0.910	0.892	1.803	0.930
97	RGM-C	SLPI NRP1	LY9 Coagulation Factor Xa	SAP IL-12 Rβ2	0.897	0.897	1.795	0.926

TABLE 6-continued

98	HGF	SLPI	C9	MMP-7	0.936	0.862	1.797	0.939
		MRC2	IL-18 Rβ	Cadherin-5				
99	Cadherin-5	C9	SLPI	MMP-7	0.936	0.877	1.813	0.934
		Kallikrein 6	HSP 90α	LY9				
100	Cadherin-5	C9	SLPI	MMP-7	0.910	0.882	1.792	0.937
		LY9	Prekallikrein	Kallistatin				

Marker	Count	Marker	Count
SLPI	100	Kallikrein 6	5
C9	85	IL-18 Rβ	5
MMP-7	83	IL-13 Rα1	5
HGF	49	IL-12 Rβ2	5
LY9	45	Hat1	5
Cadherin-5	44	ERBB1	5
RGM-C	34	Contactin-4	5
MRC2	32	C6	5
SAP	28	C5	5
α2-Antiplαsmin	18	BAFF Receptor	5
C2	13	ARSB	5
Growth hormone receptor	11	ADAM 9	5
MCP-3	9	sL-Selectin	4
NRP1	8	Contactin-1	4
Coagulation Factor Xa	8	α2-HS-Glycoprotein	4
α1-Antitrypsin	7	Thrombin/Prothrombin	4
Prekallikrein	7	TIMP-2	4
HSP 90α	7	RBP	4
SCF sR	6	Properdin	4
Troponin T	5	PCI	4
Kallistatin	5	MIP-5	4

TABLE 7

100 Panels of 8 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers				Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	HGF	SLPI	C9	MMP-7	0.962	0.872	1.833	0.935
	MRC2	Properdin	RGM-C	ADAM 9				
2	Cadherin-5	C9	SLPI	MMP-7	0.923	0.892	1.815	0.945
	C2	RGM-C	α2-Antiplαsmin	ARSB				
3	HGF	SLPI	C9	MMP-7	0.962	0.897	1.859	0.938
	MRC2	MCP-3	BAFF Receptor	α2-Antiplαsmin				
4	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.862	1.823	0.943
	HGF	MMP-7	Coagulation Factor Xa	C5				
5	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.872	1.833	0.944
	HGF	MMP-7	Coagulation Factor Xa	C6				
6	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.897	1.859	0.951
	RGM-C	MMP-7	HGF	Contactin-4				
7	Cadherin-5	C9	SLPI	MMP-7	0.949	0.882	1.831	0.942
	SAP	HGF	Kallikrein 6	ERBB1				
8	Cadherin-5	C9	SLPI	MMP-7	0.962	0.877	1.838	0.946
	SAP	HGF	Contactin-1	Growth hormone receptor				
9	HGF	SLPI	C9	MMP-7	0.962	0.887	1.849	0.939
	MRC2	HSP 90α	MCP-3	α2-Antiplαsmin				
10	HGF	SLPI	C9	MMP-7	0.949	0.882	1.831	0.940
	MRC2	α2-Antiplαsmin	RGM-C	Hat1				
11	HGF	SLPI	C9	MMP-7	0.936	0.887	1.823	0.942
	MRC2	Properdin	Cadherin-5	IL-12 Rβ2				
12	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.867	1.828	0.946
	RGM-C	MMP-7	HGF	IL-13 Rα1				
13	HGF	SLPI	C9	MMP-7	0.949	0.872	1.821	0.942
	MRC2	Properdin	Cadherin-5	IL-18 Rβ				
14	RGM-C	C9	MMP-7	SLPI	0.974	0.856	1.831	0.949
	SAP	HGF	HSP 90α	Kallistatin				
15	SLPI	NRP1	LY9	C9	0.949	0.892	1.841	0.941
	RGM-C	MRC2	MMP-7	HGF				
16	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.882	1.831	0.946
	HGF	MMP-7	MRC2	MIP-5				
17	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.862	1.823	0.949
	RGM-C	MMP-7	HGF	PCI				

TABLE 7-continued

18	RGM-C	C9	MMP-7	SLPI	0.962	0.862	1.823	0.950
	SAP	HGF	MRC2	Prekallikrein				
19	HGF	SLPI	C9	MMP-7	0.949	0.882	1.831	0.942
	MRC2	Properdin	RGM-C	RBP				
20	HGF	SLPI	C9	MMP-7	0.962	0.892	1.854	0.943
	Cadherin-5	SCF sR	MCP-3	RGM-C				
21	HGF	SLPI	C9	MMP-7	0.962	0.872	1.833	0.945
	MRC2	α2-Antiplαsmin	TIMP-2	SAP				
22	HGF	SLPI	C9	MMP-7	0.974	0.862	1.836	0.948
	MRC2	HSP 90α	RGM-C	Thrombin/Prothrombin				
23	HGF	SLPI	C9	MMP-7	0.962	0.872	1.833	0.948
	MRC2	Troponin T	RGM-C	α2-Antiplαsmin				
24	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.936	0.877	1.813	0.939
	RGM-C	MMP-7	HGF	α1-Antitrypsin				
25	HGF	SLPI	C9	MMP-7	0.962	0.867	1.828	0.945
	MRC2	HSP 90α	RGM-C	α2-HS-Glycoprotein				
26	HGF	SLPI	C9	α2-Antiplαsmin	0.974	0.877	1.851	0.949
	SAP	MMP-7	sL-Selectin	Cadherin-5				
27	RGM-C	C9	MMP-7	SLPI	0.949	0.877	1.826	0.937
	SAP	HGF	Contactin-4	ADAM 9				
28	HGF	SLPI	C9	MMP-7	0.936	0.877	1.813	0.939
	MRC2	sL-Selectin	α2-Antiplαsmin	ARSB				
29	HGF	SLPI	C9	MMP-7	0.962	0.872	1.833	0.939
	MRC2	α2-Antiplαsmin	RGM-C	BAFF Receptor				
30	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.882	1.844	0.946
	HGF	MMP-7	Coagulation Factor Xa	C2				
31	HGF	SLPI	C9	MMP-7	0.949	0.872	1.821	0.945
	MRC2	Properdin	RGM-C	C5				
32	HGF	SLPI	C9	MMP-7	0.962	0.872	1.833	0.945
	MRC2	HSP 90α	RGM-C	C6				
33	Cadherin-5	C9	SLPI	MMP-7	0.949	0.877	1.826	0.944
	SAP	HGF	Properdin	ERBB1				
34	HGF	SLPI	C9	α2-Antiplαsmin	0.974	0.862	1.836	0.942
	SAP	MMP-7	Contactin-1	Growth hormone receptor				
35	RGM-C	C9	MCP-3	SLPI	0.936	0.892	1.828	0.927
	MRC2	α2-Antiplαsmin	HGF	Hat1				
36	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.936	0.887	1.823	0.945
	HGF	MMP-7	MRC2	IL-12 Rβ2				
37	HGF	SLPI	C9	MMP-7	0.962	0.867	1.828	0.944
	MRC2	Coagulation Factor Xa	RGM-C	IL-13 Rα1				
38	HGF	SLPI	C9	MMP-7	0.936	0.877	1.813	0.947
	MRC2	α2-Antiplαsmin	RGM-C	IL-18 Rβ				
39	RGM-C	C9	MMP-7	SLPI	0.974	0.867	1.841	0.946
	SAP	HGF	MRC2	Kallikrein 6				
40	HGF	SLPI	C9	MMP-7	0.962	0.867	1.828	0.946
	MRC2	HSP 90α	RGM-C	Kallistatin				
41	Cadherin-5	C9	SLPI	MMP-7	0.936	0.903	1.838	0.942
	LY9	RGM-C	MRC2	NRP1				
42	HGF	SLPI	C9	MMP-7	0.962	0.862	1.823	0.942
	MRC2	HSP 90α	RGM-C	MIP-5				
43	Cadherin-5	C9	SLPI	MMP-7	0.910	0.897	1.808	0.947
	SAP	RGM-C	Prekallikrein	PCI				
44	Cadherin-5	C9	SLPI	MMP-7	0.936	0.892	1.828	0.941
	sL-Selectin	HGF	MRC2	RBP				
45	HGF	SLPI	C9	MMP-7	0.949	0.897	1.846	0.939
	MRC2	MCP-3	Cadherin-5	SCF sR				
46	RGM-C	C9	MCP-3	SLPI	0.949	0.877	1.826	0.938
	MRC2	HGF	MMP-7	TIMP-2				
47	RGM-C	C9	MMP-7	SLPI	0.962	0.862	1.823	0.945
	LY9	HGF	MRC2	Thrombin/Prothrombin				
48	HGF	SLPI	C9	MMP-7	0.962	0.862	1.823	0.947
	MRC2	Troponin T	RGM-C	sL-Selectin				
49	HGF	SLPI	C9	MMP-7	0.923	0.887	1.810	0.925
	MRC2	MCP-3	BAFF Receptor	α1-Antitrypsin				
50	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.877	1.826	0.944
	HGF	MMP-7	Contactin-1	α2-HS-Glycoprotein				
51	RGM-C	C9	MMP-7	SLPI	0.962	0.862	1.823	0.935
	SAP	Coagulation Factor Xa	HGF	ADAM 9				
52	HGF	SLPI	C9	MMP-7	0.936	0.872	1.808	0.945
	MRC2	α2-Antiplαsmin	RGM-C	ARSB				
53	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.882	1.844	0.948
	HGF	C2	MMP-7	HSP 90α				
54	RGM-C	C9	MMP-7	SLPI	0.962	0.851	1.813	0.943
	SAP	HGF	Contactin-4	C5				

TABLE 7-continued

55	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.877	1.826	0.945
	HGF	MMP-7	Contactin-1	C6				
56	LY9	SLPI	MMP-7	C2	0.949	0.867	1.815	0.933
	Coagulation Factor Xa	Cadherin-5	HGF	ERBB1				
57	RGM-C	C9	MMP-7	SLPI	0.974	0.862	1.836	0.944
	SAP	HGF	Contactin-4	Growth hormone receptor				
58	HGF	SLPI	C9	MMP-7	0.949	0.877	1.826	0.934
	MRC2	Hat1	LY9	C2				
59	Cadherin-5	C9	SLPI	MMP-7	0.936	0.877	1.813	0.944
	SAP	HGF	Properdin	IL-12 Rβ2				
60	Cadherin-5	C9	SLPI	MMP-7	0.936	0.887	1.823	0.949
	C2	RGM-C	IL-13 Rα1	Coagulation Factor Xa				
61	Cadherin-5	C9	SLPI	MMP-7	0.949	0.862	1.810	0.944
	SAP	HGF	Contactin-1	IL-18 Rβ				
62	HGF	SLPI	C9	MMP-7	0.974	0.862	1.836	0.942
	MRC2	HSP 90α	RGM-C	Kallikrein 6				
63	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.877	1.826	0.953
	RGM-C	MMP-7	HGF	Kallistatin				
64	HGF	SLPI	C9	MMP-7	0.923	0.892	1.815	0.942
	MRC2	Properdin	Cadherin-5	MIP-5				
65	RGM-C	C9	MMP-7	SLPI	0.974	0.872	1.846	0.947
	SAP	HGF	Contactin-4	NRP1				
66	Coagulation Factor Xa	SLPI	C9	Cadherin-5	0.910	0.897	1.808	0.946
	MMP-7	RGM-C	sL-Selectin	PCI				
67	Cadherin-5	C9	SLPI	MMP-7	0.936	0.887	1.823	0.938
	SAP	RGM-C	Prekallikrein	ADAM 9				
68	RGM-C	C9	MMP-7	SLPI	0.949	0.877	1.826	0.944
	SAP	HGF	MRC2	RBP				
69	HGF	SLPI	C9	MMP-7	0.949	0.892	1.841	0.938
	Cadherin-5	SCF sR	MCP-3	Coagulation Factor Xa				
70	HGF	SLPI	C9	MMP-7	0.949	0.877	1.826	0.941
	MRC2	α2-Antiplαsmin	TIMP-2	NRP1				
71	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.962	0.862	1.823	0.950
	RGM-C	MMP-7	HGF	Thrombin/Prothrombin				
72	HGF	SLPI	C9	MMP-7	0.949	0.872	1.821	0.947
	MRC2	Troponin T	RGM-C	Properdin				
73	RGM-C	C9	MMP-7	SLPI	0.949	0.862	1.810	0.940
	SAP	HGF	HSP 90α	α1-Antitrypsin				
74	SLPI	NRP1	LY9	C9	0.923	0.897	1.821	0.938
	RGM-C	MRC2	MMP-7	α2-HS-Glycoprotein				
75	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.936	0.872	1.808	0.945
	RGM-C	MMP-7	HGF	ARSB				
76	HGF	SLPI	C9	MMP-7	0.949	0.882	1.831	0.935
	MRC2	MCP-3	BAFF Receptor	sL-Selectin				
77	RGM-C	C9	MMP-7	SLPI	0.962	0.851	1.813	0.939
	LY9	HGF	MRC2	C5				
78	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.877	1.826	0.945
	HGF	MMP-7	C6	Contactin-1				
79	Cadherin-5	C9	SLPI	MMP-7	0.949	0.867	1.815	0.935
	Kallikrein 6	HSP 90α	RGM-C	ERBB1				
80	HGF	SLPI	C9	α2-Antiplαsmin	0.962	0.872	1.833	0.946
	SAP	MMP-7	Growth hormone receptor	Cadherin-5				
81	Cadherin-5	C9	SLPI	MMP-7	0.923	0.897	1.821	0.940
	SAP	HGF	Contactin-1	Hat1				
82	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.936	0.877	1.813	0.947
	RGM-C	MMP-7	HGF	IL-12 Rβ2				
83	SLPI	NRP1	Cadherin-5	C9	0.923	0.897	1.821	0.929
	LY9	Contactin-1	IL-13 Rα1	SAP				
84	HGF	SLPI	C9	MMP-7	0.936	0.867	1.803	0.942
	MRC2	Coagulation Factor Xa	Cadherin-5	IL-18 Rβ				
85	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.949	0.872	1.821	0.948
	HGF	MMP-7	MRC2	Kallistatin				
86	HGF	SLPI	C9	MMP-7	0.949	0.867	1.815	0.942
	MRC2	Coagulation Factor Xa	Cadherin-5	MIP-5				
87	HGF	SLPI	C9	MMP-7	0.949	0.856	1.805	0.939
	MRC2	α2-Antiplαsmin	TIMP-2	PCI				
88	LY9	C9	SLPI	Prekallikrein	0.936	0.887	1.823	0.933
	MMP-7	SAP	ADAM 9	C2				
89	α2-Antiplαsmin	C9	SLPI	Cadherin-5	0.936	0.887	1.823	0.943
	HGF	MMP-7	MRC2	RBP				
90	RGM-C	C9	MCP-3	SLPI	0.949	0.887	1.836	0.942
	MRC2	HGF	MMP-7	SCF sR				

TABLE 7-continued

91	SLPI MMP-7	NRP1 MRC2	LY9 HGF	SAP Thrombin/Prothrombin	0.949	0.872	1.821	0.935
92	HGF MRC2	SLPI Properdin	C9 RGM-C	MMP-7 Troponin T	0.949	0.872	1.821	0.947
93	SCF sR Cadherin-5	C9 HGF	SLPI SAP	MCP-3 α1-Antitrypsin	0.910	0.897	1.808	0.920
94	HGF MRC2	SLPI HSP 90α	C9 MCP-3	MMP-7 α2-HS-Glycoprotein	0.949	0.872	1.821	0.930
95	Cadherin-5 C2	C9 RGM-C	SLPI IL-13 Rα1	MMP-7 ARSB	0.923	0.882	1.805	0.940
96	α2-Antiplαsmin HGF	C9 MMP-7	SLPI BAFF Receptor	Cadherin-5 SAP	0.949	0.882	1.831	0.937
97	α2-Antiplαsmin RGM-C	C9 MMP-7	SLPI HGF	Cadherin-5 C5	0.949	0.862	1.810	0.950
98	α2-Antiplαsmin HGF	C9 MMP-7	SLPI C6	Cadherin-5 Contactin-4	0.949	0.877	1.826	0.945
99	MRC2 NRP1	C9 MMP-7	SLPI HGF	LY9 ERBB1	0.949	0.867	1.815	0.931
100	RGM-C SAP	C9 HGF	MMP-7 MRC2	SLPI Growth hormone receptor	0.962	0.872	1.833	0.943

Marker	Count	Marker	Count
SLPI	100	Growth hormone receptor	5
C9	98	ERBB1	5
MMP-7	97	C6	5
HGF	89	C5	5
RGM-C	54	BAFF Receptor	5
MRC2	53	ARSB	5
Cadherin-5	50	ADAM 9	5
α2-Antiplαsmin	38	α2-HS-Glycoprotein	4
SAP	28	α1-Antitrypsin	4
MCP-3	12	Troponin T	4
HSP 90α	12	Thrombin/Prothrombin	4
LY9	11	TIMP-2	4
Coagulation Factor Xa	11	RBP	4
Properdin	10	Prekallikrein	4
Contactin-1	8	PCI	4
NRP1	8	MIP-5	4
C2	8	Kallistatin	4
sL-Selectin	6	Kallikrein 6	4
Contactin-4	6	IL-18 Rβ	4
SCF sR	5	IL-12 Rβ2	4
IL-13 Rα1	5	Hat1	4

TABLE 8

100 Panels of 9 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers					Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	RGM-C	C9 HGF	MCP-3 MMP-7	SLPI sL-Selectin	MRC2 ADAM 9	0.962	0.897	1.859	0.939
2	RGM-C	C9 HGF	MMP-7 MRC2	SLPI NRP1	SAP ARSB	0.962	0.877	1.838	0.945
3	HGF	SLPI α2-Antiplαsmin	C9 RGM-C	MMP-7 BAFF Receptor	MRC2 MCP-3	0.962	0.897	1.859	0.942
4	α2-Antiplαsmin	C9 C2	SLPI MMP-7	Cadherin-5 Contactin-4	HGF RGM-C	0.962	0.903	1.864	0.952
5	α2-Antiplαsmin	C9 MMP-7	SLPI HGF	Cadherin-5 Contactin-4	RGM-C C5	0.962	0.887	1.849	0.951
6	α2-Antiplαsmin	C9 MMP-7	SLPI HGF	Cadherin-5 SAP	RGM-C C6	0.962	0.892	1.854	0.954
7	RGM-C	C9 HGF	MMP-7 Contactin-4	SLPI MCP-3	SAP Coagulation Factor Xa	0.974	0.882	1.856	0.942
8	RGM-C	C9 HGF	MMP-7 HSP 90α	SLPI α2-Antiplαsmin	SAP ERBB1	0.974	0.877	1.851	0.947
9	RGM-C	C9 HGF	MMP-7 Contactin-4	SLPI Growth hormone receptor	SAP Contactin-1	0.974	0.872	1.846	0.947

TABLE 8-continued

10	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.892	1.841	0.944
		α2-Antiplαsmin	RGM-C	Hat1	SAP				
11	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.877	1.838	0.952
		MMP-7	HGF	SAP	IL-12 Rβ2				
12	α2-Antiplαsmin	C9	SLPI	Cadherin-5	HGF	0.962	0.877	1.838	0.945
		C2	MMP-7	HSP 90α	IL-13 Rα1				
13	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.872	1.833	0.942
		Properdin	RGM-C	RBP	IL-18 Rβ				
14	Cadherin-5	C9	SLPI	MMP-7	SAP	0.962	0.882	1.844	0.949
		HGF	Kallikrein 6	RGM-C	MRC2				
15	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.882	1.844	0.952
		MMP-7	HGF	Contactin-4	Kallistatin				
16	RGM-C	C9	MMP-7	SLPI	LY9	0.949	0.897	1.846	0.944
		HGF	MRC2	C2	NRP1				
17	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.974	0.882	1.856	0.953
		MMP-7	HGF	SAP	MIP-5				
18	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.882	1.844	0.949
		MMP-7	HGF	Contactin-4	PCI				
19	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.887	1.849	0.946
		HGF	MMP-7	SAP	Prekallikrein				
20	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.908	1.856	0.944
		HGF	MMP-7	Cadherin-5	SCF sR				
21	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.877	1.838	0.942
		HGF	MMP-7	SAP	TIMP-2				
22	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.882	1.844	0.950
		α2-Antiplαsmin	RGM-C	sL-Selectin	Thrombin/ Prothrombin				
23	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.877	1.838	0.947
		HGF	MRC2	NRP1	Troponin T				
24	HGF	SLPI	C9	MMP-7	Cadherin-5	0.936	0.887	1.823	0.929
		SCF sR	MCP-3	Coagulation Factor Xa	α1-Antitrypsin				
25	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.913	1.849	0.939
		MCP-3	Cadherin-5	SCF sR	α2-HS-Glycoprotein				
26	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.892	1.854	0.939
		Properdin	RGM-C	ADAM 9	SAP				
27	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.877	1.838	0.945
		HGF	Contactin-4	α2-Antiplαsmin	ARSB				
28	HGF	SLPI	C9	α2-Antiplαsmin	SAP	0.974	0.882	1.856	0.940
		MMP-7	BAFF Receptor	RGM-C	Contactin-4				
29	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.882	1.844	0.952
		MMP-7	HGF	SAP	C5				
30	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.952
		MMP-7	HGF	Contactin-4	C6				
31	Cadherin-5	C9	SLPI	MMP-7	SAP	0.949	0.887	1.836	0.938
		HGF	Coagulation Factor Xa	MCP-3	ERBB1				
32	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.892	1.841	0.946
		α2-Antiplαsmin	Growth hormone receptor	Cadherin-5	C6				
33	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.887	1.836	0.939
		α2-Antiplαsmin	RGM-C	Hat1	NRP1				
34	α2-Antiplαsmin	C9	SLPI	Cadherin-5	HGF	0.962	0.872	1.833	0.946
		MMP-7	Coagulation Factor Xa	SAP	IL-12 Rβ2				
35	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.903	1.838	0.938
		MCP-3	Cadherin-5	SCF sR	IL-13 Rα1				
36	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.867	1.828	0.945
		Properdin	RGM-C	HSP 90α	IL-18 Rβ				
37	RGM-C	C9	MMP-7	SLPI	SAP	0.974	0.867	1.841	0.948
		HGF	MRC2	Kallikrein 6	sL-Selectin				
38	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.892	1.841	0.953
		α2-Antiplαsmin	RGM-C	Cadherin-5	Kallistatin				
39	RGM-C	C9	MMP-7	SLPI	LY9	0.962	0.882	1.844	0.945
		HGF	MRC2	C2	MIP-5				
40	HGF	SLPI	C9	MMP-7	Cadherin-5	0.949	0.892	1.841	0.941
		SCF sR	MCP-3	RGM-C	PCI				
41	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.913	1.849	0.941
		MCP-3	Cadherin-5	SCF sR	Prekallikrein				
42	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.897	1.846	0.936
		MCP-3	Cadherin-5	SCF sR	RBP				
43	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.897	1.833	0.947
		α2-Antiplαsmin	TIMP-2	SAP	sL-Selectin				
44	HGF	SLPI	C9	MMP-7	MRC2	0.974	0.867	1.841	0.950
		HSP 90α	RGM-C	Thrombin/ Prothrombin	α2-Antiplαsmin				

TABLE 8-continued

45	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.887	1.836	0.941
		HGF	MMP-7	sL-Selectin	Troponin T				
46	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.949	0.872	1.821	0.929
		MMP-7	BAFF Receptor	SAP	α1-Antitrypsin				
47	Cadherin-5	C9	SLPI	MMP-7	C2	0.962	0.882	1.844	0.951
		RGM-C	α2-Antiplasmin	HGF	α2-HS-Glycoprotein				
48	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.974	0.892	1.867	0.955
		MMP-7	Contactin-1	RGM-C	SAP				
49	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.897	1.846	0.935
		HSP 90α	Cadherin-5	MCP-3	ADAM 9				
50	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.949	0.887	1.836	0.943
		MMP-7	Contactin-4	Cadherin-5	ARSB				
51	RGM-C	C9	MMP-7	SLPI	SAP	0.987	0.851	1.838	0.950
		HGF	HSP 90α	α2-Antiplasmin	C5				
52	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.872	1.833	0.947
		HGF	HSP 90α	Kallistatin	ERBB1				
53	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.877	1.838	0.947
		MMP-7	Growth hormone receptor	Cadherin-5	Contactin-1				
54	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.936	0.897	1.833	0.941
		MMP-7	MRC2	SAP	Hat1				
55	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.897	1.833	0.950
		α2-Antiplasmin	RGM-C	Cadherin-5	IL-12 Rβ2				
56	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.877	1.838	0.948
		MMP-7	HGF	Contactin-4	IL-13 Rα1				
57	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.862	1.823	0.946
		HSP 90α	RGM-C	C2	IL-18 Rβ				
58	Cadherin-5	C9	SLPI	MMP-7	SAP	0.962	0.877	1.838	0.951
		HGF	Kallikrein 6	RGM-C	Contactin-1				
59	Cadherin-5	C9	SLPI	MMP-7	LY9	0.936	0.908	1.844	0.938
		RGM-C	MRC2	NRP1	RBP				
60	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.949
		MMP-7	HGF	Contactin-4	MIP-5				
61	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.877	1.838	0.944
		MMP-7	Coagulation Factor Xa	C2	PCI				
62	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.882	1.844	0.941
		HSP 90α	SAP	NRP1	Prekallikrein				
63	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.882	1.831	0.951
		α2-Antiplasmin	TIMP-2	SAP	RGM-C				
64	Cadherin-5	C9	SLPI	MMP-7	LY9	0.923	0.913	1.836	0.946
		RGM-C	MRC2	NRP1	Thrombin/ Prothrombin				
65	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.872	1.833	0.938
		HGF	Contactin-4	MCP-3	Troponin T				
66	Cadherin-5	C9	SLPI	MMP-7	SAP	0.949	0.872	1.821	0.929
		HGF	Coagulation Factor Xa	MCP-3	α1-Antitrypsin				
67	HGF	SLPI	C9	MMP-7	Cadherin-5	0.949	0.892	1.841	0.937
		SCF sR	MCP-3	Coagulation Factor Xa	α2-HS-Glycoprotein				
68	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.882	1.844	0.935
		Properdin	RGM-C	ADAM 9	HSP 90α				
69	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.936	0.887	1.823	0.941
		C2	MMP-7	Contactin-4	ARSB				
70	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.887	1.849	0.940
		MMP-7	BAFF Receptor	SAP	C2				
71	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.877	1.838	0.938
		HSP 90α	MCP-3	α2-Antiplasmin	C5				
72	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.877	1.838	0.948
		C2	MMP-7	HSP 90α	C6				
73	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.872	1.833	0.945
		HSP 90α	RGM-C	C2	ERBB1				
74	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.877	1.838	0.947
		HGF	MRC2	Growth hormone receptor	α2-Antiplasmin				
75	RGM-C	C9	MCP-3	SLPI	MRC2	0.936	0.892	1.828	0.933
		HGF	MMP-7	Contactin-1	Hat1				
76	HGF	SLPI	C9	MMP-7	MRC2	0.923	0.908	1.831	0.939
		MCP-3	Cadherin-5	SCF sR	IL-12 Rβ2				
77	RGM-C	C9	MMP-7	SLPI	SAP	0.974	0.856	1.831	0.945
		HGF	HSP 90α	Kallistatin	IL-13 Rα1				
78	RGM-C	C9	MMP-7	SLPI	SAP	0.949	0.872	1.821	0.944
		HGF	MRC2	NRP1	IL-18 Rβ				
79	Cadherin-5	C9	SLPI	MMP-7	SAP	0.974	0.862	1.836	0.950
		HGF	Kallikrein 6	RGM-C	Properdin				

TABLE 8-continued

80	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.877	1.838	0.938
		SCF sR	MCP-3	RGM-C	MIP-5				
81	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.872	1.833	0.952
		MMP-7	HGF	SAP	PCI				
82	RGM-C	C9	MMP-7	SLPI	SAP	0.949	0.892	1.841	0.953
		HGF	MRC2	Properdin	Prekallikrein				
83	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.882	1.844	0.939
		HGF	MMP-7	SAP	RBP				
84	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.882	1.831	0.943
		HGF	MMP-7	sL-Selectin	TIMP-2				
85	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.872	1.833	0.946
		HSP 90α	NRP1	Thrombin/ Prothrombin	RGM-C				
86	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.867	1.828	0.947
		HGF	Contactin-4	α2-Antiplαsmin	Troponin T				
87	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.872	1.821	0.942
		MMP-7	HGF	SAP	α1-Antitrypsin				
88	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.887	1.836	0.943
		HGF	MMP-7	SCF sR	α2-HS-Glycoprotein				
89	RGM-C	C9	MMP-7	SLPI	SAP	0.949	0.892	1.841	0.939
		HGF	Contactin-4	MCP-3	ADAM 9				
90	Cadherin-5	C9	SLPI	MMP-7	SAP	0.936	0.887	1.823	0.937
		HGF	Contactin-1	MCP-3	ARSB				
91	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.897	1.846	0.942
		HGF	MMP-7	Cadherin-5	BAFF Receptor				
92	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.872	1.833	0.940
		HGF	Contactin-1	MCP-3	C5				
93	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.903	1.838	0.938
		MCP-3	Cadherin-5	SCF sR	C6				
94	Cadherin-5	C9	SLPI	MMP-7	SAP	0.936	0.897	1.833	0.940
		HGF	Contactin-1	MCP-3	ERBB1				
95	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.877	1.838	0.944
		HGF	MRC2	Growth hormone receptor	Contactin-4				
96	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.867	1.828	0.937
		α2-Antiplαsmin	RGM-C	Hat1	IL-13 Rα1				
97	α2-Antiplαsmin	C9	SLPI	Cadherin-5	HGF	0.936	0.887	1.823	0.948
		MMP-7	Contactin-1	RGM-C	IL-12 Rβ2				
98	HGF	SLPI	C9	MMP-7	Cadherin-5	0.949	0.872	1.821	0.940
		SCF sR	MCP-3	RGM-C	IL-18 Rβ				
99	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.887	1.836	0.937
		HSP 90α	Cadherin-5	MCP-3	Kallikrein 6				
100	HGF	SLPI	C9	MMP-7	Cadherin-5	0.949	0.892	1.841	0.944
		SCF sR	MCP-3	RGM-C	Kallistatin				

Marker	Count	Marker	Count
SLPI	100	IL-18 Rβ	5
MMP-7	100	IL-13 Rα1	5
C9	100	IL-12 Rβ2	5
HGF	98	Hat1	5
RGM-C	72	Growth hormone receptor	5
Cadherin-5	54	ERBB1	5
MRC2	51	C6	5
SAP	47	C5	5
α2-Antiplαsmin	44	BAFF Receptor	5
MCP-3	34	ARSB	5
Contactin-4	17	ADAM 9	5
HSP 90α	16	α2-HS-Glycoprotein	4
SCF sR	14	α1-Antitrypsin	4
C2	11	Troponin T	4
Contactin-1	9	Thrombin/Prothrombin	4
NRP1	9	TIMP-2	4
Coagulation Factor Xa	7	RBP	4
sL-Selectin	6	Prekallikrein	4
Properdin	6	PCI	4
Kallistatin	5	MIP-5	4
Kallikrein 6	5	LY9	4

TABLE 9

100 Panels of 10 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers					Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.918	1.867	0.943
	HGF	MMP-7	Cadherin-5	SCF sR	ADAM 9				
2	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.949	0.897	1.846	0.950
	MMP-7	Contactin-4	Cadherin-5	RGM-C	ARSB				
3	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.908	1.869	0.946
	MMP-7	BAFF Receptor	RGM-C	MCP-3	MRC2				
4	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.903	1.864	0.955
	MMP-7	sL-Selectin	RGM-C	Cadherin-5	C2				
5	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.944
	MMP-7	HGF	SAP	BAFF Receptor	C5				
6	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.951
	MMP-7	HGF	Contactin-4	α2-HS-Glycoprotein	C6				
7	RGM-C	C9	MMP-7	SLPI	SAP	0.974	0.892	1.867	0.945
	HGF	Contactin-4	MCP-3	Coagulation Factor Xa	sL-Selectin				
8	Cadherin-5	C9	SLPI	MMP-7	C2	0.962	0.903	1.864	0.952
	RGM-C	α2-Antiplasmin	HGF	SAP	ERBB1				
9	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.882	1.844	0.947
	HGF	Contactin-4	Growth hormone receptor	Contactin-1	Coagulation Factor Xa				
10	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.897	1.859	0.954
	HGF	HSP 90α	α2-Antiplasmin	Contactin-1	Cadherin-5				
11	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.892	1.841	0.937
	HGF	MMP-7	sL-Selectin	SAP	Hat1				
12	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.952
	MMP-7	HGF	SAP	IL-12 Rβ2	Contactin-4				
13	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.952
	MMP-7	HGF	Contactin-4	IL-13 Rα1	SAP				
14	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.877	1.838	0.948
	Properdin	RGM-C	HSP 90α	α2-Antiplasmin	IL-18 Rβ				
15	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.887	1.849	0.940
	MCP-3	BAFF Receptor	α2-Antiplasmin	SAP	Kallikrein 6				
16	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.974	0.887	1.862	0.955
	MMP-7	HGF	SAP	Kallistatin	sL-Selectin				
17	RGM-C	C9	MMP-7	SLPI	LY9	0.962	0.892	1.854	0.946
	HGF	MRC2	C2	NRP1	SAP				
18	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.974	0.892	1.867	0.954
	MMP-7	HGF	SAP	MIP-5	Contactin-1				
19	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.952
	MMP-7	HGF	SAP	PCI	Contactin-1				
20	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.897	1.859	0.944
	HGF	MMP-7	Cadherin-5	BAFF Receptor	Prekallikrein				
21	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.913	1.862	0.942
	MCP-3	Cadherin-5	SCF sR	RBP	RGM-C				
22	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.887	1.849	0.945
	HGF	MMP-7	sL-Selectin	SAP	TIMP-2				
23	RGM-C	C9	MMP-7	SLPI	SAP	0.974	0.882	1.856	0.951
	HGF	MRC2	NRP1	sL-Selectin	Thrombin/ Prothrombin				
24	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.887	1.849	0.937
	MMP-7	BAFF Receptor	RGM-C	MCP-3	Troponin T				
25	HGF	SLPI	C9	MMP-7	Cadherin-5	0.936	0.897	1.833	0.936
	SCF sR	MCP-3	RGM-C	SAP	α1-Antitrypsin				
26	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.892	1.854	0.943
	HGF	MMP-7	SAP	Prekallikrein	ADAM 9				
27	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.892	1.841	0.950
	MMP-7	HGF	SAP	C5	ARSB				
28	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.954
	MMP-7	HGF	SAP	Properdin	C6				
29	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.897	1.859	0.946
	SCF sR	MCP-3	RGM-C	SAP	ERBB1				
30	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.882	1.844	0.942
	HGF	Contactin-4	Growth hormone receptor	Contactin-1	MCP-3				
31	RGM-C	C9	MMP-7	SLPI	LY9	0.949	0.887	1.836	0.938
	HGF	MRC2	C2	NRP1	Hat1				
32	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.949
	MMP-7	HGF	SAP	IL-12 Rβ2	C5				

TABLE 9-continued

33	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.949
	MMP-7	HGF	Contactin-4	IL-13 Rα1	C2				
34	HGF	SLPI	C9	MMP-7	Cadherin-5	0.936	0.903	1.838	0.940
	SCF sR	MCP-3	Coagulation Factor Xa	MRC2	IL-18 Rβ				
35	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.887	1.849	0.946
	SCF sR	MCP-3	RGM-C	SAP	Kallikrein 6				
36	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.887	1.849	0.947
	SCF sR	MCP-3	RGM-C	Kallistatin	SAP				
37	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.897	1.859	0.953
	MMP-7	HGF	Contactin-4	MIP-5	SAP				
38	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.882	1.844	0.951
	MMP-7	HGF	SAP	PCI	C6				
39	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.887	1.849	0.939
	MMP-7	BAFF Receptor	RGM-C	MCP-3	RBP				
40	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.887	1.849	0.952
	MMP-7	HGF	SAP	C6	TIMP-2				
41	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.974	0.877	1.851	0.940
	MMP-7	BAFF Receptor	RGM-C	MCP-3	Thrombin/ Prothrombin				
42	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.887	1.836	0.938
	MCP-3	BAFF Receptor	α2-Antiplasmin	SAP	Troponin T				
43	Cadherin-5	C9	SLPI	MMP-7	SAP	0.936	0.897	1.833	0.932
	HGF	Coagulation Factor Xa	MCP-3	SCF sR	α1-Antitrypsin				
44	Cadherin-5	C9	SLPI	MMP-7	C2	0.962	0.897	1.859	0.951
	RGM-C	α2-Antiplasmin	HGF	α2-HS-Glycoprotein	Contactin-1				
45	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.892	1.854	0.941
	Properdin	RGM-C	ADAM 9	SAP	MCP-3				
46	RGM-C	C9	MMP-7	SLPI	SAP	0.949	0.892	1.841	0.947
	HGF	MRC2	NRP1	sL-Selectin	ARSB				
47	RGM-C	C9	MMP-7	SLPI	SAP	0.974	0.877	1.851	0.947
	HGF	HSP 90α	α2-Antiplasmin	Contactin-1	ERBB1				
48	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.882	1.844	0.945
	SCF sR	MCP-3	RGM-C	SAP	Growth hormone receptor				
49	Cadherin-5	C9	SLPI	MMP-7	C2	0.936	0.897	1.833	0.947
	RGM-C	α2-Antiplasmin	HGF	SAP	Hat1				
50	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.897	1.846	0.952
	MMP-7	HGF	SAP	IL-12 Rβ2	Contactin-1				
51	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.887	1.849	0.945
	HGF	MMP-7	sL-Selectin	SAP	IL-13 Rα1				
52	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.877	1.838	0.948
	Properdin	RGM-C	HSP 90α	Cadherin-5	IL-18 Rβ				
53	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.897	1.846	0.945
	HGF	MMP-7	Cadherin-5	SCF sR	Kallikrein 6				
54	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.897	1.846	0.946
	HGF	MMP-7	Cadherin-5	sL-Selectin	Kallistatin				
55	RGM-C	C9	MCP-3	SLPI	MRC2	0.936	0.913	1.849	0.942
	HGF	MMP-7	Cadherin-5	SCF sR	LY9				
56	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.892	1.854	0.944
	SCF sR	MCP-3	RGM-C	MIP-5	SAP				
57	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.892	1.841	0.952
	MMP-7	HGF	SAP	PCI	Properdin				
58	HGF	SLPI	C9	MMP-7	Cadherin-5	0.962	0.897	1.859	0.949
	SCF sR	MCP-3	RGM-C	SAP	Prekallikrein				
59	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.897	1.846	0.952
	MMP-7	HGF	SAP	Properdin	RBP				
60	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.882	1.844	0.950
	MMP-7	Contactin-1	RGM-C	C2	TIMP-2				
61	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.903	1.851	0.946
	HGF	MMP-7	Cadherin-5	SCF sR	Thrombin/ Prothrombin				
62	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.882	1.831	0.952
	MMP-7	HGF	SAP	Kallistatin	Troponin T				
63	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.877	1.826	0.942
	MMP-7	HGF	SAP	Properdin	α1-Antitrypsin				
64	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.908	1.856	0.945
	MCP-3	Cadherin-5	SCF sR	α2-HS-Glycoprotein	RGM-C				
65	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.903	1.851	0.939
	Properdin	RGM-C	ADAM 9	HSP 90α	Cadherin-5				

TABLE 9-continued

66	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.903	1.838	0.938
	MCP-3	Cadherin-5	SCF sR	NRP1	ARSB				
67	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.897	1.846	0.948
	MMP-7	HGF	Contactin-4	MRC2	C5				
68	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.882	1.844	0.939
	MMP-7	BAFF Receptor	RGM-C	MCP-3	ERBB1				
69	Cadherin-5	C9	SLPI	MMP-7	C2	0.962	0.882	1.844	0.951
	RGM-C	α2-Antiplasmin	HGF	SAP	Growth hormone receptor				
70	HGF	SLPI	C9	MMP-7	MRC2	0.936	0.892	1.828	0.932
	MCP-3	BAFF Receptor	α2-Antiplasmin	SAP	Hat1				
71	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.949	0.897	1.846	0.952
	MMP-7	Contactin-1	RGM-C	SAP	IL-12 Rβ2				
72	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.887	1.849	0.949
	C2	MMP-7	Contactin-4	RGM-C	IL-13 Rα1				
73	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.949	0.887	1.836	0.948
	MMP-7	Contactin-1	RGM-C	Contactin-4	IL-18 Rβ				
74	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.882	1.844	0.941
	HSP 90α	MCP-3	SAP	α2-Antiplasmin	Kallikrein 6				
75	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.949	0.897	1.846	0.949
	MMP-7	MRC2	SAP	RGM-C	LY9				
76	HGF	SLPI	C9	α2-Antiplasmin	SAP	0.962	0.892	1.854	0.953
	MMP-7	sL-Selectin	RGM-C	Cadherin-5	MIP-5				
77	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.892	1.841	0.953
	MMP-7	HGF	SAP	PCI	sL-Selectin				
78	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.892	1.854	0.950
	HGF	MMP-7	SAP	Prekallikrein	α2-Antiplasmin				
79	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.897	1.846	0.943
	HGF	MMP-7	SAP	RBP	sL-Selectin				
80	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.877	1.838	0.953
	MMP-7	HGF	SAP	Kallistatin	TIMP-2				
81	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.887	1.849	0.942
	HGF	MMP-7	Contactin-1	BAFF Receptor	Thrombin/ Prothrombin				
82	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.882	1.831	0.940
	HGF	MMP-7	Contactin-1	HSP 90α	Troponin T				
83	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.936	0.887	1.823	0.937
	MMP-7	HGF	Contactin-4	MRC2	α1-Antitrypsin				
84	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.951
	MMP-7	HGF	Contactin-4	α2-HS-Glycoprotein	C2				
85	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.903	1.851	0.941
	HGF	MMP-7	Cadherin-5	BAFF Receptor	ADAM 9				
86	RGM-C	C9	MCP-3	SLPI	MRC2	0.936	0.903	1.838	0.942
	HGF	MMP-7	Cadherin-5	SCF sR	ARSB				
87	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.949	0.897	1.846	0.948
	MMP-7	HGF	Contactin-4	C5	MRC2				
88	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.892	1.854	0.954
	MMP-7	HGF	SAP	C6	sL-Selectin				
89	Cadherin-5	C9	SLPI	MMP-7	SAP	0.962	0.897	1.859	0.943
	HGF	Coagulation Factor Xa	MCP-3	SCF sR	Contactin-1				
90	RGM-C	C9	MMP-7	SLPI	SAP	0.962	0.882	1.844	0.943
	HGF	Contactin-4	MCP-3	Coagulation Factor Xa	ERBB1				
91	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.882	1.844	0.951
	MMP-7	Contactin-1	RGM-C	SAP	Growth hormone receptor				
92	RGM-C	C9	MMP-7	SLPI	LY9	0.949	0.877	1.826	0.938
	HGF	MRC2	C2	MIP-5	Hat1				
93	α2-Antiplasmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.882	1.844	0.951
	MMP-7	HGF	SAP	IL-12 Rβ2	sL-Selectin				
94	α2-Antiplasmin	C9	SLPI	Cadherin-5	HGF	0.962	0.887	1.849	0.952
	MMP-7	Contactin-1	RGM-C	SAP	IL-13 Rα1				
95	RGM-C	C9	MMP-7	SLPI	LY9	0.949	0.887	1.836	0.944
	HGF	MRC2	C2	NRP1	IL-18 Rβ				
96	HGF	SLPI	C9	MMP-7	MRC2	0.962	0.882	1.844	0.947
	Properdin	RGM-C	HSP 90α	Cadherin-5	Kallikrein 6				
97	RGM-C	C9	MCP-3	SLPI	MRC2	0.949	0.892	1.841	0.944
	HGF	MMP-7	sL-Selectin	SAP	PCI				
98	RGM-C	C9	MCP-3	SLPI	MRC2	0.962	0.887	1.849	0.945
	HGF	MMP-7	SAP	Prekallikrein	BAFF Receptor				

TABLE 9-continued

99	HGF	SLPI	C9	MMP-7	MRC2	0.949	0.897	1.846	0.940
	α2-Antiplαsmin	RGM-C	BAFF Receptor	MCP-3	RBP				
100	α2-Antiplαsmin	C9	SLPI	Cadherin-5	RGM-C	0.962	0.877	1.838	0.952
	MMP-7	HGF	SAP	Properdin	TIMP-2				

Marker	Count	Marker	Count
SLPI	100	TIMP-2	5
MMP-7	100	RBP	5
HGF	100	Prekallikrein	5
C9	100	PCI	5
RGM-C	92	MIP-5	5
SAP	68	Kallistatin	5
Cadherin-5	67	Kallikrein 6	5
α2-Antiplαsmin	56	IL-18 Rβ	5
MCP-3	45	IL-13 Rα1	5
MRC2	43	IL-12 Rβ2	5
SCF sR	18	Hat1	5
Contactin-1	16	Growth hormone receptor	5
Contactin-4	16	ERBB1	5
sL-Selectin	15	C6	5
BAFF Receptor	14	C5	5
C2	13	ARSB	5
Properdin	10	ADAM 9	5
HSP 90α	8	α2-HS-Glycoprotein	4
NRP1	6	α1-Antitrypsin	4
LY9	6	Troponin T	4
Coagulation Factor Xa	6	Thrombin/Prothrombin	4

TABLE 10

100 Panels of 11 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers				
1	SAP	MRC2	SLPI	RGM-C	MMP-7
		Cadherin-5	HGF	Prekallikrein	MCP-3
2	SAP	MMP-7	SLPI	Cadherin-5	HGF
		MRC2	RGM-C	NRP1	ARSB
3	SAP	C9	SLPI	MMP-7	HGF
		BAFF Receptor	Properdin	Cadherin-5	MCP-3
4	RGM-C	MRC2	SLPI	C9	MMP-7
		α2-Antiplαsmin	BAFF Receptor	HGF	C2
5	Cadherin-5	HGF	SLPI	C9	MMP-7
		MRC2	BAFF Receptor	MCP-3	C5
6	HGF	SCF sR	C9	SLPI	MCP-3
		SAP	sL-Selectin	MMP-7	Coagulation Factor Xa
7	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
		MCP-3	Contactin-4	RGM-C	Properdin
8	Cadherin-5	HGF	SLPI	C9	MMP-7
		SAP	α2-Antiplαsmin	RGM-C	PCI
9	HGF	LY9	SLPI	C9	C2
		MMP-7	SAP	Growth hormone receptor	Contactin-1
10	Contactin-4	MCP-3	SLPI	C9	HGF
		MMP-7	SAP	Cadherin-5	α2-Antiplαsmin
11	SAP	C9	SLPI	MMP-7	HGF
		α2-Antiplαsmin	RGM-C	LY9	Hat1
12	Cadherin-5	MMP-7	C9	RGM-C	SLPI
		MRC2	HSP 90α	ADAM 9	IL-12 Rβ2
13	SAP	C9	SLPI	MMP-7	HGF
		BAFF Receptor	Properdin	sL-Selectin	MRC2
14	MMP-7	SLPI	C9	HSP 90α	HGF
		α2-Antiplαsmin	MRC2	RGM-C	MCP-3
15	SAP	C9	SLPI	MMP-7	HGF
		Kallikrein 6	Contactin-4	Cadherin-5	MCP-3
16	Cadherin-5	HGF	SLPI	C9	MMP-7
		MRC2	Prekallikrein	SCF sR	MIP-5

TABLE 10-continued

17	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	HSP 90α	Cadherin-5	ADAM 9
18	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor
19	RGM-C	MRC2	SLPI	C9	MMP-7
		HGF	BAFF Receptor	Cadherin-5	Thrombin/Prothrombin
20	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	Properdin	RGM-C	Troponin T
21	RGM-C	MRC2	SLPI	C9	MMP-7
		ADAM 9	SAP	BAFF Receptor	α1-Antitrypsin
22	RGM-C	MCP-3	C9	MMP-7	SLPI
		HGF	Contactin-4	SAP	BAFF Receptor
23	Cadherin-5	MMP-7	SLPI	MRC2	C9
		RGM-C	HGF	ADAM 9	MCP-3
24	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	BAFF Receptor	Prekallikrein	C5
25	MMP-7	SLPI	C9	HSP 90α	α2-Antiplαsmin
		SAP	RGM-C	MCP-3	Contactin-4
26	HGF	MMP-7	α2-Antiplαsmin	C9	SLPI
		RGM-C	Cadherin-5	HSP 90α	SAP
27	MMP-7	SLPI	Contactin-1	Growth hormone receptor	SAP
		Contactin-4	MCP-3	ADAM 9	C9
28	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	Contactin-1	Hat1	RGM-C
29	SAP	MRC2	SLPI	RGM-C	MMP-7
		HSP 90α	HGF	Cadherin-5	MCP-3
30	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor
31	RGM-C	MRC2	SLPI	C9	MMP-7
		SCF sR	MCP-3	ADAM 9	SAP
32	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	Contactin-4	sL-Selectin
33	Contactin-4	MCP-3	SLPI	C9	HGF
		MMP-7	SAP	Cadherin-5	RGM-C
34	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	Contactin-4	NRP1
35	Cadherin-5	HGF	SLPI	C9	MMP-7
		RGM-C	α2-Antiplαsmin	PCI	SAP
36	SAP	C9	SLPI	MMP-7	HGF
		RBP	RGM-C	Properdin	ADAM 9
37	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	BAFF Receptor	sL-Selectin	NRP1
38	SAP	C9	SLPI	MMP-7	HGF
		NRP1	MRC2	Thrombin/Prothrombin	sL-Selectin
39	Cadherin-5	MMP-7	C9	RGM-C	SLPI
		MRC2	Troponin T	BAFF Receptor	SAP
40	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	HSP 90α	α1-Antitrypsin
41	SAP	MRC2	SLPI	RGM-C	MMP-7
		HSP 90α	HGF	Cadherin-5	MCP-3
42	MRC2	NRP1	SLPI	C9	HGF
		RGM-C	MCP-3	Contactin-4	SCF sR
43	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	BAFF Receptor	Prekallikrein	C5
44	HGF	SCF sR	C9	SLPI	MMP-7
		α2-Antiplαsmin	SAP	RGM-C	MCP-3
45	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
		MCP-3	Contactin-4	RGM-C	Cadherin-5
46	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	ERBB1	RGM-C	ADAM 9
47	RGM-C	Contactin-4	SLPI	SAP	MMP-7
		C9	HGF	MCP-3	Contactin-1
48	SAP	C9	SLPI	MMP-7	HGF
		α2-Antiplαsmin	RGM-C	LY9	Hat1
49	HGF	SCF sR	C9	SLPI	MMP-7
		SAP	MCP-3	Coagulation Factor Xa	IL-12 Rβ2
50	IL-13 Rα1	RGM-C	SLPI	C9	MMP-7
		Cadherin-5	HGF	BAFF Receptor	SAP
51	MRC2	NRP1	SLPI	C9	HGF
		Thrombin/Prothrombin	RGM-C	Contactin-1	Properdin
52	SAP	C9	SLPI	MMP-7	HGF
		Kallikrein 6	Contactin-4	Cadherin-5	MCP-3
53	Contactin-4	MCP-3	SLPI	C9	HGF
		MMP-7	SAP	Cadherin-5	RGM-C
54	Cadherin-5	HGF	SLPI	C9	MMP-7
		RGM-C	BAFF Receptor	Contactin-4	MIP-5

TABLE 10-continued

55	SAP	MMP-7	SLPI	C2	Coagulation Factor Xa
		HGF	ERBB1	RGM-C	PCI
56	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	BAFF Receptor	Properdin	RBP
57	Cadherin-5	MMP-7	C9	RGM-C	SLPI
		SAP	α2-Antiplαsmin	ERBB1	C6
58	MRC2	NRP1	SLPI	C9	HGF
		RGM-C	MCP-3	Contactin-4	SCF sR
59	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	HSP 90α	α1-Antitrypsin
60	Cadherin-5	HGF	SLPI	C9	MMP-7
		RGM-C	α2-Antiplαsmin	α2-HS-Glycoprotein	C2
61	SAP	MMP-7	SLPI	Cadherin-5	HGF
		MRC2	RGM-C	NRP1	ARSB
62	Cadherin-5	HGF	SLPI	C9	MMP-7
		RGM-C	Contactin-1	SCF sR	Contactin-4
63	Cadherin-5	HGF	SLPI	C9	MMP-7
		SAP	α2-Antiplαsmin	RGM-C	Hat1
64	RGM-C	MRC2	SLPI	C9	MMP-7
		HGF	BAFF Receptor	Cadherin-5	IL-12 Rβ2
65	HGF	SCF sR	C9	SLPI	MMP-7
		RGM-C	MCP-3	SAP	Contactin-1
66	HGF	SCF sR	C9	SLPI	MMP-7
		SAP	MCP-3	Contactin-1	RGM-C
67	SAP	C9	SLPI	MMP-7	HGF
		SCF sR	MCP-3	Contactin-4	Kallikrein 6
68	Contactin-4	MCP-3	SLPI	C9	HGF
		MMP-7	SAP	RGM-C	Contactin-1
69	SAP	MRC2	SLPI	RGM-C	MCP-3
		sL-Selectin	HGF	ADAM 9	α2-HS-Glycoprotein
70	RGM-C	MRC2	SLPI	C9	MMP-7
		MIP-5	HGF	BAFF Receptor	Cadherin-5
71	HGF	SCF sR	C9	SLPI	MMP-7
		SAP	MCP-3	RGM-C	PCI
72	Cadherin-5	HGF	SLPI	C9	MMP-7
		α2-Antiplαsmin	Contactin-1	SAP	RBP
73	SAP	MMP-7	SLPI	Cadherin-5	HGF
		C6	α2-Antiplαsmin	RGM-C	Contactin-1
74	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	Thrombin/Prothrombin	Properdin
75	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
		MCP-3	Contactin-4	RGM-C	Cadherin-5
76	SAP	C9	SLPI	MMP-7	HGF
		SCF sR	MCP-3	Contactin-4	ADAM 9
77	Cadherin-5	HGF	SLPI	C9	MMP-7
		α2-Antiplαsmin	Contactin-1	RGM-C	C2
78	Cadherin-5	MMP-7	C9	RGM-C	SLPI
		MRC2	α2-Antiplαsmin	Growth hormone receptor	SAP
79	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	α2-Antiplαsmin	Hat1
80	RGM-C	MRC2	SLPI	C9	MMP-7
		HGF	HSP 90α	Cadherin-5	IL-12 Rβ2
81	RGM-C	MRC2	SLPI	C9	MMP-7
		α2-Antiplαsmin	BAFF Receptor	HGF	Contactin-4
82	RGM-C	MRC2	SLPI	C9	MMP-7
		α2-Antiplαsmin	BAFF Receptor	HGF	Cadherin-5
83	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	HSP 90α	SCF sR
84	HSP 90α	SLPI	C9	RGM-C	MMP-7
		HGF	Kallistatin	MCP-3	Cadherin-5
85	MMP-7	LY9	SLPI	RGM-C	MRC2
		SAP	ADAM 9	Kallistatin	MCP-3
86	RGM-C	MRC2	SLPI	C9	MMP-7
		MIP-5	HGF	BAFF Receptor	Cadherin-5
87	MMP-7	SLPI	C9	α2-Antiplαsmin	RGM-C
		sL-Selectin	HGF	Coagulation Factor Xa	C2
88	MMP-7	SLPI	C9	MCP-3	MRC2
		BAFF Receptor	ADAM 9	SAP	RBP
89	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	C6	SCF sR
90	MRC2	NRP1	SLPI	C9	HGF
		RGM-C	Properdin	SAP	BAFF Receptor
91	Contactin-4	MCP-3	SLPI	C9	HGF
		MRC2	RGM-C	Troponin T	C2
92	Cadherin-5	HGF	SLPI	C9	MMP-7
		RGM-C	BAFF Receptor	SAP	α1-Antitrypsin

TABLE 10-continued

93	SAP	C9	SLPI	MMP-7	HGF
		NRP1	MRC2	Contactin-1	MCP-3
94	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	Contactin-4	Kallistatin	BAFF Receptor
95	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	Thrombin/Prothrombin	ERBB1
96	Cadherin-5	HGF	SLPI	C9	MMP-7
		α2-Antiplαsmin	SAP	RGM-C	Growth hormone receptor
97	HGF	MMP-7	α2-Antiplαsmin	C9	SLPI
		RGM-C	Contactin-1	Cadherin-5	SAP
98	Contactin-4	MCP-3	SLPI	C9	HGF
		MRC2	RGM-C	Troponin T	Cadherin-5
99	MMP-7	SLPI	C9	HSP 90α	α2-Antiplαsmin
		Contactin-1	RGM-C	MCP-3	MRC2
100	SAP	C9	SLPI	MMP-7	HGF
		MCP-3	RGM-C	HSP 90α	SCF sR

	Biomarkers	Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	Properdin ADAM 9	0.949	0.928	1.877	0.946
2	C9 MCP-3	0.962	0.892	1.854	0.946
3	RGM-C MRC2	0.962	0.918	1.879	0.945
4	MCP-3 SAP	0.962	0.908	1.869	0.946
5	Properdin RGM-C	0.949	0.913	1.862	0.942
6	RGM-C C6	0.962	0.903	1.864	0.945
7	SAP Contactin-1	0.962	0.913	1.874	0.945
8	C2 ERBB1	0.962	0.897	1.859	0.951
9	RGM-C Contactin-4	0.974	0.887	1.862	0.945
10	HSP 90α RGM-C	0.974	0.892	1.867	0.947
11	MRC2 MCP-3	0.962	0.892	1.854	0.939
12	HGF BAFF Receptor	0.962	0.897	1.859	0.936
13	RGM-C IL-13 Rα1	0.962	0.897	1.859	0.940
14	Cadherin-5 IL-18 Rβ	0.962	0.892	1.854	0.945
15	RGM-C Kallistatin	0.974	0.887	1.862	0.945
16	MCP-3 RGM-C	0.949	0.913	1.862	0.945
17	MRC2 RBP	0.962	0.903	1.864	0.936
18	MRC2 TIMP-2	0.962	0.903	1.864	0.944
19	MCP-3 Contactin-1	0.962	0.903	1.864	0.944
20	MRC2 Contactin-1	0.949	0.908	1.856	0.944
21	HGF MCP-3	0.962	0.903	1.864	0.931
22	Contactin 1 α2-HS-Glycoprotein	0.974	0.892	1.867	0.941
23	sL-Selectin ARSB	0.949	0.903	1.851	0.940
24	MRC2 ADAM 9	0.962	0.897	1.859	0.936
25	HGF C6	0.974	0.887	1.862	0.944
26	C2 ERBB1	0.962	0.897	1.859	0.952
27	HGF RGM-C	0.962	0.897	1.859	0.940
28	MRC2 Kallistatin	0.949	0.897	1.846	0.936

TABLE 10-continued

29	Properdin IL-12 Rβ2	0.936	0.923	1.859	0.941
30	MRC2 IL-13 Rα1	0.962	0.897	1.859	0.943
31	HGF IL-18 Rβ	0.962	0.892	1.854	0.941
32	MRC2 Kallikrein 6	0.962	0.897	1.859	0.945
33	HSP 90α MIP-5	0.974	0.887	1.862	0.943
34	MRC2 ADAM 9	0.962	0.903	1.864	0.939
35	Properdin Contactin-1	0.962	0.897	1.859	0.952
36	MRC2 MCP-3	0.962	0.897	1.859	0.939
37	MRC2 TIMP-2	0.962	0.897	1.859	0.936
38	RGM-C Properdin	0.962	0.903	1.864	0.952
39	HGF Properdin	0.949	0.908	1.856	0.943
40	MRC2 ADAM 9	0.962	0.892	1.854	0.931
41	Properdin α2-HS-Glycoprotein	0.949	0.918	1.867	0.942
42	MMP-7 ARSB	0.949	0.903	1.851	0.939
43	MRC2 Properdin	0.962	0.897	1.859	0.938
44	Cadherin-5 C6	0.962	0.897	1.859	0.947
45	SAP SCF sR	0.962	0.908	1.869	0.946
46	MRC2 C2	0.962	0.897	1.859	0.942
47	Growth hormone receptor C6	0.962	0.897	1.859	0.942
48	MRC2 C5	0.949	0.897	1.846	0.945
49	Cadherin-5 Contactin-1	0.949	0.903	1.851	0.942
50	Contactin-4 MCP-3	0.974	0.882	1.856	0.941
51	MMP-7 IL-18 Rβ	0.962	0.892	1.854	0.946
52	RGM-C BAFF Receptor	0.974	0.882	1.856	0.943
53	HSP 90α Kallistatin	0.974	0.892	1.867	0.945
54	MCP-3 SAP	0.974	0.887	1.862	0.943
55	Cadherin-5 Properdin	0.962	0.897	1.859	0.947
56	MRC2 Cadherin-5	0.949	0.908	1.856	0.938
57	HGF TIMP-2	0.962	0.887	1.849	0.949
58	MMP-7 Troponin T	0.949	0.908	1.856	0.941
59	MRC2 BAFF Receptor	0.962	0.887	1.849	0.931
60	Properdin Contactin-1	0.962	0.903	1.864	0.951
61	C9 Troponin T	0.962	0.887	1.849	0.950
62	MCP-3 Growth hormone receptor	0.949	0.908	1.856	0.943
63	C2 Contactin-1	0.936	0.908	1.844	0.947
64	MCP-3 Properdin	0.936	0.913	1.849	0.942
65	HSP 90α IL-13 Rα1	0.962	0.892	1.854	0.942
66	Cadherin-5 IL-18 Rβ	0.949	0.903	1.851	0.946

TABLE 10-continued

67	RGM-C ADAM 9	0.962	0.892	1.854	0.941
68	HSP 90α Kallistatin	0.974	0.887	1.862	0.943
69	MMP-7 LY9	0.949	0.913	1.862	0.939
70	SAP MCP-3	0.962	0.897	1.859	0.944
71	Cadherin-5 BAFF Receptor	0.962	0.892	1.854	0.943
72	MCP-3 MRC2	0.936	0.918	1.854	0.943
73	C9 TIMP-2	0.949	0.897	1.846	0.952
74	MRC2 Prekallikrein	0.949	0.913	1.862	0.949
75	SAP α1-Antitrypsin	0.949	0.897	1.846	0.934
76	RGM-C ARSB	0.962	0.887	1.849	0.938
77	α2-HS-Glycoprotein C5	0.962	0.897	1.859	0.950
78	HGF C2	0.949	0.908	1.856	0.951
79	MRC2 C2	0.936	0.908	1.844	0.940
80	MCP-3 Properdin	0.949	0.897	1.846	0.944
81	MCP-3 IL-13Rα1	0.962	0.892	1.854	0.941
82	MCP-3 IL-18 Rβ	0.962	0.887	1.849	0.943
83	MRC2 Kallikrein 6	0.962	0.892	1.854	0.945
84	SAP BAFF Receptor	0.974	0.887	1.862	0.942
85	HGF BAFF Receptor	0.949	0.913	1.862	0.937
86	SAP NRP1	0.962	0.897	1.859	0.942
87	Cadherin-5 PCI	0.962	0.892	1.854	0.950
88	HGF α2-Antiplαsmin	0.962	0.892	1.854	0.938
89	MRC2 TIMP-2	0.949	0.897	1.846	0.943
90	MMP-7 Thrombin/Prothrombin	0.962	0.897	1.859	0.942
91	MMP-7 SAP	0.962	0.892	1.854	0.942
92	MCP-3 Troponin T	0.949	0.892	1.841	0.931
93	RGM-C ARSB	0.949	0.897	1.846	0.942
94	RGM-C C5	0.974	0.882	1.856	0.939
95	MRC2 NRP1	0.962	0.892	1.854	0.943
96	Contactin-4 C6	0.962	0.892	1.854	0.950
97	C2 Hat1	0.936	0.908	1.844	0.947
98	MMP-7 IL-12 Rβ2	0.949	0.897	1.846	0.942

TABLE 10-continued

99	HGF IL-13 Rα1	0.962	0.892	1.854	0.944
100	MRC2 IL-18 Rβ	0.962	0.887	1.849	0.943

Marker	Count	Marker	Count
SLPI	100	Troponin T	7
MMP-7	100	Kallistatin	7
HGF	100	Coagulation Factor X2	7
C9	94	Thrombin/Prothrombin	6
RGM-C	92	IL-18 Rβ	6
SAP	81	IL-13 Rα1	6
MCP-3	77	IL-12 Rβ2	6
MRC2	60	Hat1	6
Cadherin-5	51	Growth hormone receptor	6
BAFF Receptor	31	ERBB1	6
Contactin-4	28	C5	6
α2-Antiplαsmir	27	ARSB	6
Contactin-1	23	α2-HS-Glycoprotein	5
Properdin	21	α1-Antitrypsin	5
HSP 90α	19	TIMP-2	5
SCF sR	17	RBP	5
ADAM 9	17	Prekallikrein	5
C2	14	PCI	5
NRP1	12	MIP-5	5
sL-Selectin	8	LY9	5
C6	8	Kallikrein 6	5

TABLE 11

100 Panels of 12 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers				
1	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	MRC2	MCP-3	BAFF Receptor	ADAM 9
2	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	ARSB
3	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	C5
4	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	C6
5	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	Contactin-4	RGM-C	MIP-5	BAFF Receptor
6	Cadherin-5	MMP-7	C9	RGM-C	SLPI
	SAP	Coagulation Factor Xa	C2	α2-Antiplαsmin	ERBB1
7	Cadherin-5	HGF	SLPI	C9	MMP-7
	SAP	Contactin-1	RGM-C	MCP-3	BAFF Receptor
8	RGM-C	MCP-3	C9	MMP-7	SLPI
	HGF	BAFF Receptor	Kallistatin	SAP	HSP 90α
9	MMP-7	LY9	SLPI	RGM-C	MRC2
	SAP	Cadherin-5	MCP-3	α2-Antiplαsmin	C9
10	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	Contactin-4	RGM-C	Cadherin-5	SCF sR
11	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	IL-13 Rα1
12	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	IL-18 Rβ
13	Cadherin-5	α2-Antiplαsmin	C9	SLPI	MCP-3
	RGM-C	Contactin-4	MMP-7	SAP	Kallikrein 6
14	RGM-C	MRC2	SLPI	C9	MMP-7
	sL-Selectin	HGF	ADAM 9	BAFF Receptor	SAP
15	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Cadherin-5	Prekallikrein	BAFF Receptor
16	RGM-C	MRC2	SLPI	C9	MMP-7
	BAFF Receptor	HGF	Properdin	ADAM 9	Cadherin-5
17	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Prekallikrein	HSP 90α	Cadherin-5
18	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	MRC2	MCP-3	BAFF Receptor	Thrombin/Prothrombin

TABLE 11-continued

19	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Contactin-4	NRP1	SCF sR
20	RGM-C	MRC2	SLPI	C9	MMP-7
	ADAM 9	SAP	BAFF Receptor	Cadherin-5	MCP-3
21	SAP	MRC2	SLPI	RGM-C	MCP-3
	sL-Selectin	HGF	ADAM 9	α2-HS-Glycoprotein	HSP 90α
22	SAP	C9	SLPI	MMP-7	HGF
	SCF sR	MCP-3	Contactin-4	ADAM 9	ARSB
23	RGM-C	MRC2	SLPI	C9	MMP-7
	ADAM 9	SAP	BAFF Receptor	Cadherin-5	MCP-3
24	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	C6
25	SAP	C9	SLPI	MMP-7	HGF
	NRP1	MRC2	Thrombin/Prothrombin	sL-Selectin	ERBB1
26	RGM-C	MCP-3	C9	MMP-7	SLPI
	HGF	Contactin-4	SAP	BAFF Receptor	Growth hormone receptor
27	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Hat1
28	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	IL-12 Rβ2
29	RGM-C	MRC2	SLPI	C9	MMP-7
	α2-Antiplαsmin	BAFF Receptor	HGF	ADAM 9	SAP
30	Cadherin-5	HGF	SLPI	C9	MMP-7
	α2-Antiplαsmin	Contactin-1	RGM-C	C2	IL-18 Rβ
31	RGM-C	MRC2	SLPI	C9	MMP-7
	α2-Antiplαsmin	BAFF Receptor	HGF	Cadherin-5	SAP
32	NRP1	LY9	C9	SLPI	MMP-7
	MRC2	HGF	Contactin-1	Thrombin/Prothrombin	SAP
33	RGM-C	MCP-3	C9	MMP-7	SLPI
	HGF	BAFF Receptor	Cadherin-5	SAP	MIP-5
34	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	Contactin-1	SCF sR	PCI	SAP
35	RGM-C	SLPI	RBP	C9	MMP-7
	HGF	sL-Selectin	MRC2	MCP-3	BAFF Receptor
36	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	IL-13 Rα1
37	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Kallistatin
38	MMP-7	C9	Contactin-1	SLPI	HGF
	HSP 90α	MCP-3	RGM-C	ADAM 9	MRC2
39	SAP	C9	SLPI	MMP-7	HGF
	SCF sR	MCP-3	Contactin-4	ADAM 9	ARSB
40	RGM-C	MRC2	SLPI	C9	MMP-7
	HGF	BAFF Receptor	SAP	Kallistatin	ADAM 9
41	Cadherin-5	α2-Antiplαsmin	C9	SLPI	MCP-3
	RGM-C	Contactin-4	MMP-7	SAP	Properdin
42	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	RGM-C	MRC2	ADAM 9	ERBB1
43	SAP	C9	SLPI	MMP-7	HGF
	α2-Antiplαsmin	RGM-C	LY9	Hat1	MCP-3
44	MRC2	LY9	SLPI	MMP-7	SAP
	NRP1	Thrombin/Prothrombin	Contactin-4	RGM-C	Growth hormone receptor
45	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	IL-18 Rβ
46	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Contactin-4	NRP1	SCF sR
47	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	MIP-5
48	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	Contactin-4	RGM-C	Cadherin-5	SCF sR
49	Cadherin-5	Prekallikrein	MCP-3	SLPI	SAP
	C9	HSP 90α	HGF	Kallistatin	RGM-C
50	RGM-C	MRC2	SLPI	C9	MMP-7
	SCF sR	MCP-3	ADAM 9	SAP	Properdin
51	MRC2	NRP1	SLPI	C9	HGF
	RGM-C	Properdin	SAP	BAFF Receptor	Cadherin-5
52	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Troponin T
53	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	HSP 90α	α1-Antitrypsin	BAFF Receptor
54	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	HSP 90α	Cadherin-5	α2-HS-Glycoprotein	RGM-C
55	Contactin-4	MCP-3	SLPI	C9	HGF
	MRC2	RGM-C	ADAM 9	Properdin	SAP
56	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	Contactin-4	RGM-C	Cadherin-5	SCF sR

TABLE 11-continued

57	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	BAFF Receptor	Properdin	RGM-C	C6
58	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	RGM-C	MRC2	ADAM 9	ERBB1
59	SAP	C9	SLPI	MMP-7	HGF
	α2-Antiplαsmin	RGM-C	LY9	Hat1	MCP-3
60	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	HSP 90α	Contactin-1	Properdin
61	HGF	Contactin-4	SLPI	C9	α2-Antiplαsmin
	RGM-C	BAFF Receptor	SAP	MRC2	MCP-3
62	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	IL-18 Rβ
63	Cadherin-5	α2-Antiplαsmin	C9	SLPI	MCP-3
	RGM-C	Contactin-4	MMP-7	SAP	Kallikrein 6
64	Contactin-4	MCP-3	SLPI	C9	HGF
	MMP-7	SAP	Cadherin-5	BAFF Receptor	RGM-C
65	RGM-C	MRC2	SLPI	C9	MMP-7
	BAFF Receptor	HGF	Properdin	ADAM 9	Prekallikrein
66	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	MRC2	MCP-3	BAFF Receptor	RBP
67	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Contactin-4	NRP1	BAFF Receptor
68	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Cadherin-5	C2	BAFF Receptor
69	MMP-7	Coagulation Factor Xa	C9	RGM-C	Cadherin-5
	SCF sR	HGF	SAP	MCP-3	Prekallikrein
70	RGM-C	MCP-3	C9	MMP-7	SLPI
	HGF	BAFF Receptor	Cadherin-5	SAP	α2-HS-Glycoprotein
71	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Contactin-4	NRP1	SCF sR
72	HGF	SLPI	C9	Coagulation Factor Xa	MMP-7
	MCP-3	Contactin-4	RGM-C	Kallistatin	BAFF Receptor
73	HGF	Contactin-4	SLPI	C9	α2-Antiplαsmin
	RGM-C	C6	Cadherin-5	BAFF Receptor	SAP
74	Cadherin-5	MMP-7	C9	RGM-C	SLPI
	SAP	Coagulation Factor Xa	C2	α2-Antiplαsmin	ERBB1
75	HGF	SCF sR	C9	SLPI	MCP-3
	SAP	Growth hormone receptor	Contactin-1	MMP-7	Contactin-4
76	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Hat1
77	RGM-C	MRC2	SLPI	C9	MMP-7
	HGF	BAFF Receptor	Contactin-4	Cadherin-5	IL-13 Rα1
78	SAP	MRC2	SLPI	RGM-C	MMP-7
	Cadherin-5	HGF	Prekallikrein	MCP-3	BAFF Receptor
79	MRC2	α2-Antiplαsmin	C9	SLPI	MCP-3
	MMP-7	Kallikrein 6	SAP	HSP 90α	RGM-C
80	Contactin-4	MCP-3	SLPI	C9	HGF
	MRC2	RGM-C	ADAM 9	BAFF Receptor	SAP
81	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	HSP 90α	Cadherin-5	ADAM 9	RBP
82	RGM-C	MRC2	SLPI	C9	MMP-7
	ADAM 9	SAP	BAFF Receptor	Cadherin-5	MCP-3
83	Contactin-4	MCP-3	SLPI	C9	HGF
	MRC2	RGM-C	Thrombin/Prothrombin	NRP1	Cadherin-5
84	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	Contactin-1	MRC2	ADAM 9	HSP 90α
85	RGM-C	MRC2	SLPI	C9	MMP-7
	ADAM 9	SAP	BAFF Receptor	α1-Antitrypsin	MCP-3
86	Cadherin-5	HGF	SLPI	C9	MMP-7
	RGM-C	MRC2	MCP-3	BAFF Receptor	α2-HS-Glycoprotein
87	SAP	C9	SLPI	MMP-7	HGF
	SCF sR	MCP-3	Contactin-4	ADAM 9	ARSB
88	SAP	MMP-7	α2-Antiplαsmin	SLPI	RGM-C
	HGF	BAFF Receptor	Cadherin-5	C6	SCF sR
89	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	ERBB1	RGM-C	ADAM 9	α2-HS-Glycoprotein
90	RGM-C	Contactin-4	SLPI	SAP	MMP-7
	C9	HGF	NRP1	MRC2	α2-Antiplαsmin
91	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Cadherin-5	LY9	ADAM 9
92	Contactin-4	MCP-3	SLPI	C9	HGF
	MRC2	RGM-C	ADAM 9	BAFF Receptor	SAP
93	MMP-7	SLPI	C9	HSP 90α	α2-Antiplαsmin
	Contactin-1	RGM-C	MCP-3	MRC2	IL-13 Rα1
94	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	Contactin-4	NRP1	SCF sR

TABLE 11-continued

95	RGM-C	MCP-3	C9	MMP-7	SLPI
	HGF	BAFF Receptor	Cadherin-5	SAP	HSP 90α
96	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	HSP 90α	Cadherin-5	ADAM 9	RBP
97	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Kallistatin
98	SAP	C9	SLPI	MMP-7	HGF
	NRP1	MRC2	Contactin-1	MCP-3	Thrombin/Prothrombin
99	SAP	C9	SLPI	MMP-7	HGF
	MCP-3	RGM-C	α2-Antiplαsmin	BAFF Receptor	Troponin T
100	RGM-C	MRC2	SLPI	C9	MMP-7
	ADAM 9	SAP	BAFF Receptor	α1-Antitrypsin	MCP-3

	Biomarkers	Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	Properdin SAP	0.962	0.918	1.879	0.944
2	MRC2 C2	0.949	0.908	1.856	0.942
3	MRC2 ADAM 9	0.962	0.908	1.869	0.942
4	MRC2 ADAM 9	0.949	0.918	1.867	0.940
5	SAP Contactin-1	0.974	0.897	1.872	0.941
6	HGF NRP1	0.962	0.897	1.859	0.951
7	Growth hormone receptor Kallistatin	0.974	0.892	1.867	0.943
8	Contactin-1 Cadherin-5	0.974	0.897	1.872	0.944
9	HGF Hat1	0.962	0.897	1.859	0.940
10	SAP IL-12 Rβ2	0.949	0.908	1.856	0.946
11	MRC2 Contactin-4	0.962	0.897	1.859	0.940
12	MRC2 C2	0.962	0.897	1.859	0.944
13	HGF MRC2	0.962	0.903	1.864	0.948
14	MCP-3 PCI	0.962	0.897	1.859	0.940
15	MRC2 ADAM 9	0.962	0.913	1.874	0.945
16	SAP RBP	0.962	0.913	1.874	0.939
17	MRC2 TIMP-2	0.949	0.913	1.862	0.940
18	Properdin SAP	0.962	0.918	1.879	0.947
19	MRC2 Troponin T	0.962	0.903	1.864	0.943
20	HGF α1-Antitrypsin	0.949	0.913	1.862	0.934
21	MMP-7 Cadherin-5	0.949	0.918	1.867	0.942
22	RGM-C Properdin	0.962	0.892	1.854	0.938
23	HGF C5	0.962	0.903	1.864	0.943
24	MRC2 sL-Selectin	0.949	0.918	1.867	0.946
25	RGM-C MCP-3	0.962	0.897	1.859	0.946
26	Contactin-1 ADAM 9	0.962	0.903	1.864	0.940
27	MRC2 Cadherin-5	0.949	0.903	1.851	0.939
28	MRC2 Coagulation Factor Xa	0.949	0.903	1.851	0.942
29	MCP-3 IL-13 Rα1	0.962	0.897	1.859	0.941

TABLE 11-continued

30	α2-HS-Glycoprotein Properdin	0.962	0.892	1.854	0.947
31	MCP-3 Kallikrein 6	0.962	0.903	1.864	0.947
32	RGM-C Growth hormone receptor	0.962	0.903	1.864	0.945
33	Contactin-1 Contactin-4	0.974	0.892	1.867	0.943
34	MCP-3 Coagulation Factor Xa	0.949	0.908	1.856	0.944
35	SAP Properdin	0.962	0.908	1.869	0.942
36	MRC2 TIMP-2	0.962	0.897	1.859	0.941
37	MRC2 Troponin T	0.962	0.897	1.859	0.943
38	SAP α1-Antitrypsin	0.962	0.892	1.854	0.931
39	RGM-C LY9	0.949	0.903	1.851	0.939
40	MCP-3 C5	0.962	0.903	1.864	0.941
41	HGF C6	0.949	0.913	1.862	0.949
42	SAP C2	0.962	0.892	1.854	0.942
43	MRC2 ADAM 9	0.962	0.887	1.849	0.934
44	HGF IL-12 Rβ2	0.949	0.903	1.851	0.940
45	MRC2 Cadherin-5	0.949	0.903	1.851	0.946
46	MRC2 Kallikrein 6	0.962	0.903	1.864	0.944
47	MRC2 Cadherin-5	0.962	0.903	1.864	0.944
48	SAP PCI	0.949	0.908	1.856	0.945
49	MMP-7 Contactin-4	0.962	0.908	1.869	0.946
50	HGF RBP	0.949	0.918	1.867	0.942
51	MMP-7 TIMP-2	0.949	0.908	1.856	0.942
52	MRC2 C2	0.962	0.897	1.859	0.945
53	MRC2 MIP-5	0.962	0.892	1.854	0.929
54	MRC2 BAFF Receptor	0.962	0.903	1.864	0.942
55	MMP-7 ARSB	0.949	0.903	1.851	0.938
56	SAP C5	0.962	0.903	1.864	0.946
57	MRC2 SCF sR	0.936	0.923	1.859	0.943
58	SAP MIP-5	0.962	0.892	1.854	0.939
59	MRC2 SCF sR	0.936	0.913	1.849	0.939
60	MRC2 IL-12 Rβ2	0.949	0.903	1.851	0.942
61	MMP-7 IL-13 Rα1	0.962	0.897	1.859	0.943
62	MRC2 Contactin-1	0.949	0.903	1.851	0.944
63	HGF Contactin-1	0.962	0.897	1.859	0.947
64	HSP 90α PCI	0.962	0.892	1.854	0.941
65	SAP Cadherin-5	0.962	0.908	1.869	0.943
66	Properdin SAP	0.962	0.903	1.864	0.942
67	MRC2 TIMP-2	0.962	0.892	1.854	0.938

TABLE 11-continued

68	MRC2 Troponin T	0.962	0.897	1.859	0.945
69	SLPI α1-Antitrypsin	0.949	0.903	1.851	0.936
70	Contactin-1 Contactin-4	0.962	0.903	1.864	0.944
71	MRC2 ARSB	0.949	0.903	1.851	0.941
72	SAP C5	0.974	0.887	1.862	0.940
73	MMP-7 MIP-5	0.962	0.897	1.859	0.944
74	HGF Properdin	0.962	0.892	1.854	0.951
75	RGM-C ADAM 9	0.962	0.903	1.864	0.942
76	MRC2 Kallistatin	0.949	0.897	1.846	0.937
77	MCP-3 IL-12 Rβ2	0.949	0.903	1.851	0.940
78	Properdin IL-18 Rβ	0.949	0.903	1.851	0.942
79	HGF Contactin-1	0.962	0.897	1.859	0.946
80	MMP-7 PCI	0.962	0.892	1.854	0.938
81	MRC2 Properdin	0.962	0.903	1.864	0.938
82	HGF TIMP-2	0.962	0.892	1.854	0.941
83	MMP-7 SAP	0.949	0.918	1.867	0.946
84	MCP-3 Troponin T	0.962	0.897	1.859	0.941
85	HGF C5	0.949	0.903	1.851	0.931
86	Properdin SAP	0.949	0.913	1.862	0.944
87	RGM-C Kallikrein 6	0.962	0.887	1.849	0.937
88	C9 MCP-3	0.962	0.897	1.859	0.945
89	MRC2 Contactin-1	0.962	0.892	1.854	0.939
90	Growth hormone receptor MCP-3	0.949	0.913	1.862	0.946
91	MRC2 Hat1	0.949	0.897	1.846	0.934
92	MMP-7 IL-12 Rβ2	0.949	0.903	1.851	0.940
93	HGF SAP	0.962	0.897	1.859	0.946
94	MRC2 IL-18 Rβ	0.949	0.903	1.851	0.943
95	Contactin-1 PCI	0.962	0.892	1.854	0.941
96	MRC2 RGM-C	0.962	0.903	1.864	0.940
97	MRC2 TIMP-2	0.949	0.903	1.851	0.945

TABLE 11-continued

98	RGM-C sL-Selectin	0.962	0.903	1.864	0.946
99	MRC2 Cadherin-5	0.949	0.908	1.856	0.946
100	HGF Coagulation Factor Xa	0.949	0.903	1.851	0.932

Marker	Count	Marker	Count
SLPI	100	LY9	7
MMP-7	100	sL-Selectin	6
HGF	100	α2-HS-Glycoprotein	6
RGM-C	98	α1-Antitrypsin	6
SAP	97	Troponin T	6
C9	97	Thrombin/Prothrombin	6
MCP-3	91	TIMP-2	6
MRC2	74	RBP	6
BAFF Receptor	57	Prekallikrein	6
Cadherin-5	48	PCI	6
ADAM 9	33	MIP-5	6
Contactin-4	32	Kallikrein 6	6
α2-Antiplαsmin	29	IL-18 Rβ	6
Properdin	23	IL-13 Rα1	6
Contactin-1	20	IL-12 Rβ2	6
SCF sR	17	Hat1	6
HSP 90α	15	Growth hormone receptor	6
NRP1	13	ERBB1	6
Coagulation Factor Xa	13	C6	6
Kallistatin	8	C5	6
C2	8	ARSB	6

TABLE 12

100 Panels of 13 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers			
1	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		C2	BAFF Receptor	ADAM 9
2	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	ARSB	C2
3	RGM-C	MRC2	SLPI	C9
		HGF	ADAM 9	SAP
		Prekallikrein	C5	BAFF Receptor
4	RGM-C	MCP-3	C9	MMP-7
		Contactin-1	HGF	Contactin-4
		BAFF Receptor	Coagulation Factor Xa	HSP 90α
5	HGF	SCF sR	C9	SLPI
		Cadherin-5	SAP	MCP-3
		Growth hormone receptor	sL-Selectin	C2
6	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Hat1	Cadherin-5
7	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Prekallikrein	Cadherin-5	IL-12 Rβ2
8	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	IL-13 Rα1	Cadherin-5
9	RGM-C	MRC2	SLPI	C9
		MCP-3	sL-Selectin	HGF
		BAFF Receptor	SAP	Cadherin-5
10	RGM-C	Contactin-4	SLPI	SAP
		Growth hormone receptor	C9	HGF
		Cadherin-5	ADAM 9	SCF sR

TABLE 12-continued

11	Contactin-4	MCP-3	SLPI	C9
		HSP 90α	MMP-7	SAP
		RGM-C	Kallistatin	C5
12	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	IL-13 Rα1	Cadherin-5
13	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		RGM-C	HSP 90α	Cadherin-5
14	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Contactin-1	RGM-C	PCI
15	RGM-C	MRC2	SLPI	C9
		MCP-3	HGF	BAFF Receptor
		ADAM 9	SAP	RBP
16	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Kallistatin	TIMP-2
17	RGM-C	MRC2	SLPI	C9
		MCP-3	HGF	BAFF Receptor
		Thrombin/Prothrombin	Contactin-1	IL-13 Rα1
18	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Troponin T	C2
19	RGM-C	MRC2	SLPI	C9
		HGF	ADAM 9	SAP
		Cadherin-5	MCP-3	HSP 90α
20	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		α2-HS-Glycoprotein	RGM-C	ADAM 9
21	HGF	SCF sR	C9	SLPI
		RGM-C	SAP	Growth hormone receptor
		MMP-7	Contactin-4	ADAM 9
22	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		RGM-C	C6	ADAM 9
23	SAP	C9	SLPI	MMP-7
		RGM-C	BAFF Receptor	Properdin
		MCP-3	MRC2	Coagulation Factor Xa
24	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	LY9	C2
25	MMP-7	LY9	SLPI	RGM-C
		HGF	SAP	Cadherin-5
		α2-Antiplαsmin	C9	MIP-5
26	Cadherin-5	MMP-7	C9	RGM-C
		HGF	MRC2	HSP 90α
		IL-12 Rβ2	BAFF Receptor	MCP-3
27	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	IL-18 Rβ	Cadherin-5
28	Cadherin-5	HGF	SLPI	C9
		MCP-3	RGM-C	Contactin-1
		MRC2	α2-Antiplαsmin	BAFF Receptor
29	SAP	C9	SLPI	MMP-7
		RGM-C	BAFF Receptor	Properdin
		MCP-3	MRC2	sL-Selectin
30	HGF	SLPI	C9	Coagulation Factor Xa
		SAP	MCP-3	Contactin-4
		Cadherin-5	BAFF Receptor	PCI
31	Cadherin-5	MMP-7	C9	RGM-C
		HGF	SAP	Properdin
		MCP-3	MRC2	RBP
32	RGM-C	MRC2	SLPI	C9
		MCP-3	HGF	BAFF Receptor
		Coagulation Factor Xa	Cadherin-5	SAP
33	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		Properdin	NRP1	Thrombin/Prothrombin
34	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	HSP 90α
		ADAM 9	RBP	Contactin-1

TABLE 12-continued

35	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		α1-Antitrypsin	BAFF Receptor	MIP-5
36	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	Contactin-1
		α2-HS-Glycoprotein	BAFF Receptor	α2-Antiplαsmin
37	SAP	C9	SLPI	MMP-7
		RGM-C	SCF sR	MCP-3
		ADAM 9	ARSB	LY9
38	Cadherin-5	α2-Antiplαsmin	C9	SLPI
		HGF	RGM-C	Contactin-4
		Contactin-1	SAP	Properdin
39	Cadherin-5	MMP-7	C9	RGM-C
		HGF	SAP	Coagulation Factor Xa
		α2-Antiplαsmin	ERBB1	Properdin
40	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Hat1	Cadherin-5
41	RGM-C	MRC2	SLPI	C9
		HGF	ADAM 9	SAP
		Cadherin-5	MCP-3	HSP 90α
42	HGF	Contactin-4	SLPI	C9
		MMP-7	RGM-C	C6
		MCP-3	SAP	C2
43	MMP-7	LY9	SLPI	RGM-C
		HGF	SAP	ADAM 9
		MCP-3	BAFF Receptor	Cadherin-5
44	SAP	C9	SLPI	MMP-7
		RGM-C	BAFF Receptor	Properdin
		MCP-3	MRC2	PCI
45	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	TIMP-2	Contactin-1
46	SAP	C9	SLPI	MMP-7
		RGM-C	NRP1	MRC2
		MCP-3	Thrombin/Prothrombin	Contactin-4
47	SAP	C9	SLPI	MMP-7
		RGM-C	BAFF Receptor	Properdin
		MCP-3	MRC2	α2-Antiplαsmin
48	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		α2-HS-Glycoprotein	RGM-C	ADAM 9
49	Contactin-4	MCP-3	SLPI	C9
		MMP-7	MRC2	RGM-C
		Properdin	SAP	ARSB
50	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		ERBB1	NRP1	ADAM 9
51	HGF	MMP-7	α2-Antiplαsmin	C9
		C2	RGM-C	Contactin-1
		sL-Selectin	NRP1	SAP
52	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Growth hormone receptor	Contactin-1
53	Cadherin-5	MMP-7	C9	RGM-C
		HGF	SAP	Properdin
		MCP-3	MRC2	IL-12 Rβ2
54	RGM-C	MRC2	SLPI	C9
		MCP-3	HGF	BAFF Receptor
		Coagulation Factor Xa	C2	IL-18 Rβ
55	MRC2	α2-Antiplαsmin	C9	SLPI
		HGF	MMP-7	Kallikrein 6
		HSP 90α	RGM-C	Cadherin-5
56	HSP 90α	SLPI	C9	RGM-C
		SAP	HGF	Kallistatin
		Cadherin-5	BAFF Receptor	Prekallikrein
57	HGF	SLPI	C9	Coagulation Factor Xa
		SAP	MCP-3	Contactin-4
		Cadherin-5	C2	sL-Selectin
58	Cadherin-5	MMP-7	C9	RGM-C
		HGF	SAP	Properdin
		MCP-3	MRC2	RBP
59	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		HSP 90α	Cadherin-5	RGM-C

TABLE 12-continued

60	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	Contactin-1
		α2-HS-Glycoprotein	BAFF Receptor	α2-Antiplαsmin
61	RGM-C	MRC2	SLPI	C9
		HGF	ADAM 9	SAP
		Cadherin-5	MCP-3	α1-Antitrypsin
62	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		SCF sR	ADAM 9	C2
63	MMP-7	Coagulation Factor Xa	C9	RGM-C
		SLPI	SCF sR	HGF
		Kallistatin	SAP	sL-Selectin
64	Cadherin-5	MMP-7	C9	RGM-C
		HGF	SAP	Coagulation Factor Xa
		α2-Antiplαsmin	ERBB1	NRP1
65	MMP-7	LY9	SLPI	RGM-C
		HGF	SAP	Cadherin-5
		α2-Antiplαsmin	C9	Hat1
66	Contactin-4	MCP-3	SLPI	C9
		HSP 90α	MMP-7	SAP
		RGM-C	Contactin-1	Prekallikrein
67	SAP	C9	SLPI	MMP-7
		RGM-C	BAFF Receptor	Contactin-1
		MCP-3	MRC2	ADAM 9
68	RGM-C	MRC2	SLPI	C9
		MCP-3	α2-Antiplαsmin	BAFF Receptor
		C2	SAP	HSP 90α
69	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Contactin-1	RGM-C	Kallikrein 6
70	HGF	SCF sR	C9	SLPI
		Cadherin-5	SAP	MCP-3
		Properdin	Coagulation Factor Xa	PCI
71	HGF	SCF sR	C9	SLPI
		Cadherin-5	SAP	MCP-3
		Properdin	MRC2	RBP
72	RGM-C	MRC2	SLPI	C9
		MCP-3	HGF	BAFF Receptor
		Kallistatin	ADAM 9	Prekallikrein
73	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		Prekallikrein	BAFF Receptor	Thrombin/Prothrombin
74	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		NRP1	ADAM 9	Thrombin/Prothrombin
75	RGM-C	MRC2	SLPI	C9
		HGF	ADAM 9	SAP
		α1-Antitrypsin	MCP-3	Coagulation Factor Xa
76	RGM-C	MRC2	SLPI	C9
		MCP-3	α2-Antiplαsmin	BAFF Receptor
		Cadherin-5	SAP	MIP-5
77	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	ARSB	C2
78	SAP	MMP-7	α2-Antiplαsmin	SLPI
		Contactin-4	MCP-3	C9
		BAFF Receptor	C6	Contactin-1
79	Contactin-4	MCP-3	SLPI	C9
		MMP-7	MRC2	RGM-C
		NRP1	Cadherin-5	SAP
80	Cadherin-5	HGF	SLPI	C9
		MCP-3	RGM-C	BAFF Receptor
		Kallistatin	SAP	Growth hormone receptor
81	Cadherin-5	HGF	SLPI	C9
		MCP-3	RGM-C	Contactin-1
		MRC2	NRP1	Contactin-4
82	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Prekallikrein	Cadherin-5	IL-12 Rβ2
83	MMP-7	LY9	SLPI	RGM-C
		HGF	SAP	ADAM 9
		MCP-3	BAFF Receptor	IL-13 Rα1
84	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	LY9	Contactin-4

TABLE 12-continued

85	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		sL-Selectin	BAFF Receptor	Kallikrein 6
86	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	Growth hormone receptor	Contactin-1
87	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		NRP1	ADAM 9	RBP
88	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		NRP1	SCF sR	ADAM 9
89	RGM-C	MCP-3	C9	MMP-7
		Contactin-1	HGF	Contactin-4
		BAFF Receptor	Growth hormone receptor	ADAM 9
90	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		SCF sR	ADAM 9	α2-HS-Glycoprotein
91	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	HSP 90α
		ADAM 9	Prekallikrein	RGM-C
92	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Prekallikrein	Cadherin-5	C6
93	SAP	C9	SLPI	MMP-7
		RGM-C	NRP1	MRC2
		MCP-3	Thrombin/Prothrombin	ADAM 9
94	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	IL-13 Rα1	Cadherin-5
95	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	BAFF Receptor
		HSP 90α	Cadherin-5	NRP1
96	MMP-7	SLPI	C9	HSP 90α
		MRC2	C2	MCP-3
		α2-Antiplαsmin	SAP	sL-Selectin
97	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	RGM-C
		BAFF Receptor	LY9	Contactin-4
98	Cadherin-5	HGF	SLPI	C9
		MCP-3	RGM-C	Contactin-1
		MRC2	NRP1	BAFF Receptor
99	MMP-7	SLPI	C9	MCP-3
		HGF	BAFF Receptor	ADAM 9
		Contactin-1	RGM-C	PCI
100	SAP	C9	SLPI	MMP-7
		MRC2	MCP-3	HSP 90α
		α2-HS-Glycoprotein	RGM-C	BAFF Receptor

	Biomarkers	Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	HGF Cadherin-5 Prekallikrein	0.962	0.918	1.879	0.946
2	HGF α2-Antiplαsmin C5	0.962	0.903	1.864	0.943
3	MMP-7 MCP-3 C6	0.962	0.908	1.869	0.941
4	SLPI SAP Cadherin-5	0.974	0.892	1.867	0.943
5	MMP-7 RGM-C ERBB1	0.949	0.913	1.862	0.945
6	HGF α2-Antiplαsmin LY9	0.962	0.897	1.859	0.936
7	MRC2 SAP RGM-C	0.949	0.918	1.867	0.945
8	HGF α2-Antiplαsmin ADAM 9	0.962	0.908	1.869	0.943

TABLE 12-continued

9	MMP-7 ADAM 9 IL-18 Rβ	0.962	0.892	1.854	0.942
10	MMP-7 MCP-3 Kallikrein 6	0.962	0.908	1.869	0.942
11	HGF Cadherin-5 BAFF Receptor	0.974	0.897	1.872	0.943
12	HGF α2-Antiplasmin MIP-5	0.962	0.908	1.869	0.945
13	HGF Properdin NRP1	0.962	0.913	1.874	0.942
14	MRC2 SAP sL-Selectin	0.962	0.897	1.859	0.942
15	MMP-7 Properdin Cadherin-5	0.962	0.913	1.874	0.942
16	HGF α2-Antiplasmin LY9	0.962	0.903	1.864	0.941
17	MMP-7 Cadherin-5 SAP	0.962	0.908	1.869	0.944
18	HGF α2-Antiplasmin C5	0.962	0.903	1.864	0.945
19	MMP-7 BAFF Receptor α1-Antitrypsin	0.949	0.903	1.851	0.932
20	HGF Prekallikrein Cadherin-5	0.962	0.913	1.874	0.944
21	MCP-3 Contactin-1 ARSB	0.962	0.903	1.864	0.938
22	HGF Properdin C5	0.962	0.908	1.869	0.941
23	HGF Cadherin-5 ADAM 9	0.962	0.903	1.864	0.945
24	HGF α2-Antiplasmin ERBB1	0.962	0.897	1.859	0.940
25	MRC2 MCP-3 Hat1	0.962	0.892	1.854	0.939
26	SLPI ADAM 9 Contactin-4	0.949	0.913	1.862	0.940
27	HGF α2-Antiplasmin sL-Selectin	0.949	0.903	1.851	0.946
28	MMP-7 SAP Kallikrein 6	0.962	0.908	1.869	0.946
29	HGF Cadherin-5 NRP1	0.962	0.908	1.869	0.945
30	MMP-7 RGM-C HSP 90α	0.962	0.897	1.859	0.940
31	SLPI HSP 90α ADAM 9	0.962	0.908	1.869	0.940
32	MMP-7 ADAM 9 TIMP-2	0.962	0.897	1.859	0.943

TABLE 12-continued

33	HGF Cadherin-5 BAFF Receptor	0.949	0.918	1.867	0.945
34	HGF Cadherin-5 Troponin T	0.949	0.913	1.862	0.939
35	HGF HSP 90α Cadherin-5	0.949	0.903	1.851	0.932
36	HGF RGM-C MIP-5	0.962	0.908	1.869	0.944
37	HGF Contactin-4 Properdin	0.949	0.908	1.856	0.939
38	MCP-3 MMP-7 C6	0.949	0.918	1.867	0.949
39	SLPI C2 NRP1	0.962	0.897	1.859	0.951
40	HGF α2-Antiplαsmin C5	0.949	0.903	1.851	0.939
41	MMP-7 BAFF Receptor IL-12 Rβ2	0.962	0.897	1.859	0.942
42	α2-Antiplαsmin Cadherin-5 IL-18 Rβ	0.949	0.903	1.851	0.946
43	MRC2 Kallistatin Kallikrein 6	0.962	0.903	1.864	0.938
44	HGF Cadherin-5 HSP 90α	0.949	0.908	1.856	0.941
45	HGF α2-Antiplαsmin IL-13 Rα1	0.962	0.897	1.859	0.942
46	HGF Contactin-1 ADAM 9	0.962	0.903	1.864	0.941
47	HGF Cadherin-5 Troponin T	0.949	0.913	1.862	0.946
48	HGF Prekallikrein α1-Antitrypsin	0.949	0.903	1.851	0.931
49	HGF ADAM 9 C5	0.949	0.908	1.856	0.940
50	HGF Thrombin/Prothrombin Cadherin-5	0.962	0.897	1.859	0.943
51	SLPI Cadherin-5 Growth hormone receptor	0.962	0.908	1.869	0.952
52	HGF α2-Antiplαsmin Hat1	0.949	0.903	1.851	0.936
53	SLPI HSP 90α BAFF Receptor	0.949	0.908	1.856	0.942
54	MMP-7 SAP α2-Antiplαsmin	0.962	0.887	1.849	0.943
55	MCP-3 SAP MIP-5	0.974	0.887	1.862	0.947
56	MMP-7 MCP-3 Contactin-1	0.962	0.908	1.869	0.944
57	MMP-7 RGM-C PCI	0.949	0.908	1.856	0.945

TABLE 12-continued

58	SLPI HSP 90α BAFF Receptor	0.962	0.908	1.869	0.941
59	HGF Prekallikrein TIMP-2	0.949	0.908	1.856	0.943
60	HGF RGM-C Troponin T	0.962	0.897	1.859	0.942
61	MMP-7 BAFF Receptor HSP 90α	0.949	0.903	1.851	0.932
62	HGF HSP 90α ARSB	0.949	0.908	1.856	0.939
63	Cadherin-5 MCP-3 C6	0.962	0.903	1.864	0.947
64	SLPI C2 sL-Selectin	0.962	0.897	1.859	0.951
65	MRC2 MCP-3 ADAM 9	0.949	0.903	1.851	0.936
66	HGF Cadherin-5 IL-12 Rβ2	0.949	0.908	1.856	0.946
67	HGF α2-Antiplαsmin IL-13 Rα1	0.962	0.908	1.869	0.942
68	MMP-7 HGF IL-18 Rβ	0.962	0.887	1.849	0.943
69	MRC2 SAP Coagulation Factor Xa	0.962	0.897	1.859	0.942
70	MMP-7 RGM-C Contactin-1	0.949	0.908	1.856	0.945
71	MMP-7 RGM-C ADAM 9	0.949	0.918	1.867	0.943
72	MMP-7 SAP TIMP-2	0.949	0.908	1.856	0.943
73	HGF Cadherin-5 ADAM 9	0.962	0.903	1.864	0.947
74	HGF Contactin-4 Troponin T	0.962	0.897	1.859	0.940
75	MMP-7 BAFF Receptor Troponin T	0.949	0.897	1.846	0.931
76	MMP-7 HGF α2-HS-Glycoprotein	0.962	0.908	1.869	0.945
77	HGF α2-Antiplαsmin Contactin-1	0.949	0.908	1.856	0.943
78	RGM-C HGF Cadherin-5	0.949	0.913	1.862	0.947
79	HGF Thrombin/Prothrombin ERBB1	0.949	0.908	1.856	0.945
80	MMP-7 Contactin-4 Properdin	0.962	0.903	1.864	0.942

TABLE 12-continued

81	MMP-7 SAP Hat1	0.936	0.913	1.849	0.937
82	MRC2 SAP Coagulation Factor Xa	0.949	0.908	1.856	0.943
83	MRC2 Kallistatin Cadherin-5	0.962	0.908	1.869	0.937
84	HGF α2-Antiplαsmin IL-18 Rβ	0.962	0.887	1.849	0.939
85	HGF α2-Antiplαsmin Cadherin-5	0.962	0.897	1.859	0.947
86	HGF α2-Antiplαsmin PCI	0.949	0.908	1.856	0.942
87	HGF Contactin-4 SCF sR	0.962	0.903	1.864	0.939
88	HGF Contactin-4 TIMP-2	0.949	0.908	1.856	0.940
89	SLPI SAP α1-Antitrypsin	0.949	0.897	1.846	0.931
90	HGF HSP 90α NRP1	0.962	0.903	1.864	0.940
91	HGF Cadherin-5 ARSB	0.949	0.908	1.856	0.943
92	MRC2 SAP RGM-C	0.949	0.913	1.862	0.945
93	HGF Contactin-1 ERBB1	0.962	0.892	1.854	0.940
94	HGF α2-Antiplαsmin Hat1	0.949	0.897	1.846	0.936
95	HGF Prekallikrein IL-12 Rβ2	0.949	0.908	1.856	0.939
96	HGF RGM-C IL-18 Rβ	0.962	0.887	1.849	0.947
97	HGF α2-Antiplαsmin Kallikrein 6	0.962	0.897	1.859	0.939
98	MMP-7 SAP MIP-5	0.962	0.908	1.869	0.944

TABLE 12-continued

99	MRC2 SAP HSP 90α	0.962	0.892	1.854	0.939
100	HGF Cadherin-5 RBP	0.962	0.903	1.864	0.940

Marker	Count	Marker	Count
SLPI	100	SCF sR	11
MMP-7	100	LY9	10
HGF	100	Thrombin/Prothrombin	8
SAP	99	Kallistatin	8
C9	98	Growth hormone receptor	8
RGM-C	97	α2-HS-Glycoprotein	7
MCP-3	97	RBP	7
MRC2	80	PCI	7
BAFF Receptor	68	MIP-5	7
Cadherin-5	65	Kallikrein 6	7
ADAM 9	44	IL-18 Rβ	7
α2-Antiplαsmin	35	IL-13 Rα1	7
Contactin-1	26	IL-12 Rβ2	7
HSP 90α	26	Hat1	7
Contactin-4	23	ERBB1	7
Properdin	18	C6	7
NRP1	17	C5	7
C2	15	ARSB	7
Prekallikrein	14	α1-Antitrypsin	6
Coagulation Factor Xa	13	Troponin T	6
sL-Selectin	11	TIMP-2	6

TABLE 13

100 Panels of 14 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers			
1	RGM-C	MRC2	SLPI	C9
	SAP	BAFF Receptor	HGF	Properdin
		Cadherin-5	NRP1	Contactin-4
2	MMP-7	SLPI	C9	Properdin
	HGF	MCP-3	HSP 90α	RGM-C
		SAP	ADAM 9	SCF sR
3	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
		Cadherin-5	HSP 90α	C2
4	Cadherin-5	α2-Antiplαsmin	C9	SLPI
	HGF	RGM-C	Contactin-4	MMP-7
		SAP	Properdin	C6
5	RGM-C	MRC2	SLPI	C9
	MCP-3	α2-Antiplαsmin	BAFF Receptor	HGF
		SAP	HSP 90α	Coagulation Factor Xa
6	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	RGM-C
		sL-Selectin	C2	ERBB1
7	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		Kallistatin	LY9	Cadherin-5
8	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		BAFF Receptor	ADAM 9	RBP
9	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
		SAP	IL-13 Rα1	MIP-5
10	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
		SAP	IL-13 Rα1	Contactin-1
11	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
		α2-Antiplαsmin	BAFF Receptor	MIP-5

TABLE 13-continued

12	HGF	SLPI	C9	Coagulation Factor Xa
	SAP	MCP-3	Contactin-4	RGM-C
		C2	sL-Selectin	Contactin-1
13	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		Kallistatin	C5	BAFF Receptor
14	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
		NRP1	ADAM 9	Thrombin/Prothrombin
15	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
		RBP	RGM-C	Contactin-1
16	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
		MCP-3	α1-Antitrypsin	HSP 90α
17	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		Kallistatin	C5	Contactin-1
18	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
		Prekallikrein	RGM-C	MIP-5
19	MMP-7	SLPI	C9	HSP 90α
	MRC2	C2	MCP-3	RGM-C
		SAP	LY9	Kallistatin
20	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	Contactin-4	SAP
		Growth hormone receptor	Cadherin-5	Kallistatin
21	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		LY9	Contactin-1	Cadherin-5
22	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
		Cadherin-5	C2	RGM-C
23	RGM-C	MRC2	SLPI	C9
	MCP-3	α2-Antiplαsmin	BAFF Receptor	HGF
		SAP	Cadherin-5	MIP-5
24	RGM-C	Contactin-4	SLPI	SAP
	Growth hormone receptor	C9	HGF	MCP-3
		ADAM 9	SCF sR	Contactin-1
25	Cadherin-5	HGF	SLPI	C9
	C2	SAP	α2-Antiplαsmin	RGM-C
		ERBB1	HSP 90α	NRP1
26	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	Contactin-4	SAP
		Growth hormone receptor	Cadherin-5	Kallistatin
27	Contactin-4	MCP-3	SLPI	C9
	MMP-7	MRC2	RGM-C	Thrombin/Prothrombin
		Cadherin-5	SAP	ADAM 9
28	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		BAFF Receptor	ADAM 9	Troponin T
29	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
		MCP-3	α1-Antitrypsin	HSP 90α
30	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
		SAP	α2-HS-Glycoprotein	MIP-5
31	SAP	C9	SLPI	MMP-7
	RGM-C	SCF sR	MCP-3	Contactin-4
		Growth hormone receptor	Contactin-1	ADAM 9
32	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	sL-Selectin
		RGM-C	Thrombin/Prothrombin	C6
33	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		RGM-C	Coagulation Factor Xa	C5
34	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		Hat1	Cadherin-5	LY9
35	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		ADAM 9	Thrombin/Prothrombin	HSP 90α
36	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		Kallistatin	C5	BAFF Receptor

TABLE 13-continued

37	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Properdin
		IL-13 Rα1	Contactin-1	α2-Antiplαsmin
38	Cadherin-5	MMP-7	C9	RGM-C
	HGF	SAP	Coagulation Factor Xa	C2
		ERBB1	NRP1	sL-Selectin
39	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
		NRP1	BAFF Receptor	RBP
40	HGF	SCF sR	C9	SLPI
	RGM-C	SAP	Growth hormone receptor	Contactin-1
		Contactin-4	ADAM 9	TIMP-2
41	RGM-C	MRC2	SLPI	C9
	MCP-3	α2-Antiplαsmin	BAFF Receptor	HGF
		SAP	Cadherin-5	Troponin T
42	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	RGM-C
		sL-Selectin	C2	Contactin-4
43	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		Kallistatin	C5	BAFF Receptor
44	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Contactin-4
		SCF sR	ADAM 9	Properdin
45	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		Growth hormone receptor	Contactin-1	C6
46	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		Growth hormone receptor	Cadherin-5	Kallistatin
47	MMP-7	SLPI	C9	HSP 90α
	MRC2	C2	MCP-3	RGM-C
		SAP	Prekallikrein	α2-HS-Glycoprotein
48	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
		BAFF Receptor	MIP-5	MRC2
49	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	Kallikrein 6	SAP
		RGM-C	Cadherin-5	Contactin-1
50	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	BAFF Receptor	Cadherin-5
		HSP 90α	C2	Prekallikrein
51	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		BAFF Receptor	MIP-5	RBP
52	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
		Cadherin-5	RGM-C	α2-HS-Glycoprotein
53	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		BAFF Receptor	ADAM 9	Troponin T
54	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	sL-Selectin
		RGM-C	Thrombin/Prothrombin	Cadherin-5
55	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Contactin-4
		SCF sR	ADAM 9	ARSB
56	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
		MCP-3	HSP 90α	C5
57	RGM-C	Contactin-4	SLPI	SAP
	Coagulation Factor Xa	MCP-3	C2	HGF
		Properdin	Cadherin-5	Contactin-1
58	Cadherin-5	HGF	SLPI	C9
	Contactin-1	SAP	MCP-3	Kallistatin
		C5	RGM-C	α2-HS-Glycoprotein
59	NRP1	LY9	C9	SLPI
	RGM-C	MRC2	HGF	Contactin-1
		SAP	Cadherin-5	ADAM 9
60	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
		Cadherin-5	HSP 90α	IL-12 Rβ2
61	MMP-7	SLPI	C9	HSP 90α
	HGF	Contactin-1	RGM-C	MCP-3
		IL-13 Rα1	SAP	C2

TABLE 13-continued

62	MMP-7	LY9	SLPI	RGM-C
	HGF	SAP	ADAM 9	Kallistatin
		BAFF Receptor	Cadherin-5	Kallikrein 6
63	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	BAFF Receptor	SAP
		Prekallikrein	ADAM 9	MRC2
64	Contactin-4	MCP-3	SLPI	C9
	MMP-7	MRC2	RGM-C	ADAM 9
		Cadherin-5	RBP	SAP
65	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	ADAM 9
		Kallistatin	SAP	RBP
66	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		NRP1	Thrombin/Prothrombin	Contactin-4
67	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
		MCP-3	α1-Antitrypsin	HSP 90α
68	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	sL-Selectin
		Properdin	Growth hormone receptor	Cadherin-5
69	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
		ADAM 9	Prekallikrein	HSP 90α
70	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	Contactin-4	SAP
		Coagulation Factor Xa	Growth hormone receptor	ADAM 9
71	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
		BAFF Receptor	ADAM 9	NRP1
72	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
		NRP1	BAFF Receptor	Properdin
73	RGM-C	MRC2	SLPI	C9
	SAP	BAFF Receptor	HGF	Properdin
		Cadherin-5	HSP 90α	RBP
74	HGF	MMP-7	α2-Antiplαsmin	C9
	C2	RGM-C	Contactin-1	Cadherin-5
		NRP1	SAP	Growth hormone receptor
75	Cadherin-5	HGF	SLPI	C9
	Properdin	RGM-C	MRC2	MCP-3
		ADAM 9	SAP	SCF sR
76	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
		ADAM 9	C5	HSP 90α
77	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
		ADAM 9	Prekallikrein	TIMP-2
78	RGM-C	MRC2	SLPI	C9
	SAP	BAFF Receptor	HGF	Properdin
		Cadherin-5	HSP 90α	RBP
79	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
		MCP-3	α1-Antitrypsin	HSP 90α
80	RGM-C	Contactin-4	SLPI	SAP
	Growth hormone receptor	C9	HGF	MCP-3
		ADAM 9	SCF sR	Contactin-1
81	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	MCP-3
		C5	HSP 90α	BAFF Receptor
82	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
		RGM-C	BAFF Receptor	MIP-5
83	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	RGM-C
		sL-Selectin	C2	Contactin-4
84	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
		Kallistatin	LY9	C5
85	SAP	C9	SLPI	MMP-7
	RGM-C	BAFF Receptor	Properdin	Cadherin-5
		MRC2	IL-12 Rβ2	ADAM 9
86	Cadherin-5	MMP-7	C9	RGM-C
	HGF	SAP	HSP 90α	α2-Antiplαsmin
		MCP-3	Contactin-1	IL-13 Rα1

TABLE 13-continued

87	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
		NRP1	BAFF Receptor	Properdin
88	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
		ADAM 9	C5	IL-13 Rα1
89	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
		Kallistatin	C5	BAFF Receptor
90	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	BAFF Receptor	Contactin-4
		SAP	Growth hormone receptor	TIMP-2
91	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
		RGM-C	NRP1	HSP 90α
92	SAP	C9	SLPI	MMP-7
	RGM-C	NRP1	MRC2	Contactin-1
		HSP 90α	Thrombin/Prothrombin	BAFF Receptor
93	HGF	SCF sR	C9	SLPI
	RGM-C	SAP	Growth hormone receptor	Contactin-1
		Contactin-4	ADAM 9	ARSB
94	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Properdin
		C6	ADAM 9	C5
95	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
		RGM-C	IL-13 Rα1	Coagulation Factor Xa
96	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
		ADAM 9	RBP	C5
97	MMP-7	LY9	SLPI	RGM-C
	HGF	SAP	Cadherin-5	MCP-3
		C9	Hat1	ADAM 9
98	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
		Prekallikrein	RGM-C	IL-12 Rβ2
99	Cadherin-5	MMP-7	C9	RGM-C
	HGF	SAP	Properdin	HSP 90α
		MRC2	C2	Prekallikrein
100	RGM-C	MRC2	SLPI	C9
	HGF	SCF sR	MCP-3	ADAM 9
		Properdin	Kallikrein 6	sL-Selectin

	Biomarkers	Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	MMP-7 ADAM 9 MCP-3	0.962	0.913	1.874	0.943
2	MRC2 C5 ARSB	0.949	0.913	1.862	0.940
3	MRC2 Prekallikrein RGM-C	0.962	0.913	1.874	0.945
4	MCP-3 Contactin-1 α2-HS-Glycoprotein	0.949	0.923	1.872	0.948
5	MMP-7 C2 MIP-5	0.974	0.897	1.872	0.944
6	MMP-7 Growth hormone receptor MIP-5	0.949	0.913	1.862	0.943
7	HGF BAFF Receptor Hat1	0.962	0.903	1.864	0.937
8	HGF Prekallikrein IL-12 Rβ2	0.962	0.908	1.869	0.943
9	MCP-3 RGM-C Cadherin-5	0.974	0.892	1.867	0.943
10	MCP-3 RGM-C IL-18 Rβ	0.962	0.892	1.854	0.940

TABLE 13-continued

11	MMP-7 MRC2 Kallikrein 6	0.962	0.908	1.869	0.945
12	MMP-7 Cadherin-5 PCI	0.949	0.913	1.862	0.945
13	HGF RGM-C TIMP-2	0.962	0.897	1.859	0.941
14	MMP-7 MRC2 BAFF Receptor	0.962	0.913	1.874	0.944
15	HGF ADAM 9 Troponin T	0.962	0.908	1.869	0.941
16	MMP-7 Cadherin-5 LY9	0.949	0.897	1.846	0.929
17	HGF RGM-C ARSB	0.962	0.897	1.859	0.943
18	HGF ADAM 9 C6	0.949	0.918	1.867	0.944
19	HGF α2-Antiplαsmin ERBB1	0.962	0.897	1.859	0.945
20	SLPI BAFF Receptor ADAM 9	0.962	0.908	1.869	0.943
21	HGF BAFF Receptor Hat1	0.962	0.903	1.864	0.937
22	HGF HSP 90α IL-12 Rβ2	0.962	0.908	1.869	0.944
23	MMP-7 C2 IL-18 Rβ	0.949	0.903	1.851	0.945
24	MMP-7 Cadherin-5 Kallikrein 6	0.962	0.903	1.864	0.942
25	MMP-7 PCI Contactin-1	0.962	0.897	1.859	0.950
26	SLPI BAFF Receptor TIMP-2	0.949	0.908	1.856	0.941
27	HGF NRP1 HSP 90α	0.962	0.908	1.869	0.943
28	HGF Prekallikrein Contactin-1	0.962	0.903	1.864	0.945
29	MMP-7 Cadherin-5 Thrombin/Prothrombin	0.949	0.897	1.846	0.933
30	MCP-3 RGM-C Contactin-1	0.974	0.897	1.872	0.943
31	HGF Kallikrein 6 ARSB	0.962	0.897	1.859	0.936
32	HGF NRP1 Contactin-4	0.962	0.903	1.864	0.942
33	HGF BAFF Receptor Kallistatin	0.974	0.892	1.867	0.942
34	HGF BAFF Receptor C5	0.962	0.903	1.864	0.937

TABLE 13-continued

35	HGF Prekallikrein IL-12 Rβ2	0.962	0.903	1.864	0.945
36	HGF RGM-C IL-13 Rα1	0.974	0.892	1.867	0.940
37	HGF RGM-C IL-18 Rβ	0.949	0.903	1.851	0.941
38	SLPI α2-Antiplαsmin PCI	0.962	0.897	1.859	0.950
39	MMP-7 MRC2 MIP-5	0.962	0.913	1.874	0.942
40	MCP-3 MMP-7 LY9	0.949	0.908	1.856	0.939
41	MMP-7 C2 ADAM 9	0.962	0.903	1.864	0.945
42	MMP-7 Growth hormone receptor α1-Antitrypsin	0.936	0.908	1.844	0.934
43	HGF RGM-C α2-HS-Glycoprotein	0.974	0.897	1.872	0.941
44	HGF NRP1 ARSB	0.949	0.908	1.856	0.939
45	HGF BAFF Receptor IL-13 Rα1	0.962	0.903	1.864	0.941
46	HGF BAFF Receptor Hat1	0.949	0.903	1.851	0.937
47	HGF BAFF Receptor IL-12 Rβ2	0.962	0.903	1.864	0.941
48	MMP-7 Cadherin-5 IL-18 Rβ	0.962	0.887	1.849	0.943
49	MCP-3 HSP 90α BAFF Receptor	0.962	0.903	1.864	0.946
50	SLPI SAP PCI	0.949	0.908	1.856	0.943
51	HGF Prekallikrein ADAM 9	0.962	0.908	1.869	0.943
52	HGF HSP 90α TIMP-2	0.949	0.908	1.856	0.941
53	HGF Prekallikrein Kallistatin	0.949	0.913	1.862	0.945
54	HGF NRP1 α1-Antitrypsin	0.936	0.908	1.844	0.933
55	HGF NRP1 C2	0.949	0.908	1.856	0.939
56	MMP-7 Cadherin-5 C6	0.962	0.903	1.864	0.942
57	MMP-7 C9 C5	0.949	0.918	1.867	0.946
58	MMP-7 BAFF Receptor ERBB1	0.962	0.897	1.859	0.941

TABLE 13-continued

59	MMP-7 Thrombin/Prothrombin Hat1	0.936	0.913	1.849	0.934
60	MRC2 Prekallikrein RGM-C	0.962	0.903	1.864	0.944
61	α2-Antiplαsmin MRC2 IL-18 Rβ	0.962	0.887	1.849	0.944
62	MRC2 MCP-3 Contactin-1	0.962	0.903	1.864	0.937
63	MMP-7 Contactin-4 PCI	0.936	0.918	1.854	0.943
64	HGF BAFF Receptor MIP-5	0.962	0.903	1.864	0.941
65	MMP-7 Cadherin-5 TIMP-2	0.949	0.908	1.856	0.940
66	HGF Properdin Troponin T	0.949	0.913	1.862	0.947
67	MMP-7 Cadherin-5 C5	0.949	0.892	1.841	0.932
68	MMP-7 MCP-3 ARSB	0.949	0.908	1.856	0.941
69	MMP-7 C2 C6	0.962	0.903	1.864	0.942
70	SLPI BAFF Receptor Kallistatin	0.962	0.903	1.864	0.940
71	HGF C2 ERBB1	0.962	0.897	1.859	0.942
72	MMP-7 MRC2 Hat1	0.936	0.913	1.849	0.938
73	MMP-7 ADAM 9 IL-12 Rβ2	0.962	0.903	1.864	0.936
74	SLPI sL-Selectin IL-18 Rβ	0.962	0.887	1.849	0.949
75	MMP-7 BAFF Receptor Kallikrein 6	0.949	0.913	1.862	0.943
76	MMP-7 Kallistatin PCI	0.962	0.892	1.854	0.938
77	MMP-7 Kallistatin Cadherin-5	0.949	0.908	1.856	0.944
78	MMP-7 ADAM 9 Troponin T	0.949	0.913	1.862	0.939
79	MMP-7 Cadherin-5 NRP1	0.949	0.892	1.841	0.931
80	MMP-7 Cadherin-5 ARSB	0.949	0.908	1.856	0.940

TABLE 13-continued

81	MMP-7 Prekallikrein C6	0.962	0.903	1.864	0.941
82	HGF α2-HS-Glycoprotein Coagulation Factor Xa	0.962	0.903	1.864	0.943
83	MMP-7 Growth hormone receptor ERBB1	0.949	0.908	1.856	0.945
84	HGF BAFF Receptor Hat1	0.949	0.897	1.846	0.935
85	HGF MCP-3 Prekallikrein	0.949	0.913	1.862	0.944
86	SLPI BAFF Receptor MRC2	0.962	0.903	1.864	0.945
87	MMP-7 MRC2 IL-18 Rβ	0.949	0.897	1.846	0.943
88	MMP-7 Kallistatin Kallikrein 6	0.974	0.887	1.862	0.937
89	HGF RGM-C PCI	0.962	0.892	1.854	0.941
90	MMP-7 Kallistatin HSP 90α	0.962	0.892	1.854	0.939
91	MRC2 Contactin-1 Troponin T	0.962	0.897	1.859	0.939
92	HGF MCP-3 α1-Antitrypsin	0.949	0.892	1.841	0.931
93	MCP-3 MMP-7 C5	0.962	0.892	1.854	0.940
94	HGF RGM-C MIP-5	0.962	0.903	1.864	0.941
95	MRC2 Contactin-1 Prekallikrein	0.962	0.903	1.864	0.942
96	MMP-7 C2 ERBB1	0.962	0.892	1.854	0.939
97	MRC2 α2-Antiplαsmin C5	0.949	0.897	1.846	0.937
98	HGF ADAM 9 C2	0.949	0.913	1.862	0.945

TABLE 13-continued

99	SLPI MCP-3 IL-18 Rβ	0.949	0.897	1.846	0.947
100	MMP-7 SAP BAFF Receptor	0.949	0.913	1.862	0.941

Marker	Count	Marker	Count
SLPI	100	Growth hormone	16
SAP	100	receptor	
RGM-C	100	SCF sR	13
MMP-7	100	MIP-5	13
HGF	100	sL-Selectin	10
C9	99	LY9	10
MCP-3	94	Thrombin/	9
MRC2	74	Prothrombin	
Cadherin-5	73	RBP	9
BAFF Receptor	70	IL-13 Rα1	9
ADAM 9	51	Kallikrein 6	8
HSP 90α	43	IL-18 Rβ	8
Contactin-1	36	IL-12 Rβ2	8
Contactin-4	28	Hat1	8
α2-Antiplαsmin	23	ERBB1	8
C2	23	Coagulation Factor Xa	8
Kallistatin	22	C6	8
Prekallikrein	20	ARSB	8
C5	20	α2-HS-Glycoprotein	7
NRP1	19	α1-Antitrypsin	7
Properdin	17	Troponin T	7
		TIMP-2	7
		PCI	7

TABLE 14

100 Panels of 15 Biomarkers for Diagnosing Ovarian Cancer from Benign Pelvic Masses

	Biomarkers			
1	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	MIP-5	ADAM 9	NRP1
2	SAP	C9	SLPI	MMP-7
	RGM-C	BAFF Receptor	Properdin	Cadherin-5
	MRC2	Kallistatin	ADAM 9	Prekallikrein
3	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	Cadherin-5	C2	RGM-C	C5
4	RGM-C	MRC2	SLPI	C9
	MCP-3	α2-Antiplαsmin	BAFF Receptor	HGF
	SAP	Kallikrein 6	Kallistatin	HSP 90α
5	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	BAFF Receptor	Kallistatin	C5	ADAM 9
6	Cadherin-5	MMP-7	C9	RGM-C
	HGF	MRC2	α2-Antiplαsmin	Growth hormone receptor
	C2	Kallistatin	LY9	C5
7	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	LY9	Contactin-1	Cadherin-5	Hat1
8	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Prekallikrein	IL-12 Rβ2
9	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
	BAFF Receptor	MIP-5	MRC2	IL-13 Rα1
10	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Prekallikrein	IL-18 Rβ
11	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	MRC2
	BAFF Receptor	SAP	IL-12 Rβ2	HSP 90α

TABLE 14-continued

12	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	Contactin-1	RGM-C
	RBP	ADAM 9	Prekallikrein	Cadherin-5
13	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	IL-13 Rα1	Cadherin-5	SCF sR	MIP-5
14	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	Cadherin-5	C2	RGM-C	TIMP-2
15	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	NRP1	BAFF Receptor	Properdin	MIP-5
16	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Properdin
	MIP-5	Cadherin-5	Troponin T	Contactin-1
17	HGF	SCF sR	C9	SLPI
	RGM-C	SAP	Growth hormone receptor	Contactin-1
	Contactin-4	ADAM 9	sL-Selectin	Cadherin-5
18	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	RGM-C	ADAM 9	Contactin-1	HSP 90α
19	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
	Kallistatin	C5	BAFF Receptor	ARSB
20	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	RGM-C
	sL-Selectin	C2	Contactin-4	ERBB1
21	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Hat1	Cadherin-5	LY9	C5
22	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
	SAP	IL-13 Rα1	Contactin-1	IL-18 Rβ
23	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
	RGM-C	Kallikrein 6	Cadherin-5	RBP
24	Cadherin-5	HGF	SLPI	C9
	C2	SAP	α2-Antiplαsmin	RGM-C
	Contactin-4	Coagulation Factor Xa	C6	sL-Selectin
25	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	NRP1	BAFF Receptor	MIP-5	TIMP-2
26	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	sL-Selectin
	RGM-C	Thrombin/Prothrombin	Cadherin-5	HSP 90α
27	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Prekallikrein	IL-12 Rβ2
28	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
	RGM-C	NRP1	HSP 90α	α2-HS-Glycoprotein
29	Contactin-4	MCP-3	SLPI	C9
	HSP 90α	MMP-7	SAP	Cadherin-5
	Kallistatin	C5	BAFF Receptor	ARSB
30	MMP-7	SLPI	C9	HSP 90α
	MRC2	C2	MCP-3	RGM-C
	SAP	LY9	Contactin-1	C5
31	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Growth hormone receptor	Cadherin-5	Kallistatin	C5
32	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Properdin	C5
33	RGM-C	Contactin-4	SLPI	SAP
	Growth hormone receptor	C9	HGF	MCP-3
	ADAM 9	SCF sR	Kallikrein 6	Properdin
34	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Growth hormone receptor	Cadherin-5	Kallistatin	C5
35	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	MRC2
	BAFF Receptor	SAP	IL-12 Rβ2	HSP 90α

TABLE 14-continued

36	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
	BAFF Receptor	MIP-5	MRC2	NRP1
37	SAP	C9	SLPI	MMP-7
	RGM-C	NRP1	MRC2	Contactin-1
	HSP 90α	Thrombin/Prothrombin	sL-Selectin	α2-HS-Glycoprotein
38	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	Cadherin-5	C2	RGM-C	Troponin T
39	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	MIP-5	ADAM 9	HSP 90α
40	HGF	SCF sR	C9	SLPI
	RGM-C	SAP	Growth hormone receptor	Contactin-1
	Contactin-4	ADAM 9	Kallistatin	Kallikrein 6
41	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	LY9	Contactin-4	Cadherin-5	ADAM 9
42	Cadherin-5	MMP-7	C9	RGM-C
	HGF	MRC2	NRP1	BAFF Receptor
	SAP	HSP 90α	MCP-3	MIP-5
43	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Kallistatin	LY9	C5	ADAM 9
44	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	α2-Antiplαsmin	BAFF Receptor	MIP-5	IL-13 Rα1
45	MMP-7	LY9	SLPI	RGM-C
	HGF	SAP	ADAM 9	Kallistatin
	BAFF Receptor	Cadherin-5	Prekallikrein	C2
46	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	NRP1	BAFF Receptor	MIP-5	HSP 90α
47	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	MRC2
	BAFF Receptor	SAP	HSP 90α	RBP
48	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Properdin
	C6	ADAM 9	C5	RBP
49	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	Cadherin-5	NRP1	Thrombin/Prothrombin	RGM-C
50	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Troponin T	IL-12 Rβ2
51	MMP-7	LY9	SLPI	RGM-C
	HGF	SAP	ADAM 9	Kallistatin
	BAFF Receptor	Cadherin-5	Prekallikrein	C5
52	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	HSP 90α
	ADAM 9	C2	NRP1	ARSB
53	MMP-7	SLPI	C9	MCP-3
	HGF	BAFF Receptor	ADAM 9	SAP
	RGM-C	NRP1	Coagulation Factor Xa	sL-Selectin
54	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Contactin-4
	ADAM 9	MIP-5	HSP 90α	C2
55	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Kallistatin	LY9	Cadherin-5	Hat1
56	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
	SAP	IL-13 Rα1	C5	Cadherin-5
57	HGF	MMP-7	α2-Antiplαsmin	C9
	C2	RGM-C	Contactin-1	Cadherin-5
	NRP1	SAP	Growth hormone receptor	IL-18 Rβ
58	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
	ADAM 9	C5	Kallikrein 6	Coagulation Factor Xa
59	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
	BAFF Receptor	MIP-5	MRC2	NRP1
60	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	BAFF Receptor	Contactin-4
	SAP	Growth hormone receptor	TIMP-2	HSP 90α

TABLE 14-continued

61	MRC2	NRP1	SLPI	C9
	MMP-7	RGM-C	Properdin	SAP
	Cadherin-5	HSP 90α	Thrombin/Prothrombin	MCP-3
62	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Prekallikrein	IL-13 Rα1
63	RGM-C	Contactin-4	SLPI	SAP
	Growth hormone receptor	C9	HGF	MCP-3
	ADAM 9	SCF sR	sL-Selectin	C5
64	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	ADAM 9	C5	BAFF Receptor	Thrombin/Prothrombin
65	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	Coagulation Factor Xa
	Contactin-1	RGM-C	Properdin	C6
66	MMP-7	SLPI	C9	HSP 90α
	MRC2	C2	MCP-3	RGM-C
	SAP	LY9	Kallistatin	ERBB1
67	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Hat1	Cadherin-5	LY9	C5
68	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
	RBP	RGM-C	BAFF Receptor	Kallistatin
69	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	Contactin-1	SAP
	α2-Antiplαsmin	BAFF Receptor	Kallikrein 6	C5
70	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	α2-Antiplαsmin	MRC2
	LY9	Contactin-1	SAP	α2-HS-Glycoprotein
71	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	ADAM 9
	Kallistatin	SAP	RBP	TIMP-2
72	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	MCP-3
	C5	HSP 90α	BAFF Receptor	Troponin T
73	Cadherin-5	HGF	SLPI	C9
	Properdin	MRC2	BAFF Receptor	MCP-3
	RGM-C	ADAM 9	SAP	Troponin T
74	MMP-7	LY9	SLPI	RGM-C
	HGF	SAP	ADAM 9	Kallistatin
	BAFF Receptor	Cadherin-5	Prekallikrein	C5
75	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	MCP-3
	C5	BAFF Receptor	C6	MIP-5
76	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	Contactin-4	SAP
	Growth hormone receptor	Cadherin-5	C2	ADAM 9
77	NRP1	LY9	C9	SLPI
	RGM-C	MRC2	HGF	Contactin-1
	SAP	Cadherin-5	ADAM 9	MCP-3
78	MRC2	α2-Antiplαsmin	C9	SLPI
	HGF	MMP-7	HSP 90α	BAFF Receptor
	SAP	IL-13 Rα1	C5	Contactin-4
79	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	Cadherin-5
	BAFF Receptor	ADAM 9	Prekallikrein	IL-18 Rβ
80	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	LY9	Contactin-1	Cadherin-5	Kallikrein 6
81	Cadherin-5	HGF	SLPI	C9
	Properdin	MRC2	BAFF Receptor	MCP-3
	RGM-C	ADAM 9	SAP	Troponin T
82	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
	ADAM 9	Prekallikrein	TIMP-2	Cadherin-5
83	Cadherin-5	HGF	SLPI	C9
	Properdin	RGM-C	MRC2	MCP-3
	ADAM 9	SAP	Contactin-4	Growth hormone receptor
84	Cadherin-5	HGF	SLPI	C9
	Properdin	MRC2	BAFF Receptor	MCP-3
	RGM-C	ADAM 9	SAP	α2-HS-Glycoprotein
85	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	sL-Selectin
	RGM-C	Contactin-4	Cadherin-5	ADAM 9

TABLE 14-continued

86	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	HSP 90α	Cadherin-5
	RBP	RGM-C	BAFF Receptor	sL-Selectin
87	RGM-C	MRC2	SLPI	C9
	MCP-3	HGF	BAFF Receptor	SAP
	ADAM 9	sL-Selectin	C5	NRP1
88	HGF	SCF sR	C9	SLPI
	Cadherin-5	SAP	MCP-3	RGM-C
	sL-Selectin	C2	ERBB1	MIP-5
89	SAP	MRC2	SLPI	RGM-C
	Properdin	Cadherin-5	HGF	Prekallikrein
	ADAM 9	C5	HSP 90α	C2
90	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
	MCP-3	HSP 90α	IL-12 Rβ2	Kallistatin
91	RGM-C	MRC2	SLPI	C9
	HGF	ADAM 9	SAP	BAFF Receptor
	MCP-3	C5	IL-13 Rα1	Contactin-1
92	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	BAFF Receptor	Prekallikrein
	RGM-C	ADAM 9	Cadherin-5	Coagulation Factor Xa
93	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
	BAFF Receptor	C2	MRC2	Kallikrein 6
94	RGM-C	MCP-3	C9	MMP-7
	Contactin-1	HGF	BAFF Receptor	Cadherin-5
	HSP 90α	C2	Prekallikrein	Coagulation Factor Xa
95	SAP	C9	SLPI	MMP-7
	MRC2	MCP-3	RGM-C	α2-Antiplαsmin
	Kallistatin	LY9	C5	ADAM 9
96	HSP 90α	SLPI	C9	RGM-C
	SAP	HGF	Kallistatin	MCP-3
	BAFF Receptor	MIP-5	MRC2	NRP1
97	Cadherin-5	HGF	SLPI	C9
	MCP-3	RGM-C	α2-Antiplαsmin	MRC2
	LY9	Contactin-1	SAP	α2-HS-Glycoprotein
98	Cadherin-5	HGF	SLPI	C9
	NRP1	MRC2	BAFF Receptor	ADAM 9
	SAP	sL-Selectin	MCP-3	Kallistatin
99	RGM-C	MRC2	SLPI	C9
	MCP-3	sL-Selectin	HGF	ADAM 9
	SAP	Cadherin-5	C6	NRP1
100	Cadherin-5	HGF	SLPI	C9
	Properdin	RGM-C	MRC2	MCP-3
	ADAM 9	SAP	MIP-5	C5

	Biomarkers	Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	HGF Prekallikrein Contactin-4	0.962	0.918	1.879	0.943
2	HGF MCP-3 ARSB	0.949	0.913	1.862	0.944
3	HGF HSP 90α ADAM 9	0.962	0.913	1.874	0.945
4	MMP-7 Cadherin-5 C6	0.962	0.908	1.869	0.945
5	MMP-7 Coagulation Factor Xa HSP 90α	0.974	0.897	1.872	0.943
6	SLPI SAP ERBB1	0.962	0.903	1.864	0.945
7	HGF BAFF Receptor C5	0.962	0.903	1.864	0.937
8	HGF C2 HSP 90α	0.962	0.908	1.869	0.944
9	MMP-7 Cadherin-5 Coagulation Factor Xa	0.974	0.897	1.872	0.942

TABLE 14-continued

10	HGF C2 Contactin-1	0.949	0.908	1.856	0.944
11	MMP-7 ADAM 9 PCI	0.949	0.908	1.856	0.941
12	HGF BAFF Receptor MIP-5	0.962	0.913	1.874	0.944
13	HGF BAFF Receptor C6	0.962	0.908	1.869	0.944
14	HGF HSP 90α C5	0.949	0.918	1.867	0.943
15	MMP-7 MRC2 Thrombin/Prothrombin	0.949	0.918	1.867	0.944
16	HGF RGM-C C5	0.962	0.913	1.874	0.943
17	MCP-3 MMP-7 α1-Antitrypsin	0.936	0.908	1.844	0.932
18	HGF α2-HS-Glycoprotein Cadherin-5	0.962	0.913	1.874	0.943
19	HGF RGM-C α2-HS-Glycoprotein	0.962	0.897	1.859	0.939
20	MMP-7 Growth hormone receptor MIP-5	0.949	0.913	1.862	0.943
21	HGF BAFF Receptor MIP-5	0.962	0.897	1.859	0.935
22	MCP-3 RGM-C C6	0.962	0.892	1.854	0.941
23	MRC2 Contactin-1 HSP 90α	0.962	0.903	1.864	0.940
24	MMP-7 MCP-3 PCI	0.949	0.908	1.856	0.945
25	MMP-7 MRC2 Prekallikrein	0.949	0.913	1.862	0.943
26	HGF NRP1 C5	0.962	0.903	1.864	0.944
27	HGF C2 Troponin T	0.962	0.908	1.869	0.945
28	MRC2 Contactin-1 α1-Antitrypsin	0.949	0.892	1.841	0.929
29	HGF RGM-C Properdin	0.962	0.897	1.859	0.941
30	HGF α2-Antiplαsmin ERBB1	0.962	0.892	1.854	0.945
31	HGF BAFF Receptor Hat1	0.949	0.903	1.851	0.936
32	HGF C2 IL-18 Rβ	0.949	0.903	1.851	0.943
33	MMP-7 Cadherin-5 C5	0.949	0.913	1.862	0.942

TABLE 14-continued

34	HGF BAFF Receptor PCI	0.949	0.908	1.856	0.941
35	MMP-7 ADAM 9 RBP	0.962	0.908	1.869	0.942
36	MMP-7 Cadherin-5 TIMP-2	0.962	0.897	1.859	0.939
37	HGF MCP-3 BAFF Receptor	0.962	0.903	1.864	0.943
38	HGF HSP 90α IL-12 Rβ2	0.962	0.908	1.869	0.944
39	HGF Prekallikrein α1-Antitrypsin	0.936	0.903	1.838	0.933
40	MCP-3 MMP-7 ARSB	0.962	0.897	1.859	0.937
41	HGF BAFF Receptor Coagulation Factor Xa	0.962	0.908	1.869	0.942
42	SLPI C2 ERBB1	0.962	0.892	1.854	0.941
43	HGF BAFF Receptor Hat1	0.949	0.903	1.851	0.935
44	MMP-7 MRC2 HSP 90α	0.974	0.897	1.872	0.944
45	MRC2 MCP-3 IL-18 Rβ	0.949	0.903	1.851	0.939
46	MMP-7 MRC2 PCI	0.949	0.908	1.856	0.940
47	MMP-7 ADAM 9 MIP-5	0.962	0.908	1.869	0.940
48	HGF RGM-C TIMP-2	0.949	0.908	1.856	0.939
49	HGF HSP 90α IL-12 Rβ2	0.962	0.903	1.864	0.944
50	HGF Prekallikrein Kallistatin	0.949	0.918	1.867	0.945
51	MRC2 MCP-3 α1-Antitrypsin	0.936	0.903	1.838	0.928
52	HGF SCF sR Kallistatin	0.949	0.908	1.856	0.940
53	MRC2 Contactin-1 Cadherin-5	0.962	0.908	1.869	0.945
54	HGF Prekallikrein ERBB1	0.949	0.903	1.851	0.941
55	HGF BAFF Receptor C5	0.949	0.903	1.851	0.937
56	MCP-3 RGM-C MIP-5	0.974	0.897	1.872	0.943
57	SLPI sL-Selectin α2-HS-Glycoprotein	0.962	0.887	1.849	0.948

TABLE 14-continued

58	MMP-7 Kallistatin MIP-5	0.974	0.887	1.862	0.939
59	MMP-7 Cadherin-5 PCI	0.949	0.908	1.856	0.940
60	MMP-7 Kallistatin Contactin-1	0.949	0.908	1.856	0.940
61	HGF BAFF Receptor Kallistatin	0.962	0.903	1.864	0.945
62	HGF C2 Troponin T	0.962	0.903	1.864	0.942
63	MMP-7 Cadherin-5 α1-Antitrypsin	0.936	0.903	1.838	0.932
64	HGF Prekallikrein ARSB	0.949	0.908	1.856	0.944
65	MMP-7 C2 C5	0.949	0.918	1.867	0.947
66	HGF α2-Antiplαsmin ADAM 9	0.949	0.903	1.851	0.941
67	HGF BAFF Receptor Contactin-4	0.949	0.903	1.851	0.936
68	HGF ADAM 9 IL-18 Rβ	0.962	0.887	1.849	0.941
69	MMP-7 MRC2 HSP 90α	0.962	0.897	1.859	0.945
70	MMP-7 SCF sR PCI	0.936	0.918	1.854	0.942
71	MMP-7 Cadherin-5 LY9	0.949	0.908	1.856	0.939
72	MMP-7 Prekallikrein Cadherin-5	0.962	0.903	1.864	0.944
73	MMP-7 C5 α1-Antitrypsin	0.923	0.913	1.836	0.932
74	MRC2 MCP-3 ARSB	0.949	0.908	1.856	0.938
75	MMP-7 Prekallikrein HSP 90α	0.962	0.903	1.864	0.940
76	SLPI BAFF Receptor ERBB1	0.949	0.903	1.851	0.940
77	MMP-7 Thrombin/Prothrombin Hat1	0.936	0.913	1.849	0.936
78	MCP-3 RGM-C Cadherin-5	0.962	0.908	1.869	0.943
79	HGF C2 RBP	0.949	0.897	1.846	0.941
80	HGF BAFF Receptor MIP-5	0.962	0.897	1.859	0.940
81	MMP-7 C5 PCI	0.949	0.903	1.851	0.942

TABLE 14-continued

82	MMP-7 Kallistatin HSP 90α	0.949	0.908	1.856	0.942
83	MMP-7 BAFF Receptor α1-Antitrypsin	0.923	0.913	1.836	0.930
84	MMP-7 C5 HSP 90α	0.962	0.908	1.869	0.942
85	HGF NRP1 ARSB	0.949	0.908	1.856	0.941
86	HGF ADAM 9 C6	0.962	0.903	1.864	0.940
87	MMP-7 Kallistatin Coagulation Factor Xa	0.962	0.908	1.869	0.942
88	MMP-7 Growth hormone receptor Kallistatin	0.949	0.903	1.851	0.943
89	MMP-7 MCP-3 Hat1	0.923	0.923	1.846	0.936
90	MMP-7 Cadherin-5 RBP	0.962	0.908	1.869	0.941
91	MMP-7 Cadherin-5 HSP 90α	0.962	0.903	1.864	0.941
92	HGF α2-HS-Glycoprotein IL-18 Rβ	0.949	0.897	1.846	0.941
93	MMP-7 Cadherin-5 LY9	0.962	0.897	1.859	0.939
94	SLPI SAP PCI	0.949	0.903	1.851	0.941
95	HGF BAFF Receptor TIMP-2	0.949	0.908	1.856	0.942
96	MMP-7 Cadherin-5 Thrombin/Prothrombin	0.962	0.903	1.864	0.994
97	MMP-7 SCF sR α1-Antitrypsin	0.923	0.913	1.836	0.933
98	MMP-7 RGM-C ARSB	0.949	0.908	1.856	0.941

TABLE 14-continued

99	MMP-7 BAFF Receptor Contactin-4	0.962	0.903	1.864	0.941
100	MMP-7 BAFF Receptor ERBB1	0.949	0.903	1.851	0.941

Marker	Count	Marker	Count
SLPI	100	Contactin-4	18
SAP	100	Properdin	15
RGM-C	100	sL-Selectin	14
MMP-7	100	Growth hormone receptor	13
HGF	100		
MCP-3	98	SCF sR	11
C9	96	RBP	10
Cadherin-5	86	Coagulation Factor Xa	10
MRC2	85		
BAFF Receptor	82	α2-HS-Glycoprotein	9
ADAM 9	57	ERBB1	9
HSP 90α	46	C6	9
C5	38	ARSB	9
Kallistatin	33	α1-Antitrypsin	8
Contactin-1	32	Troponin T	8
Prekallikrein	27	Thrombin/ Prothrombin	8
C2	25		
α2-Antiplαsmin	24	TIMP-2	8
MIP-5	24	PCI	8
NRP1	21	Kallikrein 6	8
LY9	19	IL-18 Rβ	8
		IL-13 Rα1	8
		IL-12 Rβ2	8
		Hat1	8

TABLE 15

Biomarker Designation	Solution K_d(M)	Assay LLOQ (M)	Up or Down Regulated
α1-Antitrypsin	2×10^{-9}	2×10^{-11}	Up
α2-Antiplasmin	8×10^{-9}	6×10^{-13}	Down
α2-HS-Glycoprotein	1×10^{-8}	4×10^{-13}	Down
ADAM 9	4×10^{-9} (pool)	NM	Down
ARSB	3×10^{-9}	NM	Down
BAFF Receptor	5×10^{-9} (pool)	NM	Down
C2	1×10^{-10}	5×10^{-14}	Up
C5	1×10^{-9}	4×10^{-12}	Up
C6	7×10^{-12} (pool)	1×10^{-12}	Up
C9	1×10^{-11}	1×10^{-14}	Up
Cadherin-5	2×10^{-9}	4×10^{-12}	Down
Coagulation Factor Xa	2×10^{-10}	4×10^{-13}	Down
Contactin-1	5×10^{-11}	8×10^{-14}	Down
Contactin-4	3×10^{-10}	8×10^{-13}	Down
ERBB1	1×10^{-10}	1×10^{-14}	Down
Growth hormone receptor	3×10^{-9}	5×10^{-12}	Down
Hat1	1×10^{-9}	NM	Down
HGF	4×10^{-10}	NM	Up
HSP 90α	1×10^{-10}	1×10^{-12}	Up
IL-12 Rβ2	2×10^{-9} (pool)	NM	Down
IL-13 Rα1	3×10^{-9}	NM	Up
IL-18 Rβ	6×10^{-11}	NM	Up
Kallikrein 6	4×10^{-9} (pool)	NM	Up

TABLE 15-continued

Biomarker Designation	Solution K_d(M)	Assay LLOQ (M)	Up or Down Regulated
Kallistatin	2×10^{-11} (pool)	7×10^{-14}	Down
LY9	1×10^{-9}	NM	Down
MCP-3	6×10^{-9}	2×10^{-12}	Down
MIP-5	9×10^{-9} (pool)	2×10^{-10}	Up
MMP-7	7×10^{-11}	3×10^{-13}	Up
MRC2	2×10^{-9}	1×10^{-13}	Down
NRP1	9×10^{-11}	1×10^{-14}	Up
PCI	1×10^{-10}	1×10^{-12}	Down
Prekallikrein	2×10^{-11} (pool)	3×10^{-13}	Down
Properdin	2×10^{-11}	2×10^{-12}	Down
RBP	1×10^{-8} (pool)	9×10^{-11}	Down
RGM-C	3×10^{-11}	NM	Down
SAP	7×10^{-10}	3×10^{-13}	Up
SCF sR	5×10^{-11}	3×10^{-12}	Down
SLPI	2×10^{-11}	9×10^{-13}	Up
sL-Selectin	2×10^{-10} (pool)	2×10^{-13}	Down
Thrombin/Prothrombin	5×10^{-11}	7×10^{-13}	Down
TIMP-2	1×10^{-10}	6×10^{-11}	Down
Troponin T	2×10^{-10}	5×10^{-11}	Down

TABLE 16

Aptamer Designation	μ_c	σ_c^2	μ_d	σ_d^2	KS	p-value	AUC
α1-Antitrypsin	3386	7.20E+05	5948	5.92E+06	0.62	2.03E−19	0.86
α2-Antiplasmin	19115	3.68E+06	16103	5.43E+06	0.54	3.02E−15	0.80
α2-HS-Glycoprotein	1747	6.19E+04	1474	8.61E+04	0.44	3.51E−10	0.75
ADAM 9	1844	2.17E+04	1685	1.71E+04	0.47	2.39E−11	0.78
ARSB	6297	2.92E+05	5808	2.21E+05	0.42	3.47E−09	0.76
BAFF Receptor	3265	6.02E+04	3079	3.34E+04	0.38	7.61E−08	0.71
C2	107229	9.91E+07	117783	1.89E+08	0.43	1.64E−09	0.73
C5	14468	4.15E+06	16477	5.22E+06	0.40	1.89E−08	0.74
C6	92660	1.73E+08	107328	2.82E+08	0.41	9.22E−09	0.76
C9	161177	9.17E+08	208251	9.01E+08	0.61	6.01E−19	0.86
Cadherin-5	9561	2.58E+06	8221	1.89E+06	0.35	1.96E−06	0.74
Coagulation Factor Xa	18670	1.12E+07	15407	9.80E+06	0.43	7.64E−10	0.76
contactin-1	37472	4.81E+07	29895	7.16E+07	0.41	7.23E−09	0.75
Contactin-4	14963	9.29E+06	12268	8.16E+06	0.41	9.22E−09	0.73
ERBB1	52741	6.94E+07	41543	6.56E+07	0.53	1.08E−14	0.81
Growth hormone receptor	1057	1.90E+04	942	7.06E+03	0.39	3.02E−08	0.76
Hat1	1019	1.07E+04	928	6.33E+03	0.42	2.11E−09	0.75
HGF	668	4.07E+03	735	4.67E+03	0.41	5.67E−09	0.75
HSP 90α	40733	3.01E+08	55087	3.31E+08	0.38	7.61E−08	0.71
IL-12 Rβ2	1217	1.42E+04	1099	1.56E+04	0.41	9.22E−09	0.75
IL-13 Rα1	614	6.40E+03	697	8.92E+03	0.42	3.47E−09	0.74
IL-18 Rβ	449	1.30E+03	488	1.48E+03	0.44	3.51E−10	0.76
Kallikrein 6	256	1.67E+03	298	2.15E+03	0.42	2.11E−09	0.75
Kallistatin	111611	3.01E+08	85665	5.64E+08	0.48	5.89E−12	0.82
LY9	983	2.19E+04	845	1.46E+04	0.43	9.86E−10	0.75
MCP-3	703	4.88E+03	642	2.71E+03	0.43	9.86E−10	0.75
MIP-5	1531	4.55E+05	2123	7.95E+05	0.33	5.35E−06	0.72
MMP-7	3057	2.61E+06	5936	1.74E+07	0.44	2.70E−10	0.74
MRC2	16105	1.78E+07	12716	1.09E+07	0.39	3.82E−08	0.72
NRP1	5314	1.41E+06	6450	9.96E+05	0.43	9.86E−10	0.74
PCI	31852	4.29E+07	22140	8.05E+07	0.53	1.48E−14	0.80
Prekallikrein	122660	3.23E+08	100877	2.99E+08	0.52	7.01E−14	0.80
Properdin	65527	1.10E+08	55599	1.25E+08	0.41	1.17E−08	0.74
RBP	5193	1.21E+06	4088	1.36E+06	0.45	1.22E−10	0.73
RGM-C	21625	2.11E+07	17527	9.18E+06	0.43	1.64E−09	0.78
SAP	142805	7.07E+08	167146	7.28E+08	0.38	7.61E−08	0.75
SCF sR	12432	1.09E+07	9472	5.69E+06	0.44	2.70E−10	0.76
SLPI	25007	2.07E+07	35986	1.22E+08	0.59	1.02E−17	0.85
sL-Selectin	30048	3.31E+07	24163	2.50E+07	0.43	9.86E−10	0.79
Thrombin/Prothrombin	62302	1.67E+07	58099	1.80E+07	0.45	1.59E−10	0.75
TIMP-2	15793	3.16E+06	13796	2.64E+06	0.49	1.04E−12	0.79
Troponin T	1972	3.68E+04	1767	2.58E+04	0.47	1.81E−11	0.78

TABLE 17

Sensitivity & Specificity for Exemplary Combinations of BAFF Receptors

#									Sensitivity	Specificity	Sensitivity + Specificity	AUC
1	BAFF Receptor								0.744	0.564	1.308	0.7
2	BAFF Receptor	RGM-C							0.821	0.733	1.554	0.81
3	BAFF Receptor	RGM-C	HGF						0.833	0.744	1.577	0.84
4	BAFF Receptor	RGM-C	HGF	SLPI					0.846	0.8	1.646	0.89
5	BAFF Receptor	RGM-C	HGF	SLPI	C9				0.885	0.81	1.695	0.92
6	BAFF Receptor	RGM-C	HGF	SLPI	C9	α2-Antiplasmin			0.91	0.846	1.756	0.92
7	BAFF Receptor	RGM-C	HGF	SLPI	C9	α2-Antiplasmin	SAP		0.923	0.846	1.769	0.93
8	BAFF Receptor	RGM-C	HGF	SLPI	C9	α2-Antiplasmin	SAP	MMP-7	0.974	0.856	1.83	0.94

TABLE 17-continued

Sensitivity & Specificity for Exemplary Combinations of BAFF Receptors

#											Sensitivity	Specificity	Sensitivity + Specificity	AUC
9	BAFF Receptor	RGM-C	HGF	SLPI	C9	α2-Antiplasmin	SAP	MMP-7	MCP-3		0.962	0.882	1.884	0.94
10	BAFF Receptor	RGM-C	HGF	SLPI	C9	α2-Antiplasmin	SAP	MMP-7	MCP-3	HSP 90α	0.974	0.882	1.856	0.94

TABLE 18

Parameters derived from training set for naïve Bayes classifier.

Biomarker	μ_c	σ_c^2	μ_d	σ_d^2
HGF	668	4.07E+03	735	4.67E+03
SLPI	25007	2.07E+07	35986	1.22E+08
C9	161177	9.17E+08	208251	9.01E+08
α2-Antiplasmin	19115	3.68E+06	16103	5.43E+06
SAP	142805	7.07E+08	167146	7.28E+08
MMP-7	3057	2.61E+06	5936	1.74E+07
BAFF Receptor	3265	6.02E+04	3079	3.34E+04
RGM-C	21625	2.11E+07	17527	9.18E+06
MCP-3	703	4.88E+03	642	2.71E+03
MRC2	16105	1.78E+07	12716	1.09E+07

TABLE 19

Number of Samples by Site

	Benign	Cancer
Site 1	114	87
Site 2	81	55
TOTAL	195	142

TABLE 20

Biomarkers of Ovarian Cancer from All Site Analysis (Aggregated Data)

α2-Antiplasmin
α2-HS-Glycoprotein
ADAM 9
C2
C5
C6
C9
Coagulation Factor Xa
Contactin-1
Contactin-4
ERBB1
HGF
IL-12 Rβ2
Kallistatin
Ly9
MCP-3
MMP-7
NRP1
Properdin
RGM-C
SCF sR
SLPI
sL-Selectin
Thrombin/Prothrombin
Troponin T

TABLE 21

Biomarkers of Ovarian Cancer Within Sites

α1-Antitrypsin
α2-Antiplasmin
BAFF Receptor
C2
C6
C9
Cadherin-5
Contactin-1
Contactin-4
Growth hormone receptor
HGF
HSP 90α
IL-13 Rα1
MCP-3
LY9
MIP-5
MRC2
NRP1
Prekallikrein
RGM-C
SAP
SCF sR
SLPI
sL-Selectin

TABLE 22

Biomarkers of Ovarian Cancer from Blended Data Analysis

α2-Antiplasmin
ARSB
C2
C6
C9
Contactin-1
Contactin-4
ERBB1
Hat1
HGF
IL-12 Rβ2
IL-13 Rα1
IL-18 Rβ
Kallikrein 6
Kallistatin
LY9
MCP-3
NRP1
PCI
Prekallikrein
RBP
RGM-C
SCF sR
SLPI
sL-Selectin
Thrombin/Prothrombin
TIMP-2

TABLE 23

Calculation details for naïve Bayes classifier.

Biomarker	RFU	$-\frac{1}{2}\left(\frac{x_i-\mu_{c,i}}{\sigma_{c,i}}\right)^2$	$-\frac{1}{2}\left(\frac{x_i-\mu_{d,i}}{\sigma_{d,i}}\right)^2$	$\ln\frac{\sigma_{d,i}}{\sigma_{c,i}}$	Ln(likelihood)	likelihood
HGF	701	−0.134	−0.125	0.069	0.060	1.062
SLPI	34158	−2.018	−0.014	0.886	−1.118	0.327
C9	182792	−0.255	−0.360	−0.009	0.096	1.101
α2−Antiplasmin	19531	−0.023	−1.081	0.195	1.253	3.500
SAP	170310	−0.535	−0.007	0.015	−0.513	0.599
MMP−7	896	−0.894	−0.730	0.948	0.784	2.190
BAFF Receptor	3207	−0.028	−0.242	−0.294	−0.079	0.924
RGM−C	22545	−0.020	−1.371	−0.415	0.936	2.550
MCP−3	733	−0.095	−1.537	−0.294	1.148	3.152
MRC2	12535	−0.357	−0.001	−0.246	−0.601	0.548

What is claimed is:

1. A method for diagnosing that an individual does or does not have ovarian cancer, the method comprising:

detecting, in a biological sample from an individual, biomarker values that each correspond to one of at least N biomarkers selected from Table 1, wherein said individual is classified as having or not having ovarian cancer based on said biomarker values, and wherein N=2-42.

2. The method of claim **1**, wherein detecting the biomarker values comprises performing an in vitro assay.

3. The method of claim **2**, wherein said in vitro assay comprises at least one capture reagent corresponding to each of said biomarkers, and further comprising selecting said at least one capture reagent from the group consisting of aptamers, antibodies, and a nucleic acid probe.

4. The method of claim **3**, wherein said at least one capture reagent is an aptamer.

5. The method of claim **2**, wherein the in vitro assay is selected from the group consisting of an immunoassay, an aptamer-based assay, a histological or cytological assay, and an mRNA expression level assay.

6. The method of claim **1**, wherein each biomarker value is evaluated based on a predetermined value or a predetermined range of values.

7. The method claim **1**, wherein the biological sample is ovarian tissue and wherein the biomarker values derive from a histological or cytological analysis of said ovarian tissue.

8. The method of claim **1**, wherein the biological sample is selected from the group consisting of whole blood, plasma, and serum.

9. The method of claim **1**, wherein the biological sample is plasma.

10. The method of claim **1**, wherein the individual is a human.

11. The method of claim **1**, wherein N=2-15.

12. The method of claim **1**, wherein N=2-10.

13. The method of claim **1**, wherein N=3-10.

14. The method of claim **1**, wherein N=4-10.

15. The method of claim **1**, wherein N=5-10.

16. The method of claim **1**, wherein the individual has a pelvic mass.

17. A computer-implemented method for indicating a likelihood of ovarian cancer, the method comprising:

retrieving on a computer biomarker information for an individual, wherein the biomarker information comprises biomarker values that each correspond to one of at least N biomarkers selected from Table 1;

performing with the computer a classification of each of said biomarker values; and

indicating a likelihood that said individual has ovarian cancer based upon a plurality of classifications, and wherein N=2-42.

18. A computer program product for indicating a likelihood of ovarian cancer, the computer program product comprising:

a computer readable medium embodying program code executable by a processor of a computing device or system, the program code comprising:

code that retrieves data attributed to a biological sample from an individual, wherein the data comprises biomarker values that each correspond to one of at least N biomarkers selected from Table 1, wherein said biomarkers were detected in the biological sample; and

code that executes a classification method that indicates an ovarian cancer status of the individual as a function of said biomarker values; and wherein N=2-42.

19. The computer program product of claim **18**, wherein said classification method uses a probability density function.

20. The computer program product of claim **19**, wherein said classification method uses two or more classes.

21. The method of claim **17**, wherein indicating the likelihood that the individual has ovarian cancer comprises displaying the likelihood on a computer display.

22. A method for diagnosing that an individual does or does not have ovarian cancer, the method comprising:

detecting, in a biological sample from an individual, biomarker values that each correspond to a panel of biomarkers selected from Table 1, wherein said individual is classified as having or not having ovarian cancer, and wherein the panel of biomarkers has a sensitivity+specificity value of 1.64 or greater.

23. The method of claim **22**, wherein the panel has a sensitivity+specificity value of 1.69 or greater.

24. The method of claim **22**, wherein the individual has a pelvic mass.

* * * * *

专利名称(译)	卵巢癌生物标志物及其用途		
公开(公告)号	US20100086948A1	公开(公告)日	2010-04-08
申请号	US12/574341	申请日	2009-10-06
[标]申请(专利权)人(译)	私募蛋白质体公司		
申请(专利权)人(译)	SOMALOGIC INC.		
当前申请(专利权)人(译)	SOMALOGIC INC.		
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摘要(译)

本申请包括用于检测和诊断卵巢癌的生物标记物，方法，装置，试剂，系统和试剂盒。在一个方面，本申请提供了可以单独使用或以各种组合使用以诊断卵巢癌或允许将盆腔肿块鉴别诊断为良性或恶性的生物标记物。另一方面，提供了用于诊断个体卵巢癌的方法，其中所述方法包括在来自个体的生物样品中检测至少一种生物标志物值，所述生物标志物值对应于选自表1中提供的生物标志物组的至少一种生物标志物。其中，基于至少一种生物标记值，确定个体被分类为患有卵巢癌，或者确定患有卵巢癌的个体的可能性。