



Office de la Propriété  
Intellectuelle  
du Canada

Un organisme  
d'Industrie Canada

Canadian  
Intellectual Property  
Office

An agency of  
Industry Canada

CA 2789411 A1 2011/08/18

(21) **2 789 411**

(12) **DEMANDE DE BREVET CANADIEN  
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2011/02/13  
(87) Date publication PCT/PCT Publication Date: 2011/08/18  
(85) Entrée phase nationale/National Entry: 2012/08/09  
(86) N° demande PCT/PCT Application No.: IL 2011/000153  
(87) N° publication PCT/PCT Publication No.: 2011/099012  
(30) Priorité/Priority: 2010/02/12 (US61/303,691)

(51) Cl.Int./Int.Cl. *G01N 33/564* (2006.01)  
(71) Demandeur/Applicant:  
YEDA RESEARCH AND DEVELOPMENT, CO., LTD., IL  
(72) Inventeurs/Inventors:  
COHEN, IRUN R., IL;  
DOMANY, EYTAN, IL;  
SHENTAL, NOAM, IL;  
FATTAL, ITTAI, IL  
(74) Agent: SHAPIRO COHEN

(54) Titre : DIAGNOSTIC DU LUPUS ERYTHEMATEUX SYSTEMIQUE (LES)  
(54) Title: DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

(57) **Abrégé/Abstract:**

The present invention relates to methods and kits for diagnosing systemic lupus erythematosus (SLE) in a subject. Particularly, the present invention relates to a specific antibody profile useful in diagnosing SLE in a subject.



## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau

(43) International Publication Date  
18 August 2011 (18.08.2011)



(10) International Publication Number  
**WO 2011/099012 A1**

(51) International Patent Classification:  
*G01N 33/564* (2006.01)

(21) International Application Number:  
PCT/IL2011/000153

(22) International Filing Date:  
13 February 2011 (13.02.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
61/303,691 12 February 2010 (12.02.2010) US

(71) Applicant (for all designated States except US): **YEDA RESEARCH AND DEVELOPMENT CO. LTD.** [IL/IL]; at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **COHEN, Irun R.** [IL/IL]; 11 Hankin Street, 76354 Rehovot (IL). **DOMANY, Eytan** [IL/IL]; c/o Yeda Research and Development Co. Ltd., at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). **SHENTAL, Noam** [IL/IL]; c/o Yeda Research and Development Co. Ltd., at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). **FATTAL, Ittai** [IL/IL]; c/o Yeda Research and Development Co. Ltd., at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(74) Agents: **WEBB, Cynthia** et al.; P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

(57) Abstract: The present invention relates to methods and kits for diagnosing systemic lupus erythematosus (SLE) in a subject. Particularly, the present invention relates to a specific antibody profile useful in diagnosing SLE in a subject.



**WO 2011/099012 A1**

## DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

### FIELD OF THE INVENTION

The present invention relates to methods and kits for diagnosing systemic lupus erythematosus (SLE) in a subject. Particularly, the present invention relates to a specific antibody profile useful in diagnosing SLE in a subject.

### BACKGROUND OF THE INVENTION

Systemic lupus erythematosus (SLE) is an inflammatory disorder of autoimmune etiology, occurring predominantly in young women. SLE can affect many of the body's organ systems, including kidneys, skin, joints, nervous system, serous membranes, blood cells and vessels. Although the specific cause of SLE is unknown, multiple factors are associated with the development of the disease, including genetic, racial, hormonal, and environmental factors.

SLE course is usually chronic, relapsing, and unpredictable. Untreated SLE can be fatal as it progresses from attack of skin and joints to internal organs, including lung, heart, and kidneys, thus making early and accurate diagnosis of and/or assessment of risk of developing SLE particularly critical. SLE mainly appears as a series of flare-ups, with intervening periods of little or no disease manifestation. Kidney damage, measured by the amount of protein in the urine, is one of the most acute areas of damage associated with pathogenicity in SLE, and accounts for at least 50% of the mortality and morbidity of the disease.

SLE is characterized by the production of unusual autoantibodies in the blood. Over 100 different self-molecules have been found to bind autoantibodies in different patients (Sherer et al., 2004, Semin. Arthritis. Rheum. 34:501-37), forming immune complexes which circulate the blood and eventually deposit in tissues. These immune complex depositions cause chronic inflammation and eventually tissue damage. The autoantibodies also have direct pathogenic effects contributing to hemolytic anemia and thrombocytopenia.

Typically, a diagnosis of SLE can be made on the basis of eleven criteria defined by the American College of Rheumatology (ACR). These criteria include malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder (e.g., leucopenia, lymphopenia, hemolytic anemia or

thrombocytopenia), immunologic disorder and anti-nuclear antibodies (ANA) (Tan et al., 1997, *Arthritis Rheum* 1997, 40:1725). A subject can be clinically diagnosed with SLE if he meets at least four of the eleven criteria. Nevertheless, SLE is still possible even in case when less than four criteria are present.

5           While anti-nuclear antibodies and autoantibodies to dsDNA, phospholipids and Sm proteins are among the eleven criteria used for diagnosing SLE (Tan et al., 1997, *Arthritis Rheum* 1997, 40:1725), many patients diagnosed with SLE lack these autoantibodies, especially when they are in clinical remission.

10           One of the most difficult challenges in clinical management of complex autoimmune diseases such as SLE is the accurate and early identification of the disease in a patient. In addition, no reliable diagnostic markers have been identified that enable clinicians or others to accurately define pathophysiological aspects of SLE, clinical activity, response to therapy, or prognosis.

#### The antigen chip

15           Antigen microarrays are recently developed tools for the high-throughput characterization of the immune response (Robinson et al., 2002, *Nat Med* 8, 295-301), and have been used to analyze immune responses in vaccination and in autoimmune disorders (Robinson et al., 2002; Robinson et al., 2003, *Nat Biotechnol.* 21, 1033-9; Quintana et al., 2004; Kanter et al., 2006, *Nat Med* 12, 138-43). It has been hypothesized, that patterns of  
20           multiple reactivities may be more revealing than single antigen-antibody relationships (Quintana et al., 2006, *Lupus* 15, 428-30) as shown in previous analyses of autoimmune repertoires of mice (Quintana et al., 2004; Quintana et al., 2001, *J Autoimmun* 17, 191-7) and humans (Merbl et al., 2007, *J Clin Invest* 117, 712-8; Quintana et al., 2003, *J Autoimmun* 21, 65-75) in health and disease. Thus, autoantibody repertoires have the  
25           potential to provide both new insights into the pathogenesis of the disease and to serve as immune biomarkers (Cohen, 2007, *Nat Rev Immunol.* 7, 569-74) of the disease process.

          PCT Pub. No. WO 02/08755 to some of the inventors of the present invention is directed to a method, system and an article of manufacture for clustering and thereby identifying predefined antigens reactive with undetermined immunoglobulins of sera  
30           derived from patient subjects in need of diagnosis of disease or monitoring of treatment. The '755 publication discloses the use of antigen arrays for identifying antigens reactive with immunoglobulins of sera derived from subjects afflicted with various diseases. Further disclosed are diagnostic methods, and systems useful in these methods, employing

the step of clustering a subset of antigens of a plurality of antigens, said subset of antigens being reactive with a plurality of antibodies being derived from a plurality of patients having an impaired immune system and suffering from a disease, and associating or deassociating the antibodies of a subject with the resulting cluster.

5 U.S. Pat. App. Pub. No. 2005/0260770 to some of the inventors of the present invention discloses an antigen array system and diagnostic uses thereof. The application provides a method of diagnosing an immune disease, and particularly type 1 diabetes, or a predisposition thereto in a subject, comprising determining a capacity of immunoglobulins of the subject to specifically bind each antigen probe of an antigen probe set.

10 PCT Pub. No. WO 10/128506 to some of the inventors of the present invention relates to methods of identifying the development of a cardiovascular disease in an individual, specifically, for recognizing the development of an acute myocardial infarction (AMI) process in an individual.

None of the prior art discloses an antigen array that can provide a specific, reliable,  
15 accurate and discriminatory assay for diagnosing SLE. Such discriminatory assays would be highly valuable in identification of the presence of SLE in patients or the determination of susceptibility to develop the disease and in tailoring adequate therapeutic approach for each patient.

There remains a need for improved diagnostic methods and kits useful in diagnosing  
20 SLE in a subject.

### **SUMMARY OF THE INVENTION**

The present invention provides methods and kits for diagnosing systemic lupus erythematosus (SLE) in a subject, antigen probe arrays for practicing such a diagnosis, and  
25 antigen probe sets for generating such arrays.

The present invention is based in part on the unexpected results obtained when testing the antibody reactivity of SLE patients using an antigen array. The analysis resulted in the identification of unique autoantibody reactivity patterns. Unexpectedly, the unique autoantibody patterns persist independently of disease activity and are present also in  
30 subjects with long-term clinical remission (e.g., subjects in renal remission). Advantageously, the reactivity pattern showed strikingly high sensitivity and high specificity for SLE. Furthermore, a healthy control subject who had the SLE antibody pattern was later found to develop clinical SLE.

Thus, the present invention provides unique antigen-autoantibody reactivity patterns relevant to SLE. While several single antigens (e.g., hyaluronic acid) were identified as being sufficient on their own to adequately diagnose SLE, specific combinations of these antigens, as detailed in Table 1 herein below, were significantly more accurate and reliable in discriminating SLE patients and control subjects than each antigen alone.

**Table 1 - Antigens discriminating SLE and healthy controls**

| <b>Antigen</b>                                         | <b>SEQ ID NO:</b> | <b>CAS number</b> |
|--------------------------------------------------------|-------------------|-------------------|
| Hyaluronic acid                                        |                   | 9067-32-7         |
| CD99                                                   | SEQ ID NO: 1      |                   |
| double strand DNA (dsDNA)                              |                   | 73049-39-5        |
| single strand DNA (ssDNA)                              |                   | 91080-16-9        |
| Epstein-Barr virus (EBV)                               | SEQ ID NO: 2      |                   |
| Myeloperoxidase (MPO)                                  | SEQ ID NO: 3      |                   |
| Insulin-like growth factor binding protein-1 (IGFBP-1) | SEQ ID NO: 4      |                   |
| Cardiolipin                                            |                   | 383907-10-6       |
| Collagen Type III (Collagen III)                       |                   | 9007-34-5         |
| Collagen Type IV (Collagen IV)                         |                   | 9007-34-5         |
| actin                                                  |                   | 51005-14-2        |
| Bone morphogenetic protein-4 (BMP4)                    | SEQ ID NO: 5      |                   |
| CMV (CMV Pp150)                                        | SEQ ID NO: 6      |                   |
| F50                                                    | SEQ ID NO: 7      |                   |
| Hepatocyte Growth Factor (HGF)                         | SEQ ID NO: 8      |                   |
| Horseradish peroxide (HRP)                             |                   | 9003-99-0         |
| HSP60p18                                               | SEQ ID NO: 9      |                   |
| Rubella virus (RV)                                     | SEQ ID NO: 10     |                   |
| S100 calcium-binding protein A4 (S100A4)               | SEQ ID NO: 11     |                   |
| CITED1                                                 | SEQ ID NO: 12     |                   |
| Fragile Histidine Triad (FHIT)                         | SEQ ID NO: 13     |                   |

According to a first aspect, the present invention provides a method of diagnosing systemic lupus erythematosus (SLE) in a subject, the method comprising:

- 5 (i) determining the reactivity of IgG and IgM antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT, thereby determining the reactivity pattern of the sample to the plurality of antigens, and
- 10 (ii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

In one embodiment, the plurality of antigens comprises at least three antigens. In 15 another embodiment, the plurality of antigens comprises at least four antigens. In another embodiment, the plurality of antigens comprises at least five antigens. In another embodiment, the plurality of antigens comprises at least six antigens. In another embodiment, the plurality of antigens comprises at least seven antigens. In another embodiment, the plurality of antigens comprises at least eight antigens. According to an 20 exemplary embodiment the plurality of antigens consist of IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin and collagen III. Each possibility represents a separate embodiment of the invention.

As used herein, the “reactivity of antibodies in a sample” to “a plurality of antigens” 25 refers to the immune reactivity of each antibody in the sample to a specific antigen selected from the plurality of antigens. The immune reactivity of the antibody to the antigen, i.e. its ability to specifically bind the antigen, may be used to determine the amount of the antibody in the sample. Specifically, determining the “reactivity of IgG and IgM antibodies in a sample” refers to the reactivity of at least one IgG antibody in the sample to a specific 30 antigen selected from the plurality of antigens, and at least one IgM antibody to another specific antigen selected from the plurality of antigens.

The reactivity pattern of the sample thus reflects the levels of each one of the tested antibodies in the sample, thereby providing a quantitative assay. In a particular

embodiment, the reactivity is quantitatively determined. Thus, for instance, the reactivity of an antibody to an antigen may be increased or decreased. In certain embodiments, the reactivity of at least one antibody to a specific antigen (from the plurality of antigens) is up-regulated. Preferably, the reactivity of the IgG antibodies is up-regulated (i.e., increased). In  
5 additional embodiments, the reactivity of at least one antibody to a specific antigen is down-regulated. Preferably, the reactivity of the IgM antibodies is down-regulated (i.e., decreased). In yet another particular embodiment, a significant quantitative difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

10 Typically, determining the reactivity of antibodies in the sample to the plurality of antigens is performed using an immunoassay. Advantageously, the plurality of antigens may be used in the form of an antigen array.

In another embodiment the method comprises determining the reactivity of at least one IgG antibody and at least one IgM antibody in said sample to said plurality of antigens.

15 In another embodiment, the method comprises determining the reactivity of a plurality of IgG antibodies and a plurality of IgM antibodies in said sample to said plurality of antigens.

According to an additional embodiment, the method comprises determining the reactivity of IgG antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, EBV, ssDNA, dsDNA,  
20 BMP4, F50, HGF and HSP60p18. According to an exemplary embodiment, the method comprises determining the reactivity of IgG antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, EBV, ssDNA and dsDNA. In a specific embodiment, the reactivity of an IgG antibody to an antigen (selected from the plurality of antigens) is up-regulated. According to these  
25 embodiments, a significant up-regulation between the reactivity pattern of said sample obtained from the subject compared to the reactivity pattern of a control sample is an indication that the subject is afflicted with SLE.

According to some embodiments, the method comprises determining the reactivity of IgM antibodies in the sample obtained from the subject to a plurality of antigens selected  
30 from the group consisting of: CD99, IGFBP1, MPO, cardiolipin, Collagen III, collagen IV, actin, CMV, HRP, RV, S100A4, CITED1 and FHIT. According to an exemplary embodiment, the method comprises determining the reactivity of IgM antibodies in the serum sample obtained from the subject to a plurality of antigens selected from the group

consisting of: CD99, MPO, IGFBP1, cardiolipin and Collagen III. According to another embodiment, the method comprises determining the reactivity of IgM antibodies in the serum sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, IGFBP1, MPO and cardiolipin. According to yet another embodiment, the method comprises determining the reactivity of IgM antibodies in the serum sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, MPO and Collagen III. In a specific embodiment, the reactivity of an IgM antibody to an antigen (from the plurality of antigens) is down-regulated. In a particular embodiment, a significant down regulation between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

According to another embodiment, the method comprises determining the reactivity of IgM antibodies in the serum sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, MPO and Collagen III. In a particular embodiment, a significant down regulation between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE in renal remission.

According to another embodiment, the sample obtained from the subject is a biological fluid. According to some embodiments, the sample is selected from the group consisting of plasma, serum, blood, cerebrospinal fluid, synovial fluid, sputum, urine, saliva, tears, lymph specimen, or any other biological fluid known in the art. According to certain embodiments, the sample obtained from the subject is selected from the group consisting of plasma, serum and blood. According to one embodiment, the sample is a serum sample. Each possibility represents a separate embodiment of the invention.

According to certain embodiments of the methods of the present invention, the control is selected from the group consisting of a sample from at least one healthy individual, a panel of control samples from a set of healthy individuals, and a stored set of data from healthy individuals. Typically, a healthy individual is a subject not afflicted with SLE or any other form of lupus. In another embodiment, a healthy individual is a subject not afflicted with an autoimmune disease.

According to some embodiments, the method further comprises diluting the sample e.g. 1:10 or more before determining the reactivity of antibodies in the sample.

A “significant difference” between reactivity patterns refers, in different

embodiments, to a statistically significant difference, or in other embodiments to a significant difference as recognized by a skilled artisan.

Advantageously, the methods of the invention may employ the use of learning and pattern recognition analyzers, clustering algorithms and the like, in order to discriminate  
5 between reactivity patterns of healthy control subjects to those of patients having SLE. As such, this term specifically includes a difference measured by, for example, determining the reactivity of antibodies in a test sample to a plurality of antigens, and comparing the resulting reactivity pattern to the reactivity patterns of negative and positive control samples (e.g. samples obtained from control subjects which are not afflicted with SLE or  
10 patients afflicted with SLE, respectively) using such algorithms and/or analyzers. The difference may also be measured by comparing the reactivity pattern of the test sample to a predetermined classification rule obtained in such manner.

According to certain embodiments, the present invention provides a kit for the diagnosis of SLE, comprising a plurality of antigens selected from the group consisting of  
15 IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT.

In a particular embodiment, the plurality of antigens consists of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin and Collagen III. In yet another  
20 particular embodiment, the plurality of antigens consists of: hyaluronic acid, CD99, MPO, IGFBP1 and Collagen III.

According to the principles of the invention the kits comprise a plurality of antigens also referred to herein as antigen probe sets. These antigen probe sets comprising a plurality of antigens are reactive specifically with the sera of subjects having SLE. According to the  
25 principles of the invention, the plurality of antigens may advantageously be used in the form of an antigen array. According to some embodiments the antigen array is conveniently arranged in the form of an antigen chip.

In other embodiments, the kit may further comprise means for determining the reactivity of antibodies in a sample to the plurality of antigens. For example, the kit may  
30 contain reagents, detectable labels and/or containers which may be used for measuring specific binding of antibodies to the antigen probes of the invention. In a particular embodiment, said kit is in the form of an antigen array. In other embodiments, said kit may further comprise negative and/or positive control samples. For example, a negative control

sample may contain a sample from at least one healthy individual (e.g., an individual not-afflicted with SLE). A positive control may contain a sample from at least one individual afflicted with SLE, or a subtype of SLE (e.g., an SLE subject in renal remission or an SLE subject with active lupus nephritis), which is being diagnosed. Other non-limiting  
5 examples are a panel of control samples from a set of healthy individuals or diseased individuals, or a stored set of data from control individuals.

In other embodiments, the kit may further comprise means for comparing reactivity patterns of antibodies in different samples to the plurality of antigens.

According to another aspect, the present invention provides an antigen probe set  
10 comprising the antigen probes selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT. In a particular embodiment, the antigen probe set comprises the antigen probes selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO,  
15 cardiolipin and Collagen III.

According to another aspect, the present invention provides an article of manufacture comprising the antigen probe set of the present invention.

According to another aspect, the present invention provides use of the antigen probe set for the preparation of a diagnostic composition for diagnosing SLE in a subject in need  
20 thereof. In one embodiment, the diagnostic composition is useful for determining the reactivity of antibodies in a sample, thereby determining the reactivity pattern of the sample to said plurality of antigens, wherein a significant difference between the reactivity pattern of said sample compared to a reactivity pattern of a control sample is an indication for SLE.

According to another aspect, the present invention provides a method of diagnosing  
25 SLE in a subject, the method comprising:

- (i) determining the reactivity of antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, MPO, collagen III, thereby determining the reactivity pattern of the sample to the plurality of antigens, and  
30
- (ii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

According to one embodiment, the plurality of antigens further comprises at least one, at least two or at least three antigens selected from dsDNA, ssDNA, EBV and cardiolipin. Each possibility represents a separate embodiment of the invention.

According to another embodiment, determining the reactivity of antibodies in the sample comprises determining the reactivity of IgG and IgM antibodies in said sample. In a particular embodiment, step (i) comprises determining the reactivity of IgG antibodies in a sample to hyaluronic acid antigen and determining the reactivity of IgM antibodies in the sample to a plurality of antigens selected from the group consisting of: CD99, MPO, IGFBP1, collagen III, thereby determining the reactivity pattern of the sample to the plurality of antigens.

Other objects, features and advantages of the present invention will become clear from the following description and drawings.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1** shows the antibody reactivity of individual subjects to the antigen reactivities that characterize patients with SLE. Sera from healthy controls (black squares) and from SLE subjects in renal remission (open circles), acute lupus nephritis (open triangles), or without renal involvement (asterisk) were tested for antibody reactivities to the designated antigen. The relative amount of antibody reactivity is shown on the Y axis. The X axis orders the subjects according to their relative reactivity.

**Figure 2** depicts a three-dimensional principal component analysis (PCA). The PCA was based on the seven antigen reactivities that distinguish healthy controls from patients with SLE in renal remission (Table 6). The star represents the signature of a subject who was healthy at the time of serum collection but who later developed SLE, healthy controls are represented as black squares, SLE subjects in renal remission are depicted as open circles, subjects with acute lupus nephritis are depicted as triangles and SLE subjects without renal involvement are represented as asterisk.

## **DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides methods of diagnosing systemic lupus erythematosus (SLE) in a subject using antigen probe arrays for practicing such a diagnosis, and identifies specific antigen probe sets for generating such arrays. According

to some embodiments, the present invention relates to an autoantibody-based biomarker test for early diagnosis of SLE, including SLE in renal remission.

Without wishing to be bound by any particular theory or mechanism of action, the invention is based in part on the finding that the antibody reactivity profile in serum of SLE patients was clearly distinct of healthy control individuals. Although serum autoantibodies have been extensively investigated in SLE, the unique antibody immune signatures as described herein have not been described before. Advantageously, the unique antibody signatures of the present invention provide highly sensitive and specific assays for diagnosing SLE.

According to some embodiments, the present invention provides a method of diagnosing systemic lupus erythematosus (SLE) in a subject, the method comprising:

- (i) determining the reactivity of antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT, thereby determining the reactivity pattern of the sample to the plurality of antigens, and
- (ii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

In a preferred embodiment, the method comprises determining the reactivity of IgG and IgM antibodies in the sample to said plurality of antigens. In another embodiment the method comprises determining the reactivity of at least one IgG antibody and at least one IgM antibody in said sample to said plurality of antigens.

The reactivity pattern of the sample reflects the levels of each one of the tested antibodies in the sample, thereby providing a quantitative assay. In a preferred embodiment, the antibodies are quantitatively determined. In yet another preferred embodiment, a significant quantitative difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE. In specific embodiments, up-regulation of the reactivity of an antibody, specifically an IgG antibody, in a sample to an antigen refers to an increase (i.e., elevation) of about at least two, about at least three, about at least four, or about at least five times

higher (i.e., greater) than the reactivity levels of the antibody to the antigen in the control. In another embodiment, down-regulation of the reactivity of an antibody, specifically an IgM antibody, in a sample to an antigen refers to a decrease (i.e., reduction) of about at least two, about at least three, about at least four, or about at least five times lower than the reactivity  
5 levels of the antibody to the antigen in the control.

As exemplified herein below, antigen analysis of autoantibodies (e.g., using microarray analysis) can identify serum autoantibody patterns associated with different clinical forms of SLE; the signatures were based on collective autoantibody patterns, not single autoantibody reactivities. These informative patterns included IgG autoantibodies as  
10 well as IgM autoantibodies. Moreover, the informative patterns included decreases as well as increases of autoantibody reactivities relative to those found in healthy controls.

In a particular embodiment, the method comprises:

(i) determining the reactivity of IgG antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid,  
15 EBV, ssDNA and dsDNA;

(ii) determining the reactivity of IgM antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, MPO, IGFBP1, cardiolipin and Collagen III; thereby determining the reactivity pattern of the sample to the plurality of antigens; and

20 (iii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant increase between the reactivity pattern of the IgG antibodies in said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE, or wherein a significant decrease between the reactivity pattern of the IgM antibodies in said sample obtained from the subject  
25 compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

In yet another embodiment, a significant increase between the reactivity pattern of the IgG antibodies in said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE, and a significant  
30 decrease between the reactivity pattern of the IgM antibodies in said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

### **Antigen probes and antigen probe sets**

According to further embodiments, the invention provides antigen probes and antigen probe sets useful for diagnosing SLE, as detailed herein.

According to the principles of the invention, the invention further provides a plurality of antigens also referred to herein as antigen probe sets. These antigen probe sets comprising a plurality of antigens are reactive specifically with the sera of subjects having SLE. According to the principles of the invention, the plurality of antigens may advantageously be used in the form of an antigen array. According to some embodiments the antigen array is conveniently arranged in the form of an antigen chip.

10 A "probe" as used herein means any compound capable of specific binding to a component. According to one aspect, the present invention provides an antigen probe set comprising a plurality of antigens selected from the group consisting of: CD99, IGFBP1, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT. According to 15 certain embodiments, the antigen probe set comprises a subset of the antigens of the present invention. In a particular embodiment, the subset of antigen consists of CD99, IGFBP1, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin and collagen III. The reactivity of antibodies to the plurality of antigens of the invention may be determined according to techniques known in the art. Further, the antigens used in the present invention are known 20 in the art and are commercially available, e.g., from Sigma Aldrich or Prospecc.

#### **Hyaluronic acid**

Hyaluronic acid is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. In a particular embodiment, the hyaluronic acid antigen of the present invention is a human hyaluronic acid, e.g. from the 25 umbilical cord of human placenta (commercially available, e.g., from Sigma Aldrich, catalog number H1876; CAS number 9067-32-7). Additional non limiting examples of hyaluronic acid have a CAS number selected from: 31799-91-4 or 9067-32-7.

#### **EBV**

The Epstein-Barr virus (EBV), also called Human herpes virus 4 (HHV-4), is a virus 30 of the herpes family. The reactivity of antibodies to the EBV antigen may be determined according to techniques known in the art. In a particular embodiment, the EBV antigen of the present invention contains the HHV-4 Early Antigen Type D (GeneBank: CAD53407.1), C-terminus regions residing at amino acids 306-390 as set forth in SEQ ID

NO: 2. In one embodiment, said EBV consists of SEQ ID NO: 2, or a analog or fragment thereof. The EBV antigen is commercially available, e.g., from Prospec, catalog number CMV-272.

Double strand deoxyribonucleic acid (dsDNA)

5 Anti-dsDNA antibodies are highly specific for SLE; they are present in 70% of cases, whereas they appear in only 0.5% of people without SLE. The reactivity of antibodies to the dsDNA antigen may be determined according to techniques known in the art. In a particular embodiment, dsDNA has a CAS number of 73049-39-5. The dsDNA antigen is commercially available, e.g., from Sigma Aldrich, catalog number D1501.

10 Single strand deoxyribonucleic acid (ssDNA)

The reactivity of antibodies to the ssDNA antigen may be determined according to techniques known in the art. In a particular embodiment, ssDNA has a CAS number of 91080-16-9. The ssDNA antigen is commercially available, e.g., from Sigma Aldrich, catalog number D8899.

15 CD99

CD99 (also known as E2 antigen, T-cell surface glycolprotein) is expressed on the cell membrane of some lymphocytes, cortical thymocytes, and granulosa cells of the ovary. The antigen is also expressed by most pancreatic islet cells, Sertoli cells of the testis, and some endothelial cells. In a particular embodiment, the CD99 antigen of the present invention is a human CD99 (NP\_002405 or NP\_001116370.1). The amino acid sequence of human CD99 isoform a precursor is set forth in SEQ ID NO:1. In one embodiment, said CD99 antigen consists of SEQ ID NO: 1, or an analog or fragment thereof. Said CD99 antigen is commercially available, e.g., from Prospec, catalog number PRO-294.

Myeloperoxidase (MPO)

25 MPO is an important enzyme used by granulocytes during phagocytic lysis of foreign particles engulfed. In normal tissues and in a variety of myeloproliferative disorders myeloid cells of both neutrophilic and eosinophilic types, at all stages of maturation, exhibit strong cytoplasmic reactivity for MPO. In a particular embodiment, the MPO antigen of the present invention is a human MPO (NP\_000241.1). The amino acid sequence of human MPO is set forth in SEQ ID NO:3. In one embodiment, said MPO antigen consists of SEQ ID NO: 3, or an analog or fragment thereof. Said MPO antigen is commercially available, e.g., from Prospec, catalog number ENZ-334.

Insulin-like growth factor binding protein (IGFBP)-1

IGFBP1 is a member of the insulin-like growth factor binding protein (IGFBP) family and encodes a protein with an IGFBP domain and a thyroglobulin type-I domain. The protein binds both insulin-like growth factors (IGFs) I and II and circulates in the plasma. Human IGFBP1 may be recombinantly produced as a single polypeptide chain  
5 containing 218 amino acids, having a molecular mass of 28.8 KDa. In a particular embodiment, the IGFBP1 antigen of the present invention is a human IGFBP1 (NP\_000587.1)). The amino acid sequence of human IGFBP1 is set forth in SEQ ID NO:4. In one embodiment, said IGFBP1 antigen consists of SEQ ID NO: 4, or an analog or fragment thereof. Said IGFBP1 antigen is commercially available, e.g., from Prospec,  
10 catalog number CYT-299.

#### Cardiolipin

Cardiolipin (1,3-bis(sn-3'-phosphatidyl)-sn-glycerol) is a mitochondrial phospholipid that is found in mammalian tissues in low concentrations, most often in the inner mitochondrial membrane. Cardiolipin inhibits cell attachment of fibronectin,  
15 vitronectin and Type I collagen. In a particular embodiment, the cardiolipin antigen of the present invention is a bovine cardiolipin. The reactivity of antibodies to the cardiolipin antigen may be determined according to techniques known in the art. In a particular embodiment, cardiolipin has a CAS number of 383907-10-6. The bovine cardiolipin antigen is commercially available, e.g., from Sigma Aldrich, catalog number C0563.

#### Collagen Type III

Type III collagen is the second most abundant collagen in human tissues and occurs particularly in tissues exhibiting elastic properties, such as skin, blood vessels and various internal organs. Mutations of type III collagen cause the most severe form of Ehlers-Danlos syndrome, EDS IV, which affect arteries, internal organs, joints and skin, and may cause  
25 sudden death when the large arteries rupture. In a particular embodiment, the type III collagen antigen of the present invention is a Bornstein and Traub Type III collagen, e.g., from human placenta. The reactivity of antibodies to the collagen-III antigen may be determined according to techniques known in the art. In a particular embodiment, collagen-III has a CAS number of 9007-34-5. The collagen-III antigen is commercially available,  
30 e.g., from Sigma Aldrich, catalog number C4407.

#### Collagen Type IV

Unlike most collagens, type IV collagen is an exclusive member of the basement membranes and through a complex inter- and intramolecular interactions form

supramolecular networks that influence cell adhesion, migration, and differentiation (Khoshnoodi et al., Microsc Res Tech. 2008 May;71(5):357-70). In a particular embodiment, the type IV collagen antigen of the present invention is a Bornstein and Traub Type IV collagen, e.g., from human placenta. The reactivity of antibodies to the collagen-IV antigen may be determined according to techniques known in the art. In a particular embodiment, collagen-IV has a CAS number of 9007-34-5. The collagen-IV antigen is commercially available, e.g., from Sigma Aldrich, catalog number C7521.

#### Actin

Actin is a 43 kDa protein that is very highly conserved between species. There are three main actin isotypes ( $\alpha$ ,  $\beta$  and  $\gamma$ ), which show >90% amino-acid (aa) homology between isotypes and >98% homology within members of a particular isotypic group. Actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, and the establishment and maintenance of cell junctions and cell shape. In a particular embodiment, the actin antigen of the present invention is a bovine actin. The reactivity of antibodies to the actin antigen may be determined according to techniques known in the art. In a particular embodiment, actin has a CAS number of 51005-14-2. The bovine actin antigen is commercially available, e.g., from Sigma Aldrich, catalog number A3653.

#### Bone morphogenetic protein-4 (BMP4)

BMP4 plays an important role in the onset of endochondral bone formation in humans. A reduction in BMP4 expression has been associated with a variety of bone diseases, including the heritable disorder Fibrodysplasia Ossificans Progressiva. Human BMP4 (e.g., NP\_570912.2) may be recombinant produced in E.Coli as a monomeric, non-glycosylated, polypeptide chain (molecular mass of 13009 Dalton). In a particular embodiment, the BMP4 antigen of the present invention is a human BMP4 (commercially available, e.g., from Prospec, catalog number CYT-361). In a specific embodiment, the BMP4 antigen has the amino acid sequence as set forth in SEQ ID NO: 5 (SPKHHSQRARKKNKNCRRHSLYVDFSDVGWNDWIVAPPGYQAFYCHGDCPFPLADHLNSTNHAIVQTLVNSVNSSIPKACCVPTELSAISMLYLDEYDKVVLKNYQEMVVEGCGCR, or an analog or fragment thereof.

#### CMV

CMV (CytomegaloVirus) belongs to the Betaherpesvirinae subfamily of Herpesviridae which includes herpes simplex virustypes 1 and 2, varicella-zoster virus, and

Epstein-Barrvirus. CMV is a double-stranded linear DNA virus with 162 hexagonal protein capsomeres surrounded by a lipid membrane. CMV has the largest genome of the herpes viruses, ranging from 230-240 kilobase pairs. Human CMV is composed of unique and inverted repeats that include the existence of 4 genome isomers caused by inversion of L-S genome components (class E). The CMV antigen used in the examples herein below is an E.Coli derived recombinant protein containing the CMV Pp150 (UL32) immunodominant regions residing at amino acids 1011-1048. Said CMV Pp150 (e.g., GI:224496137 or GI:224496135) is known to those skilled in the art. The amino acid sequence of said CMV Pp150 is set forth in SEQ ID NO:6. In one embodiment, said CMV Pp150 consists of SEQ ID NO: 6, or an analog or fragment thereof. The CMV antigen of the present invention is commercially available, e.g., from Prospec, catalog number CMV-216.

#### F50

The F50 antigen used in the examples herein below is an oligopeptide having the amino acid sequence CVKGGTTKIFLVGDYSSSAE as set forth as SEQ ID NO: 7. In one embodiment, said F50 antigen consists of SEQ ID NO: 7, or an analog or fragment thereof.

#### Hepatocyte Growth Factor (HGF)

HGF is a multifunctional growth factor which regulates both cell growth and cell motility. It exerts a strong mitogenic effect on hepatocytes and primary epithelial cells. Human HGF may be recombinantly produced in Baculovirus as a heterodimer, non-glycosylated, polypeptide chain consisting an  $\alpha$ -chain of 463 amino acids and  $\beta$ -chain of 234 amino acids having a total molecular mass of 78.0 KDa. In a particular embodiment, the HGF antigen of the present invention is a human HGF (AAA64297.1). The amino acid sequence of said human HGF is set forth in SEQ ID NO:8. In one embodiment, said HGF consists of SEQ ID NO: 8, or an analog or fragment thereof. Said HGF antigen is commercially available, e.g., from Prospec, catalog number CYT-244.

#### Horseradish peroxidase (HRP)

Horseradish peroxidase is a single chain polypeptide containing four disulfide bridges. It is a glycoprotein containing 18% carbohydrate. The carbohydrate composition consists of galactose, arabinose, xylose, fucose, mannose, mannosamine, and galactosamine depending upon the specific isozyme. HRP belongs to the ferroporphyrin group of peroxidases and may be isolated from horseradish roots (*Amoracia rusticana*). The reactivity of antibodies to the HRP antigen may be determined according to techniques known in the art. In a particular embodiment, HRP has a CAS number of

9003-99-0. The HRP antigen is commercially available, e.g., from Sigma Aldrich, catalog number P6782.

#### HSP60p18

5 The HSP60p18 antigen used in the examples herein below is an oligopeptide having the amino acid sequence QSIVPALEIANAHRKPLVIIA as set forth as SEQ ID NO: 9. In one embodiment, said HSP60p18 antigen consists of SEQ ID NO: 9, or an analog or fragment thereof.

#### Rubella virus (RV)

10 RV is an enveloped positive-strand RNA virus of the family Togaviridae. In a specific embodiment, the RV antigen is an E.Coli derived recombinant protein containing the RV Capsid C regions, amino acids 1-123. The amino acid sequence of the RV Capsid C is known to those skilled in the art, e.g., NP\_740662.1. The amino acid sequence of amino acids 1-123 of RV capsid c is set forth in SEQ ID NO:10. In one embodiment, said RV antigen consists of SEQ ID NO: 10, or an analog or fragment thereof. Said RV antigen is  
15 commercially available, e.g., from Prospec, catalog number RUB-293.

#### S100A4

S100A4 (also known as S100 calcium-binding protein A4, Metastasin, Calvasculin) is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100A is composed of an alpha and beta chain. S100A4 may function in motility, invasion,  
20 and tubulin polymerization. In a specific embodiment, the S100A4 antigen of the invention is a human S100A4 (CAG29341.1). The amino acid sequence of human S100A4 is set forth in SEQ ID NO:11. In one embodiment, said S100A4 antigen consists of SEQ ID NO: 11, or an analog or fragment thereof. Said S100A4 antigen is commercially available, e.g., from Prospec, catalog number PRO-307.

#### 25 CITED1

The human Cbp/p300-interacting transactivator 1 (CITED1, also known as Melanocyte-specific protein 1 or MSG1) is a protein having 193 amino acids (NP\_001138359.1). The amino acid sequence of human CITED1 is set forth in SEQ ID NO:12. In one embodiment, said CITED1 antigen consists of SEQ ID NO: 12, or an analog  
30 or fragment thereof. CITED1 antigen is commercially available, e.g., from Prospec, catalog number PRO-295.

#### FHIT

FHIT (Fragile Histidine Triad) is a member of the histidine triad gene family,

involved in purine metabolism. The FHIT protein is a tumor suppressor with reduced or no expression in numerous types of cancer. In a specific embodiment, the FHIT antigen of the invention is a human FHIT (ABM66093.1). The amino acid sequence of human FHIT is set forth in SEQ ID NO:13. In one embodiment, said FHIT antigen consists of SEQ ID NO: 13, or an analog or fragment thereof. Said FHIT antigen is commercially available, e.g., from Prospecc, catalog number PRO-297.

Preferably, the plurality of antigens comprises a set of the antigens of the present invention. Yet in other embodiments, the plurality of antigens (or the antigen probe set) comprises or consists of a subset thereof, e.g. at least 3, 4, 5, 6, 7, 8, 9, 10, or 11 different antigens each selected from the antigens of the present invention, wherein each possibility represents a separate embodiment of the invention. Such subsets may be selected so as to result in optimal sensitivity and/or specificity of the diagnostic assay. In other embodiments, the probe set comprises up to 10, or in other embodiments up to 15, 20, 30, 40 or 50 different antigens. It should be noted, that while such probe sets are considered sufficient for reliably identifying a subject with SLE, the antigen probe sets of the invention may conveniently be used, in certain embodiments, in the form of antigen arrays comprising a greater number of antigens, e.g. about 25 antigens or more.

According to a particular embodiment, the antigen probe set of the invention comprise a plurality of antigens selected from CD99, IGFBP1, hyaluronic acid, MPO, EBV, ssDNA, dsDNA, and cardiolipin, as detailed herein, for the diagnosis of SLE.

In another embodiment, the plurality of antigens consists of hyaluronic acid, EBV, ssDNA and dsDNA. As disclosed herein, this subset of antigens showed up-regulated reactivities with IgG antibodies in serum obtained from subjects with SLE. In another embodiment, the plurality of antigens consists of CD99, MPO, IGFBP1 and cardiolipin. As disclosed herein, this subset of antigens showed down-regulated reactivities with IgM antibodies in serum obtained from subjects with SLE.

In another embodiment, a probe set consisting of the hyaluronic acid, EBV, ssDNA, dsDNA, CD99, MPO and collagen III antigens is sufficient to discriminate between subjects with SLE in renal remission, and healthy individuals that are not afflicted with SLE. In another embodiment, the plurality of antigens consists of hyaluronic acid, EBV, ssDNA and dsDNA. As disclosed herein, this subset of antigens showed up-regulated reactivities with IgG antibodies in serum obtained from subjects with SLE, particularly SLE

in renal remission. In another embodiment, the plurality of antigens consists of CD99, MPO and collagen III. As disclosed herein, this subset of antigens showed down-regulated reactivities with IgM antibodies in serum obtained from subjects with SLE, such as in renal remission.

5           Antigen probes to be used in the assays of the invention may be purified or synthesized using methods well known in the art. For example, an antigenic protein or peptide may be produced using known recombinant or synthetic methods, including, but not limited to, solid phase (e.g. Boc or f-Moc chemistry) and solution phase synthesis methods (Stewart and Young, 1963; Meienhofer, 1973; Schroder and Lupke, 1965; Sambrook *et al.*,  
10   2001). One of skill in the art will possess the required expertise to obtain or synthesize the antigen probes of the invention. Some of the antigen probes are also commercially available, e.g. from Sigma (St. Louis, MO, USA), Prospec (Ness-Ziona, Israel), Abnova (Taipei City, Taiwan), Matreya LLC (Pleasant Gap, PA, USA), Avanti Polar Lipids (Alabaster, AL, USA), Calbiochem (San Diego, CA, USA), Chemicon (Temecula, CA,  
15   USA), GeneTex (San Antonio, TX, USA), Novus Biologicals (Littleton, CO, USA) Assay Designs (Ann Arbor, MI, USA), ProSci Inc. (Poway, CA, USA), EMD Biosciences (San Diego, CA, USA), Cayman Chemical (Ann Arbor, MI, USA), HyTest (Turku, Finland), Meridian Life Science (Memphis, TN USA) and Biodesign International (Saco, ME, USA), as detailed herein below.

20           It should be noted, that the invention utilizes antigen probes as well as homologs, fragments and derivatives thereof, as long as these homologs, fragments and derivatives are immunologically cross-reactive with these antigen probes. The term “immunologically cross-reactive” as used herein refers to two or more antigens that are specifically bound by the same antibody. The term “homolog” as used herein refers to a peptide which having at  
25   least 70%, at least 75%, at least 80%, at least 85% or at least 90% identity to the antigen's amino acid sequence. Cross-reactivity can be determined by any of a number of immunoassay techniques, such as a competition assay (measuring the ability of a test antigen to competitively inhibit the binding of an antibody to its known antigen).

          The term “fragment” as used herein refers to a portion of a polypeptide, or  
30   polypeptide analog which remains immunologically cross-reactive with the antigen probes, e.g., to immunospecifically recognize the target antigen. The fragment may have the length of about 40%, about 50%, about 60%, about 70%, about 80%, about 85%, about 90% or about 95% of the respective antigen.

The term peptide typically refers to a polypeptide of up to about 50 amino acid residues in length. According to particular embodiments, the antigenic peptides of the invention may be 10-50 amino acids in length and are typically about 10-30 or about 15-25 amino acids in length.

5 The term encompasses native peptides (either degradation products, synthetically synthesized peptides, or recombinant peptides), peptidomimetics (typically, synthetically synthesized peptides), and the peptide analogues peptoids and semipeptoids, and may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to: N-terminus modifications; C-terminus modifications; peptide bond modifications, including  
10 but not limited to  $\text{CH}_2\text{-NH}$ ,  $\text{CH}_2\text{-S}$ ,  $\text{CH}_2\text{-S=O}$ ,  $\text{O=C-NH}$ ,  $\text{CH}_2\text{-O}$ ,  $\text{CH}_2\text{-CH}_2$ ,  $\text{S=C-NH}$ ,  $\text{CH=CH}$ , and  $\text{CF=CH}$ ; backbone modifications; and residue modifications.

The antigens of the invention may be used having a terminal carboxy acid, as a carboxy amide, as a reduced terminal alcohol or as any pharmaceutically acceptable salt,  
15 e.g., as metal salt, including sodium, potassium, lithium or calcium salt, or as a salt with an organic base, or as a salt with a mineral acid, including sulfuric acid, hydrochloric acid or phosphoric acid, or with an organic acid e.g., acetic acid or maleic acid.

Functional derivatives consist of chemical modifications to amino acid side chains and/or the carboxyl and/or amino moieties of said peptides. Such derivatized molecules  
20 include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The  
25 imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those polypeptides, which contain one or more naturally occurring or modified amino acid derivatives of the twenty standard amino acid residues. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine;  
30 homoserine may be substituted for serine; and ornithine may be substituted for lysine.

The amino acid residues described herein are in the "L" isomeric form, unless otherwise indicated. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the peptide substantially retains the desired antibody

specificity.

Suitable analogs may be readily synthesized by now-standard peptide synthesis methods and apparatus or recombinant methods. All such analogs will essentially be based on the antigens of the invention as regards their amino acid sequence but will have one or more amino acid residues deleted, substituted or added. When amino acid residues are substituted, such conservative replacements which are envisaged are those which do not significantly alter the structure or antigenicity of the polypeptide. For example basic amino acids will be replaced with other basic amino acids, acidic ones with acidic ones and neutral ones with neutral ones. In addition to analogs comprising conservative substitutions as detailed above, analogs comprising non-conservative amino acid substitutions are further contemplated, as long as these analogs are immunologically cross reactive with a peptide of the invention.

In other aspects, there are provided nucleic acids encoding these peptides, vectors comprising these nucleic acids and host cells containing them. These nucleic acids, vectors and host cells are readily produced by recombinant methods known in the art (see, e.g., Sambrook *et al.*, 2001). For example, an isolated nucleic acid sequence encoding an antigen of the invention can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Nucleic acid sequences include natural nucleic acid sequences and homologs thereof, including, but not limited to, natural allelic variants and modified nucleic acid sequences in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a functional peptide of the present invention.

The lipid antigens to be used in the assays of the invention may be purified or synthesized using methods well known in the art (see, for example, Biochemistry of Lipids, Lipoproteins, and Membranes, 4<sup>sup</sup>.th Ed. (2002; Vance D E and Vance, J E, editors; Elsevier, Amsterdam, Boston); Enzymes in Lipid Modification (2000; Bornsheuer, U T, editor; Wiley-VCH, Weinheim, N.Y.); Lipid Synthesis and Manufacture (1999; Gunstone, F D, editor; Sheffield Academic Press, Sheffield, England; CRC Press, Boca Raton, Fla.); Lipid Biochemistry, 5<sup>sup</sup>.th Ed (2002; Gurr, M I, Harwood, J L, and Frayn, K N, editors; Blackwell Science, Oxford, Malden, Mass). In another embodiment, the lipid antigens to

be used in the assays of the invention may be commercially purchased as detailed herein below.

### **Diagnostic methods**

5 According to some embodiments, the invention provides diagnostic methods useful for the detection of SLE.

According to some embodiments, the methods of the invention are effected by determining the reactivity of antibodies in a sample obtained from a test subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, CD99, IGFBP1, 10 EBV, ssDNA, dsDNA, MPO, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT, thereby determining the reactivity pattern of the sample to the plurality of antigens, and comparing the reactivity pattern of said sample to a control reactivity pattern. In one embodiment, a significant difference between the reactivity pattern of said sample compared to a reactivity pattern of a 15 control sample indicates that the subject is afflicted with SLE.

As used herein, the “reactivity of antibodies in a sample” to “a plurality of antigens” refers to the immune reactivity of each antibody in the sample to a specific antigen selected from the plurality of antigens. The immune reactivity of the antibody to the antigen, i.e. its ability to specifically bind the antigen, may be used to determine the 20 amount of the antibody in the sample, thereby providing a quantitative assay. The calculated levels of each one of the tested antibodies in the sample are selectively referred to as the reactivity pattern of the sample to these antigens.

An antibody “directed to” an antigen, as used herein is an antibody which is capable of specifically binding the antigen. Determining the levels of antibodies directed to 25 a plurality of antigens includes measuring the level of each antibody in the sample, wherein each antibody is directed to a specific antigen selected from: hyaluronic acid, EBV, ssDNA, dsDNA, CD99, MPO, IGFBP1, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT. This step is typically performed using an immunoassay, as detailed herein.

30 In other embodiments, determining the reactivity of antibodies in said sample to said plurality of antigens, (and the levels of each one of the tested antibodies in the sample) is performed by a process comprising:

(i) contacting the sample, under conditions such that a specific antigen-antibody

complex may be formed, with an antigen probe set comprising said plurality of antigens, and

(ii) quantifying the amount of antigen-antibody complex formed for each antigen probe.

5       The amount of antigen-antibody complex is indicative of the level of the tested antibody in the sample (or the reactivity of the sample with the antigen).

10       In certain embodiments, the test sample and control samples comprise IgG and/or IgM antibodies. Particularly, the test sample and control samples may comprise IgG and IgM antibodies. In yet another preferred embodiment, the test and control samples comprise a plurality of IgG antibodies and a plurality of IgM antibodies. In another embodiment, the reactivity of at least one antibody to a specific antigen from the plurality of antigens of the invention is up-regulated. In another embodiment, the reactivity of at least one antibody to a specific antigen is down-regulated.

15       According to another aspect, the present invention provides a method of assessing whether a subject is at risk of developing SLE, the method comprising: (i) determining the reactivity of IgG and IgM antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, CD99, EBV, ssDNA, dsDNA, MPO, IGFBP1, cardiolipin, collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT, thereby determining the reactivity pattern of the sample to the plurality of antigens, and (ii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

25       According to an exemplary embodiment the plurality of antigens consist of hyaluronic acid, CD99, EBV, ssDNA, dsDNA, MPO, IGFBP1, cardiolipin and collagen III. According to another embodiment the plurality of antigens consist of hyaluronic acid, CD99, MPO, IGFBP1 and collagen III.

30       In some embodiments, the methods of the present invention employ an antigen microarray system for informatically characterizing informative patterns of antibodies as specific biomarkers for subtypes of SLE, as detailed herein.

Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of

"true positives"). Diseased individuals not detected by the assay are "false negatives". Subjects who are not diseased and who test negative in the assay are termed "true negatives". The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion of those without the disease who test  
5 positive.

In some embodiments, the plurality of antigens is selected to exhibit at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sensitivity, combined with at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% specificity. In some embodiments, both the sensitivity and specificity are at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%.  
10

In one embodiment, the method distinguishes SLE with a sensitivity of at least 70% at a specificity of at least 85% when compared to control subjects (e.g., a healthy individual not afflicted with SLE). In another embodiment, the method distinguishes SLE with a sensitivity of at least 80% at a specificity of at least 90% when compared to control  
15 subjects. In another embodiment, the method distinguishes SLE with a sensitivity of at least 90% at a specificity of at least 90% when compared to control subjects.

#### **Antibodies, samples and immunoassays**

Antibodies, or immunoglobulins, comprise two heavy chains linked together by  
20 disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains (CH). Each light chain has a variable domain (VL) at one end and a constant domain (CL) at its other end, the light chain variable domain being aligned with the variable domain of the heavy chain and the  
25 light chain constant domain being aligned with the first constant domain of the heavy chain (CH1). The variable domains of each pair of light and heavy chains form the antigen binding site.

The isotype of the heavy chain (gamma, alpha, delta, epsilon or mu) determines immunoglobulin class (IgG, IgA, IgD, IgE or IgM, respectively). The light chain is either of  
30 two isotypes (kappa,  $\kappa$  or lambda,  $\lambda$ ) found in all antibody classes.

It should be understood that when the terms "antibody" or "antibodies" are used, this is intended to include intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments thereof such as the Fab or F(ab')<sub>2</sub>

fragments. Further included within the scope of the invention (for example as immunoassay reagents, as detailed herein) are chimeric antibodies; recombinant and engineered antibodies, and fragments thereof.

Exemplary functional antibody fragments comprising whole or essentially whole  
5 variable regions of both light and heavy chains are defined as follows:

(i) Fv, defined as a genetically engineered fragment consisting of the variable region of the light chain and the variable region of the heavy chain expressed as two chains;

(ii) single-chain Fv ("scFv"), a genetically engineered single-chain molecule including the variable region of the light chain and the variable region of the heavy chain,  
10 linked by a suitable polypeptide linker.

(iii) Fab, a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule, obtained by treating whole antibody with the enzyme papain to yield the intact light chain and the Fd fragment of the heavy chain, which consists of the variable and CH1 domains thereof;

15 (iv) Fab', a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule, obtained by treating whole antibody with the enzyme pepsin, followed by reduction (two Fab' fragments are obtained per antibody molecule); and

(v) F(ab')<sub>2</sub>, a fragment of an antibody molecule containing a monovalent antigen-binding portion of an antibody molecule, obtained by treating whole antibody with the enzyme pepsin (i.e., a dimer of Fab' fragments held together by two disulfide bonds).  
20

The term "antigen" as used herein is a molecule or a portion of a molecule capable of being bound by an antibody. The antigen is typically capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one  
25 or more epitopes. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens. An "antigenic peptide" is a peptide which is capable of specifically binding an antibody.

In another embodiment, detection of the capacity of an antibody to specifically bind  
30 an antigen probe may be performed by quantifying specific antigen-antibody complex formation. The term "specifically bind" as used herein means that the binding of an antibody to an antigen probe is not competitively inhibited by the presence of non-related molecules.

In certain embodiments, the method of the present invention is performed by determining the capacity of an antigen of the invention to specifically bind antibodies of the IgG isotype, or, in other embodiments, antibodies of the IgM, isolated from a subject.

5 Methods for obtaining suitable antibody-containing biological samples from a subject are well within the ability of those of skill in the art. Typically, suitable samples comprise whole blood and products derived therefrom, such as plasma and serum. In other embodiments, other antibody-containing samples may be used, e.g. CSF, urine and saliva samples.

10 Numerous well known fluid collection methods can be utilized to collect the biological sample from the subject in order to perform the methods of the invention.

In accordance with the present invention, any suitable immunoassay can be used with the subject peptides. Such techniques are well known to the ordinarily skilled artisan and have been described in many standard immunology manuals and texts. In certain  
15 preferable embodiments, determining the capacity of the antibodies to specifically bind the antigen probes is performed using an antigen probe array-based method. Preferably, the array is incubated with suitably diluted serum of the subject (e.g. diluted 1:10) so as to allow specific binding between antibodies contained in the serum and the immobilized antigen probes, washing out unbound serum from the array, incubating the washed array  
20 with a detectable label-conjugated ligand of antibodies of the desired isotype, washing out unbound label from the array, and measuring levels of the label bound to each antigen probe.

According to some aspects the methods of the present invention may be practiced using antigen arrays as disclosed in WO 02/08755 and U.S. 2005/0260770 to some of the  
25 inventors of the present invention. WO 02/08755 is directed to a system and an article of manufacture for clustering and thereby identifying predefined antigens reactive with undetermined immunoglobulins of sera derived from patient subjects in need of diagnosis of disease or monitoring of treatment. Further disclosed are diagnostic methods, and systems useful in these methods, employing the step of clustering a subset of antigens of a  
30 plurality of antigens, said subset of antigens being reactive with a plurality of antibodies being derived from a plurality of patients, and associating or disassociating the antibodies of a subject with the resulting cluster. U.S. Pat. App. Pub. No. 2005/0260770 to some of the inventors of the present invention discloses an antigen array system and diagnostic uses

thereof. The application provides a method of diagnosing an immune disease, particularly diabetes type 1, or a predisposition thereto in a subject, comprising determining a capacity of immunoglobulins of the subject to specifically bind each antigen probe of an antigen probe set. The teachings of said disclosures are incorporated in their entirety as if fully set forth herein.

In other embodiments, various other immunoassays may be used, including, without limitation, enzyme-linked immunosorbent assay (ELISA), flow cytometry with multiplex beads (such as the system made by Luminex), surface plasmon resonance (SPR), elipsometry, and various other immunoassays which employ, for example, laser scanning, light detecting, photon detecting via a photo-multiplier, photographing with a digital camera based system or video system, radiation counting, fluorescence detecting, electronic, magnetic detecting and any other system that allows quantitative measurement of antigen-antibody binding.

Various methods have been developed for preparing arrays suitable for the methods of the present invention. State-of-the-art methods involves using a robotic apparatus to apply or "spot" distinct solutions containing antigen probes to closely spaced specific addressable locations on the surface of a planar support, typically a glass support, such as a microscope slide, which is subsequently processed by suitable thermal and/or chemical treatment to attach antigen probes to the surface of the support. Conveniently, the glass surface is first activated by a chemical treatment that leaves a layer of reactive groups such as epoxy groups on the surface, which bind covalently any molecule containing free amine or thiol groups. Suitable supports may also include silicon, nitrocellulose, paper, cellulosic supports and the like.

Preferably, each antigen probe, or distinct subset of antigen probes of the present invention, which is attached to a specific addressable location of the array is attached independently to at least two, more preferably to at least three separate specific addressable locations of the array in order to enable generation of statistically robust data.

In addition to antigen probes of the invention, the array may advantageously include control antigen probes or other standard chemicals. Such control antigen probes may include normalization control probes. The signals obtained from the normalization control probes provide a control for variations in binding conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a given binding antibody-probe ligand interaction to vary. For example, signals, such as fluorescence intensity, read from

all other antigen probes of the antigen probe array are divided by the signal (e.g., fluorescence intensity) from the normalization control probes thereby normalizing the measurements. Normalization control probes can be bound to various addressable locations on the antigen probe array to control for spatial variation in antibody-ligand probe efficiency. Preferably, normalization control probes are located at the corners or edges of the array to control for edge effects, as well as in the middle of the array.

The labeled antibody ligands may be of any of various suitable types of antibody ligand. Preferably, the antibody ligand is an antibody which is capable of specifically binding the Fc portion of the antibodies of the subject used. For example, where the antibodies of the subject are of the IgM isotype, the antibody ligand is preferably an antibody capable of specifically binding to the Fc region of IgM antibodies of the subject.

The ligand of the antibodies of the subject may be conjugated to any of various types of detectable labels. Preferably the label is a fluorophore, most preferably Cy3. Alternately, the fluorophore may be any of various fluorophores, including Cy5, fluorescein isothiocyanate (FITC), phycoerythrin (PE), rhodamine, Texas red, and the like. Suitable fluorophore-conjugated antibodies specific for antibodies of a specific isotype are widely available from commercial suppliers and methods of their production are well established.

Antibodies of the subject may be isolated for analysis of their antigen probe binding capacity in any of various ways, depending on the application and purpose. While the subject's antibodies may be suitably and conveniently in the form of blood serum or plasma or a dilution thereof (e.g. 1:10 dilution), the antibodies may be subjected to any desired degree of purification prior to being tested for their capacity to specifically bind antigen probes. The method of the present invention may be practiced using whole antibodies of the subject, or antibody fragments of the subject which comprises an antibody variable region.

### **Data analysis**

In some embodiments, the methods of the invention may employ the use of learning and pattern recognition analyzers, clustering algorithms and the like, in order to discriminate between reactivity patterns of subjects having a subtype of SLE to control subjects. For example, the methods may include determining the reactivity of antibodies in a test sample to a plurality of antigens, and comparing the resulting pattern to the reactivity patterns of negative and positive control samples using such algorithms and/or analyzers.

Thus, in another embodiment, a significant difference between the reactivity pattern

of a test sample compared to a reactivity pattern of a control sample, wherein the difference is computed using a learning and pattern recognition algorithm, indicates that the subject is afflicted with SLE. For example, the algorithm may include, without limitation, supervised or non-supervised classifiers including statistical algorithms including, but not limited to, principal component analysis (PCA), partial least squares (PLS), multiple linear regression (MLR), principal component regression (PCR), discriminant function analysis (DFA) including linear discriminant analysis (LDA), and cluster analysis including nearest neighbor, artificial neural networks, coupled two-way clustering algorithms, multi-layer perceptrons (MLP), generalized regression neural network (GRNN), fuzzy inference systems (FIS), self-organizing map (SOM), genetic algorithms (GAS), neuro-fuzzy systems (NFS), adaptive resonance theory (ART).

In certain embodiments, one or more algorithms or computer programs may be used for comparing the amount of each antibody quantified in the test sample against a predetermined cutoff (or against a number of predetermined cutoffs). Alternatively, one or more instructions for manually performing the necessary steps by a human can be provided.

Algorithms for determining and comparing pattern analysis include, but are not limited to, principal component analysis, Fischer linear analysis, neural network algorithms, genetic algorithms, fuzzy logic pattern recognition, and the like. After analysis is completed, the resulting information can, for example, be displayed on display, transmitted to a host computer, or stored on a storage device for subsequent retrieval.

Many of the algorithms are neural network based algorithms. A neural network has an input layer, processing layers and an output layer. The information in a neural network is distributed throughout the processing layers. The processing layers are made up of nodes that simulate the neurons by the interconnection to their nodes. Similar to statistical analysis revealing underlying patterns in a collection of data, neural networks locate consistent patterns in a collection of data, based on predetermined criteria.

Suitable pattern recognition algorithms include, but are not limited to, principal component analysis (PCA), Fisher linear discriminant analysis (FLDA), soft independent modeling of class analogy (SIMCA), K-nearest neighbors (KNN), neural networks, genetic algorithms, fuzzy logic, and other pattern recognition algorithms. In some embodiments, the Fisher linear discriminant analysis (FLDA) and canonical discriminant analysis (CDA) as well as combinations thereof are used to compare the output signature and the available data from the database.

In other embodiments, principal component analysis is used. Principal component analysis (PCA) involves a mathematical technique that transforms a number of correlated variables into a smaller number of uncorrelated variables. The smaller number of uncorrelated variables is known as principal components. The first principal component or eigenvector  
5 accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. The main objective of PCA is to reduce the dimensionality of the data set and to identify new underlying variables.

Principal component analysis compares the structure of two or more covariance matrices in a hierarchical fashion. For instance, one matrix might be identical to another  
10 except that each element of the matrix is multiplied by a single constant. The matrices are thus proportional to one another. More particularly, the matrices share identical eigenvectors (or principal components), but their eigenvalues differ by a constant. Another relationship between matrices is that they share principal components in common, but their eigenvalues differ. The mathematical technique used in principal component analysis is called  
15 eigenanalysis. The eigenvector associated with the largest eigenvalue has the same direction as the first principal component. The eigenvector associated with the second largest eigenvalue determines the direction of the second principal component. The sum of the eigenvalues equals the trace of the square matrix and the maximum number of eigenvectors equals the number of rows of this matrix.

20 In another embodiment, the algorithm is a classifier. One type of classifier is created by "training" the algorithm with data from the training set and whose performance is evaluated with the test set data. Examples of classifiers used in conjunction with the invention are discriminant analysis, decision tree analysis, receiver operator curves or split and score analysis.

25 The term "decision tree" refers to a classifier with a flow-chart-like tree structure employed for classification. Decision trees consist of repeated splits of a data set into subsets. Each split consists of a simple rule applied to one variable, e.g., "if value of "variable 1" larger than "threshold 1"; then go left, else go right". Accordingly, the given feature space is partitioned into a set of rectangles with each rectangle assigned to one class.

30 The terms "test set" or "unknown" or "validation set" refer to a subset of the entire available data set consisting of those entries not included in the training set. Test data is applied to evaluate classifier performance.

The terms "training set" or "known set" or "reference set" refer to a subset of the

respective entire available data set. This subset is typically randomly selected, and is solely used for the purpose of classifier construction.

### **Diagnosing SLE**

5           The methods of the invention are useful in diagnosing systemic lupus erythematosus (SLE). In some embodiments, SLE is cutaneous SLE. In additional embodiments, said SLE is subacute cutaneous SLE or SCLE. Other forms of lupus may also be diagnosed using the methods and kits of the invention, such as nephritis, extrarenal, cerebritis, pediatric, non-renal, discoid (DLE), and alopecia. Each possibility is a separate  
10       embodiment of the invention. "Lupus" as used herein is an autoimmune disease or disorder involving antibodies that attack connective tissue.

          As used herein the term "diagnosing" or "diagnosis" refers to the process of identifying a medical condition or disease (e.g., SLE) by its signs, symptoms, and in particular from the results of various diagnostic procedures, including e.g. detecting the  
15       reactivity of antibodies in a biological sample (e.g. serum) obtained from an individual, to a plurality of antigens. Furthermore, as used herein the term "diagnosing" or "diagnosis" encompasses screening for a disease, detecting a presence or a severity of a disease, distinguishing a disease from other diseases including those diseases that may feature one or more similar or identical symptoms, providing prognosis of a disease, monitoring  
20       disease progression or relapse, as well as assessment of treatment efficacy and/or relapse of a disease, disorder or condition, as well as selecting a therapy and/or a treatment for a disease, optimization of a given therapy for a disease, monitoring the treatment of a disease, and/or predicting the suitability of a therapy for specific patients or subpopulations or determining the appropriate dosing of a therapeutic product in patients or  
25       subpopulations.

          As demonstrated herein below (Example 4), a sample was obtained from a subject when she was in a healthy state and was negative for standard anti-DNA antibodies, but 8 months later she began suffering from non-specific symptoms and was eventually found, after 7 more months, to fulfill the criteria for a diagnosis of SLE. Thus the antigen-  
30       microarray signature might also detect pre-clinical SLE. In a particular embodiment, the method and kits of the invention are useful for diagnosing pre-clinical SLE. In yet another particular embodiment, the method and kits of the invention are useful for early detection of SLE.

In one embodiment, the subject being diagnosed according to the methods of the invention is symptomatic. In other embodiments, the subject is asymptomatic.

The diagnostic procedure can be performed in vivo or in vitro, preferably in vitro.

#### 5      Criteria for diagnosing SLE

The 1982 American College of Rheumatology (ACR) criteria summarize features necessary to diagnose SLE. The presence of 4 of the 11 criteria yields a sensitivity of 85% and a specificity of 95% for SLE. Patients with SLE may present with any combination of clinical features and serologic evidence of lupus.

10

- Serositis - Pleurisy, pericarditis on examination or diagnostic ECG or imaging
- Oral ulcers - Oral or nasopharyngeal, usually painless; palate is most specific
- Arthritis - Nonerosive, two or more peripheral joints with tenderness or swelling
- Photosensitivity - Unusual skin reaction to light exposure
- 15      • Blood disorders - Leukopenia ( $<4 \times 10^3$  cells/ $\mu\text{L}$  on more than one occasion), lymphopenia ( $<1500$  cells/ $\mu\text{L}$  on more than one occasion), thrombocytopenia ( $<100 \times 10^3$  cells/ $\mu\text{L}$  in the absence of offending medications), hemolytic anemia
- Renal involvement - Proteinuria ( $>0.5$  g/d or 3+ positive on dipstick testing) or cellular casts
- 20      • ANAs - Higher titers generally more specific ( $>1:160$ ); must be in the absence of medications associated with drug-induced lupus
- Immunologic phenomena - dsDNA; anti-Smith (Sm) antibodies; antiphospholipid antibodies (anticardiolipin immunoglobulin G [IgG] or immunoglobulin M [IgM] or lupus anticoagulant); biologic false-positive serologic test results for syphilis, lupus
- 25      erythematosus (LE) cells (omitted in 1997)
- Neurologic disorder - Seizures or psychosis in the absence of other causes
- Malar rash - Fixed erythema over the cheeks and nasal bridge, flat or raised
- Discoid rash - Erythematous raised-rimmed lesions with keratotic scaling and follicular plugging, often scarring

30

Two of the most commonly used instruments for SLE diagnosis are the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and the Systemic Lupus Activity Measure (SLAM).

### SLE Disease Activity Index (SLEDAI)

The SLEDAI is an index that measures disease activity by weighting the importance of each organ system involved. The SLEDAI includes 24 items, representing  
5 nine organ systems. The variables are obtained by history, physical examination and laboratory assessment. Each item is weighted from 1 to 8 based on the significance of the organ involved. For example, mouth ulcers are scored as 2, while seizures are scored as 8. The laboratory parameters that are included in the SLEDAI include white blood cell count, platelet count, urinalysis, serum C3, C4 and anti-dsDNA. The total maximum score is 105.

### Systemic Lupus Activity Measure (SLAM)

The SLAM includes 32 items representing 11 organ systems. The items are scored not only as present/absent, but graded on a scale of 1 to 3 based on severity. The total possible score for the SLAM is 86. Both the SLEDAI and the SLAM have been shown to  
15 be valid, reliable, and sensitive to change over time (Liang et al. 1989, Arth Rheum 32:1107-18), and are widely used in research protocols and clinical trials. These indices are particularly useful for examining the value of newly proposed serologic or inflammatory markers of disease activity in SLE.

Despite the obvious utility of these instruments, there are some drawbacks. First,  
20 there is not always complete agreement between the SLAM and the SLEDAI in the same set of patients. There are several possible reasons for these discrepancies. Unlike the SLEDAI, the SLAM includes constitutional symptoms such as fatigue and fever, which may or may not be considered attributable to active SLE; this activity index relies on physician interpretation. In addition, the SLEDAI does not capture mild degrees of activity  
25 in some organ systems and does not have descriptors for several types of activity, such as hemolytic anemia.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting  
30 the broad scope of the invention.

## EXAMPLES

### Materials and Methods

5

#### Human Subjects

The study was approved by the institutional review board of each participating clinical unit; informed consent was obtained from all participants.

Three groups of SLE patients and a control group were studied: 15 patients in renal remission; 14 patients with active lupus nephritis; 11 patients without renal involvement; and 16 age-matched and sex-matched healthy controls. Blood samples and clinical data were collected from SLE patients arriving at the Rheumatology Unit and Hematology Department of the Sheba Medical Center, Israel; the Rheumatology Unit at the Hadassah Medical Center, Ein Kerem, Jerusalem, Israel; and the Cellular Biology and Immunogenetics Unit at the Corporacion para Investigaciones Biologicas, Medellín, Colombia. All patients fulfilled the American College of Rheumatology criteria for SLE (Tan et al: Arthritis. Rheum. 25:1271, 1982; updated by MC Hochberg, Arthritis. Rheum. 1997; 40:1725.). SLE patients with active lupus nephritis were defined by a SLEDAI of  $\geq 8$  and one of the following: new onset proteinuria of  $\geq 1\text{g}$ ; an increase in the urinary protein:creatinine ratio  $\geq 2$ ; or an increase of  $\geq 50\%$  of the baseline serum creatinine. SLE patients in renal remission were individuals who were once diagnosed as having active lupus nephritis as defined above, but were now with a systemic lupus erythematosus disease activity index (SLEDAI)  $\leq 4$  and one of the following: a return to baseline serum creatinine with a decrease in proteinuria to within 25% of their baseline level, or a return to baseline proteinuria and a return of serum creatinine to within 25% of their baseline level. All patients in remission remained stable for at least 6 months; the mean time in remission was 8 years; the range was 3 months to 30 years. Patients without known renal involvement were known not to have suffered from kidney involvement in the past and during a follow up of at least 1 year. The mean time from diagnosis was 7 years; the range was from 0.5 to 27 years. Additional patient data are shown in Table 2 (values are presented as mean  $\pm$  standard error or percent).

Table 2- The clinical characteristics of the SLE patients

| <b>Characteristic</b>               | <b>Renal Involvement</b> |                         |                      |
|-------------------------------------|--------------------------|-------------------------|----------------------|
|                                     | <b>None (n=11)</b>       | <b>Remission (n=15)</b> | <b>Active (n=14)</b> |
| <b>Sex (% Female)</b>               | 100%                     | 87%                     | 86%                  |
| <b>Age (years)</b>                  | 40.6±4.4                 | 36.1±3.2                | 32.4±2.5             |
| <b>SLEDAI</b>                       | 1.8±1                    | 1.1±0.4                 | 14.6±2.2             |
| <b>Anti DNA Ab (%positive)</b>      | 18%                      | 40%                     | 86%                  |
| <b>Complement (%decreased)</b>      | 9%                       | 7%                      | 83%                  |
| <b>Creatinine mg/dL</b>             | 0.75±0.03                | 0.97±0.08               | 1.2±0.26             |
| <b>Duration of disease (months)</b> | 84±29                    | 150±23                  | 118±25               |

### **Antigen Microarrays and Serum Testing**

5           Antigen microarray chips were prepared as described in Merbl et al. 2007 J. Clin. Invest. 117:712-8 and Quintana et al. 2006, Lupus 15:428-30; and Quintana et al. 2004, Proc. Natl. Acad. Sci. U S A. 101: 14615-21.

694 antigens, each at its optimal concentration (mostly at 1 mg/ml), were spotted in triplicate on Epoxy-activated glass substrates using a 48-pin robot (Microgrid 600, 10 Genomics Solutions, Ann Arbor, MI, USA). These antigens included proteins, synthetic peptides from the sequences of selected proteins, nucleotides, phospholipids, and other self and non-self molecules. The microarrays were then blocked for 1 hour at 37°C with 1% bovine serum albumin. Test serum in blocking buffer (1:10 dilution) was incubated under a coverslip for 1 hour at 37°C. The arrays were then washed and incubated for 1 hour at 37°C 15 with a 1:500 dilution of two detection antibodies, mixed together: a goat anti-human IgG Cy3-conjugated antibody, and a goat anti-human IgM Cy5-conjugated antibody, (both purchased from Jackson ImmunoResearch Laboratories Inc. West Grove, PA, USA). Image acquisition was performed by laser (Agilent Technologies, Santa Clara, CA, USA) and the results were analyzed by Quantarray software (Packard BioChip Technologies, 20 Billerica, MA, USA) and by additionally developed software. The quantitative range of signal intensity of binding to each antigen spot was 0–65,000; this range of detection made

it possible to obtain reliable data at the 1:10 dilution of test samples (Quintana et al., Proc. Natl. Acad. Sci. USA. 105:18889-94). Anti-DNA antibodies were also measured separately using ELISA (QUANTA-Lite, Inova, San Diego, CA) and the Farr assay (Wold et al., Science 161: 806).

5

### Image Analysis and Data Processing

Technically faulty spots were manually excluded by visual inspection of each slide. The foreground and background intensities of multiple spots of each antigen were then averaged, and a log-base-10 value of the difference between the foreground and the background was calculated; differences less than 500 were clamped to 500 and then log transformed. To control for differences between different slides, the average laser intensity value of each slide (in the corresponding IgM or IgG channel) was then subtracted. The value of each antigen was then shifted such that its minimal value over the entire dataset equals zero. The resulting value was taken as the *antigen reactivity* of the antibodies binding to that spotted antigen. Antigens that showed zero reactivity in more than 80% of the slides were excluded, as were antigens whose coefficient of variation across slides was lower than 20%. In this way, the 694 IgM and the 694 IgG antigen reactivities were reduced to about 930 reactivities, almost evenly split between IgM and IgG channels.

20

### Statistical Analysis

Statistical analysis was done to identify antigen reactivities and groups of antigen reactivities that could distinguish between the different groups of subjects. The different comparisons were performed in the same manner, designated generically here as groups A and B, each consisting of  $n_A$  and  $n_B$  subjects (each subject being a separate microarray slide). To estimate the quality of the differentiation between groups A and B, a leave-one-out (LOO) procedure was applied (Duda et al., 2001, Pattern Classification. John Wiley and Sons, Inc., New York. 654 pp): One subject out of  $n_A+n_B$  was left aside, and the rest of the  $n_A+n_B-1$  subjects were used to select candidate antigens for separating groups A and B. A T-test between groups A and B (excluding the one subject) was applied, and the  $d$  antigens that passed a false discovery rate (FDR) threshold of 5% were selected (Benjamini et al., 1995, Journal of the Royal Statistical Society B. 57:289-300). These antigens were then used to classify the left-out subject using the K-nearest-neighbors algorithm (Duda et al., 2001), with  $K=3$ . More specifically, the left-out subject seeks its 3 nearest subject data

30

points in the d-dimensional space containing the other  $n_A+n_B-1$  subjects, and performs its classification according to the majority class of these 3 subjects. This procedure was repeated for all  $n_A+n_B$  subjects; the performance, which appears in Table 3 was simply the number of misclassifications, quantified as specificity (1-false positive rate) and sensitivity (1-false negative rate) measures.

The composition of the list of separating antigen reactivities can vary depending on the specific subject that is left out in the particular LOO cross-validation run. To further identify antigen reactivities that play an important role in separating groups A and B, the antigen reactivities that appeared in the candidate lists in at least 90% of the LOO tests was selected. The LOO procedure was repeated with each of these selected antigen reactivities, acting alone, by classifying the left-out subject using a simple threshold criterion: the reactivity values of the  $n_A+n_B-1$  training subjects that threshold value which maximized the specificity was found, and then classified the left-out point using this threshold. The average LOO specificity and sensitivity of this classification over the  $n_A+n_B$  subjects appear for each of the selected antigens in Tables 4 and 6. To test the performance of combinations of antigen reactivities, the particular combination was projected onto a one-dimensional space using principal component analysis (PCA), and the combination was treated as with the above single reactivities.

In addition to the LOO test, the subjects in groups A and B, were taken together with their list of differentiating antigen reactivities, and used to classify a test set C via three nearest neighbors. For example, the entire list of frequent antigen reactivities that separated healthy controls from SLE subjects in renal remission was used to classify the other two SLE groups – those in renal relapse and those without renal involvement. Figure 2 displays a projection of these antigen reactivities onto a 3-dimensional space using a principal component analysis (PCA) representation (Duda et al., 2001).

### EXAMPLE 1

Antigen microarray reactivities differentiate SLE subjects from healthy controls

Table 3 shows a global analysis of the 930 total antibody reactivities of the healthy control subjects compared with those of three SLE groups based on a leave one out (LOO) test: all SLE subjects; SLE subjects in renal remission; and SLE subjects with active lupus

nephritis. The average LOO sensitivity and specificity are presented for a K=3 nearest neighbor classifier.

The analysis shows that the microarray reactivities clearly separated the three groups of SLE subjects from the healthy controls. A comparison between healthy controls and SLE subjects without renal involvement does not appear in the table because no antigen exceeded an FDR level of 5% in some of the LOO tests. Moreover, the various sub-groups of SLE subjects, with the limited numbers available for testing, also could not be separated from one another because none of the antigen reactivities passed an FDR level of 5% in any of the LOO tests. .

**Table 3- Performance of the antigen microarray on different classification tasks**

| <b>Healthy controls compared to:</b>     | <b>Sensitivity</b> | <b>Specificity</b> |
|------------------------------------------|--------------------|--------------------|
| All SLE subjects                         | 90%                | 81%                |
| SLE subjects in renal remission          | 93%                | 100%               |
| SLE patients with active lupus nephritis | 86%                | 100%               |

## **EXAMPLE 2**

SLE subjects manifest both up-regulation and down-regulation of individual reactivities

Table 5 lists the particular antibody reactivities that distinguished all the SLE patients as a group from the healthy control subjects. Eight IgG antibody reactivities were up-regulated in the SLE group as compared to control: dsDNA, ssDNA, HyaluronicAcid, EBV, BMP4, F50, HGF and hsp60p18. Further, thirteen IgM antibody reactivities were down-regulated in the SLE group as compared to control: MPO, IGFBP1, CD99, Cardiolipin, actin, CMV, Collagen-III, Collagen-IV, HRP, RV, S100A4, CITED1 and FHIT.

Table 4 lists the particular antibody reactivities that distinguished all the SLE patients as a group from the healthy control subjects. Figure 1 shows the relative amounts of antibody reactivities to the antigens listed in Table 4 in each serum. The differences between the two groups for each of these antigens exceeded an FDR level of 5% ( $p < 0.0007$ ). Four IgG antibody reactivities were up-regulated in the SLE group: classic reactivities to dsDNA and ssDNA, reactivity to Epstein-Barr Virus (EBV), which has been

previously found to be strongly associated with SLE (Barzilai et al., 2007, Ann. N Y Acad. Sci. 1108:567-77), and a surprising reactivity to hyaluronic acid (Figure 1).

Table 4- Antibody reactivities that individually distinguish the healthy control subjects from all the SLE patients

| Antigen                      | Immunoglobulin Isotype | Sensitivity (%) | Specificity (%) |
|------------------------------|------------------------|-----------------|-----------------|
| <b>SLE Up-regulated</b>      |                        |                 |                 |
| dsDNA                        | IgG                    | 58              | 87              |
| ssDNA                        | IgG                    | 75              | 94              |
| HyaluronicAcid               | IgG                    | 86              | 86              |
| EBV                          | IgG                    | 70              | 88              |
| <b>SLE Down-regulated</b>    |                        |                 |                 |
| MPO                          | IgM                    | 78              | 88              |
| IGFBP1                       | IgM                    | 59              | 82              |
| CD99                         | IgM                    | 61              | 93              |
| Cardiolipin                  | IgM                    | 60              | 81              |
| <b>All eight antigens</b>    | IgM + IgG              | 93              | 88              |
| <b>All four IgG antigens</b> | IgG                    | 90              | 88              |
| <b>All four IgM antigens</b> | IgM                    | 68              | 88              |

Four novel antigen reactivities, all IgM, were found to be down-regulated in SLE: Insulin-like growth factor binding protein 1 (IGFBP1), CD99, Cardiolipin and myeloperoxidase (MPO). The IgM antibody reactivities of SLE subjects to these antigens tended to be low or undetectable compared with the healthy controls (Figure 1). In contrast to the decreased IgM reactivities to MPO and to cardiolipin, increased IgG antibodies to these antigens have been associated with SLE and other vasculitis-related diseases (Barzilai et al., 2007; Sen and Isenberg, 2003 Lupus 12:651-8; Love, P.E. and Santoro, S.A. 1990, Ann. Intern. Med. 112:682-98; Moreland et al., 1991. Clin. Immunol. Immunopathol. 60:412-8).

Table 4 shows that, except for IgG reactivity to hyaluronic acid, the other individual reactivities showed sensitivity for SLE of < 80%. However, the specificities of each reactivity, whether increased IgG or decreased IgM, were > 80%. The combination of all eight reactivities increased the sensitivity to > 90%; the combination of four IgG increased reactivities was more sensitive than the combination of the four IgM decreased reactivities: 90% compared with 68%, respectively. The specificities of each of the combined sets were equal at 88%.

Table 5- Antibody reactivities that individually distinguish the healthy control subjects from all the SLE patients

| Antigen                   | Immunoglobulin Isotype | Sensitivity (%) | Specificity (%) |
|---------------------------|------------------------|-----------------|-----------------|
| <b>SLE Up-regulated</b>   |                        |                 |                 |
| dsDNA                     | IgG                    | 58              | 87              |
| ssDNA                     | IgG                    | 75              | 94              |
| HyaluronicAcid            | IgG                    | 86              | 86              |
| EBV                       | IgG                    | 70              | 88              |
| BMP4                      | IgG                    | 38              | 88              |
| F50                       | IgG                    | 38              | 88              |
| HGF                       | IgG                    | 69              | 82              |
| hsp60p18                  | IgG                    | 49              | 87              |
| <b>SLE Down-regulated</b> |                        |                 |                 |
| MPO                       | IgM                    | 78              | 88              |
| IGFBP1                    | IgM                    | 59              | 82              |
| CD99                      | IgM                    | 61              | 93              |
| Cardiolipin               | IgM                    | 60              | 81              |
| actin                     | IgM                    | 58              | 77              |
| CMV                       | IgM                    | 53              | 87              |
| CollagenIII               | IgM                    | 64              | 83              |
| CollagenIV                | IgM                    | 57              | 77              |

|        |     |    |    |
|--------|-----|----|----|
| HRP    | IgM | 44 | 88 |
| RV     | IgM | 38 | 88 |
| S100A4 | IgM | 36 | 88 |
| CITED1 | IgM | 45 | 88 |
| FHIT   | IgM | 23 | 88 |

### EXAMPLE 3

#### SLE subjects in remission maintain an SLE signature

5

An important question is whether clinical renal remission is associated with a return of the SLE antibody pattern to a healthy state. Table 6 shows that SLE patients in clinical remission still maintained an SLE signature. These patients manifested significantly up-regulated IgG reactivities to the same four antigens that characterized the general set of SLE subjects: dsDNA, ssDNA, hyaluronic acid and EBV. Moreover, those in remission manifested down-regulation of three IgM reactivities, of which two were characteristic of the SLE group as a whole: decreased IgM reactivity to CD99 and to MPO were present in both groups, but those in remission manifested decreased IgM reactivity to collagen III rather than to cardiolipin and to IGFBP1 (Figure 1).

10

15

Table 6 also shows that combining the four increased IgG and the three decreased IgM reactivities led to 100% sensitivity and 94% specificity. Thus, a combination of reactivities may provide a higher degree of accuracy than any of the component reaction alone. Note too that the set of combined decreased IgM reactivities performed as well as did the set of combined increased IgM reactivities appears to be a characteristic of SLE.

20

Table 6- Antibody reactivities that individually distinguish the healthy control subjects from the SLE patients in renal remission

| Antigen                 | Ig Isotype | Sensitivity (%) | Specificity (%) |
|-------------------------|------------|-----------------|-----------------|
| <b>SLE Up-regulated</b> |            |                 |                 |
| ssDNA                   | IgG        | 87              | 94              |
| dsDNA                   | IgG        | 47              | 87              |
| HyaluronicAcid          | IgG        | 93              | 86              |

|                           |           |     |    |
|---------------------------|-----------|-----|----|
| EBV                       | IgG       | 73  | 88 |
| <b>SLE Down-regulated</b> |           |     |    |
| MPO                       | IgM       | 87  | 94 |
| Collagen III              | IgM       | 73  | 83 |
| CD99                      | IgM       | 77  | 93 |
| All seven antigens        | IgM + IgG | 100 | 94 |
| All four IgG antigens     | IgG       | 93  | 94 |
| All three IgM antigens    | IgM       | 93  | 94 |

#### EXAMPLE 4

The SLE remission signature also characterizes other SLE groups

5 To determine whether the list of antigen reactivities characteristic of SLE in remission might be applicable to SLE generally, we used the seven antigens that separated subjects in remission from healthy controls (Table 6) to classify the 14 SLE patients with active lupus nephritis and the 11 SLE patients without renal involvement.

10 These 25 SLE patients were classified via a three nearest neighbours algorithm, based on 15 SLE patients in remission and 16 healthy controls. Twenty-three of these 25 SLE subjects were correctly classified, generating a sensitivity of 92%. Only 2 out of the 26 SLE patients were misclassified (not shown).

15 Figure 2 displays a 3-dimensional PCA representation (projected from the space spanned by the 7 separating antigens) of healthy control subjects and those with various sub-groups of SLE. The healthy controls were clearly separated by the seven antigen reactivities from the SLE subjects in remission. Moreover, the individuals in long-term remission, in acute lupus nephritis, or without renal involvement were completely overlapping. In other words, the remission list of antigen reactivities in Table 3 constitutes an SLE antibody signature that includes SLE subjects with active lupus nephritis and those  
20 without renal involvement. Remarkably, the subject marked by a blue star, who was projected into the SLE domain of Figure 2; serum from this subject was obtained when she was in a healthy state and was negative for standard anti-DNA antibodies, but 8 months later she began suffering from non-specific symptoms and was eventually found, after 7 more months, to fulfill the criteria for a diagnosis of SLE. Thus the antigen-microarray  
25 signature also detects pre-clinical SLE.

## References

Sherer, Y., Gorstein, A., Fritzler, M.J., Shoenfeld, Y. 2004. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. *Semin. Arthritis. Rheum.* 34:501-37.

5 Criteria published by EM Tan et al: *Arthritis. Rheum.* 25:1271, 1982; updated by MC Hochberg, *Arthritis. Rheum.* 1997; 40:1725.

Quintana, F.J., Farez, M.F., Viglietta, V., Iglesias, A.H., Merbl, Y., Izquierdo, G., Lucas, M., Basso, A.S., Khoury, S.J., Lucchinetti, C.F., Cohen, I.R., Weiner, H.L. 2008. Antigen microarrays identify unique serum autoantibody signatures associated with  
10 different clinical forms and pathologic subtypes of multiple sclerosis. *Proc. Natl. Acad. Sci. U S A.* 105:18889-94.

Merbl, Y., Zucker-Toledano, M., Quintana, F.J., Cohen, I.R. 2007. Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. *J. Clin. Invest.* 117:712-8.

15 Barzilai, O., Sherer, Y., Ram, M., Izhaky, D., Anaya, J.M., Shoenfeld, Y. 2007 Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report. *Ann. N Y Acad. Sci.* 1108:567-77.

Manolova, I., Dancheva, M., Halacheva, K. 2002. Predominance of IgG1 and IgG3 subclasses of autoantibodies to neutrophil cytoplasmic antigens in patients with systemic  
20 lupus erythematosus. *Rheumatol. Int.* 21:227-33.

Sen, D., Isenberg, D.A. 2003. Antineutrophil cytoplasmic autoantibodies in systemic lupus erythematosus. *Lupus* 12:651-8.

Love, P.E., Santoro, S.A. 1990. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders.  
25 Prevalence and clinical significance. *Ann. Intern. Med.* 112:682-98.

Moreland, L.W., Gay, R.E., Gay, S. 1991. Collagen autoantibodies in patients with vasculitis and systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* 60:412-8.

Isenberg, D.A., Colaco, C.B., Dudeney, C., Todd-Pokropek, A., Snaith, M.L. 1986. The relationship of anti-DNA antibody idiotypes and anti-cardiolipin antibodies to disease  
30 activity in systemic lupus erythematosus. *Medicine* 65:46-55.

Li, Q.Z., Zhou, J., Wandstrat, A.E., Carr-Johnson, F., Branch, V., Karp, D.R., Mohan, C., Wakeland, E.K., Olsen, N.J. 2007. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. *Clin. Exp.*

Immunol. 147:60-70.

Li, Q.Z., Xie, C., Wu, T., Mackay, M., Aranow, C., Putterman, C., Mohan, C. 2005. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. J. Clin. Invest. 115:3428-39.

5 Quintana, F.J., Merbl, Y., Sahar, E., Domany, E., Cohen, I.R. 2006. Antigen-chip technology for accessing global information about the state of the body. Lupus 15:428-30.

Quintana, F.J., Hagedorn, P.H., Elizur, G., Merbl, Y., Domany, E., Cohen, I.R. 2004. Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes. Proc. Natl. Acad. Sci. U S A. 101  
10 Suppl 2: 14615-21.

Wold, R.T., Young, F.E., Tan, E.M., Farr, R.S. 1968. Deoxyribonucleic acid antibody: A method to detect its primary interaction with deoxyribonucleic acid. Science 161: 806.

Duda, R.O., Hart, P.E., Stork, D.G. 2001. Pattern Classification. John Wiley and  
15 Sons, Inc., New York. 654 pp.

Benjamini, Y., Hochberg, Y. 1995. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society B. 57:289-300.

20 The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the  
25 meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

**CLAIMS**

1. A method of diagnosing systemic lupus erythematosus (SLE) in a subject, the method comprising:

- 5 (i) determining the reactivity of IgG and IgM antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT, thereby determining the reactivity pattern of the sample to the plurality of antigens; and
- (ii) comparing the reactivity pattern of said sample to a control reactivity pattern;

10 wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the reactivity pattern of a control sample is an indication that the subject is afflicted with SLE.

2. The method of claim 1, wherein the plurality of antigens comprise at least three antigens.

15 3. The method of claim 1, wherein the plurality of antigens comprise at least four antigens.

4. The method of claim 1, wherein the plurality of antigens comprise at least five antigens.

5. The method of claim 1, wherein the plurality of antigens comprise at least six antigens.

6. The method of claim 1, wherein the plurality of antigens comprise at least seven  
20 antigens.

7. The method of claim 1, wherein the plurality of antigens comprise at least eight antigens.

8. The method of claim 1, wherein the plurality of antigens consist of IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin and collagen III.

25 9. The method of claim 1, comprising determining the reactivity of a plurality of IgG antibodies and a plurality of IgM antibodies in said sample to said plurality of antigens.

10. The method of claim 1, comprising determining the reactivity of IgG antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, BMP4, EBV, ssDNA, dsDNA, F50, HGF and HSP60p18.

30 11. The method of claim 10, comprising determining the reactivity of IgG antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: hyaluronic acid, EBV, ssDNA and dsDNA.

12. The method of any one of claims 10-11, wherein a significant up-regulation between

the reactivity pattern of said sample obtained from the subject compared to the reactivity pattern of a control sample is an indication that the subject is afflicted with SLE.

13. The method of claim 1, comprising determining the reactivity of IgM antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, IGFBP1, MPO, cardiolipin, Collagen III, collagen IV, actin, CMV, horseradish peroxide, RV, S100A4, CITED1 and FHIT.

14. The method of claim 13, comprising determining the reactivity of IgM antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, IGFBP1, MPO, cardiolipin and Collagen III.

15. The method of claim 13, comprising determining the reactivity of IgM antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, IGFBP1, MPO and cardiolipin.

16. The method of any one of claims 13-15, wherein a significant down-regulation between the reactivity pattern of said sample obtained from the subject compared to the reactivity pattern of a control sample is an indication that the subject is afflicted with SLE.

17. The method of claim 13, comprising determining the reactivity of IgM antibodies in the sample obtained from the subject to a plurality of antigens selected from the group consisting of: CD99, MPO and Collagen III.

18. The method claim 17, wherein a significant down-regulation between the reactivity pattern of said sample obtained from the subject compared to the reactivity pattern of a control sample is an indication that the subject is afflicted with SLE in renal remission.

19. A method of diagnosing SLE in a subject, the method comprising:

(i) determining the reactivity of antibodies in a sample obtained from the subject to a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, MPO, and collagen III, thereby determining the reactivity pattern of the sample to the plurality of antigens, and

(ii) comparing the reactivity pattern of said sample to a control reactivity pattern, wherein a significant difference between the reactivity pattern of said sample obtained from the subject compared to the control reactivity pattern is an indication that the subject is afflicted with SLE.

20. The method of claim 19, wherein determining the reactivity of antibodies in a sample comprises determining the reactivity of IgG and IgM antibodies in said sample.

21. The method of claim 19, wherein the plurality of antigens further comprises at least one

antigen selected from dsDNA, ssDNA, EBV and cardiolipin.

22. The method of any one of claims 1 or 19, wherein the sample is a serum sample.

23. The method of any one of claims 1 or 19, wherein the control is selected from the group consisting of a sample from at least one healthy individual, a panel of control samples from

5 a set of healthy individuals, and a stored set of data from control individuals.

24. The method of any one of claims 1 or 19, further comprising diluting the sample 1:10 before determining the reactivity of antibodies in the sample.

25. The method of any one of claims 1 or 19, wherein said plurality of antigens is used in the form of an antigen array.

10 26. A kit for the diagnosis SLE in a subject comprising: a plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, horseradish peroxide, HSP60p18, RV, S100A4, CITED1 and FHIT.

27. The kit of claim 26, wherein said kit is in the form of an antigen array.

15 28. The kit of claim 26, further comprising means for determining the reactivity of antibodies in a sample to the plurality of antigens.

29. The kit of claim 26, further comprising means for comparing reactivity patterns of antibodies in different samples to the plurality of antigens.

30. An antigen probe set comprising a plurality of antigen probes selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, HRP, HSP60p18, RV, S100A4, CITED1 and FHIT.

31. An article of manufacture comprising the antigen probe set of any claim 30.

32. Use of an antigen probe set for the preparation of a diagnostic composition for  
25 diagnosing SLE, the antigen probe set comprising the plurality of antigens selected from the group consisting of: IGFBP1, CD99, hyaluronic acid, EBV, ssDNA, dsDNA, MPO, IGFBP1, cardiolipin, Collagen III, collagen IV, actin, BMP4, CMV, F50, HGF, horseradish peroxide, HSP60p18, RV, S100A4, CITED1 and FHIT.

33. The use of claim 32, wherein the diagnostic composition is used for determining the  
30 reactivity of antibodies in a sample, thereby determining the reactivity pattern of the sample to the plurality of antigens, wherein a significant difference between the reactivity pattern of said sample compared to a reactivity pattern of a control sample is an indication for SLE.

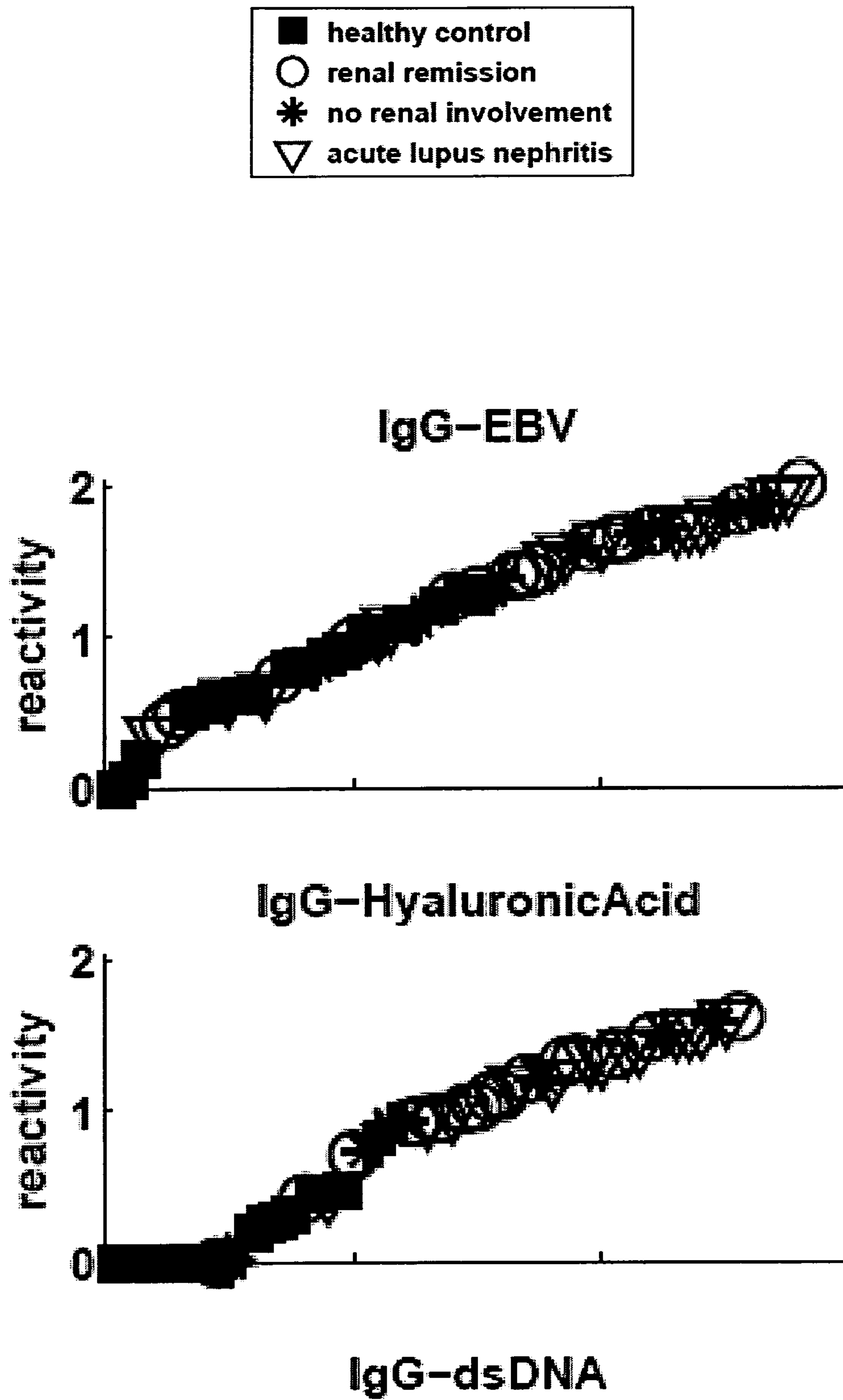


Figure 1

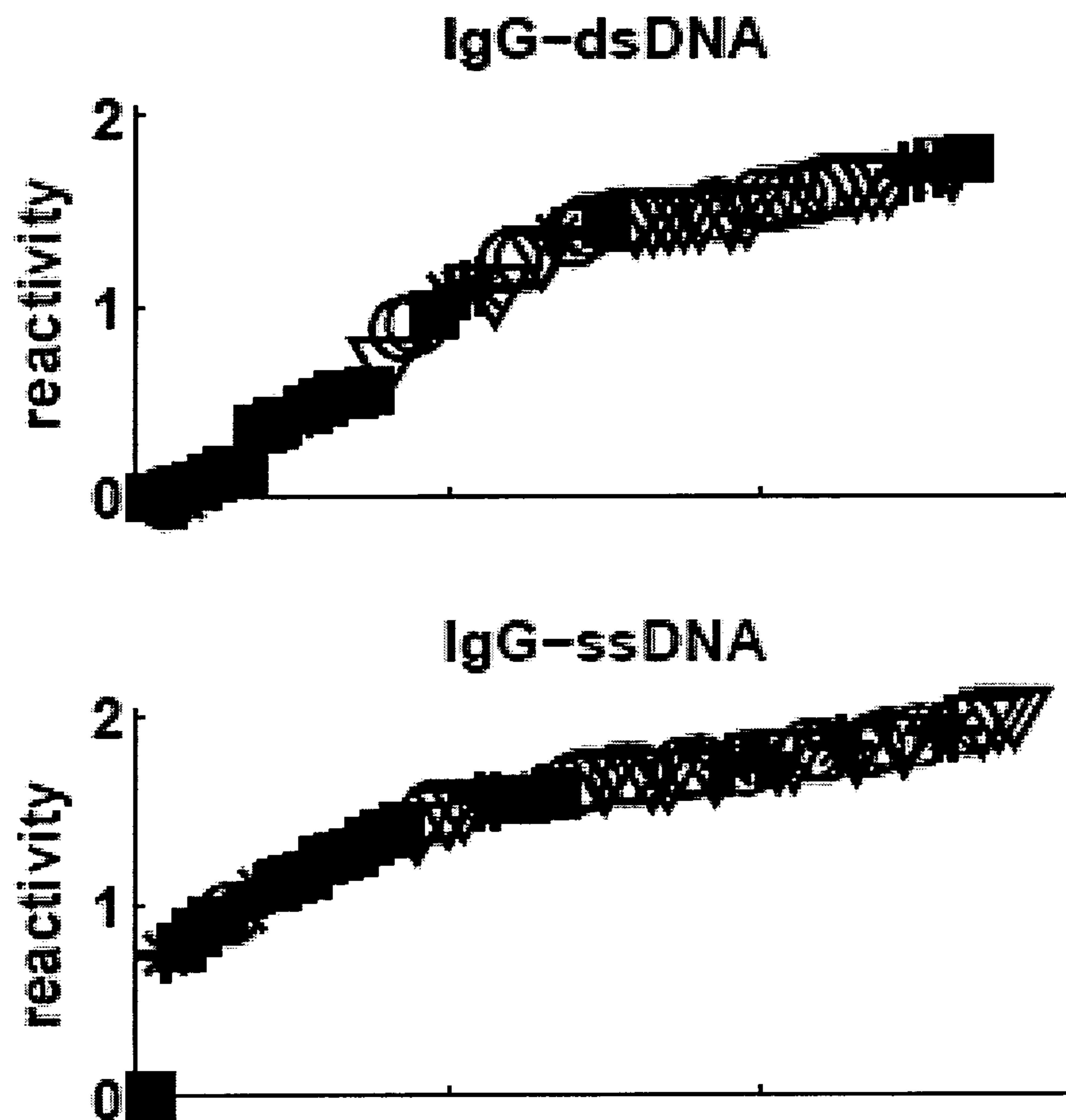


Figure 1 (Cont. 1)

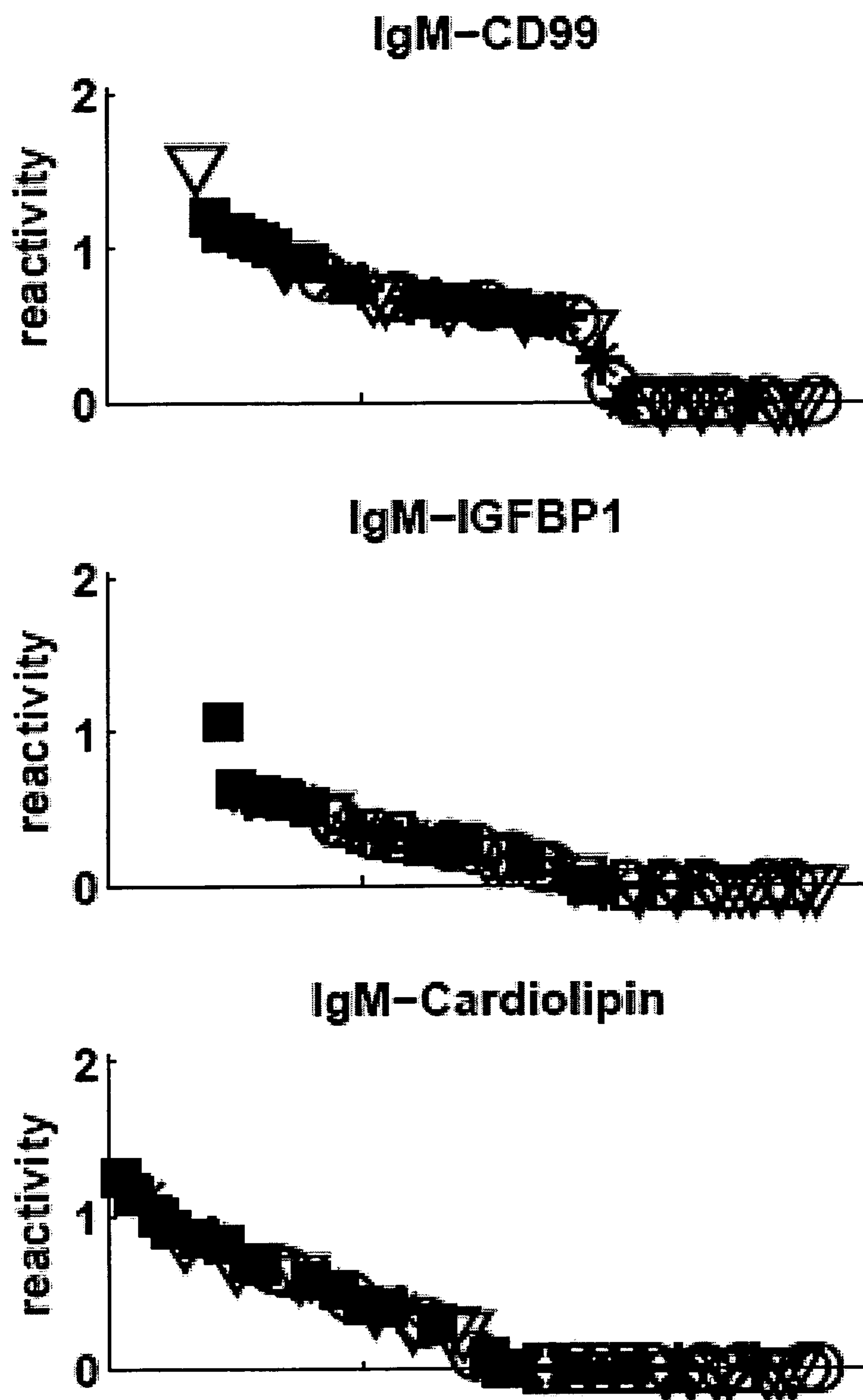


Figure 1 (Cont. 2)

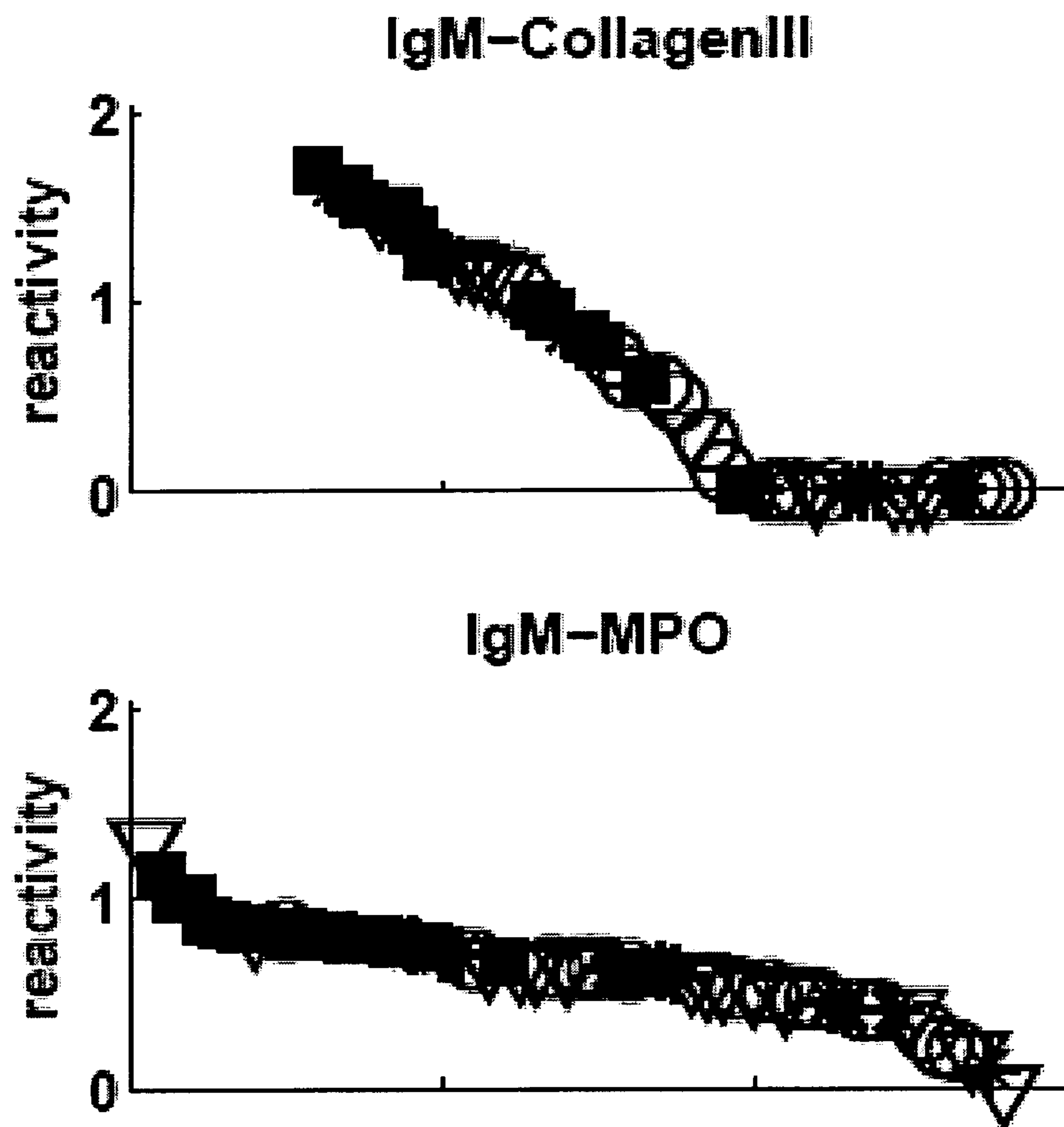


Figure 1 (Cont. 3)

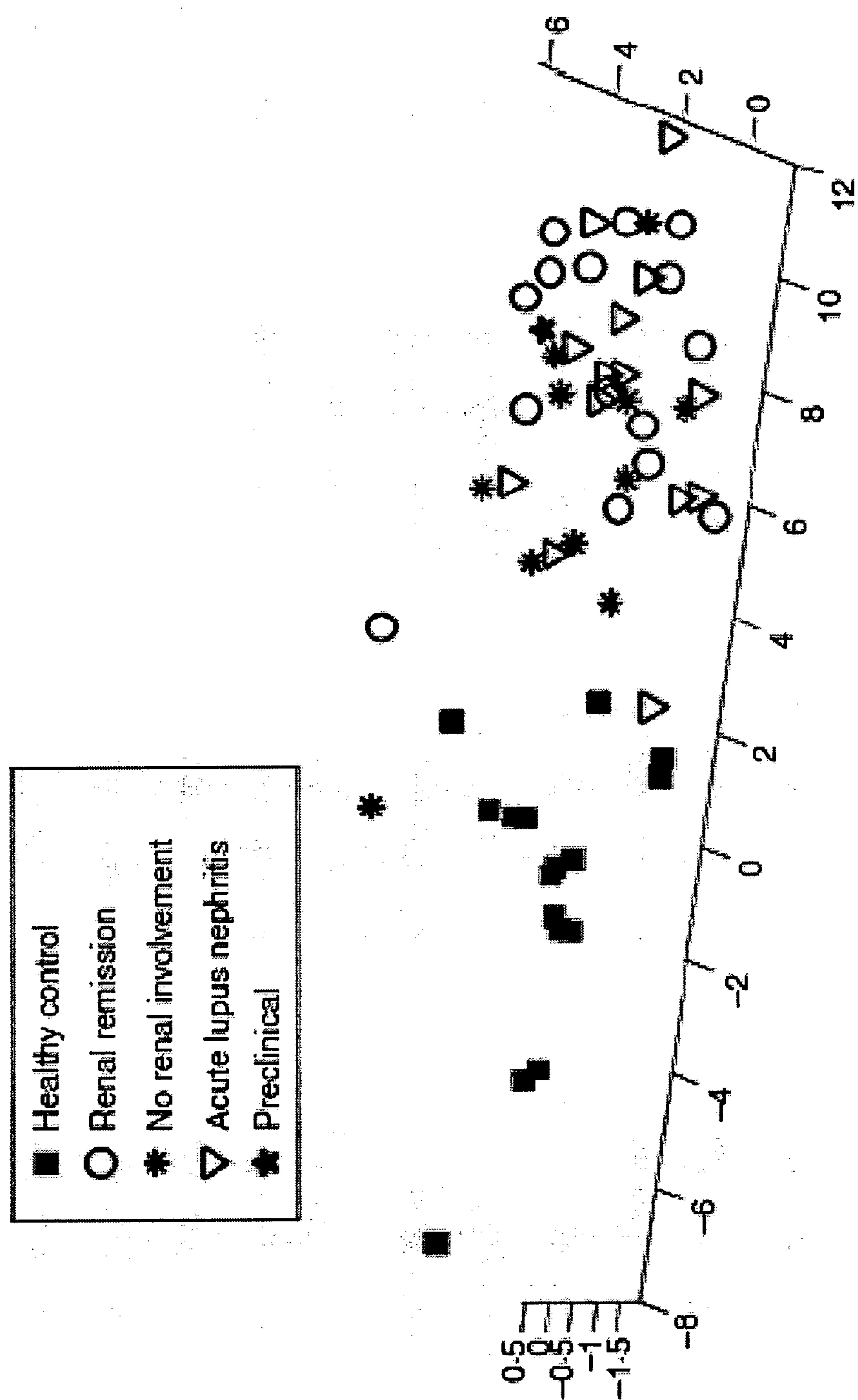


Figure 2