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(54) **Title:** COMPOSITIONS AND METHODS FOR DETECTING LOA LOA

(57) **Abstract:** Disclosed are methods of detecting the presence of *Loa loa* in a biological sample using one or more antigens, each having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20. Related compositions, specific binding partners, and test kits are also disclosed.



COMPOSITIONS AND METHODS FOR DETECTING LOA LOA

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/153,654, filed April 28, 2015, which is incorporated by reference in its entirety herein.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED
ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 56,829 Byte ASCII (Text) file named "723930SeqListing_ST25.txt," dated April 27, 2016.

BACKGROUND OF THE INVENTION

[0003] *Loa loa* (African eyeworm) is a filarial nematode estimated to infect 3-13 million people in central and western Africa, and is the causative agent for loiasis. Infected individuals may exhibit a range of relatively benign symptoms. However, those who are lifelong inhabitants of endemic areas are generally asymptomatic, even with the presence of large numbers of microfilariae circulating throughout their bloodstreams. Loiasis is, nevertheless, a public health concern due to the occurrence of greater than 1,000 severe adverse reactions (including fatal encephalopathies) in *Loa*-infected individuals receiving ivermectin as a result of mass drug administration (MDA) programs aimed at the elimination of onchocerciasis and lymphatic filariasis. Consequently, disruption of further MDA has occurred in certain communities where these diseases are co-endemic.

[0004] The mechanism of *Loa*-related post-ivermectin encephalopathy is unclear, but the risk appears to be greatest with patients having blood microfilariae (mf) counts greater than about >8000 mf/ml (for non-neurological adverse events) and >25000 mf/ml (for severe, neurological adverse events). Reducing or preventing disruption of MDA therefore requires that patients with high-levels of circulating *L. loa* microfilaremia be identified and excluded from treatment. Currently available methods for detecting *Loa loa* such as, for example, microscopic evaluation of blood samples, real time quantitative polymerase chain reaction (PCR)/real time (RT)-PCR, and loop-mediated isothermal amplification (LAMP) may require

sophisticated instrumentation, which may be impractical for widespread screening. Accordingly, there exists a need for improved methods for detecting the presence of *L. loa*.

BRIEF SUMMARY OF THE INVENTION

[0005] An embodiment of the invention provides a method of detecting the presence of *Loa loa* in a biological sample, the method comprising assaying the biological sample to determine the presence of one or more antigens in the biological sample, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least one of the antigens is indicative of the presence of *Loa loa* in the biological sample.

[0006] Another embodiment of the invention provides a method comprising assaying the biological sample to determine the presence of one or more antibodies in the biological sample, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least one of the antibodies is indicative of the presence of *Loa loa* in the biological sample.

[0007] Still another embodiment of the invention provides a composition comprising an immunologically-stimulatory concentration of one or more isolated or purified antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20 and a physiologically-acceptable carrier.

[0008] Still another embodiment of the invention provides a specific binding partner that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-20.

[0009] An embodiment of the invention provides a method for producing an antibody that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-20, the method comprising administering a composition comprising an immunologically-stimulatory concentration of the inventive antigen to an animal under conditions sufficient for the animal to develop an immune response to the antigen.

[0010] Another embodiment of the invention provides a test kit comprising (a) one or more specific binding partner(s), each of which specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20; (b) one or more substrate(s) onto which (a) is bound or affixed; (c) one or more reagent(s) for facilitating

binding of the amino acid sequence(s) to (a); and (d) one or more reagent(s) for detecting the amino acid sequence(s) specifically bound to (a).

[0011] Still another embodiment of the invention provides a test kit comprising (a) one or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20; (b) one or more substrate(s) onto which (a) is bound or affixed; (c) one or more reagent(s) for facilitating binding of one or more antibodies to (a); and (d) one or more reagent(s) for detecting the antibody or antibodies specifically bound to (a).

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0012] Figure 1 is a graph showing the change (Δ) in optical density (O.D.) as a function of known LOAG_17808 antigen concentrations ($\mu\text{g/ml}$) in the serum of *L. loa*-infected patients as measured by an enzyme-linked immunosorbent assay (ELISA). The stars represent the amount of antigen measured in two unrelated *L. loa*-infected patients.

[0013] Figures 2A and 2B are graphs showing the specificity and sensitivity of two antigens, LOAG_16297 (SEQ ID NO: 4) (A) and LOAG_17808 (SEQ ID NO: 14) (B), when used to measure IgG antibodies against these two Ruc-antigen fusion proteins in sera from *L. loa*-infected patients, uninfected control patients, or patients infected with *Wuchereria bancrofti* (Wb) or *Onchocerca volvulus* (Ov). The dashed lines indicate the cutoff value for positivity (800 LU for LOAG_16297 (A) and 3100 LU for LOAG_17808 (B)).

[0014] Figures 3A and 3B are graphs showing the percent (%) inhibition of LOAG_16297 (SEQ ID NO: 4) (A) or LOAG_17808 (SEQ ID NO: 14) (B) at various doses ($\mu\text{g/ml}$) when added to phosphate buffered saline (PBS) or human AB sera (ABS) at two different dilutions (ABS-1/5 or ABS-1/20). Data are expressed as percent inhibition in a luciferase immunoprecipitation system (LIPS)-based assay.

[0015] Figure 4 is a graph showing the percent (%) inhibition by various dosages ($\mu\text{g/ml}$) of LOAG_16297 (SEQ ID NO: 4) in a LIPS-based assay. The stars represent the values (concentrations) of the specific LOAG_16297 antigens in the serum of five *L. loa*-infected patients. The triangles represent the absence of LOAG_16297 in the serum of normal individuals that are not infected with *L. loa*.

[0016] Figure 5 is a graph showing the reactivities (measured as light units/ μL of serum ($\times 10^3$)) of the four fusion proteins LOAG_16297, LOAG_17808, LOAG_05915, and LOAG_18552 to their specific antibodies in Rb anti-protein serum, Rb anti-protein IgG, Rb

anti-mf IgG, or Rb pre-bleed. “Rb anti-protein serum” refers to antisera raised against the two most immunogenic peptides of each protein. “Rb anti-protein IgG” refers to the IgG purified from those antisera. “Rb anti-mf IgG” refers to purified IgG anti-*L. loa* mf somatic antigen. “Rb pre-bleed” refers to antisera collected prior to immunization. The protein names are indicated under the X-axis.

[0017] Figures 6A-6C are graphs showing the levels of IgG specific to LOAG_16297 (A), LOAG_17808 (B) and SXP-1 (C) as measured by light units (LU) (Log_{10} luminometer U/ μL) assessed by LIPS and compared between *Loa*-infected and uninfected controls. The horizontal solid line represents the median level for each group and the horizontal dotted line indicates the threshold of sensitivity/specificity of the assay determined by using a ROC analysis. Each individual is represented by a single dot with closed circles being used for the *Loa*-infected and the open circles for the uninfected individuals.

[0018] Figure 7 is a graph showing the percent inhibition as a function of spiked recombinant protein ($\mu\text{g/ml}$) in human AB serum for LOAG_16297 (squares) and LOAG_17808 (circles).

[0019] Figures 8A-8B are graphs showing the quantities of LOAG_16297 (A) and LOAG_17808 (B) estimated for 31 *L. loa*-, 15 *W. bancrofti*-, 15 *O. volvulus*-infected, and 25 uninfected (control) individuals extrapolated from standard curves as represented in Figure 7. The horizontal solid black line in each group indicates the geometric mean in ng/ml of protein, and each individual value is represented by an individual dot.

[0020] Figures 9A-9B are graphs showing the correlation between detected quantities of protein (estimated, ng/ml) in *L. loa* mf-infected individuals and the corresponding mf count ($\times 10^3/\text{ml}$).

DETAILED DESCRIPTION OF THE INVENTION

[0021] It has been discovered that twenty antigens having the amino acid sequences of SEQ ID NOs: 1-20, respectively, are derived from *Loa loa* microfilariae. SEQ ID NOs: 1-20 are hereinafter collectively referred to as “*Loa loa* antigens.”

[0022] It is believed that these *Loa loa* antigens are unique to the microfilariae of *Loa loa* and that these antigens are not found in related filarial parasites. It has also been discovered that these *Loa loa* antigens are present in biological samples from patients that are infected with *Loa loa* and are absent from the biological samples of patients that are not infected with

Loa loa. Accordingly, it is contemplated that these *Loa loa* antigens may be useful biomarkers for detecting and quantifying *Loa loa* in a biological sample.

[0023] The inventive methods and compositions provide many advantages. For example, the inventive methods and compositions may detect *Loa loa* more rapidly than other techniques of detecting *Loa loa* such as, for example, microscopic evaluation of blood samples, RT-PCR, and LAMP. In addition, the inventive methods and compositions may not require the sophisticated instrumentation required by other techniques such as, for example, microscopic evaluation of blood samples, RT-PCR, and LAMP. Accordingly, the inventive methods and compositions may be more practical than these techniques for wide-spread screening, particularly in resource-limited geographical regions.

[0024] An embodiment of the invention provides a method of detecting the presence of *Loa loa* in a biological sample. The method may comprise assaying the biological sample to determine the presence of one or more antigens in the biological sample, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20. In an embodiment of the invention, each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14. The assaying may involve using a specific binding partner that preferentially binds to one of the inventive *Loa loa* antigens, or two or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) binding partners that each preferentially binds to a different inventive *Loa loa* antigen. For example, the assaying can involve the use of a first specific binding partner that can preferentially, e.g., specifically, bind to an antigen having the amino acid sequence of SEQ ID NO: 4, a second specific binding partner that can preferentially/specifically bind to an antigen having the amino acid sequence of SEQ ID NO: 14, or both of said first and said second specific binding partners, and so on. In this regard, in an embodiment of the invention, the method comprises assaying the biological sample to determine the presence of two or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 in the biological sample, wherein the presence of at least two of the antigens is indicative of the presence of *Loa loa* in the biological sample. In an embodiment of the invention, each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-14. In an embodiment of the invention, the two antigens have the amino acid sequences of SEQ ID NOs: 4 and 14, respectively.

[0025] In an embodiment of the invention, the method comprises (a) contacting the biological sample with one or more specific binding partner(s), each of which specifically

binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming one or more complexes; and (b) detecting the one or more complexes, wherein detection of the one or more complexes is indicative of *Loa loa* in the biological sample. In an embodiment of the invention, each specific binding partner binds to a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-14.

[0026] In still another embodiment of the invention, method comprises (a) contacting the biological sample with two or more specific binding partner(s), each of which specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming two or more complexes; and (b) detecting the two or more complexes, wherein detection of the two or more complexes is indicative of *Loa loa* in the biological sample. In an embodiment of the invention, each specific binding partner specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14. In an embodiment of the invention, the two specific binding partners specifically bind to antigens having the amino acid sequences of SEQ ID NOs: 4 and 14, respectively.

[0027] Contacting may comprise physically contacting the biological sample with the specific binding partner(s) under conditions that facilitate the formation of one or more complexes including the binding partner(s) bound, respectively, to any of the *Loa loa* antigens described herein. The method may further comprise washing any unbound specific binding partner and any unbound antigen from the one or more complexes.

[0028] Detecting the one or more complexes can be carried out through any number of ways known in the art. For instance, the one or more binding partners can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0029] Using such specific binding partners that can specifically/preferentially bind to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 (or two or more specific binding partners, each specifically binding to a different *Loa loa* antigen), the assaying can use immunochemical methods to detect the presence of one (or two or more) antigens of SEQ ID NOs: 1-20 in the biological sample. Such types of assays are known to persons of ordinary skill in the art and include methods such as immunoprecipitation, immunonephelometry, radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescent immunoassay (FIA), luciferase immunoprecipitation system

(LIPS), and lateral flow immunochromatographic assay (also referred to as “lateral flow test”) and the like.

[0030] Preferably, the assay employed in the inventive method using one or more specific binding partner(s) is an enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA) (e.g., sandwich ELISA). In this regard, an embodiment of the invention provides a method comprising (a) contacting the biological sample with a first specific binding partner that specifically binds to one of the antigens, thereby forming a first complex; (b) contacting the first complex with a second specific binding partner that specifically binds to the first complex, thereby forming a second complex; and (c) detecting the second complex, wherein detection of the second complex is indicative of the presence of *Loa loa* in the biological sample. In an embodiment of the invention, the first specific binding partner is a first antibody, or an antigen binding fragment thereof, and the second specific binding partner is a second antibody, or an antigen binding fragment thereof, as described herein with respect to other aspects of the invention.

[0031] The contacting of the biological sample with a first specific binding partner and forming a first complex may be carried out as described herein with respect to other aspects of the invention. Contacting the first complex with a second specific binding partner may comprise physically contacting the first complex with the second specific binding partner under conditions that facilitate the formation of a second complex including the second binding partner bound to the first complex. The method may further comprise washing any unbound binding partner and any unbound antigen from the second complex. Detecting the second complex may be carried out as described herein with respect to other aspects of the invention.

[0032] The presence of one (or more) antigens of SEQ ID NOs: 1-20 in the biological sample indicates that *Loa loa* is present in the biological sample and in the mammal from which the biological sample was obtained. In an embodiment of the invention, the presence of one (or more) antigens of SEQ ID NOs: 1-14 in the biological sample indicates that *Loa loa* is present in the biological sample and in the mammal from which the biological sample was obtained.

[0033] The biological sample may be any suitable biological sample from any mammal. In an embodiment of the invention, the biological sample is whole blood, serum, plasma, urine, or saliva.

[0034] As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human. Preferably, the biological sample is a human biological sample.

[0035] A “specific binding partner” is a molecule that can bind with measurably higher affinity to one of the *Loa loa* antigens than to other molecules. In an embodiment of the invention, the specific binding partner is an antibody (also referred to herein as an “immunoglobulin”) or an antigen-binding fragment of the antibody. The antibody for use in the inventive method can be of any type. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form.

[0036] In an embodiment, the specific binding partner is an antigen binding fragment of any of the antibodies described herein. The antigen binding fragment can be any fragment of the antibody that has at least one antigen binding site. In an embodiment, the antigen binding fragment is a Fab fragment (Fab), F(ab')₂ fragment, diabody, triabody, tetrabody, single-chain variable region fragment (scFv), or disulfide-stabilized variable region fragment (dsFv). A single-chain variable region fragment (scFv), which is a truncated Fab fragment including the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic antigen, can be generated using routine recombinant DNA technology techniques (see, e.g., Murphy et al. (eds.), *Janeway's Immunobiology*, 8th Ed., Garland Science, New York, NY (2011)). Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology. The antibody fragments of the invention, however, are not limited to these exemplary types of antibody fragments.

[0037] It is contemplated that a mammal infected with *Loa loa* may produce one or more antibodies, each of which specifically binds to an antigen having a different amino acid

sequence selected from the group consisting of SEQ ID NOs: 1-20. It is contemplated that a mammal infected with *Loa loa* may produce one or more antibodies, each of which specifically binds to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14. Accordingly, it is believed that detecting antibodies in the biological sample that specifically bind to one or more of the *Loa loa* antigens described herein may indicate the presence of *Loa loa* in the biological sample.

[0038] In this regard, another embodiment of the invention provides a method of detecting the presence of *Loa loa* in a biological sample, the method comprising assaying the biological sample to determine the presence of one or more antibodies in the biological sample, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least one of the antibodies is indicative of the presence of *Loa loa* in the biological sample. In an embodiment of the invention, each antibody specifically binds to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14.

[0039] The assaying for the presence of antibodies may be carried out as described herein with respect to other aspects of the invention. In an embodiment of the invention, the method comprises (a) contacting the biological sample with one or more antigens, each having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming one or more complexes with one or more antibodies, each antibody specifically binding to a different antigen, and (b) detecting the one or more complexes, wherein detection of the one or more complexes is indicative of *Loa loa* in the biological sample. In an embodiment of the invention, each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14. The contacting may be carried out as described herein with respect to other aspects of the invention. The method may further comprise washing unbound antibody or unbound antigen, as described herein with respect to other aspects of the invention.

[0040] Detecting the complex can be carried out through any number of ways known in the art. For instance, the inventive *Loa loa* antigens can be labeled with a detectable label as described herein with respect to other aspects of the invention.

[0041] Two or more antibodies, each antibody binding to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, may be detected in the biological sample. In this regard, an embodiment of the invention provides a method comprising assaying the biological sample to determine the presence of two or more antibodies in the

biological sample, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least two of the antibodies is indicative of the presence of *Loa loa* in the biological sample. In an embodiment of the invention, each antibody binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs 1-14. In an embodiment of the invention, the two antibodies specifically bind to antigens having the amino acid sequences of SEQ ID NOs: 4 and 14, respectively.

[0042] In an embodiment of the invention, the antigen is a first antigen, and the method further comprises (a) contacting the biological sample with a second antigen having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming a second complex with a second antibody that specifically binds to the second antigen, and (b) detecting the second complex, wherein detection of the second complex is indicative of *Loa loa* in the biological sample, and wherein the first and second antigens have different amino acid sequences. In an embodiment of the invention, the method comprises contacting the biological sample with a second antigen having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14. In an embodiment of the invention, the two antigens have the amino acid sequences of SEQ ID NOs: 4 and 14, respectively. The contacting and detecting may be carried out as described herein with respect to other aspects of the invention.

[0043] In an embodiment of the invention, the methods of detecting *Loa loa* described herein may further comprise quantifying the number of *Loa loa* organisms present in the biological sample. For example, the method may comprise quantifying the number of *Loa loa* microfilariae in the biological sample. Quantification of the *Loa loa* may be carried out using routine techniques. In an embodiment of the invention, quantifying the *Loa loa* comprises determining the amount of detectable label present. For example, the method may comprise determining the amount of fluorescence or luminescence provided by the detectable label. In an embodiment of the invention, quantification may comprise carrying out any of the methods of detecting *Loa loa* described herein in (1) a test biological sample in which the quantity of *Loa loa* is unknown and (2) a positive control biological sample having a known quantity of *Loa loa*. The quantity of *Loa loa* in the test biological sample may be estimated by comparing the quantity of label detected in the test biological sample to that detected in the positive control biological sample.

[0044] In another aspect, the invention provides reagents for generating antibodies that can specifically (e.g., preferentially) bind to any one of SEQ ID NOs: 1-20. In this respect, the invention provides a composition comprising an immunologically-stimulatory concentration of at least one isolated or purified antigen, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20 and a physiologically-acceptable carrier. An embodiment of the invention provides a composition comprising an immunologically-stimulatory concentration of at least one isolated or purified antigen, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-14 and a physiologically-acceptable carrier. The composition can be administered to an animal (typically a mouse, goat, rabbit, or other animal commonly employed for generating antibodies). The immunologically-stimulatory concentration of the composition is sufficiently concentrated to challenge the immune system of the animal with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of SEQ ID NOs: 1-20 present in the composition. The physiological carrier can be a buffered saline or other media typically employed to administer antigenic substances for the production of antibodies. The composition also can comprise one or more adjuvants to assist the animal in mounting an immune reaction to one or more of SEQ ID NOs: 1-20 present in the composition to increase the likelihood that antibodies capable of specifically binding to such antigens will be generated. In an embodiment of the invention, the composition also can comprise one or more adjuvants to assist the animal in mounting an immune reaction to one or more of SEQ ID NOs: 1-14.

[0045] Many assays for determining an immunologically-stimulatory concentration are known in the art. For purposes of the invention, an assay, which comprises comparing the extent to which antibodies are secreted by B cells in the mammal upon administration of a given concentration of the one or more of SEQ ID NOs: 1-20, respectively, to a mammal among a set of mammals in which each is given a different concentration of the one or more of SEQ ID NOs: 1-20, respectively, could be used to determine a starting concentration to be administered to a mammal. The extent to which antibodies are secreted by B cells upon administration of a certain concentration can be assayed by methods known in the art.

[0046] Thus, using such immunological compositions, the invention provides a method of producing one or more antibodies, each antibody specifically (e.g., preferentially) binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 by administering (e.g., by intravenous injection or other suitable route) the

composition comprising the immunologically-stimulatory concentration of the one or more of SEQ ID NOs: 1-20, respectively, and a physiologically-acceptable carrier to an animal under conditions sufficient for the animal to develop an immune response to the one or more of SEQ ID NOs: 1-20, respectively. An embodiment of the invention provides a method of producing one or more antibodies, each antibody specifically (e.g., preferentially) binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14 by administering (e.g., by intravenous injection or other suitable route) the composition comprising the immunologically-stimulatory concentration of the one or more of SEQ ID NOs: 1-14, respectively, and a physiologically-acceptable carrier to an animal under conditions sufficient for the animal to develop an immune response to the one or more of SEQ ID NOs: 1-14, respectively. In this context, the animal's immune response includes the generation of one or more antibodies, each antibody specifically (e.g., preferentially) binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20.

[0047] Standard immunological techniques can be employed in producing antibodies that can specifically (e.g., preferentially) bind to any one of SEQ ID NOs: 1-20, respectively, in accordance with the present invention. For example, once the animal is inoculated and has developed the immune response (producing one or more antibodies, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20), serum can be harvested from the animal which comprises the antibody or antibodies of interest. The serum can be further processed, if desired, to concentrate or stabilize the antibody or antibodies. The resulting composition is typically a polyclonal antibody composition.

[0048] Alternatively, once the animal is inoculated and has developed the immune response (producing one or more antibodies, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 or producing one or more antibodies, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14), one or more splenocytes can be harvested from the animal, which can then be fused with one or more immortal cell(s) (e.g., myeloma cells) to form one or more hybridomas. The hybridoma is then cultured (and typically proliferated into a population), such that it then secretes the antibodies that can specifically (e.g., preferentially) bind to one or more of SEQ ID NOs: 1-20, respectively, into the culture media in which the hybridoma is grown. The

culture medium can then be harvested and further processed, if desired, to concentrate or stabilize the antibody or antibodies. Also, the hybridomas can be cultured initially, or subcultured, at a sufficiently dilute density to establish clonal populations, which can facilitate the production of monoclonal antibodies. Standard hybridoma methods are described in, e.g., Murphy, *supra*, and *Antibodies: A Laboratory Manual*, 2nd Ed., CSH Press (2013).

[0049] The affinity of binding of the resulting antibodies can be assessed, for example, by exposing a substrate coated or impregnated with one of SEQ ID NOs: 1-20 to the putative specific binding partner (e.g., an antibody) and exposing a negative control (e.g., a blank substrate and/or one coated with or impregnated with another molecule (e.g., albumin)) to the putative specific binding partner under like conditions. It will be understood that the substrate(s) can be the same, such as a Western blot having spots or bands of several molecules. After exposure to the putative specific binding partner, the substrate(s) can be washed to remove non-specific binding and then the presence of the specific binding partner bound to one of SEQ ID NOs: 1-20 can be verified, e.g., by using a labeled secondary antibody or other suitable technique.

[0050] The antibodies produced in accordance with the present invention can be derivitized to produce other specific binding partners for specifically binding one of SEQ ID NOs: 1-20 (e.g., an antigen binding portion of the antibody as described herein with respect to other aspects of the invention). Also, if desired, the antibodies and other specific binding partners that can specifically bind one of SEQ ID NOs: 1-20 can be bound to or conjugated with a detectable label as described herein with respect to other aspects of the invention.

[0051] Phage display furthermore can be used to generate an antibody. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular biology and recombinant DNA techniques. See, for instance, Green et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 4th Edition, Cold Spring Harbor Laboratory Press, New York (2012) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, NY (2007). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete or partial antibody is reconstituted comprising the selected variable domain. Nucleic acid sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that antibodies

having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Murphy et al., *supra*).

[0052] Another embodiment of the invention provides a specific binding partner that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-20. Another embodiment of the invention provides a specific binding partner that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-14. Preferably, the specific binding partner is an antibody, or antigen-binding fragment thereof.

[0053] The inventive antibody (or antibodies) and other specific binding partner(s) for specifically binding one of SEQ ID NOs: 1-20 can be employed as reagents in standard immunochemical assays for the detection of SEQ ID NOs: 1-20, respectively, such as from a biological sample as described herein. Thus, another embodiment of the present invention provides a composition comprising one or more antibodies or other specific binding partner(s) that can specifically (e.g., preferentially) bind to SEQ ID NOs: 1-20, respectively, in isolated form or including a carrier. Another embodiment of the present invention provides a composition comprising one or more antibodies or other specific binding partner(s) that can specifically (e.g., preferentially) bind to SEQ ID NOs: 1-14, respectively, in isolated form or including a carrier. The carrier can be aqueous and include buffers, preservatives, chelating agents, if desired, to enhance stability and to facilitate their use in immunochemical assays.

[0054] The inventive antigens, antibodies, and specific binding partners described herein can be of synthetic or natural origin, and can be isolated or purified to any degree. The terms “isolated” and “purified,” as used herein, means having been removed from its natural environment. The term “purified” or “isolated” means having been increased in purity and does not require absolute purity or isolation; rather, it is intended as a relative term. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

[0055] An embodiment of the invention also provides a test kit comprising the inventive antibody (or antibodies) and other specific binding partner(s) that can specifically (e.g., preferentially) bind to SEQ ID NOs: 1-20, respectively. An embodiment of the invention also provides a test kit comprising the inventive antibody (or antibodies) and other specific binding partner(s) that can specifically (e.g., preferentially) bind to SEQ ID NOs: 1-14, respectively. The test kit can include, for example, one or more substrate(s) onto which the

specific binding partner(s) is/are bound or affixed, one or more reagent(s) for facilitating binding of one or more of SEQ ID NOs: 1-20 (or one or more of SEQ ID NOs: 1-14) within a sample to the specific binding partner(s), one or more reagent(s) for detecting the amino acid sequence(s) specifically bound to the specific binding partner(s) (e.g., one or more secondary antibody(ies) that can specifically bind to the antigen or specific binding partner(s), which can be conjugated to an enzymatic substrate moiety, fluorescent moiety, or radioactive moiety, as well as suitable enzymes and apparatus for detecting the fluorescence or radioactivity, respectively), and other materials and reagents commonly employed in immunochemical diagnostic test kits and apparatus.

[0056] Another embodiment of the invention also provides a test kit comprising one or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 that can specifically bind to one or more antibodies in a biological sample. Another embodiment of the invention also provides a test kit comprising one or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14 that can specifically bind to one or more antibodies in a biological sample. The test kit can include, for example, one or more substrate(s) onto which the antigen(s) is/are bound or affixed, one or more reagent(s) for facilitating binding of one or antibodies within a sample to one or more of SEQ ID NOs: 1-20, respectively, one or more reagent(s) for detecting the antibody specifically bound to the antigen (e.g., one or more secondary antibody (antibodies)) that can specifically bind to the antigen or antibody, as described herein with respect to other aspects of the invention, and other materials and reagents commonly employed in immunochemical diagnostic test kits and apparatus.

[0057] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLES

[0058] The following materials and methods were employed for the experiments described in Examples 1-8.

Study Population and samples

[0059] Samples were collected from subjects as part of registered protocols approved by the Institutional Review Boards of the National Institute of Allergy and Infectious Diseases

for the filarial-infected patients (NCT00001345) and for the healthy donors (NCT00090662). Written informed consent was obtained from all subjects.

[0060] Urine from one microfilaremic (17,000 mf/ml) *L. loa*-infected patient assessed at the NIH Clinical Center and one normal North American donor (who had never traveled outside the USA) was used for the profiling of specific *L. loa* mf proteins by using RPLC-MS/MS (reverse phase liquid chromatography tandem mass spectrometry).

[0061] Plasma samples used to validate the utility of potential biomarkers were from *L. loa*-infected individuals (n=31 [26 being microfilaremic and 5 amicrofilaremic]). Samples used as controls included subjects with *W. bancrofti* (mf+; n=15) from India and the Cook Islands (both non-endemic for *L. loa*), those with *O. volvulus* (mf+; n=15) infection from Ecuador (non-endemic for *L. loa*) and those from North America (n=31) that had no history of exposure to filarial or other helminths and who had never traveled outside of North America. The parasitological diagnosis of all infections was made based on the demonstration of mf in the blood (for *W. bancrofti* and *L. loa*) or in the skin (for *O. volvulus*) using standard techniques (Moody et al., *Clin. Lab. Haematol.*, 22: 189-201 (2000); Dickerson et al., *J. Parasitol.*, 76: 829-833 (1990)) or by finding adult parasites in the tissues (e.g. the eye for *L. loa*).

Sample preparation prior to mass spectrometric analysis

[0062] Urine samples were processed according to a workflow adapted from Nagaraj et al., *J. Proteome Res.*, 10: 637-645 (2011). Briefly, urine samples were centrifuged for 15 minutes (mins) at 4 °C and the supernatant was concentrated using a spin-filter with a molecular weight cut off 3 KDa. Proteins were precipitated by acetone precipitation and subsequently treated in 10 mM Tris-HCl at 95 °C for 5 mins. The samples were then reduced, alkylated and double digested with Lys-C in combination with trypsin overnight at 37 °C. Tryptic peptides were further desalted, lyophilized and reconstituted in 25% acetonitrile with 0.1% formic acid and further fractionated using strong cation exchange (SCX) chromatography. The SCX fractions of the urine samples were pooled into 32 fractions, lyophilized and reconstituted in 0.1% TFA (trifluoroacetic acid) to be analyzed by LCMS (liquid chromatography–mass spectrometry).

Nanobore reversed-phase liquid chromatography tandem MS

[0063] Nanobore RPLC-MS/MS was performed using an Agilent 1200 nanoflow LC system coupled online with a LTQ ORBITRAP VELOS mass spectrometer. The RPLC column (75 μm i.d. x 10 cm) were slurry-packed in-house with 5 μm , 300 Å pore size C-18 stationary phase into fused silica capillaries with a flame pulled tip. The mass spectrometer was operated in a data-dependent mode in which each full MS scan was followed by twenty MS/MS scans wherein the twenty most abundant molecular ions were dynamically selected for collision-induced dissociation (CID) using a normalized collision energy of 35%.

Protein identification and quantification

[0064] The RPLC-MS/MS data were searched using SEQUEST software through Bioworks interface against a *L. loa* database downloaded from Broad Institute (version 2.2). Dynamic modifications of methionine oxidation as well as fixed modification of carbamidomethyl cysteine were also included in the database search. Only tryptic peptides with up to two missed cleavage sites meeting a specific SEQUEST scoring criteria [$\Delta C_n \geq 0.1$ and charge state dependent cross correlation ($X_{\text{corr}} \geq 1.9$ for $[M+H]^1+$, ≥ 2.2 for $[M+2H]^2+$ and ≥ 3.5 for $[M+3H]^3+$] were considered as legitimate identifications.

Transcriptomics data

[0065] mRNA expression levels (putative proteins) of the mf state of *L. loa* were obtained using RNAseq as part of the *L. loa* genome project described in Desjardins et al., *Nat. Genet.*, 45: 495-500 (2013).

Protein/peptide selection for immunoassays

[0066] *L. loa* mf proteins identified only in the infected urine (absent in the uninfected urine) were down-selected for immunoassays based on comparison of sequence homologies against human, *L. loa* and other related filarial species (*B. malayi*, *O. volvulus*, and *W. bancrofti*) or any other relevant nematode for which genome is available.

[0067] Proteins that showed no or little homology to non-*Loa* sequences were selected for identification of immunogenic peptides using PROTEAN software (Lasergene Suite). Among these, the 2 peptides that were potentially the most immunogenic and *Loa*-specific

(no significant hit to human or other filarial nematodes) per protein were chosen. These peptides were synthesized by the NIAID Peptide Facility as unconjugated free peptides and conjugated to KLH (keyhole limpet hemocyanin), the latter used to produce specific polyclonal antibodies in rabbits.

Generation of rabbit polyclonal antibodies

[0068] KLH-conjugated peptides were used to raise polyclonal antisera in rabbits using standard protocols as described in (Suarez-Pantaleon et al., *Anal. Chim. Acta.*, 761: 186-193 (2013)). In addition, polyclonal antisera were raised against a somatic extract of *L. loa* mf using the same standardized protocols. After assessing the reactivity of each of the antisera to its appropriate free peptide by ELISA, the IgG was purified from the sera using Protein A/G (Pierce, Rockford, IL, USA) columns. These purified IgG antibodies were used as capture antibodies in the luciferase immune-precipitation systems (LIPS) assay for antigen detection.

Fusion proteins and COS-1 cells transfection

[0069] Fusion proteins were made for each of the *in silico* selected proteins by cloning the full-length gene expressing the protein of interest into a FLAG-epitope-tagged mammalian *Renilla reniformis* luciferase (Ruc)-containing expression vector pREN2 (Burbelo et al., *BMC Biotechnol.*, 5: 22 (2005)). Extracts (lysates) containing the light-emitting Ruc-antigen fusions were prepared from 100-mm² dishes of 48-hours transfected Cos-1 cells as previously described (Burbelo et al., *BMC Biotechnol.*, 5: 22 (2005); Burbelo et al., *J. Vis. Exp.*, doi:10.3791/1549 (2009)) and frozen until use for LIPS.

LIPS-based antibody and antigen detection systems

[0070] For evaluating antibody titers, a standard LIPS antibody-based assay was used (Burbelo et al., *J. Clin. Microbiol.*, 46: 2298-2304 (2008); Burbelo et al., *J. Vis. Exp.*, doi:10.3791/1549 (2009); Burbelo et al., *Transl. Res.*, 165: 325-335 (2015)). Briefly, 100 µl of the assay master mix (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1% Triton X-100), 1 µl of undiluted plasma/serum, and 2x10⁶ luminescence units (LU) of the Ruc-antigen fusion protein were added to each well of a 96-well polypropylene plate. This plate was then incubated for 10 minutes at room temperature. Next, 7 µl of a 30% suspension of

ULTRALINK protein A/G beads (Pierce, Rockford, IL, USA) in phosphate-buffered saline (PBS) was added to the bottom of a 96-well filter high throughput-screening plate (Millipore, Bedford, MA, USA). The 100- μ l antigen-antibody reaction mixture from each microtiter well of the 96-well polypropylene plate was then transferred to the well of the filter plate which was further incubated for 10-15 minutes at room temperature. The filter plate containing the mixture was then applied to a vacuum manifold. The retained protein A/G beads were washed with the assay master mix and with PBS (pH = 7.4) and the plate was blotted and LU measured in a Berthold LB 960 Centro microplate luminometer, using a coelenterazine substrate mixture (Promega, Madison, WI, USA).

[0071] For quantifying antigens, the original LIPS antibody-testing format was modified for use in a competitive LIPS assay. In competitive LIPS antigen detection, the *Renilla* luciferase (Ruc) fusion constructs of the antigen of interest are incubated with serum containing unfused antigen. These antigens are then immobilized on agarose beads containing antigen-specific IgG. After washing, the amount of specific antigen present is determined by the inhibition of the Ruc fusion construct by the unfused antigen after adding luciferase substrate.

[0072] Having first coupled the purified antigen-specific IgG to ULTRALINK beads (Pierce, Rockford, IL, USA), 5 μ l of a 50% suspension (in PBS) of these beads (specific IgG-ULTRALINK beads) were added to the bottom of a 96-well filter plate. Glycine-treated plasma/sera (Henrard et al., *J. Clin. Microbiol.*, 33: 72-75 (1995)) diluted 1/5 was added to the beads for 30 minutes at room temperature. Then, an optimized number of specific LU of Ruc-antigen fusions was added in each well and incubated for 10 minutes at room temperature. Specific IgG-ULTRALINK beads were washed with the assay master mix, then with PBS. The plate was blotted and LU measured with a Berthold LB 960 Centro microplate luminometer. The percent inhibition was calculated for each sample, and the quantity of specific protein in each sample estimated by using a standard curve designed using known concentrations of each protein in 1/5 diluted human AB serum (Figure 7).

[0073] All samples were run in duplicate. All LU data presented were corrected for background by subtracting LU values of beads incubated with Ruc-antigens but no sera.

Statistical Analysis

[0074] Figures and statistical analyses, including specificity and sensitivity calculations (ROC analysis) and correlations (Spearman rank) were performed using Prism 6.0 (GraphPad

Software, Inc., San Diego, CA, USA). Fischer's exact test was used to compare the percent positivity between groups, and the non-parametric Mann–Whitney test were used to estimate difference in antigen amounts between two groups. All differences were considered significant at the $p < 0.05$ level.

EXAMPLE 1

[0075] This example demonstrates the identification of *Loa loa* antigens.

[0076] Purified *Loa loa* microfilariae (mf) were obtained from one patient. RNA sequences were obtained from the purified *L. loa* mf. The RNA sequences encoded 15,444 putative *L. loa* mf proteins. The bioinformatics of the 15,444 proteins were analyzed by comparison to proteins in general protein databases (Protein Data Bank (PDB) and Swiss-Prot) and specific protein databases (*Caenorhabditis elegans*, *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*). Of the 15,444 proteins, 20 were present in mf excretory-secretory (ES) products. These 20 proteins had little to no homology to any of the other proteins in the databases.

[0077] Shotgun proteomics using trypsin digestion was performed on the samples shown in Table 1A, followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. *L. loa*-specific proteins, mf ES proteins, and/or abundant proteins were identified, as shown in Table 1A. Mass spectrometry analyses of urine from the *L. loa*-infected individual resulted in the identification of spectra matching to 70 *L. loa* proteins, of which 18 proteins were detectable by at least 2 unique peptides and not present in normal uninfected urine (Table 1A and 1B). All of these 18 proteins were identified to be *L. loa* mf proteins. Their corresponding transcript expression (Desjardins et al., *Nat. Genet.*, 45:495-500 (2013)) (FPKM) ranged from 2.07 to 3,841.10. Eight (44.4%) of the 18 *L. loa* urine specific proteins were annotated as “hypothetical” proteins with unknown function (Table 1B).

TABLE 1A

	Urine	Serum	ES (<i>in vitro</i> culture)
<i>L. loa</i>-infected patients	n=1	n=8	ES products from 20 x 10 ⁶ mf isolated by apheresis
Uninfected control patients	n=1	n=5	-
<i>L. loa</i>-specific proteins	m=18	m=170	-
mf ES proteins	-	-	1,273
abundant proteins	-	-	204
“n” refers to the number of patients “m” refers to the number of different proteins			

TABLE 1B

Protein ID	Description	Peptide count	MW (kDa)	FKPM	Homology to Human		
					E-value	% Coverage	Homologue?
LOAG_00073	Heat shock protein 90	2	69.4	3841.1	0E+00	99	YES
LOAG_01395	WD repeats and SOF1 domain-containing protein	2	51.0	101.3	5E-174	97	YES
LOAG_01611	Hypothetical protein	2	64.5	29.8	1E-85	74	YES
LOAG_02628	Low-density lipoprotein receptor repeat class B containing protein	2	196.0	33.7	4E-129	86	YES
LOAG_03988	Hypothetical protein	2	54.0	152.3	2E-08	92	YES
LOAG_04876	Peptidase M16 inactive domain-containing protein	2	47.4	40.7	2E-43	93	YES
LOAG_05583	U4/U6 small nuclear ribonucleoprotein hPtp4	2	56.6	43.4	2E-157	99	YES
LOAG_05701	14-3-3-like protein 2	2	28.3	2392.0	1E-147	94	YES
LOAG_05915	Hypothetical protein	2	104.3	14.7	2E-02	33	NO
LOAG_06631	Troponin	2	171.1	8.8	1E-05	13	YES
LOAG_09325	Hypothetical protein	2	33.0	68.3	1E-13	27	YES
LOAG_10011	Hypothetical protein	2	13.6	3257.3	7E-66	98	YES
LOAG_16297	Hypothetical protein	2	14.3	0.4	5E-04	67	NO
LOAG_17249	Pyruvate kinase	2	59.0	609.7	0E+00	99	YES
LOAG_17808	PWWP domain-containing protein	2	69.8	13.9	9E-04	5	NO
LOAG_18456	Cullin-associated NEDD8-dissociated protein 1	2	124.1	45.5	0E+00	98	YES
LOAG_18552	Hypothetical protein	2	106.5	2.1	1E-03	44	NO
LOAG_19057	Hypothetical protein	3	30.2	89.2	5E-128	77	YES

FKPM represents the relative mRNA expression level obtained using RNAseq (Desjardins et al., *Nat. Genet.*, 45:495-500 (2013)) and MW stands for "molecular weight".

[0078] The bioinformatics of the *L. loa*-specific proteins identified in Table 1A were analyzed by comparison to proteins in the general and specific protein databases described above in this Example.

[0079] Of the 170 *L. loa*-specific proteins identified in serum in Table 1A, 15 were mostly present in the mf ES products and had little or no homology to any of the other proteins in the databases.

[0080] Of the 18 *L. loa*-specific proteins identified in urine in Table 1A, three were present in the mf ES products, and four additional proteins had little or no homology to any of the other proteins in the databases. Further details about the four selected urine proteins are provided in Table 2.

TABLE 2

Protein Name (SEQ ID NO)	LOAG_05915 (SEQ ID NO: 1)	LOAG_18552 (SEQ ID NO: 15)	LOAG_17808 (SEQ ID NO: 14)	LOAG_16297 (SEQ ID NO: 4)
Description	Hypothetical protein (2736 nucleotides (nt))	Hypothetical protein (2682 nt)	PWWP domain-containing protein (1878 nt)	Hypothetical protein (375 nt)
Sequence size (number of amino acid residues)	911	893	625	124
molecular weight (MW)	104.378	106.549	69.931	14.258
secretome P	0.237	0.216	0.386	0.742
FPKM*	14.65	2.07	13.87	0.37
Predicted helices	1	0	0	0
Present in the LLMF ES**	yes	yes	yes	no

*fragments per kilobase of exon per million fragments mapped

***L. loa* microfilariae excretory-secretory products

[0081] Of the many proteins present in mf ES products, in the serum, in the urine, or in the transcriptome, 20 proteins (SEQ ID NO: 1-20, respectively) were chosen for further study based on their specificity and abundance.

[0082] Filtering the data for proteins with little or no sequence homology with human proteins shortlisted four *L. loa* proteins: LOAG_05915 (SEQ ID NO: 1), LOAG_16297 (SEQ ID NO: 4), LOAG_17808 (SEQ ID NO: 14) and LOAG_18552 (SEQ ID NO: 15) (Tables 1B and 2). These four proteins were then assessed for having homologues in the other filariae sequenced to date, *B. malayi*, *W. bancrofti*, and *O. volvulus*.

EXAMPLE 2

[0083] This example demonstrates the detection of the protein of LOAG_17808 (SEQ ID NO: 14) in the serum of two *L. loa*-infected patients using a capture ELISA assay.

[0084] Rabbit anti-microfilariae ES antibody (IgG) was applied to a plate. A sample of serum from a *L. loa*-infected patient was then applied to the plate. Serum from a mouse that had previously been injected with the LOAG_17808 (SEQ ID NO: 14) was then applied to the plate. The concentration of LOAG_17808 (SEQ ID NO: 14) was determined by measuring the optical density (OD). The results are shown in Figure 1.

[0085] As shown in Figure 1, LOAG_17808 (SEQ ID NO: 14) was detected in the serum of two unrelated *L. loa*-infected patients.

EXAMPLE 3

[0086] This example demonstrates the specificity of antibody responses to LOAG_16297 (SEQ ID NO: 4) and LOAG_17808 (SEQ ID NO: 14) proteins, respectively, in humans.

[0087] A LIPS assay was carried out in which Ruc-antigen (LOAG_16297 (SEQ ID NO: 4) or LOAG_17808 (SEQ ID NO: 14) fusion proteins were incubated with sera from *L. loa*-infected patients, uninfected control patients, or patients infected with *Wuchereria bancrofti* (Wb) or *Onchocerca volvulus* (Ov). Light units were measured with a luminometer.

[0088] The results are shown in Figures 2A-2B. As shown in Figure 2A, the human antibody response to LOAG_16297 (SEQ ID NO: 4) demonstrated 100% sensitivity and 92% specificity. As shown in Figure 2B, the human antibody response to LOAG_17808 (SEQ ID NO: 14) demonstrated 100% sensitivity and 96% specificity.

EXAMPLE 4

[0089] This example demonstrates the detection of LOAG_16297 (SEQ ID NO: 4) and LOAG_17808 (SEQ ID NO: 14), respectively, by a competitive LIPS antigen assay.

[0090] Rabbit anti-protein IgG antibody coated agarose beads were applied to a plate. Various doses (0.001 to 100 µg/ml) of antigen (LOAG_16297 (SEQ ID NO: 4) or LOAG_17808) were applied to the beads. After 10 minutes, antigen-LIPS lysate was applied. After 10 minutes, the light units were measured.

[0091] The results are shown in Figures 3A and 3B. As shown in Figures 3A and 3B, LOAG_16297 (SEQ ID NO: 4) and LOAG_17808 (SEQ ID NO: 14) were detected by a competitive LIPS antigen assay.

EXAMPLE 5

[0092] This example demonstrates the immunogenicity of the four LOAG_05915 (SEQ ID NO: 1), LOAG_16297 (SEQ ID NO: 4), LOAG_17808 (SEQ ID NO: 14) and LOAG_18552 (SEQ ID NO: 15) *L. loa* proteins.

[0093] The immunogenicity of the protein antigens was assessed using hyperimmune rabbit antisera in a standard LIPS assay. As shown in Figure 5, there was minimal reactivity with the respective pre-bleed sera, and robust reactivity with the hyperimmune sera (and their purified IgG) from two of the four fusion proteins, LOAG_17808 and LOAG_16297. In addition, the LOAG_17808 fusion protein was also recognized by purified IgG antibodies raised against *L. loa* somatic mf antigen (Figure 5).

[0094] To evaluate the reactivity of these proteins in humans, sera/plasma from *L. loa*-infected (patients) and uninfected (control) subjects were used and compared to the reactivity to *L. loa* SXP-1, a previously described *L. loa* antigen (Burbelo et al., *J. Clin. Microbiol.*, 46: 2298-2304 (2008)). As expected, healthy-control samples had very low signals, with a median anti-LOAG_16297, LOAG_17808, and SXP-1 antibody titers of 236 LU, 97 LU, and 746 LU, respectively (Figures 6A-6C). For *L. loa*-infected patients, the median value was 6 times higher for LOAG_16297 (1,423 LU), 148 times higher for LOAG_17808 (14,317 LU), and 905 times for SXP-1 (674,990 LU) than the median titers of the uninfected healthy controls. The difference between *L. loa* infected patients and uninfected controls were significant for all tested fusion proteins ($P < 0.0001$). In addition, a ROC analysis shows that the two *L. loa* mf antigens were able to accurately distinguish *Loa*-infected from *Loa*-uninfected individuals: LOAG_16297 with 96.7% sensitivity and 100% specificity, using a threshold of 760 LU (Figure 6A), and LOAG_17808 with 100% sensitivity and 96.7% specificity, using a threshold of 862 LU (Figure 6B). By comparison, *L. loa* SXP-1 showed 100% of sensitivity and 100% of specificity using a threshold of 10,785 LU on the same set of 31 patients and 31 controls (Figure 6C).

EXAMPLE 6

[0095] This example demonstrates the ability to identify LOAG_16297 and LOAG_17808 using a competitive LIPS assay.

[0096] The ability to identify LOAG_16297 and LOAG_17808 in an antigen detection system was next tested using a competitive LIPS assay in *L. loa*-, *W. bancrofti*-, *O. volvulus*-infected individuals and uninfected healthy controls. Using pooled human AB serum spiked with increasing concentrations of the appropriate antigen, standard curves were generated. The standard curves allowed the percent inhibition in the competitive LIPS assay to be related to the antigen concentration present in the sera (Figure 7). These standard curves were then used to quantitate the levels of circulating protein in the serum of *Loa*-infected patients and in the control groups. For LOAG_16297 (Figure 8A), the geometric mean level of detectible protein in serum/plasma was 17.88 ng/ml in *Loa*-infected subjects, whereas it was negligible in *W. bancrofti*-, *O. volvulus*-infected and in uninfected subjects. Using a cutoff based on a ROC analysis (5 ng/ml), it can be seen that there were measurable antigen levels in 12/26 microfilaremic *Loa*-infected individuals compared to 0/5 amicrofilaremic *Loa*-infected, 0/31 uninfected ($P < 0.0001$), 0/15 *O. volvulus*- ($P = 0.004$) and 1/15 *W. bancrofti*-infected ($P = 0.03$) individuals. For LOAG_17808 (Figure 8B), the geometric mean level of protein was 36.68 ng/ml in *Loa*-infected, 21.04 ng/ml in *W. bancrofti*-infected, 1.86 ng/ml in *O. volvulus*-infected, and 4.97 ng/ml in uninfected individuals. Again using ROC analysis (with an upper threshold of 39 ng/ml), there were detectible LOAG_17808 levels in 9/26 microfilaremic *Loa*-infected subjects for 0/5 in the amicrofilaremic *Loa*-infected, 0/31 in the uninfected control ($P = 0.002$), 0/15 *O. volvulus*- ($P = 0.02$) and 4/15 in the *W. bancrofti*-infected ($P = 0.9$) groups.

EXAMPLE 7

[0097] This example compares the performance of the LOAG_16297 and LOAG_17808 LIPS to microscopy.

[0098] To further assess the performance of the LOAG_16297 and LOAG_17808-based LIPS antigen detection assays, modified competitive LIPS assays were run using plasma from 26 *Loa*-microfilaremic (*Loa*-mf+) subjects with a range of mf counts and from 25 healthy (uninfected) individuals (Table 3). Considering microcopy to be the “gold standard” for *L. loa* mf quantification, the LOAG_16297 antigen LIPS had a sensitivity of 76.9% (95%

confidence interval [95% CI]: 56.3% to 91.0%), a specificity of 96.0% (95% CI: 79.6% to 99.9%), a positive predictive value (PPV) of 95.2% (95% CI: 76.2% to 99.9%) and a negative predictive value (NPV) of 80% (95% CI: 61.4% to 92.2%). For the LOAG_17808 competitive LIPS assay, the sensitivity, specificity, PPV and NPV was 80.7 (95% CI: 60.6% to 93.5%), 37.5 (95% CI: 18.8% to 59.4%), 58.3% (95% CI: 40.8% to 74.5%), and 64.3% (95% CI: 35.1% to 87.2%).

TABLE 3

Assays	Status	Mf+	Mf-	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
LOAG_16297 LIPS	Positive	20	1	76.9% (56.3-91.0)	96.0% (79.6-99.9)	95.2% (76.2-99.9)	80.0% (61.4-92.3)
	Negative	6	24				
LOAG_17808 LIPS	Positive	21	15	80.7% (60.6-93.5)	37.5% (18.8-59.4)	58.3% (40.8-74.5)	64.3% (35.1-87.2)
	Negative	5	9				

PPV stands for positive predictive value and NPV for negative predictive value. The 95% confidence interval (95% CI) is indicated for each parameter.

EXAMPLE 8

[0099] This example demonstrates the correlation between the amount of LOAG_16297 antigen and the number of microfilariae.

[0100] To evaluate if the levels of antigen circulating in the plasma of *Loa*-infected individuals with range of mf counts were correlated with the density of mf, Spearman rank correlation was performed between the plasma concentrations of LOAG_16297 and LOAG_17808 proteins and the corresponding counts of *L. loa* mf as determined by microscopy (Figure 9A-9B). As can be seen, there was a significant positive correlation for LOAG_16297 ($r = 0.71$; $P < 0.0001$; Figure 9A) and for LOAG_17808 ($r = 0.61$; $P = 0.0002$; Figure 9B).

[0101] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0102] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise

indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0103] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A method of detecting the presence of *Loa loa* in a biological sample, the method comprising assaying the biological sample to determine the presence of one or more antigens in the biological sample, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least one of the antigens is indicative of the presence of *Loa loa* in the biological sample.

2. The method according to claim 1, comprising assaying the biological sample to determine the presence of two or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20 in the biological sample, wherein the presence of at least two of the antigens is indicative of the presence of *Loa loa* in the biological sample.

3. The method according to claim 1 or 2, comprising:

(a) contacting the biological sample with one or more specific binding partner(s), each of which specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming one or more complexes; and

(b) detecting the one or more complexes, wherein detection of the one or more complexes is indicative of *Loa loa* in the biological sample.

4. The method according to claim 3, wherein the specific binding partner is an antibody, or an antigen binding fragment thereof.

5. The method according to claim 3, comprising:

(a) contacting the biological sample with two or more specific binding partner(s), each of which specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, thereby forming two or more complexes; and

(b) detecting the two or more complexes, wherein detection of the two or more complexes is indicative of *Loa loa* in the biological sample.

6. The method according to any one of claims 1-5, comprising:

- (a) contacting the biological sample with a first specific binding partner that specifically binds to one of the antigens, thereby forming a first complex;
- (b) contacting the first complex with a second specific binding partner that specifically binds to the first complex, thereby forming a second complex; and
- (c) detecting the second complex, wherein detection of the second complex is indicative of the presence of *Loa loa* in the biological sample.

7. The method according to claim 6, wherein the first specific binding partner is a first antibody, or an antigen binding fragment thereof, and the second specific binding partner is a second antibody, or an antigen binding fragment thereof.

8. A method of detecting the presence of *Loa loa* in a biological sample, the method comprising assaying the biological sample to determine the presence of one or more antibodies in the biological sample, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least one of the antibodies is indicative of the presence of *Loa loa* in the biological sample.

9. The method of claim 8, comprising assaying the biological sample to determine the presence of two or more antibodies in the biological sample, each antibody specifically binding to an antigen having a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20, wherein the presence of at least two of the antibodies is indicative of the presence of *Loa loa* in the biological sample.

10. The method according to any one of claims 1-9, wherein the assaying is carried out by immunoprecipitation, immunonephelometry, radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescent immunoassay (FIA), luciferase immunoprecipitation system (LIPS), or lateral flow immunochromatographic assay.

11. The method of claim 10, wherein the assaying is carried out by enzyme-linked immunosorbent assay (ELISA).

12. The method of any one of claims 1-11, wherein the biological sample is a human biological sample.
13. The method of any one of claims 1-12, wherein the biological sample is whole blood, serum, or plasma.
14. The method of any one of claims 1-12, wherein the biological sample is urine.
15. The method of any one of claims 1-12, wherein the biological sample is saliva.
16. A composition comprising an immunologically-stimulatory concentration of at least one isolated or purified antigen, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20 and a physiologically-acceptable carrier.
17. The composition of claim 16, further comprising an adjuvant.
18. A method for producing an antibody that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-20, the method comprising administering the composition of claim 16 or 17 to an animal under conditions sufficient for the animal to develop an immune response to the antigen.
19. The method of claim 18, comprising harvesting serum from the animal after the animal has developed the immune response, wherein the serum comprises the antibody.
20. The method of claim 18 or 19, comprising harvesting a splenocyte from the animal after the animal has developed the immune response, fusing the splenocyte with an immortalized cell to form a hybridoma which secretes the antibody into culture medium, culturing the hybridoma, and harvesting the culture medium containing the antibody.
21. A specific binding partner that specifically binds to an antigen having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-20.

22. The specific binding partner of claim 21, wherein the specific binding partner is an antibody, or antigen-binding fragment thereof.

23. A test kit comprising:

- (a) one or more specific binding partner(s), each of which specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20;
 - (b) one or more substrate(s) onto which (a) is bound or affixed;
 - (c) one or more reagent(s) for facilitating binding of the amino acid sequence(s) to (a);
- and
- (d) one or more reagent(s) for detecting the amino acid sequence(s) specifically bound to (a).

24. A test kit comprising:

- (a) one or more antigens, each antigen having a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-20;
 - (b) one or more substrate(s) onto which (a) is bound or affixed;
 - (c) one or more reagent(s) for facilitating binding of one or more antibodies to (a);
- and
- (d) one or more reagent(s) for detecting the antibody or antibodies specifically bound to (a).

25. The method according to any one of claims 1-15, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14.

26. The method according to any one of claims 18-20, wherein the antigen has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14.

27. The composition according to claim 16 or 17, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14.

28. The specific binding partner according to claim 21 or 22, wherein the antigen has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14.

29. The test kit according to claim 23, wherein each of the one or more specific binding partners specifically binds to a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-14.

30. The test kit according to claim 24, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NO: 1-14.

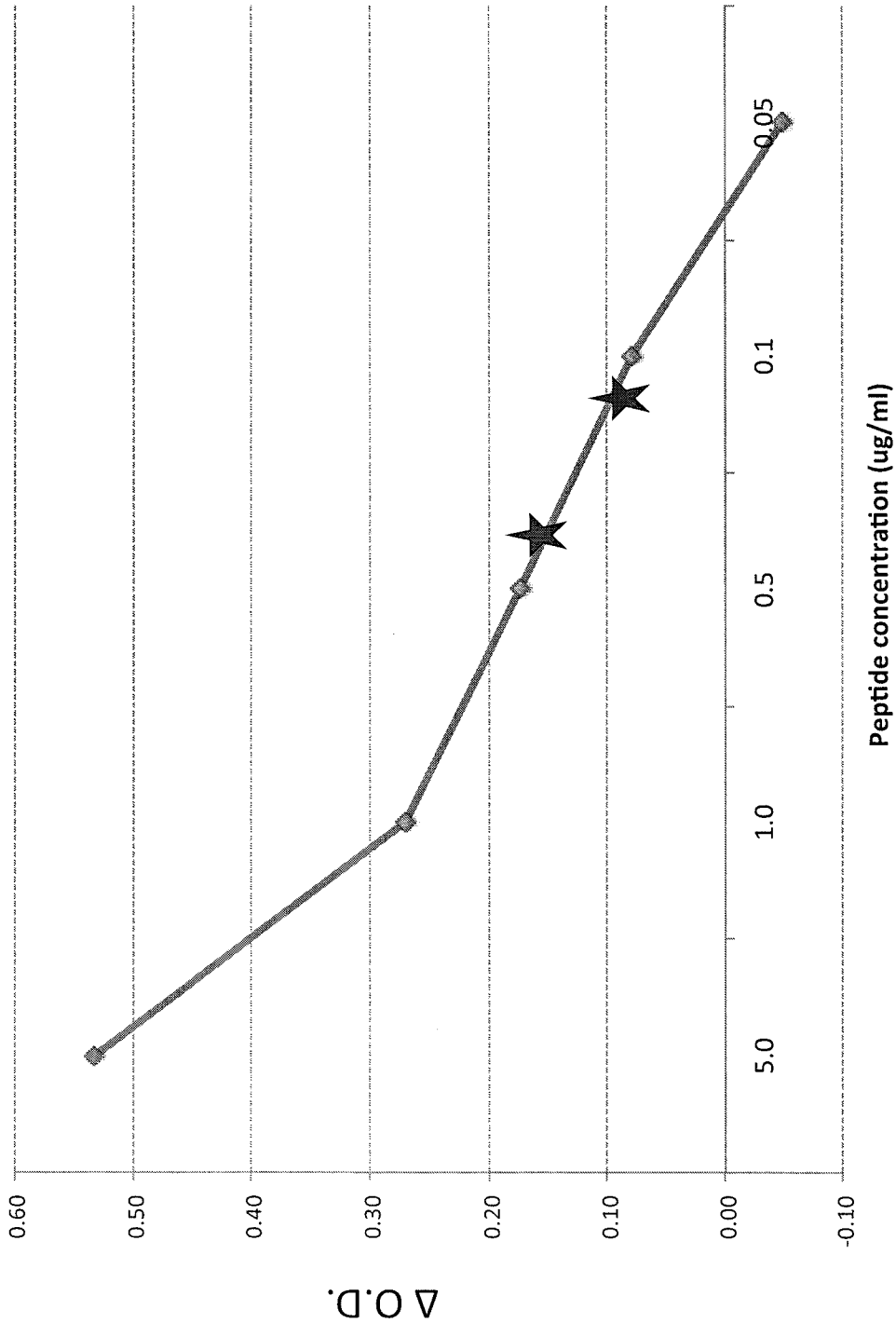


FIG. 1

FIG. 2A

LOAG_16297

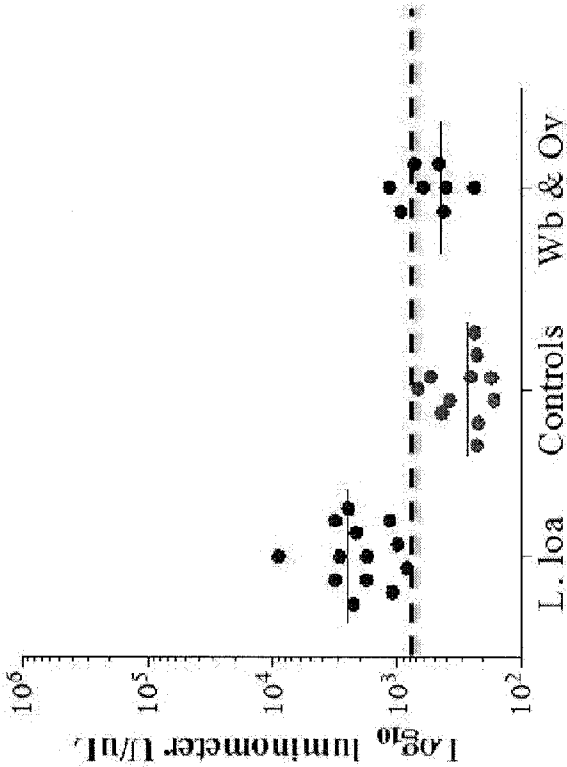


FIG. 2B

LOAG_17808

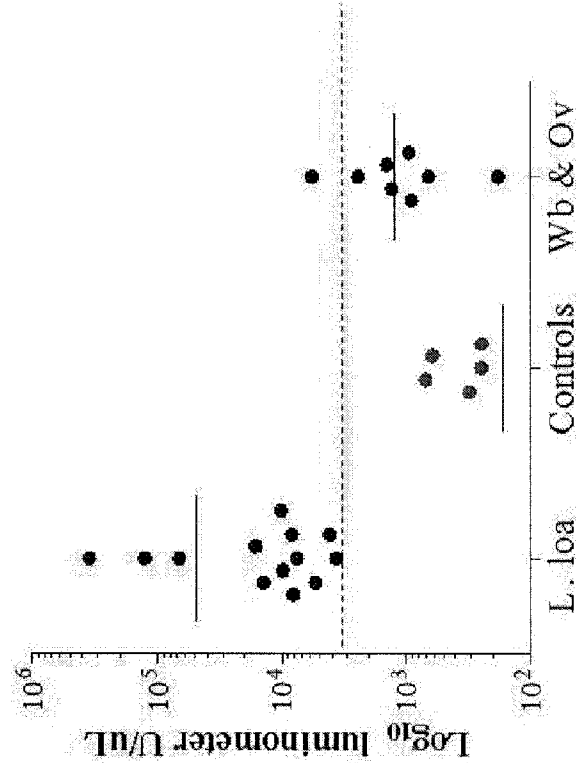


FIG. 3A

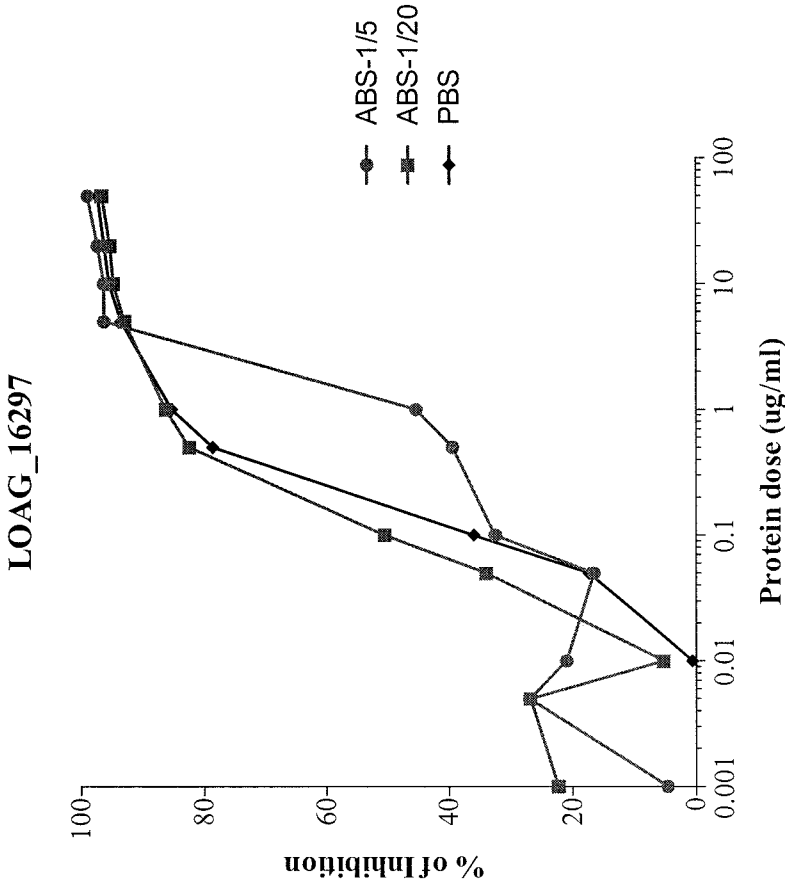
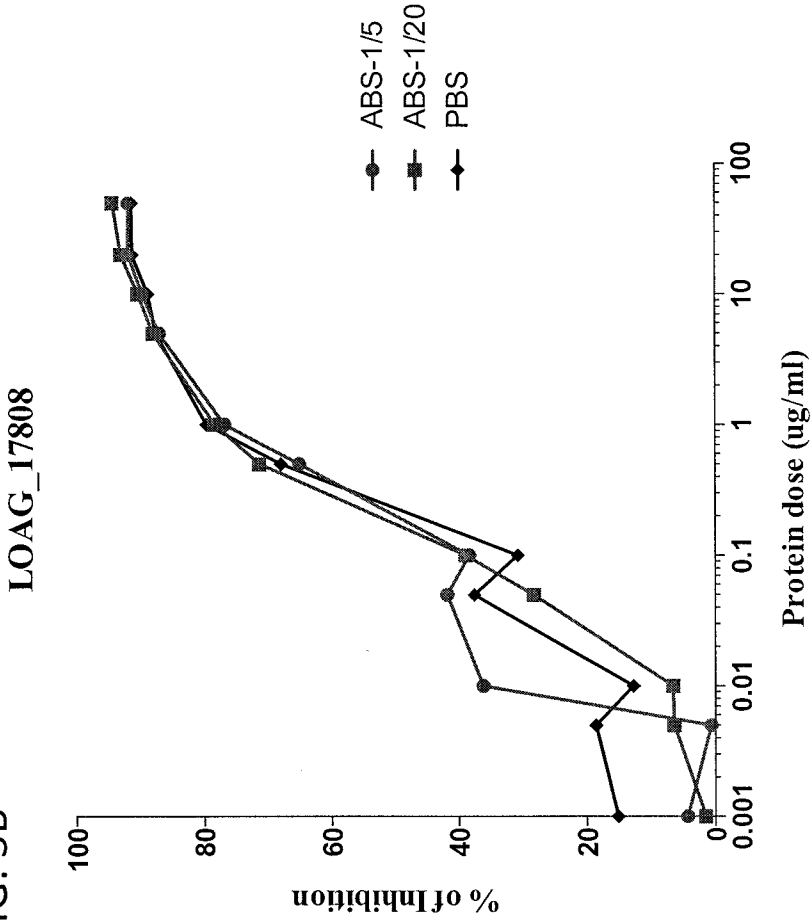


FIG. 3B



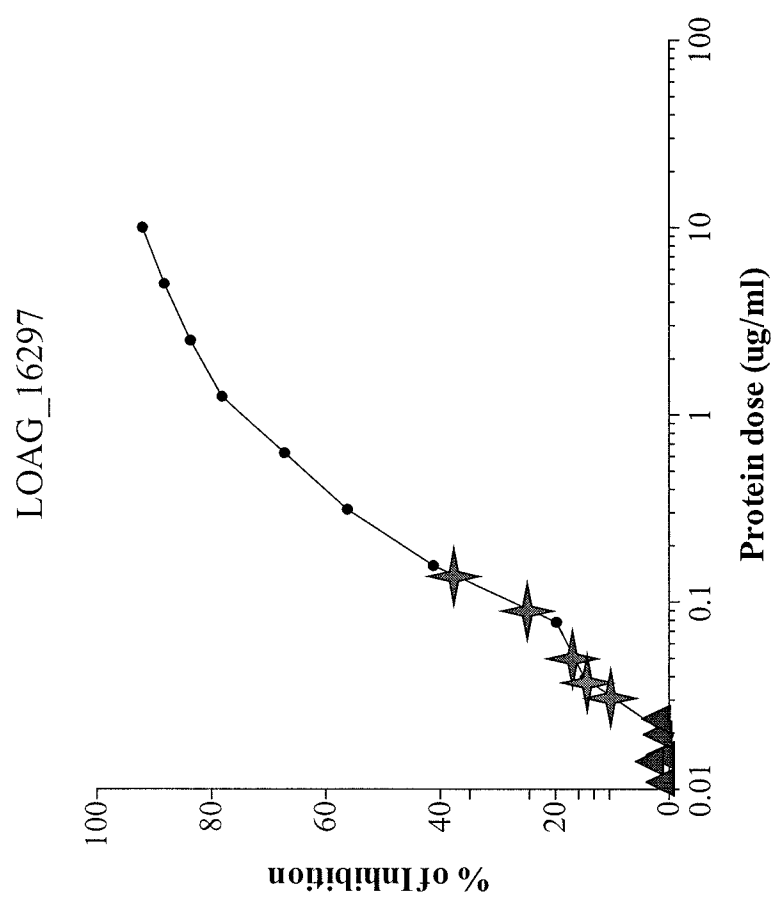


Figure 4

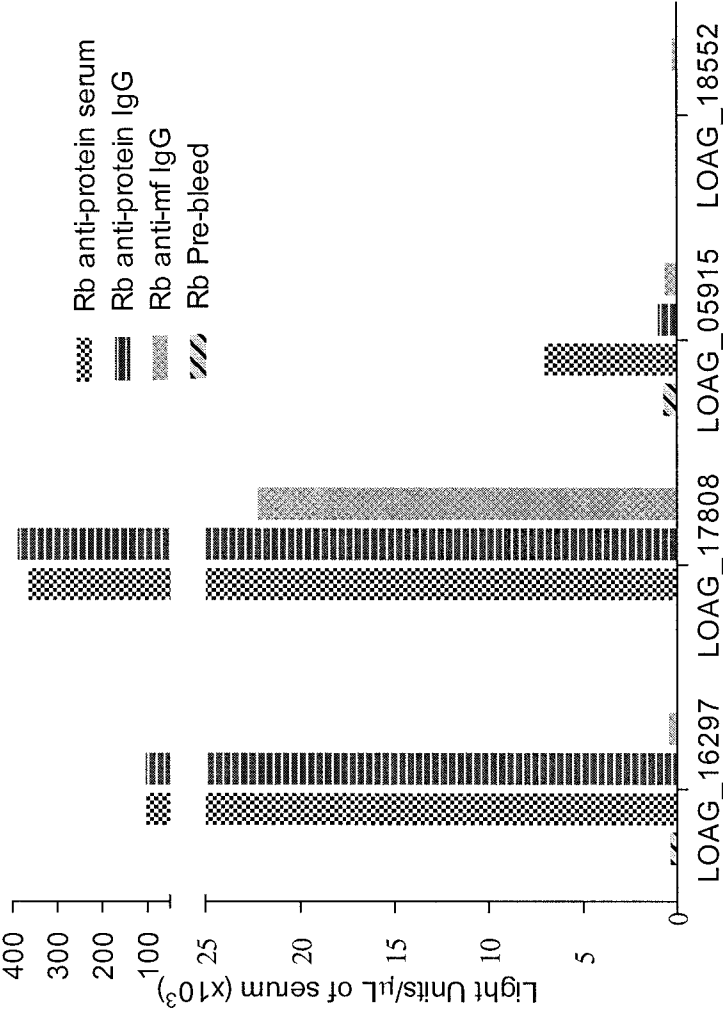


FIG. 5

FIG. 6A

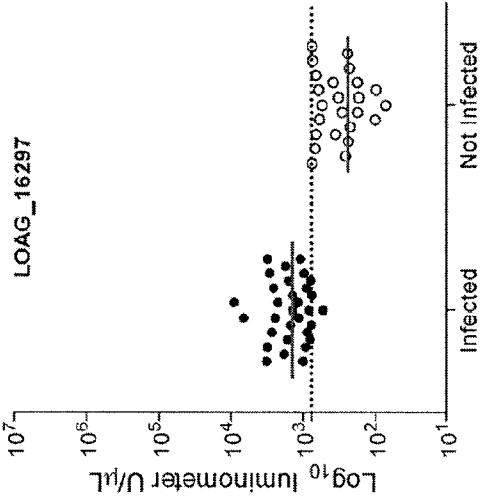


FIG. 6B

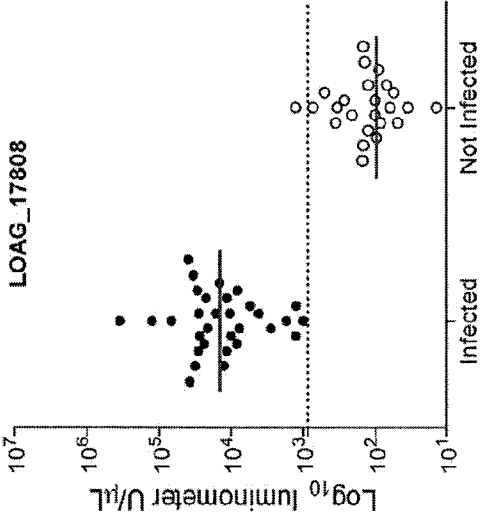
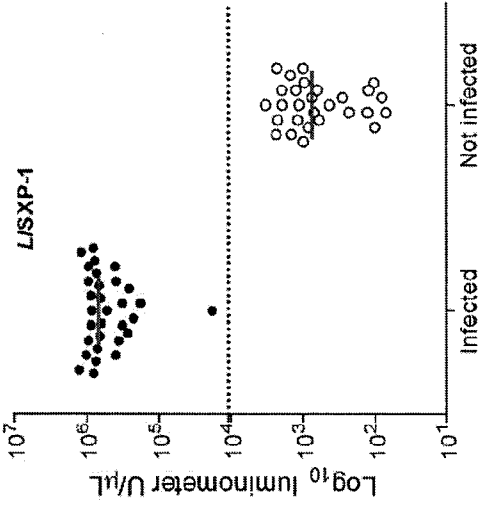


FIG. 6C



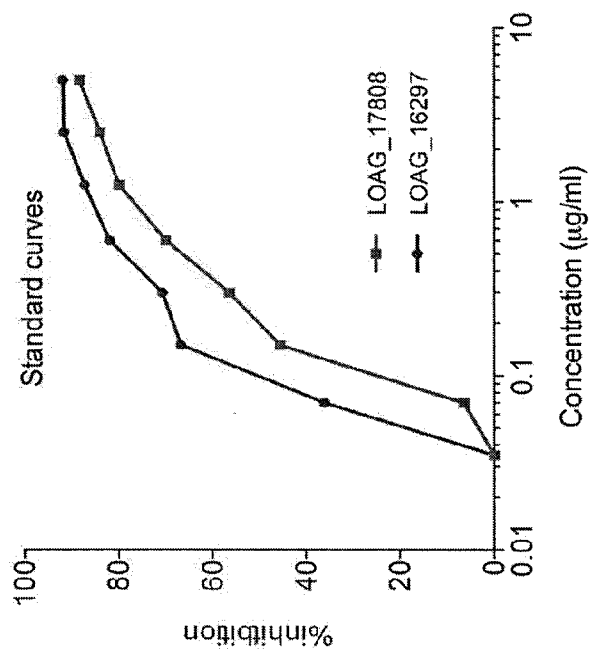


FIG. 7

FIG. 8A

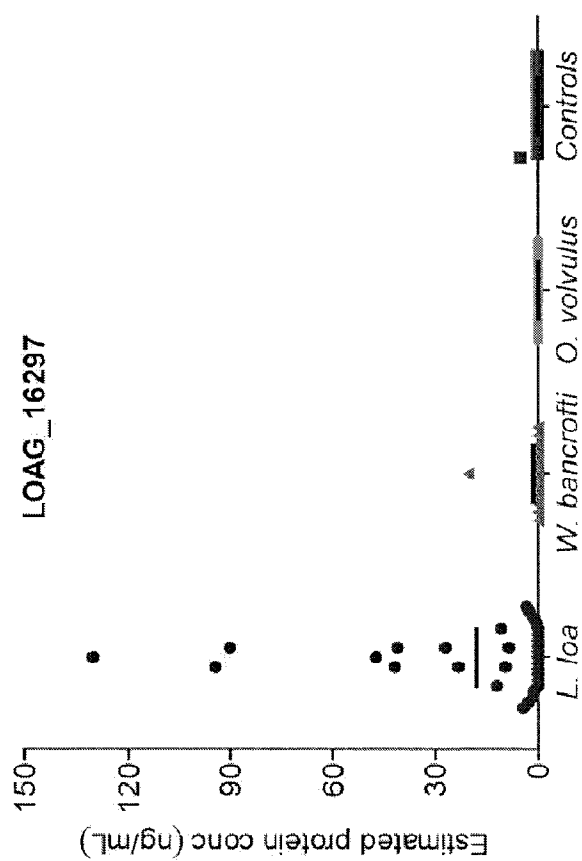


FIG. 8B

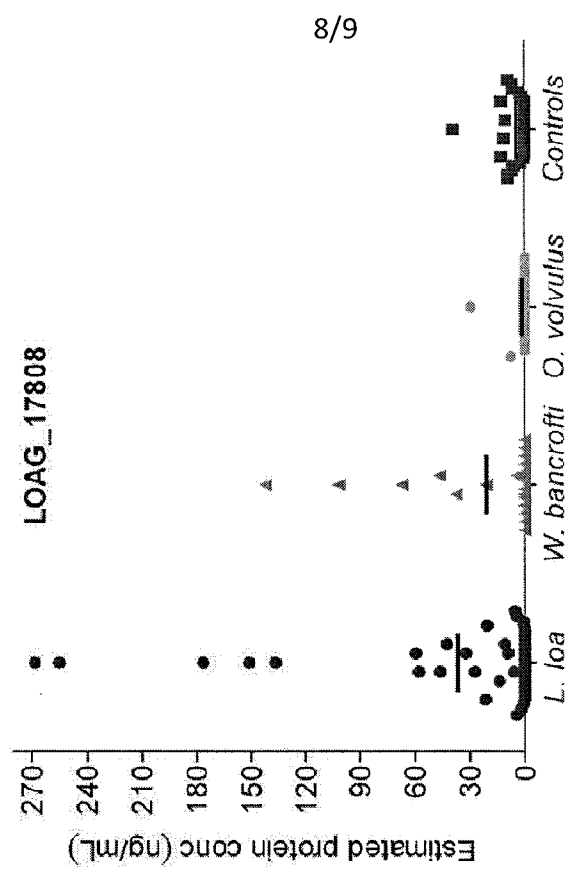


FIG. 9A

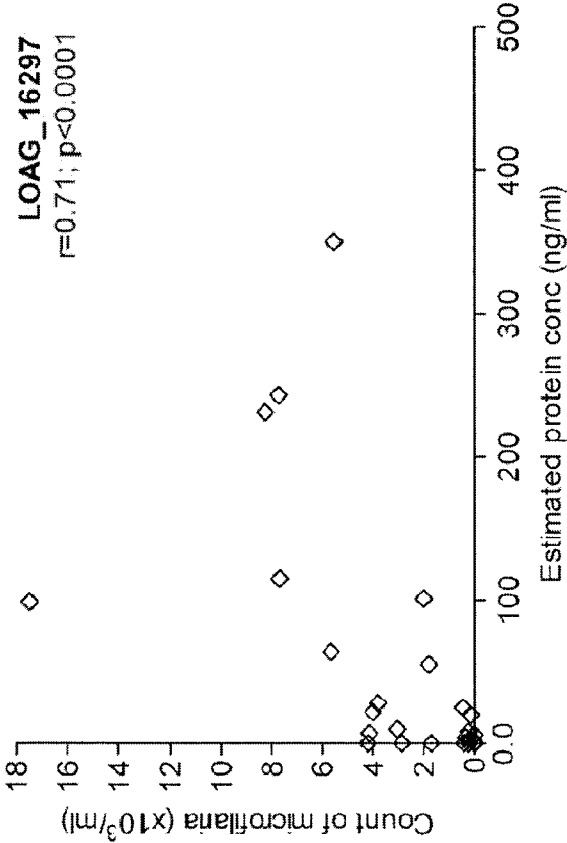
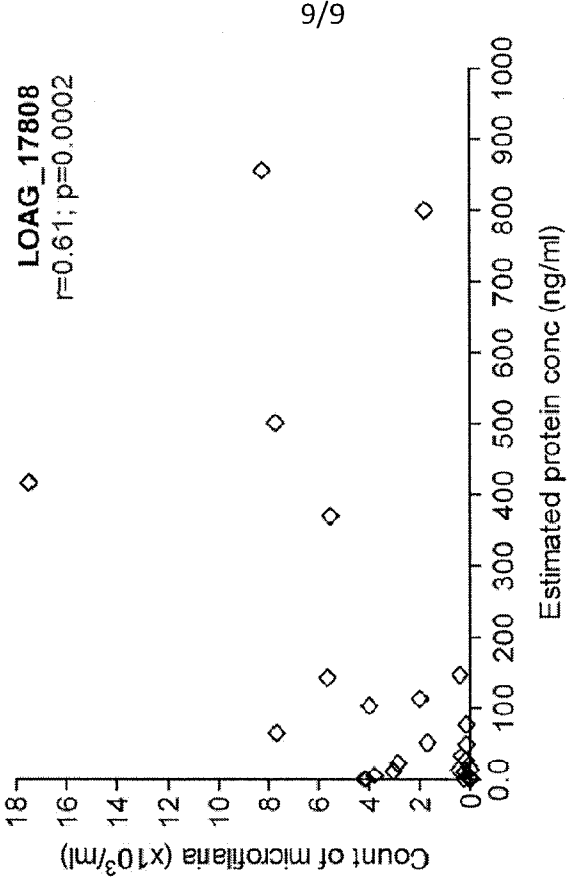


FIG. 9B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029673

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/53 C07K16/18 C07K14/435
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07K

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

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

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WALKER-DEEMIN A. ET AL.: "Detection of circulating antigens in Gabonese patients with Loa loa filariasis", TROP. MED. INT. HEALTH, vol. 1, no. 6, December 1996 (1996-12), pages 772-778, XP55286390, abstract page 773	1-7,12,13
A	KLION A.D. ET AL.: "Serum immunoglobulin G4 antibodies to the recombinant antigen, L1-SXP-1, are highly specific for Loa loa infection", J. INFECT. DIS., vol. 187, 1 January 2003 (2003-01-01), pages 128-133, XP55286409, abstract page 129, column 2	8-13
	----- -/--	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

7 July 2016

Date of mailing of the international search report

20/07/2016

Name and mailing address of the ISA/

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Authorized officer

Giry, Murielle

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/029673

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BURBELO P.D. ET AL.: "Rapid, novel, specific, high-throughput assay for diagnosis of Loa loa infection", J. CLIN. MICROBIOL., vol. 46, no. 7, July 2008 (2008-07), pages 2298-2304, XP55286434, abstract -----	8-13
A	AKUE J.P. ET AL.: "Markers of Loa loa infection in permanent residents of a loiasis endemic area of Gabon", TRANS. R. SOC. TROP. MED. HYG., vol. 90, no. 2, March 1996 (1996-03), pages 115-118, XP004542708, abstract page 115, column 2, paragraph 1 - page 116, column 2, paragraph 1 -----	8-13
A	TOURÉ F. ET AL.: "Detection of Loa loa-specific DNA in blood from occult-infected individuals", EXP. PARASITOL., vol. 86, no. 3, July 1997 (1997-07), pages 163-170, XP55014335, abstract page 164, column 2 - page 165, column 1 page 168, column 1 -----	1-30
A	FINK D.L. ET AL.: "Rapid molecular assays for specific detection and quantitation of Loa loa microfilaremia", PLOS NEGL. TROP. DIS., vol. 5, no. 8, E1299, 30 August 2011 (2011-08-30), pages 1-8, XP55014333, abstract page 2, column 1 - page 3, column 1 -----	1-30
A	DRAME P.M. ET AL.: "Loop-mediated isothermal amplification for rapid and semiquantitative detection of Loa loa infection", J. CLIN. MICROBIOL., vol. 52, no. 6, June 2014 (2014-06), pages 2071-2077, XP55286126, abstract page 2072 -----	1-30
A	WO 02/38600 A2 (CENIX BIOSCIENCE GMBH [DE]) 16 May 2002 (2002-05-16) abstract claims 1-24 ----- -/--	1-30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/029673

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/029673

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	DRAME P.M. ET AL.: "Identification and validation of Loa loa microfilaria-specific biomarkers: a rational design approach using proteomics and novel immunoassays", MBO, vol. 7, no. 1, E2132-15, 16 February 2016 (2016-02-16), pages 1-8, XP55286120, the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/029673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0238600	A2	16-05-2002	
		AU 2791702 A	21-05-2002
		CA 2428453 A1	16-05-2002
		EP 1334187 A2	13-08-2003
		JP 2004525612 A	26-08-2004
		US 2005101773 A1	12-05-2005
		WO 0238600 A2	16-05-2002
