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Nutman et al.

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

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- (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 61 days.

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C07K 16/18 (2006.01)
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 USPC 424/130.1, 139.1, 151.1, 184.1, 185.1;
 435/4, 7.1, 7.2
 See application file for complete search history.

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- (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.

28 Claims, 9 Drawing Sheets

Specification includes a Sequence Listing.

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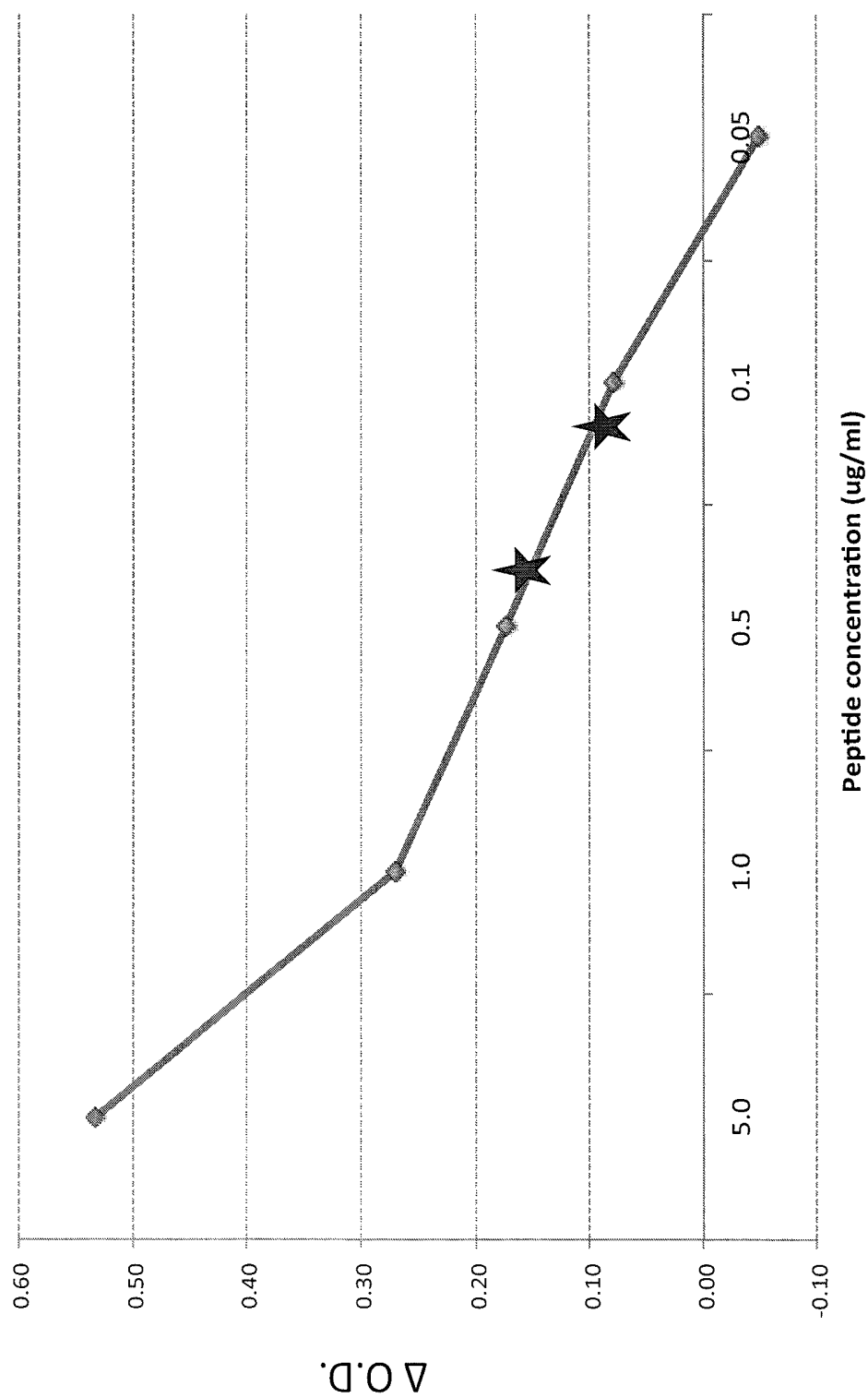


FIG. 1

LOAG_16297

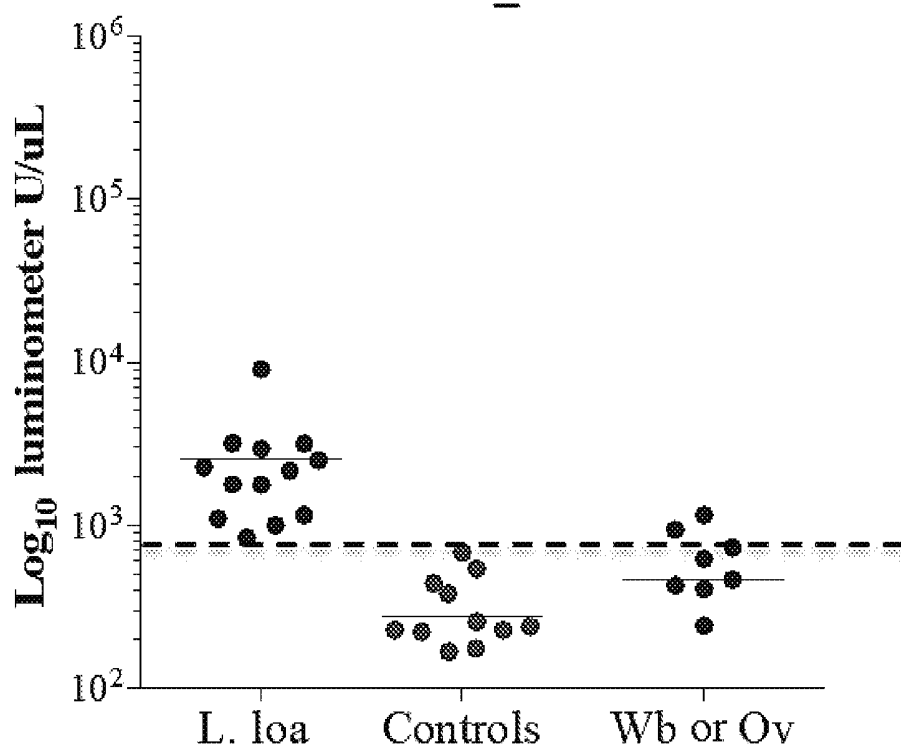


FIG. 2A

LOAG_17808

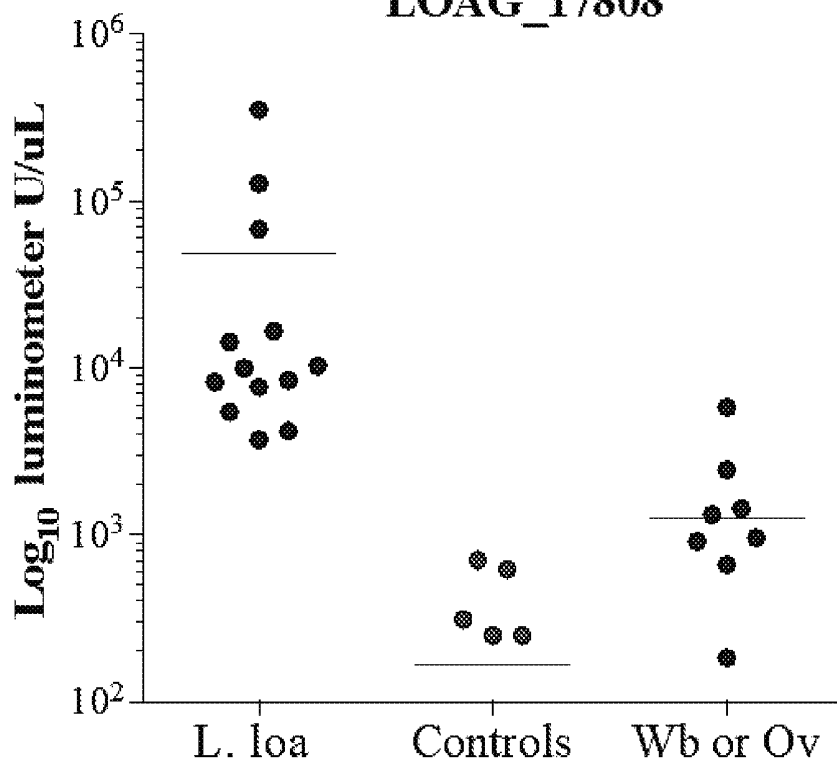


FIG. 2B

FIG. 3A

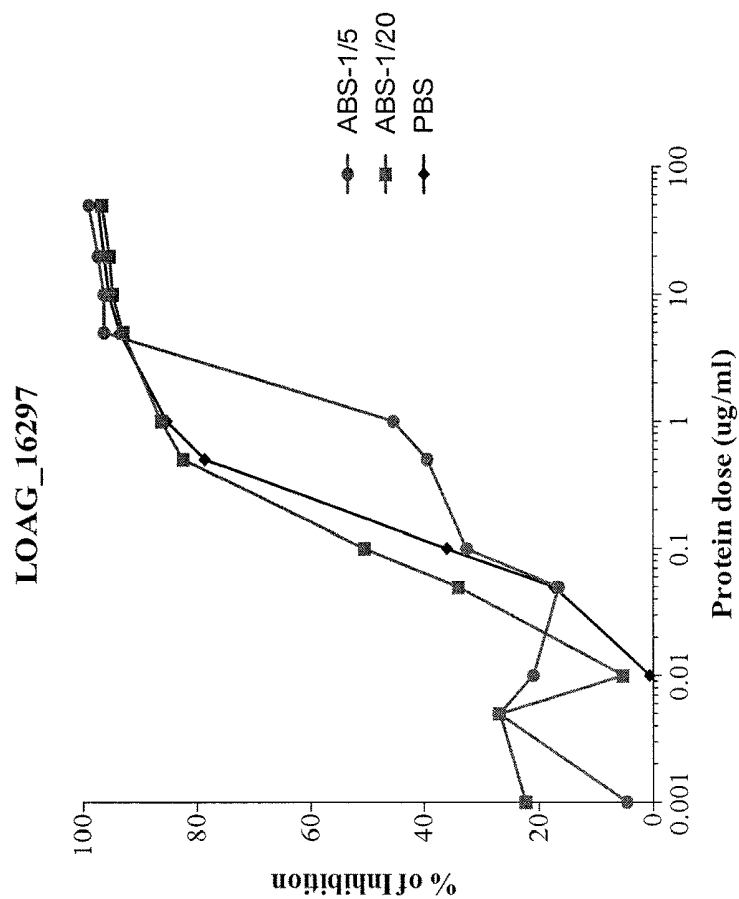
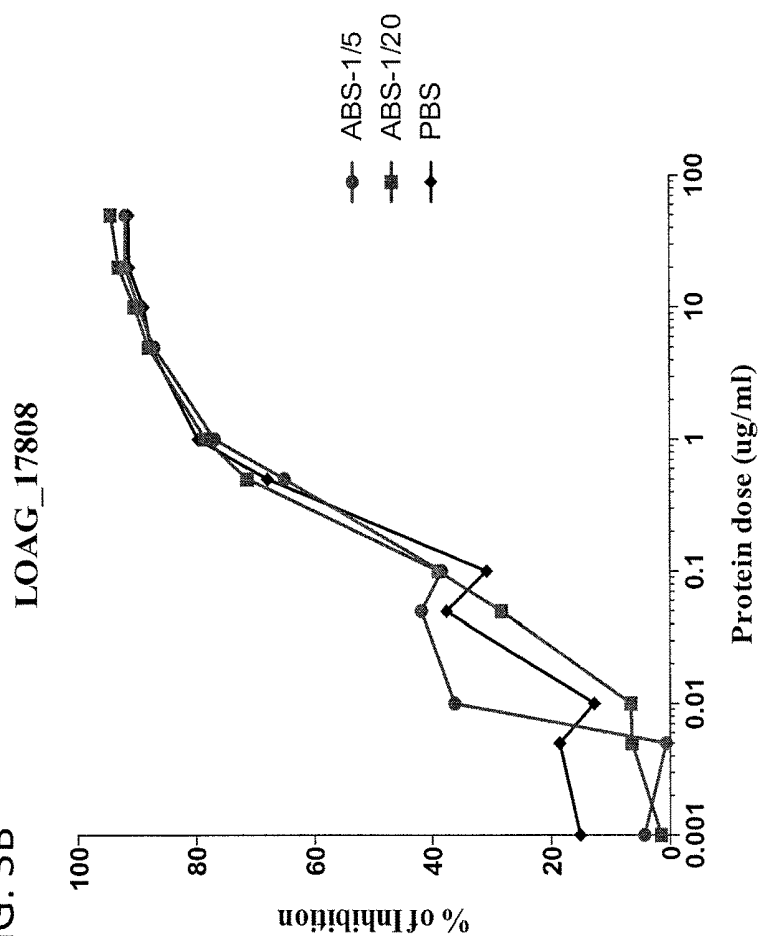


FIG. 3B



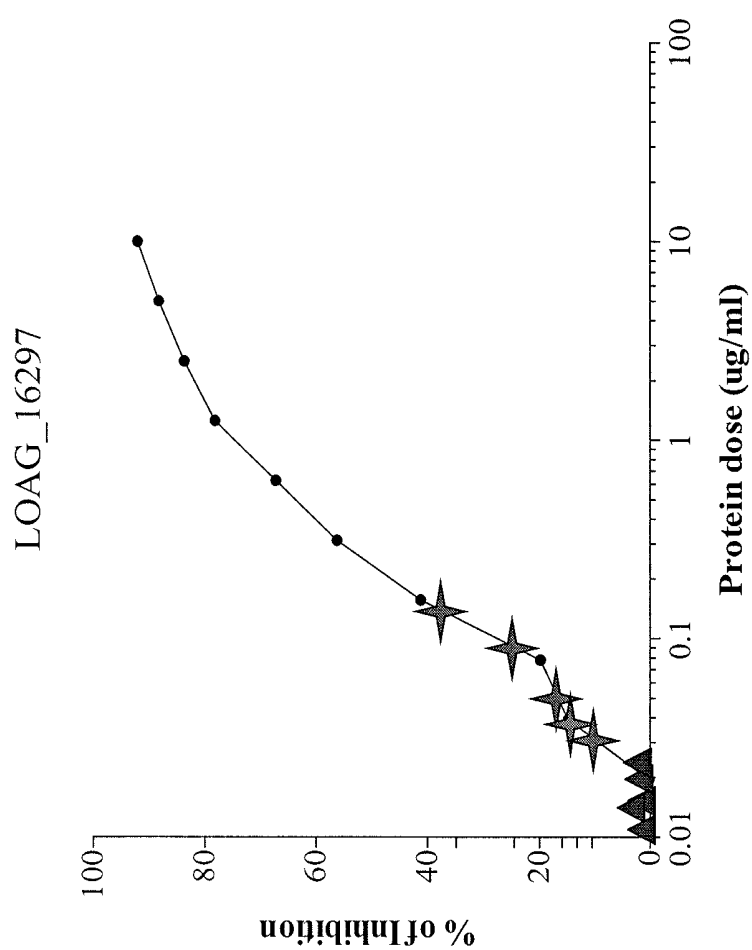


Figure 4

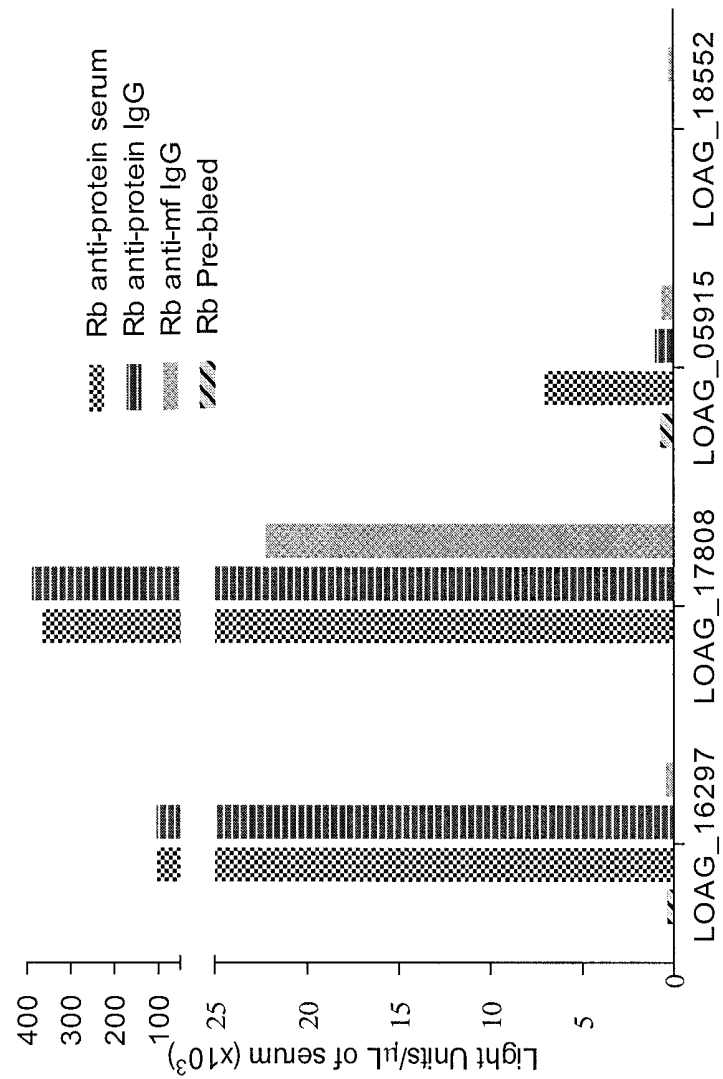


FIG. 5

FIG. 6C

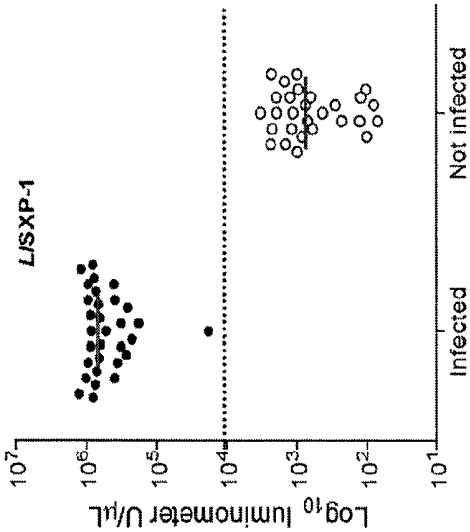


FIG. 6B

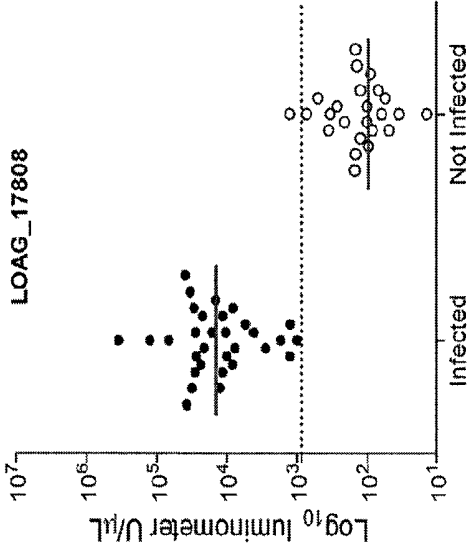
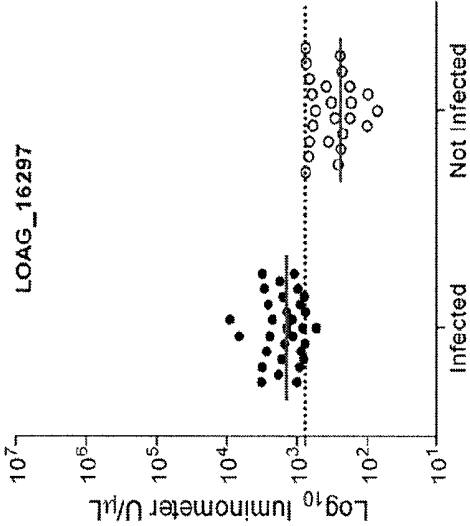


FIG. 6A



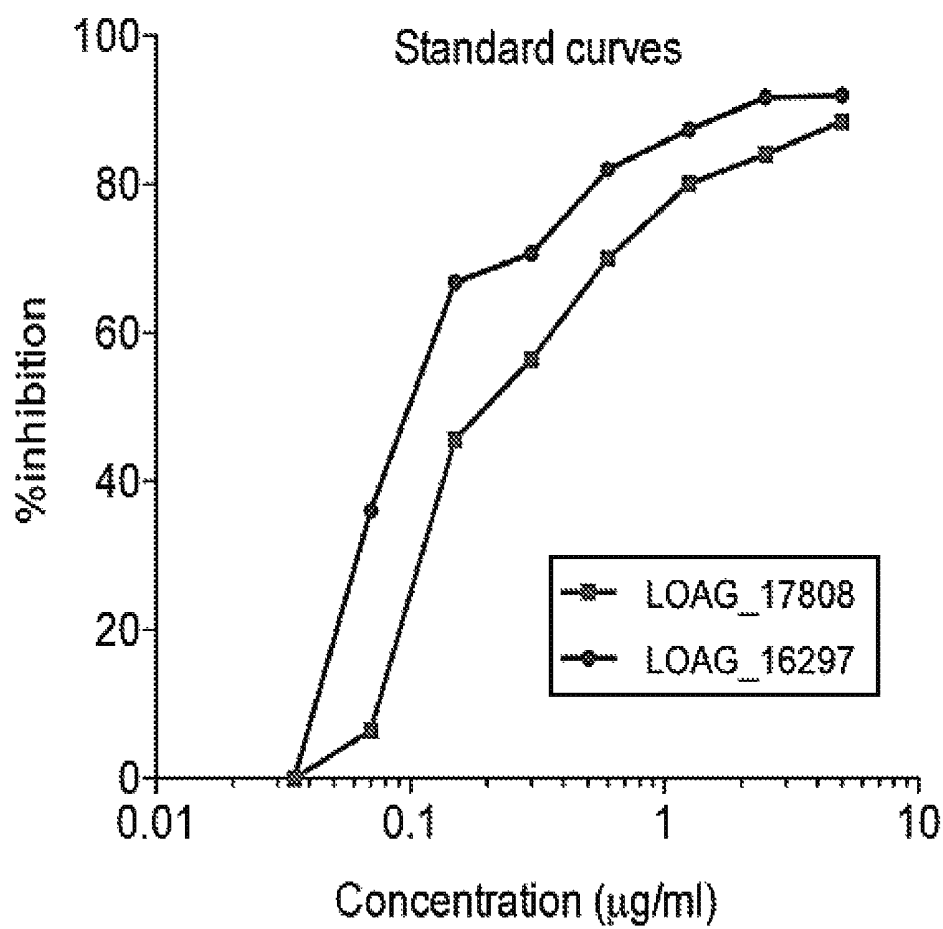


FIG. 7

FIG. 8A

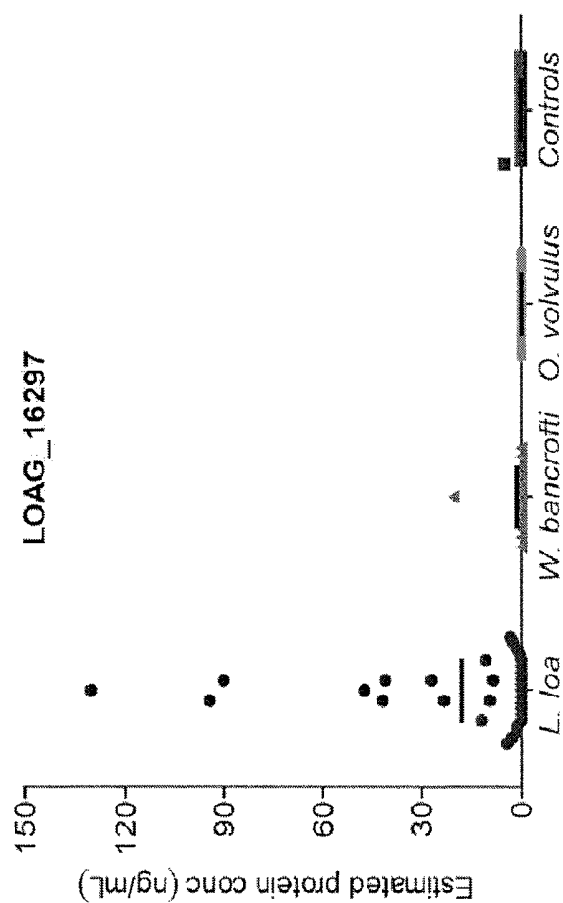


FIG. 8B

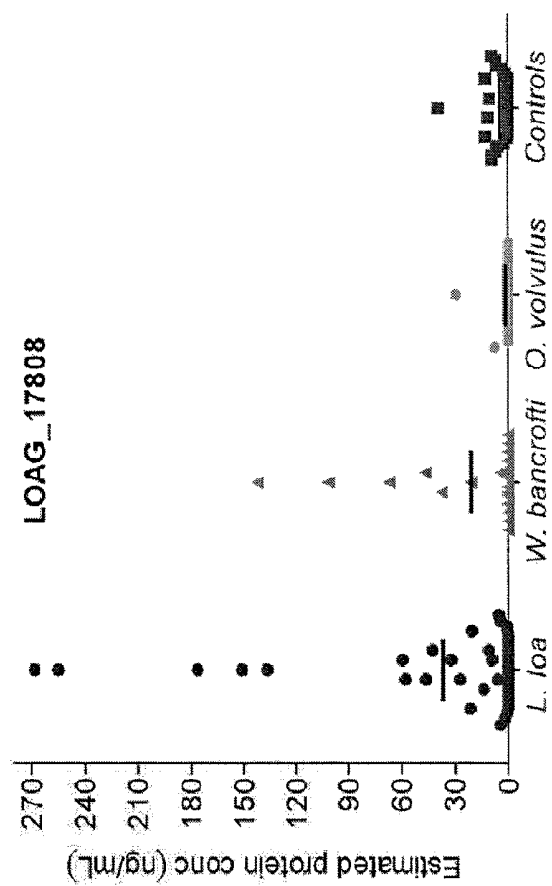


FIG. 9A

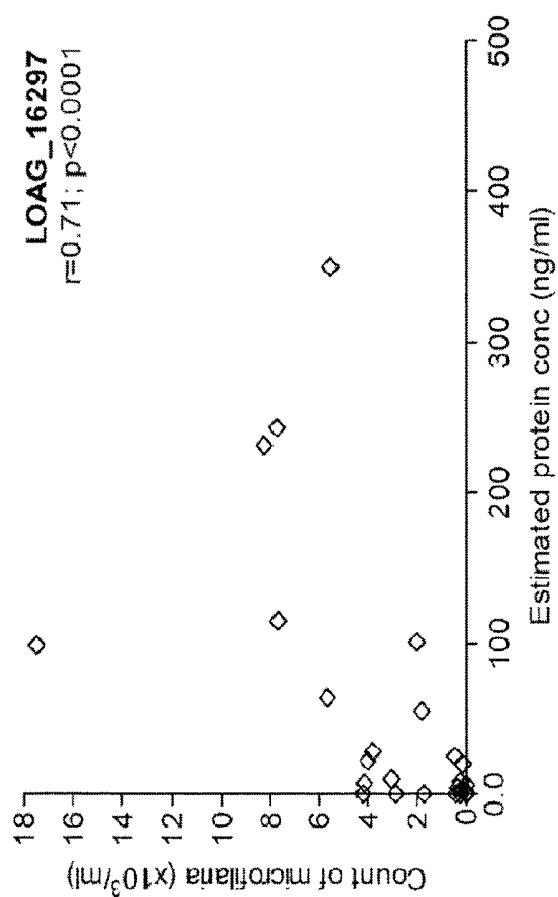
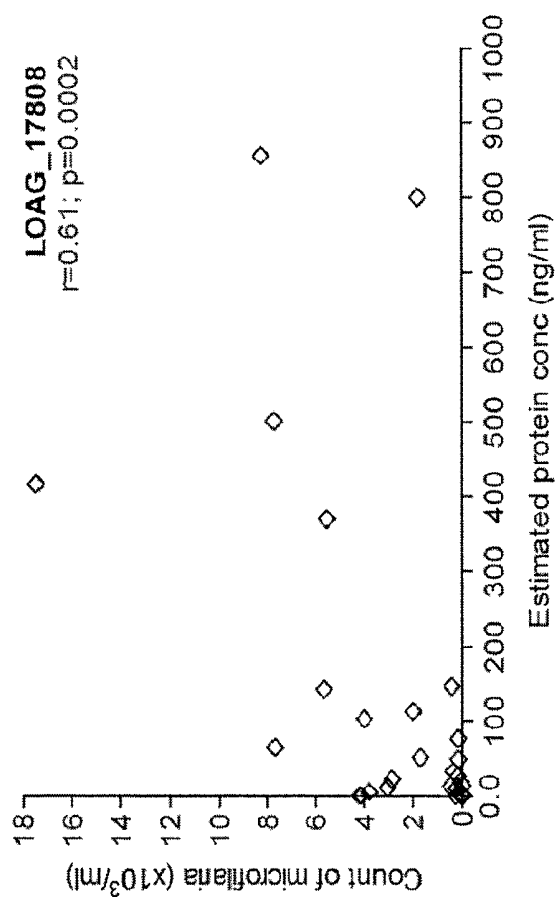


FIG. 9B



COMPOSITIONS AND METHODS FOR DETECTING *LOA LOA*

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is the U.S. national phase of International Patent Application No. PCT/US2016/029673, filed Apr. 28, 2016, which claims the benefit of U.S. Provisional Patent Application No. 62/153,654, filed Apr. 28, 2015, each of which is incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under project numbers ZIAAI0000512-27 and ZIAAI000439-30 by the National Institutes of Health, National Institute of Allergy and Infectious Diseases. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 56,876 Byte ASCII (Text) file named "735794_ST25.txt," dated Oct. 13, 2017.

BACKGROUND OF THE INVENTION

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.

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

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.

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.

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.

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.

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.

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).

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)

FIG. 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.

FIGS. 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)).

FIGS. 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.

FIG. 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*.

FIG. 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.

FIGS. 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.

FIG. 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).

FIGS. 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 FIG. 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.

FIGS. 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

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."

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.

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.

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.

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.

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.

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.

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).

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.

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.

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.

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.

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.

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, Ceboidea, or Simioida (monkeys) or of the order Anthropoidea (humans and apes). An especially preferred mammal is the human. Preferably, the biological sample is a human biological sample.

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.

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, N.Y. (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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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).

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.

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.

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).

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.

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.

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%.

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.

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.

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

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

Study Population and Samples

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.

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).

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

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

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.×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

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 ($X_{corr} \geq 1.9$ for $[M+H]^+$, ≥ 2.2 for $[M+2H]^{2+}$ and ≥ 3.5 for $[M+3H]^{3+}$] were considered as legitimate identifications.

Transcriptomics Data

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

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.

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

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, Ill., 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

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

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 μ l of the assay master mix (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1% Triton X-100), 1 μ l of undiluted plasma/serum, and 2×10^6 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 μ l of a 30% suspension of ULTRALINK protein A/G beads (Pierce, Rockford, Ill., USA) in phosphate-buffered saline

(PBS) was added to the bottom of a 96-well filter high throughput-screening plate (Millipore, Bedford, Mass., 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, Wis., USA).

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.

Having first coupled the purified antigen-specific IgG to ULTRALINK beads (Pierce, Rockford, Ill., 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 (FIG. 7).

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

Figures and statistical analyses, including specificity and sensitivity calculations (ROC analysis) and correlations (Spearman rank) were performed using Prism 6.0 (Graph-Pad Software, Inc., San Diego, Calif., 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

This example demonstrates the identification of *Loa loa* antigens.

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.

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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).

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TABLE 1A

	Urine	Serum	ES (in vitro culture)
<i>L. loa</i> -infected patients	n = 1	n = 8	ES products from 20 × 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 hPrp4	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

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

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.

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.

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

Description	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)
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

TABLE 2-continued

	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)
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

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.

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

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.

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 FIG. 1.

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

Example 3

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.

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.

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

Example 4

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.

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.

The results are shown in FIGS. 3A and 3B. As shown in FIGS. 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

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.

The immunogenicity of the protein antigens was assessed using hyperimmune rabbit antisera in a standard LIPS assay. As shown in FIG. 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 (FIG. 5).

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 (FIGS. 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 (FIG. 6A), and

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LOAG_17808 with 100% sensitivity and 96.7% specificity, using a threshold of 862 LU (FIG. 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 (FIG. 6C).

Example 6

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

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 (FIG. 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 (FIG. 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 (FIG. 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

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

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

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(Table 3). Considering microscopy 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	Positive	20	1	76.9%	96.0%	95.2%	80.0%
LIPS	Negative	6	24	(56.3-91.0)	(79.6-99.9)	(76.2-99.9)	(61.4-92.3)
LOAG_17808	Positive	21	15	80.7%	37.5%	58.3%	64.3%
LIPS	Negative	5	9	(60.6-93.5)	(18.8-59.4)	(40.8-74.5)	(35.1-87.2)

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

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

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 (FIG. 9A-9B). As can be seen, there was a significant positive correlation for LOAG_16297 ($r=0.71$; $P<0.0001$; FIG. 9A) and for LOAG_17808 ($r=0.61$; $P=0.0002$; FIG. 9B).

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.

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.

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.

SEQUENCE LISTING

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<213> ORGANISM: *Loa loa*

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Tyr Arg Ala Ala Leu Ala Ser Leu Ser Ser His Ile Asp Thr Ile Glu
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Lys Lys Ser Gly Leu Leu Gly Ala Asp His Gln Met Ser Glu Glu Leu
 100 105 110
 Ser Ala Phe Lys Ala Ser Glu Ile Asn Lys Gln Ser Val Leu Gly Asp
 115 120 125
 Leu Val Lys Lys Thr Lys Lys Asp Ser Asp Ala Ala Ser Ser Gly Asp
 130 135 140
 Thr Ala Ser Asn Ala Ser Ala Pro His Val Trp Gly Ile Gly Glu Val
 145 150 155 160
 Val Trp Leu Ser Met Pro Asn His Ser Glu Trp Pro Val Val Ile Arg
 165 170 175
 Glu Leu Lys Lys Lys Phe Ala Met Val Asp Ala Phe Pro Leu Lys Phe
 180 185 190
 Asn Cys Lys Pro Glu Arg Tyr Pro Leu Ser Ala Cys Gln Lys Phe Glu
 195 200 205
 Leu Thr Asp Lys Asn Leu Glu Ala Ala Ile Arg Lys Glu Arg Asn Cys
 210 215 220
 Glu Leu Arg Met Ala Leu Gln Ser Val Met Lys Tyr Phe Lys Arg Lys
 225 230 235 240
 Glu Gln Met Asn Asn Val Glu Lys Ser Asp Thr Glu Asp Asn Thr Ala
 245 250 255
 Lys Val Asp Asp Lys Asn Asp Ala Gln Gln Gly Cys Asp Arg Lys Asp
 260 265 270
 Lys Ser Thr Thr Gly Gly Leu Glu Glu Leu Arg Ser Val Asp Gly Glu
 275 280 285
 Ala Glu Ala Lys Leu Thr Gly Met Leu Lys Glu Gly Glu Asn Lys Arg
 290 295 300
 Asp Gly Lys Arg Arg Met Asp Lys Ser Pro Ser Pro Ala Ala Lys Arg
 305 310 315 320
 Gln Lys Phe Ser Asp Val Met Lys Asp Leu Glu Ile Glu Leu Ser Glu
 325 330 335
 Lys Leu Glu Asn Leu Ser Lys Gly Asp Leu Ala Trp Ile Asn Arg Ala
 340 345 350
 Arg Ser Gly Arg Ile Val Lys Trp Pro Ile Leu Val Leu Lys Val Asp
 355 360 365
 Asn Val Asn Lys Ser Cys Val Cys Thr Glu Leu Pro Leu Asp Asp Met
 370 375 380
 Ser Ala Asn Ala Glu Trp Cys Leu Asn Ser Glu Lys Thr Arg Val Val
 385 390 395 400
 Gln Leu Lys Asn Ile Tyr Leu Tyr Asp Thr Val Glu Ala Lys Ile Asp
 405 410 415
 Glu Ile Glu Asp Ala Glu Leu Lys Thr Ala Ile Gln Gln Ala Asp Glu
 420 425 430
 Ile Ala Glu Gly Ser Tyr Arg Pro Phe Asp Asp Glu Arg Asn Ser Tyr
 435 440 445
 Asp Lys Asn Gly Asn Ser Ser Ile Arg Glu Asn Gly Cys Lys Ala Ala
 450 455 460
 Ser Val Asn Ile Leu Ala Pro Lys Glu Ile Leu Arg Leu Cys Lys Ser
 465 470 475 480
 Glu Met Cys Ala Arg His Leu Met Ala Ile Trp Thr Gly Gln Phe Thr
 485 490 495
 Cys Thr Arg His Ala Gly Tyr Glu Pro Pro Phe Met Ala Pro Leu Gln
 500 505 510

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Phe Glu Leu Asn Thr Gly Asp Leu Leu Pro Glu Thr Asp Gly Ile Ser
 515 520 525
 Leu Val Glu His Leu Asp Ala Thr Val Ala Lys Phe Lys Asp Ala Met
 530 535 540
 Lys Ser Ser Leu Arg Arg Leu His Tyr Val Thr Thr Val Ala Val Pro
 545 550 555 560
 Glu Ala Ile Ile Phe Ala Ile Thr Gln Ile Arg Tyr Cys Ser Gly Ser
 565 570 575
 Glu Ala Asn Glu Ile Phe Glu Asn Ala Leu Leu Lys Thr Val Glu Lys
 580 585 590
 Thr Pro Ser Glu Pro Ser Asp Glu Ile Gly Ala Ala Thr Gly Phe Glu
 595 600 605
 Gln Leu Leu Lys Ala Ala Ser Lys Val Arg Cys Ala Ala Leu Gly Met
 610 615 620
 Asp
 625

<210> SEQ ID NO 4
 <211> LENGTH: 124
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 4

Met Leu Thr Ala Gly Met Val Glu Thr Arg Lys Tyr Glu Asn Arg Lys
 1 5 10 15
 Ile Thr Phe Gly Val Gln Thr Leu Ser Glu Asn Val Lys Asn Ile Gln
 20 25 30
 Val Gln Ile Val Asp Ser Ile Leu Glu Arg Ile Pro Tyr Val Ile Asn
 35 40 45
 Asn Glu Arg Glu Gln Leu Val Glu Ser Lys Trp Gly Lys Pro Tyr Val
 50 55 60
 Leu Leu Gly Val Glu Gly Ile Ala Asp Val Val Ile Arg Asn Ser Met
 65 70 75 80
 Lys His Pro Pro Gly Trp Met Ile Met Glu Thr Glu Val Glu Val Val
 85 90 95
 Glu Gly Gly Lys Arg Lys Val Ala Gln Asp Ser Asp Thr Gly Asn Arg
 100 105 110
 Asn Asp Glu Ser Tyr Lys Phe Lys Gln Leu Arg Ile
 115 120

<210> SEQ ID NO 5
 <211> LENGTH: 949
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 5

Met Ala Ser Ser Leu Met Asn His Thr Asn Gly Glu Leu Leu Trp Thr
 1 5 10 15
 Phe Phe Glu Asn Trp Leu Asp Glu Lys Leu Asp Ala Asn Glu Val Ile
 20 25 30
 Val Lys Asn Thr Asp Val Lys Asn Asn Cys Lys Arg Thr His Asp Leu
 35 40 45
 Pro Trp Lys Gly Val Thr Ile Ser Glu Ser Val Asn Lys Leu Leu Glu
 50 55 60
 Glu Leu Ile Glu Gln Leu Ile Asp Asn Tyr Val Asn Ser Trp Tyr Lys
 65 70 75 80

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Thr	Lys	Ile	Ser	Asp	Asp	Thr	Thr	Phe	Ile	Asn	Glu	Ile	Arg	Tyr	Gln	
			85						90					95		
Ile	Arg	Tyr	Ala	Val	Ala	Val	Leu	Tyr	Leu	Arg	Leu	Gln	Lys	Ile	Asp	
			100					105					110			
Leu	Ser	Ser	Thr	Ile	Leu	Phe	Glu	Ala	Val	Pro	Leu	Ala	Ala	Ile	His	
			115				120					125				
Cys	Val	Arg	Val	Glu	His	Leu	Arg	Ala	Thr	Ile	Asp	Lys	Ser	Met	Ser	
	130					135					140					
Ser	Ser	His	Leu	Val	Glu	Thr	Lys	Ile	Leu	Glu	Ala	Met	Pro	Asp	Val	
145				150						155					160	
His	Phe	Ala	Leu	Ser	Ser	Arg	Gln	Asn	Glu	Val	Asp	Tyr	Leu	Arg	Glu	
			165					170						175		
Leu	Ala	Asp	His	Leu	Ile	Gly	Leu	Ile	Met	Asp	Glu	Arg	Cys	Ile	Ala	
			180					185					190			
Gly	His	Pro	Ser	Asp	Ser	Asp	Ser	Pro	Phe	Arg	Asp	Ile	Leu	Thr	Asn	
		195					200					205				
His	Pro	Arg	Pro	Trp	Ala	Ser	His	Val	Cys	Arg	His	Phe	Leu	Arg	Glu	
	210					215					220					
Leu	Phe	Val	Phe	Ser	Phe	Phe	Leu	Pro	Thr	Met	Asp	Leu	Ile	Ala	Asp	
225					230					235					240	
Pro	Asp	Ile	Ile	Asn	Arg	Val	Leu	Ile	Phe	Leu	Phe	Asp	Ser	Asp	Val	
				245					250					255		
Leu	Asn	Cys	Pro	Pro	Leu	Ser	Gln	Glu	Ser	Arg	Gln	Val	Glu	Ile	Leu	
			260					265					270			
His	Gly	Leu	Thr	Asp	Tyr	Ser	Leu	Asn	Asp	Thr	Pro	Asp	Ser	Leu	Leu	
		275					280					285				
Gln	Leu	Lys	Leu	Ser	Asp	Met	Leu	Arg	Asp	Thr	Gly	Gln	Leu	Ile	Met	
	290					295					300					
Phe	Arg	Ala	Tyr	Leu	Asn	Asp	Ile	His	Ala	Pro	Leu	Asn	Glu	Leu	Asp	
305				310						315					320	
Phe	Leu	Val	His	Ala	Gly	Asp	Ala	His	Gly	Arg	Met	Leu	Asn	Val	Gln	
			325						330					335		
Asn	Asp	Leu	Val	Ala	Met	Ser	Glu	Leu	Gln	Tyr	Asp	Ile	Trp	Glu	Leu	
		340						345					350			
Phe	Ile	Lys	Tyr	Ile	His	Glu	Gly	Ala	Pro	Asp	Arg	Ile	Asn	Leu	Pro	
		355					360					365				
Ile	Glu	Ile	Val	Cys	Glu	Phe	Thr	Glu	Ala	Val	Glu	Lys	His	Asp	Cys	
	370					375						380				
Glu	Leu	Leu	Asp	Arg	Cys	Leu	Glu	Lys	Ala	Phe	Gln	Ile	Val	Tyr	Lys	
385					390					395					400	
Arg	Met	Gln	His	Glu	Tyr	Val	Ile	Pro	Phe	Cys	Gln	Ser	Glu	Cys	Phe	
			405						410					415		
Leu	Gly	Asn	Leu	Cys	Gly	Pro	Arg	Pro	Val	Gly	Val	Asp	Glu	Leu	Phe	
		420						425					430			
Thr	Ser	Arg	Glu	Tyr	Ser	Lys	Ser	Thr	Arg	Gly	Leu	Leu	Ser	Ala	Val	
		435					440					445				
Glu	Pro	Asn	Cys	Ser	Leu	Thr	Gln	Phe	Arg	Asn	Arg	Phe	Trp	Arg	Val	
	450					455					460					
Val	Leu	Pro	Thr	Ala	Glu	Ser	Val	Asp	Pro	Ser	Phe	Asp	His	Leu	Asn	
465					470					475					480	
Asn	Val	Ala	Leu	Ser	Glu	Thr	Asn	Ser	Gly	Ile	Glu	Pro	Ala	Asp	Asn	
			485						490					495		
Ile	Thr	Glu	Gly	Leu	Gly	Ser	Asp	Ser	Pro	Asp	Asp	Gly	Thr	Thr	Leu	

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500							505							510						
Pro	Thr	Phe	Glu	Phe	Val	Leu	Asn	Asn	Gly	Lys	Glu	Lys	Thr	Ala	Glu					
		515					520					525								
Met	Gln	Asp	Asp	Gln	Leu	Ile	Ser	Asn	Met	Pro	Asp	Glu	Glu	Val	Val					
	530					535					540									
Glu	Gln	Gln	Met	Ile	Leu	Ser	Asn	Ser	Phe	Glu	Ile	Pro	Met	Phe	Asp					
545					550					555					560					
Pro	Glu	Arg	Asp	Met	Asn	Lys	Trp	Asn	Val	Thr	Phe	His	Met	Leu	Ser					
				565					570					575						
His	Asp	Glu	Ile	Leu	Leu	Val	Val	Cys	His	Thr	Met	Tyr	Val	Tyr	Val					
		580						585					590							
Ile	Ser	Val	Glu	Arg	Phe	Asp	Val	Ser	Asp	Asp	Ile	Asp	Gln	Lys	Ser					
	595						600					605								
Leu	Ser	Ala	Thr	Ala	Ala	Pro	Gln	Lys	Trp	Ser	Val	Ile	Arg	Gln	Tyr					
	610					615					620									
Asn	Glu	Phe	Tyr	Ile	Leu	Glu	Ser	Lys	Leu	Ile	Glu	Phe	His	Gly	Ser					
625					630					635					640					
Met	Ile	Lys	Thr	Glu	Ser	Leu	Pro	Pro	Arg	Arg	Phe	Phe	Asn	Cys	Lys					
				645					650					655						
Ser	Arg	Val	Tyr	Val	Glu	Ser	Arg	Arg	Glu	Ile	Phe	Glu	Arg	Phe	Ile					
			660					665					670							
His	Leu	Leu	Thr	Lys	Gln	Arg	Ala	Leu	Lys	Gln	Ser	Asp	Leu	Leu	Tyr					
	675					680						685								
Val	Phe	Leu	Ser	Thr	Glu	Gln	Arg	Leu	Lys	Asp	Ser	Thr	Gln	Ile	Ser					
	690					695					700									
Asp	Leu	Tyr	Pro	Trp	Asn	Met	Val	Lys	Lys	Val	Pro	Gly	Lys	Phe	Ala					
705					710					715					720					
Arg	Glu	Lys	Gly	Gln	Asn	Leu	Lys	Pro	Phe	Ile	Leu	Ser	Leu	Leu	Ala					
			725						730					735						
Ala	Thr	Leu	Ile	Pro	His	Pro	Asn	Asn	Ser	Met	Glu	Ser	Ser	Val	Thr					
		740						745						750						
Ser	Cys	Ile	His	Pro	Glu	Ile	Ala	Asn	Gln	Thr	Arg	Arg	Ile	Leu	Ala					
	755						760					765								
Ser	Asp	Ile	Tyr	Gly	Asp	Asn	Cys	Pro	Ser	Ala	Gln	Thr	Asp	Phe	Ser					
	770					775						780								
Leu	Thr	Lys	Ser	Thr	Leu	Trp	Thr	Asn	Ser	Phe	Tyr	Asp	Ala	Val	Leu					
785					790					795					800					
Phe	Ile	Leu	Asn	Arg	Leu	Phe	Gly	Val	Val	Arg	Trp	Pro	Ile	Trp	Ile					
			805						810					815						
Ile	Ile	Thr	Ile	Arg	His	Leu	Met	Gly	Ser	Thr	Ile	Asp	Ala	Val	Ala					
		820						825					830							
Ala	Val	Leu	Phe	Arg	Arg	Phe	Leu	Ser	Arg	Met	Phe	Val	Asp	Ile	Asn					
	835						840					845								
Cys	Ile	Arg	Ile	Leu	Arg	Phe	Ile	Gln	Glu	Ser	Val	Phe	Gly	Phe	Asn					
	850					855						860								
Ser	Ser	Thr	Glu	Thr	Asp	Gln	Glu	Lys	Met	Leu	Arg	Met	Glu	Leu	Ala					
865					870					875					880					
Gln	His	Leu	Thr	Leu	Glu	Tyr	Leu	Gln	Glu	Gln	Leu	Pro	Val	Cys	Phe					
			885						890					895						
Ile	Lys	Leu	Ile	Gly	His	Lys	Gln	Phe	Ser	Gln	Gly	Met	Gln	Thr	Ile					
		900						905					910							
Phe	Arg	Ile	Leu	Gln	Tyr	Pro	Arg	Leu	Asn	Lys	Gln	Leu	Ala	Tyr	Val					
	915						920					925								

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Cys Leu Asp Ile Phe Ile Gln Lys Ile Phe Pro Ile Glu Asn Glu Arg
 930 935 940

Asn Leu Glu Ile Lys
 945

<210> SEQ ID NO 6
 <211> LENGTH: 104
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 6

Met Ala Asp Leu Ile Asp Ile Asp Asp Gln Arg Arg Asn Gly Pro
 1 5 10 15
 Cys Ile Leu Tyr Asp Ala Ser Trp Ala Val Trp Ser Glu Arg Gln Ala
 20 25 30
 Arg Ala Gln Lys Val Gln Leu Val Arg Thr Val Glu Gln Ile Asn Arg
 35 40 45
 Gln Thr Asp Gly Leu Val Cys Tyr Val Lys Arg Lys Asp Leu Phe Gly
 50 55 60
 Asp Leu Ser Ile Tyr Pro Trp Ala Arg Ser Ile Arg Glu Leu Val Pro
 65 70 75 80
 Ser Ser Val Leu Ala Pro His Gln Pro Leu Glu His Phe Lys Gly Lys
 85 90 95
 Glu Ile His Cys Ser Val Thr Trp
 100

<210> SEQ ID NO 7
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 7

Met Pro His Met Ser Lys Leu Ser Leu Gly Met Glu Gly Gly Ser Ser
 1 5 10 15
 Val Val Val Glu Leu Gly Ser Gly Ser Asp Leu Gln Asn Arg Pro Trp
 20 25 30
 Thr Arg Thr Phe Ser Cys Leu Lys Val Arg Val Val Pro Leu Met Gln
 35 40 45
 Asp Ile Ile Arg Asn Lys Pro Thr Val Ala
 50 55

<210> SEQ ID NO 8
 <211> LENGTH: 170
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 8

Met Lys Glu Ser Lys Leu Trp Glu Ala Ala Leu Glu Ser Ala Ile Leu
 1 5 10 15
 Ile Gly Leu Pro Ile Phe Ser Ser Pro Leu Ser Pro Leu Ser Ile Ser
 20 25 30
 Lys Ile Pro Ala Ser Ile Ala Phe Asn Thr Lys His Leu Cys Leu Ala
 35 40 45
 Leu Leu Val Ser Ala Phe Thr Ile Val Ala Phe Ser Ile Leu Val Arg
 50 55 60
 Lys Met Thr Pro Lys Ile Arg Val Arg Ser Lys Val Gly Thr Pro Ile
 65 70 75 80

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Ile Ile Arg Thr Ala Ala Thr His Lys Lys Val Arg Cys Asn Trp Pro
      85                      90                      95

Lys Ala Leu Gln Arg Pro Leu Arg Pro Val Ala Leu Lys Ile Cys Asp
      100                    105                    110

Arg Lys Ile Cys Leu Phe Leu Tyr Pro Tyr Tyr Ala Ser Phe Leu Gly
      115                    120                    125

Asn Leu Gln Pro Lys Gly Tyr Pro Gln Ser Phe Tyr Lys Ser Ile His
      130                    135                    140

Ile Ala Ile Asn Leu Asp Gly Ser Val Thr Val Met Asp Asp Gly Arg
      145                    150                    155                    160

Gly Thr Asn Leu Lys Thr Leu Leu Lys Met
      165                      170

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<210> SEQ ID NO 9
<211> LENGTH: 143
<212> TYPE: PRT
<213> ORGANISM: Loa loa

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<400> SEQUENCE: 9

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Met Lys Glu Asp Gly Lys Arg Glu Phe Val Ile His Lys Ala Ser Leu
1      5      10      15

Lys Ile Asn Asn Phe Ala Ile Gly Asn Ser Glu Arg Glu Pro Ile Trp
      20      25      30

Leu His Ser Ile Lys Ser Arg Phe His Met Gln Tyr Arg Met Pro His
      35      40      45

Glu Phe Ile Gly Leu Lys Ile Leu Pro Ile Ser Asn Ile Phe Arg Leu
      50      55      60

Leu Glu Ala Ile Val Ile Ser His Glu Val Lys His Cys Glu Met Pro
      65      70      75      80

Pro Gln Cys His His Arg Trp Arg Leu Lys Lys His Asn Ile Tyr Leu
      85      90      95

Met Ile Asn Asp Glu Asp Ser Glu Lys Ser Glu Asn Glu Met Asn Ser
      100      105      110

His Trp Leu Asn Ser Glu Ala Val Ser Trp Thr Asp Glu Thr Arg Asn
      115      120      125

Ser Phe Asp Met Met Lys Asp Thr Arg Ser Ile Val Pro Ser Ser
      130      135      140

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<210> SEQ ID NO 10
<211> LENGTH: 96
<212> TYPE: PRT
<213> ORGANISM: Loa loa

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<400> SEQUENCE: 10

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Met Pro Gly Ile Gly His Leu Glu Arg Ser Gly Ser Cys Tyr Arg Thr
1      5      10      15

Met Lys Lys Asp Leu Arg Tyr Trp Trp Ser His Trp Val Gln Lys Ile
      20      25      30

Leu Lys Asn Arg Gln Asn Gln Phe Ser Arg Gly Arg Ser Glu Ser His
      35      40      45

Arg Pro Ser Ala Lys Pro Glu Val Phe Ser Pro Ala Cys Leu Glu Ile
      50      55      60

Gln Pro Pro His Ile Phe Arg Lys Asp Ser Glu Arg Ser Cys Glu Cys
      65      70      75      80

Pro Lys His Gly Gly Ser Ser Arg Lys Asn Asn Asp Ile Gly Asn Ser
      85      90      95

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<210> SEQ ID NO 11
 <211> LENGTH: 305
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 11

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Met Leu Gly Ser Trp Lys Trp Ser His Gly Trp Ile Tyr Leu Gly Ser
1      5      10      15
Val Met Gly Ala Asp Gly Gly Thr Ile Pro Lys Arg Cys Glu Leu Val
      20      25      30
Lys Lys Lys Lys Lys Lys Glu Lys Leu Asp Lys Asn Val Ala Asn Thr
      35      40      45
Thr Arg Trp Arg Leu Cys Arg Leu Ser Gln Glu Pro Leu Lys Arg Pro
      50      55      60
Ile Val Ala Cys Arg Leu Gly Asn Leu Tyr Asn Lys Glu Glu Val Leu
65      70      75      80
Asn Ala Ile Leu Ser Lys Lys Ile Gly Glu Tyr Glu Val Ala Met His
      85      90      95
Ile Arg Gly Leu Arg Asp Ile Lys Glu Leu Lys Leu Thr Asp Ser Lys
      100     105     110
Glu Tyr Arg Glu Ser Gly Ala Asp Lys Gly Asp Val Tyr Lys Asp His
      115     120     125
Asn Ile Ala Pro Phe Cys Cys Pro Val Thr Gly Ile Ser Met Asn Gly
130     135     140
Asn His Pro Phe Thr Val Asn Trp Arg Cys Gly Cys Val Ile Ser Glu
145     150     155     160
Lys Ala Ile Glu Glu Val Lys Pro Asp Val Cys His Gly Cys Gly Gly
      165     170     175
Pro Phe Ser Lys Asp Asp Leu Ile Leu Ile Asn Pro Pro Gln Asp Val
      180     185     190
Leu Glu Ile Tyr Lys Lys Arg Glu Glu Glu Arg Leu Lys Arg Lys Leu
      195     200     205
Arg Lys Thr Ala Ser Gln Glu Pro Thr Glu Lys Lys Ser Ser Met Ser
210     215     220
Cys Lys Met Glu Ile Glu Ser Thr Gln Lys Lys Gly Ile Val Ser Glu
225     230     235     240
Arg Lys Arg Glu Ala Ala Thr Ser Ala Asp Phe Lys Asp Leu Leu Arg
      245     250     255
Lys Val Glu Lys Arg Lys Ala Thr Ser Ala Lys Ile Ser Ser Ile Gln
      260     265     270
Asp Ser Asn Ala Ser Ala Ala Tyr Lys Ser Leu Phe Thr Thr Cys Glu
      275     280     285
Glu Ala Lys His Lys Pro Glu Pro His Trp Ile Thr His Asn Pro Leu
290     295     300
Phe
305

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<210> SEQ ID NO 12
 <211> LENGTH: 100
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 12

```

Met Lys His Thr Ser Thr Leu Thr Leu Phe Phe Tyr Gly Gly Lys Arg
1      5      10      15

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Ile Asp Lys Tyr Thr Ile Thr Ser Gln Ile Arg Arg Thr Ile Ser Glu
      20                      25                      30

Lys Ala Leu Ile Phe His Lys Leu Arg Arg Lys Gly Met Asp Phe Gly
      35                      40                      45

Ser Asn Leu Lys Lys Val Pro Arg Ile Cys Ser Ser Ile Leu Asn Trp
      50                      55                      60

Ser His Leu Gln Ala Met Arg His Ile Asn Phe Val Thr Glu Lys Ser
      65                      70                      75                      80

Asn Ser Thr Met Ile Glu Leu Asn Gly Thr Val Gln Lys Asn Gln Thr
      85                      90                      95

Tyr Leu Thr Arg
      100

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<210> SEQ ID NO 13
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: Loa loa

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<400> SEQUENCE: 13

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```

Met Val Arg Ile Asp Glu Asn Ile Ile Ser Gly Ala Lys Gly Ala Ser
1      5      10      15

Thr Val Thr Pro Arg Leu Arg Ser Gly Ala Gly Arg Glu Gly Cys Val
      20      25      30

Val Val Glu Tyr Val Met Ser Phe Leu Glu Gly Asp Cys Ser Cys Asn
      35      40      45

Pro

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<210> SEQ ID NO 14
<211> LENGTH: 625
<212> TYPE: PRT
<213> ORGANISM: Loa loa

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<400> SEQUENCE: 14

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Met Gly Arg Lys Pro Val Arg Asn Ser Ala Pro Thr Ser Asp Leu Lys
1      5      10      15

Thr Gly Asp Val Val Trp Ala Pro Tyr Arg Arg Phe Pro Glu Trp Pro
      20      25      30

Ala Leu Val Arg Cys Val Tyr Pro Lys Lys Val Thr Tyr Thr Phe Leu
      35      40      45

Pro Ile Asn Glu Ser Ser Asn Leu Lys Ser Ser Ile Phe Ser Cys Pro
      50      55      60

Pro Gln Lys Leu Arg Leu Leu Thr Gly Asn Glu Ser Leu Pro Ala Gly
      65      70      75      80

Ala Lys Asn Asp Met Lys Glu Ala Tyr Lys Ala Ala Leu Glu Ile Leu
      85      90      95

Lys Lys Ser Gly Leu Leu Gly Ala Asp His Gln Met Ser Glu Glu Leu
      100      105      110

Ser Ala Phe Lys Ala Ser Glu Ile Asn Lys Gln Ser Val Leu Gly Asp
      115      120      125

Leu Val Lys Lys Thr Lys Lys Asp Ser Asp Ala Ala Ser Ser Gly Asp
      130      135      140

Thr Ala Ser Asn Ala Ser Ala Pro His Val Trp Gly Ile Gly Glu Val
      145      150      155      160

Val Trp Leu Ser Met Pro Asn His Ser Glu Trp Pro Val Val Ile Arg
      165      170      175

Glu Leu Lys Lys Lys Phe Ala Met Val Asp Ala Phe Pro Leu Lys Phe

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180							185					190				
Asn	Cys	Lys	Pro	Glu	Arg	Tyr	Pro	Leu	Ser	Ala	Cys	Gln	Lys	Phe	Glu	
		195					200					205				
Leu	Thr	Asp	Lys	Asn	Leu	Glu	Ala	Ala	Ile	Arg	Lys	Glu	Arg	Asn	Cys	
		210				215					220					
Glu	Leu	Arg	Met	Ala	Leu	Gln	Ser	Val	Met	Lys	Tyr	Phe	Lys	Arg	Lys	
					230					235					240	
Glu	Gln	Met	Asn	Asn	Val	Glu	Lys	Ser	Asp	Thr	Glu	Asp	Asn	Thr	Ala	
				245					250					255		
Lys	Val	Asp	Asp	Lys	Asn	Asp	Ala	Gln	Gln	Gly	Cys	Asp	Arg	Lys	Asp	
			260					265					270			
Lys	Ser	Thr	Thr	Gly	Gly	Leu	Glu	Glu	Leu	Arg	Ser	Val	Asp	Gly	Glu	
		275					280					285				
Ala	Glu	Ala	Lys	Leu	Thr	Gly	Met	Leu	Lys	Glu	Gly	Glu	Asn	Lys	Arg	
		290				295					300					
Asp	Gly	Lys	Arg	Arg	Met	Asp	Lys	Ser	Pro	Ser	Pro	Ala	Ala	Lys	Arg	
					310					315					320	
Gln	Lys	Phe	Ser	Asp	Val	Met	Lys	Asp	Leu	Glu	Ile	Glu	Leu	Ser	Glu	
				325					330					335		
Lys	Leu	Glu	Asn	Leu	Ser	Lys	Gly	Asp	Leu	Ala	Trp	Ile	Asn	Arg	Ala	
			340					345					350			
Arg	Ser	Gly	Arg	Ile	Val	Lys	Trp	Pro	Ile	Leu	Val	Leu	Lys	Val	Asp	
		355				360						365				
Asn	Val	Asn	Lys	Ser	Cys	Val	Cys	Thr	Glu	Leu	Pro	Leu	Asp	Asp	Met	
		370				375					380					
Ser	Ala	Asn	Ala	Glu	Trp	Cys	Leu	Asn	Ser	Glu	Lys	Thr	Arg	Val	Val	
					390					395					400	
Gln	Leu	Lys	Asn	Ile	Tyr	Leu	Tyr	Asp	Thr	Val	Glu	Ala	Lys	Ile	Asp	
			405					410						415		
Glu	Ile	Glu	Asp	Ala	Glu	Leu	Lys	Thr	Ala	Ile	Gln	Gln	Ala	Asp	Glu	
		420						425					430			
Ile	Ala	Glu	Gly	Ser	Tyr	Arg	Pro	Phe	Asp	Asp	Glu	Arg	Asn	Ser	Tyr	
		435					440					445				
Asp	Lys	Asn	Gly	Asn	Ser	Ser	Ile	Arg	Glu	Asn	Gly	Cys	Lys	Ala	Ala	
	450					455					460					
Ser	Val	Asn	Ile	Leu	Ala	Pro	Lys	Glu	Ile	Leu	Arg	Leu	Cys	Lys	Ser	
					470					475					480	
Glu	Met	Cys	Ala	Arg	His	Leu	Met	Ala	Ile	Trp	Thr	Gly	Gln	Phe	Thr	
				485					490					495		
Cys	Thr	Arg	His	Ala	Gly	Tyr	Glu	Pro	Pro	Phe	Met	Ala	Pro	Leu	Gln	
			500					505					510			
Phe	Glu	Leu	Asn	Thr	Gly	Asp	Leu	Leu	Pro	Glu	Thr	Asp	Gly	Ile	Ser	
		515					520					525				
Leu	Val	Glu	His	Leu	Asp	Ala	Thr	Val	Ala	Lys	Phe	Lys	Asp	Ala	Met	
		530				535						540				
Lys	Ser	Ser	Leu	Arg	Arg	Leu	His	Tyr	Val	Thr	Thr	Val	Ala	Val	Pro	
					550					555					560	
Glu	Ala	Ile	Ile	Phe	Ala	Ile	Thr	Gln	Ile	Arg	Tyr	Cys	Ser	Gly	Ser	
				565					570					575		
Glu	Ala	Asn	Glu	Ile	Phe	Glu	Asn	Ala	Leu	Leu	Lys	Thr	Val	Glu	Lys	
			580					585					590			
Thr	Pro	Ser	Glu	Pro	Ser	Asp	Glu	Ile	Gly	Ala	Ala	Thr	Gly	Phe	Glu	
		595					600					605				

-continued

Gln Leu Leu Lys Ala Ala Ser Lys Val Arg Cys Ala Ala Leu Gly Met
 610 615 620

Asp
 625

<210> SEQ ID NO 15
 <211> LENGTH: 893
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 15

Met Ser Tyr Asn Leu Cys Lys Ile Lys Asn Leu Asp Asn Ala Glu Arg
 1 5 10 15

Gln Ile Arg Arg Leu Glu Glu Arg Leu Arg Ala Ala Asp Leu Glu Lys
 20 25 30

Ala Ala Ile Glu Lys Ala Arg Lys Phe Leu Glu Glu Glu Ile Asn Lys
 35 40 45

Leu His Gln Gln Tyr Gln Lys Ala Thr Ala Glu Glu Glu Arg Lys Ala
 50 55 60

Arg Asp Lys Gln His Glu Ile Asn Leu Gly Leu Glu Glu Glu Tyr Lys
 65 70 75 80

Asn Arg Ile Asn Glu Leu Lys Ser Arg Ile Glu Thr Ile Gln Arg Asp
 85 90 95

Asn Thr Lys Leu Lys Thr Glu Leu Asn Thr Met Arg Asp Lys Tyr Arg
 100 105 110

Asp Thr Glu Asn Glu Tyr Asn Ile Thr Leu Arg Lys Leu Glu Glu Lys
 115 120 125

Asp Ala Ala Leu Arg His Leu Asp Glu Thr Lys Arg Gln Phe Thr Asn
 130 135 140

Glu Leu Asp Gln Gln Arg Thr Arg Tyr Asp Thr Leu Asn Ser Glu Phe
 145 150 155 160

Asp Arg Leu Asn Asn Glu Tyr Glu Asn Ala Asn Lys Thr Val Val Thr
 165 170 175

Leu Glu Gln Thr Val Arg Glu Ile Lys Gln Gln Arg Asp Glu Tyr Gly
 180 185 190

Lys Gln Lys Asp Glu Leu Ser Arg Gln Met Phe Asp Leu Lys His Gln
 195 200 205

Leu Glu Asn Glu Lys Ala Ala Arg Glu Glu Ala Glu Lys Thr Thr Ser
 210 215 220

Arg Leu Thr Gln Glu Ile Glu Lys Phe Lys Leu Gln Ile Thr Asp Tyr
 225 230 235 240

Glu Asn Gln Leu Ser Met Leu Arg Arg His Asn Asp Glu Leu Asp Thr
 245 250 255

Gln Ile Lys Ser Gly Gln Ala Lys Ile Thr Ala Ile Glu Asn Asp Leu
 260 265 270

Ile Thr Ser Gln Lys Glu Thr Val Arg Leu Asn Glu Leu Asn Ser Arg
 275 280 285

Leu Gln Arg Glu Lys Gln Asp Ala Ile Asn Gln Lys Leu Lys Ala Glu
 290 295 300

Ser Asp Ala Glu Val Trp Lys Glu Gln Ile Arg Arg Leu Glu Leu Glu
 305 310 315 320

Ile Glu Lys Leu Lys Ile Glu Asn Arg Thr Ile Thr Glu Gln Glu Glu
 325 330 335

Lys Thr Arg Asp Ala Leu Thr Lys Glu Thr Asn Arg Ala His Leu Leu

-continued

340	345	350
Gln Lys Glu Leu Glu Glu Thr Lys	Ala Glu Ile Glu Glu Leu Glu Lys	
355	360	365
Lys Ile Lys Arg Leu Glu Gln Glu Ile Glu Ser Ser Ser Arg Gly Thr		
370	375	380
Arg Lys Asp Glu Ser Glu Glu Thr Asp Lys Ile Ser Leu Gly Pro Ser		
385	390	395
400		
Gly Pro Thr Val Tyr Asp Thr Glu Ile His Glu Ile Arg Ile Arg Glu		
405	410	415
Val Asn Asp Lys Trp Lys Leu Glu Phe Asp Lys Ile Leu Gly Glu Lys		
420	425	430
Asp Glu Leu Glu Arg Arg Ile Arg Asp Leu Glu Asp Gln Ile Thr Gln		
435	440	445
Lys Asn Arg Glu Phe Glu Arg Gln Glu Thr Glu Ile Ala Glu Leu Lys		
450	455	460
Arg Lys His Gln Glu Glu Ile Asp Arg Leu Arg Ser Glu Ile Ser Gln		
465	470	475
480		
Leu His Asp Lys His Gln Asn Asp Leu Asp Asp Glu Lys Glu Gln Tyr		
485	490	495
Asn Lys Asn Leu Glu Ser Ile Lys Tyr Val Glu Asp Glu Leu Arg Asn		
500	505	510
Lys Leu Ala Glu Ala Glu Arg Lys Leu Ala Glu Ala Glu Asn Arg Glu		
515	520	525
Asn Gln Leu Glu Arg Glu Lys Val Glu Leu Lys Glu Lys Tyr Glu Gln		
530	535	540
Ala Leu Ala Gln Ile Gln Lys Leu Lys Asp Asp Leu Asp Asp Ala Arg		
545	550	555
560		
Gln Glu Ala Glu Asn Glu Ile Gln Lys Trp Lys Thr Glu Val Tyr Ser		
565	570	575
Val Arg Ser Glu Leu Lys Ala Leu Glu Thr Ser Ser Asn Ala Leu Arg		
580	585	590
Thr Gln Leu Ala Ala Ala Asn Glu Arg Ala Glu Ser Leu Asn Lys Thr		
595	600	605
Val Asn Asp Gln Asn Gly Lys Ile Arg Glu Leu Asn Thr Gln Ile Arg		
610	615	620
Arg Leu Glu Glu Glu Ile Ser Asp Leu Lys Ser Ala Ala Val Thr Arg		
625	630	635
640		
Glu Ser Asp Leu Glu Ser Ser Leu Ser Arg Leu Arg Ser Val Glu Asp		
645	650	655
Gln Tyr Ala Thr Leu Gln Ser Glu His Ala Lys Thr Arg Asn Glu Leu		
660	665	670
Glu Ile Leu Gln Arg Glu Tyr Asp Leu Leu Lys Ser Thr Asn Ile Asn		
675	680	685
Gln Glu Ser Glu Leu Glu Arg Leu Arg Asn Lys Ile Gln Gln Tyr Glu		
690	695	700
Val Thr Ile Lys Glu Gln Lys Asn Ala Leu Asp His Leu Lys Ala Glu		
705	710	715
720		
Arg Glu Arg Leu Gln Asn Ile Tyr Arg Asp Lys Val Lys Gln Leu Asp		
725	730	735
His Leu Thr Gln Leu Val Gln Ser Phe Asp Val Lys Met Asn Lys Met		
740	745	750
Arg Gln Asn Leu Arg Asp Thr Ser Asp Lys Phe Val Ala Ala Glu Thr		
755	760	765

-continued

Glu Arg Asn Ala Leu Arg Ser Glu Val Thr Lys Leu Gln Gln Glu Leu
 770 775 780
 Gln Phe Gly Lys Asp Gln Met Val Arg Arg Thr Asp Glu Tyr Gln Ser
 785 790 795 800
 Ser Leu Glu Asp Leu Ala Asn Ala His Arg Ala Ala Glu Asp Gly Arg
 805 810 815
 Leu Asn Ala Leu Gln Glu Leu Glu Ser Arg Lys Tyr Glu Leu Ala Asp
 820 825 830
 Leu Lys Ser Arg Phe Glu Asn Thr Glu Gln Arg Leu Thr Ser Leu Gln
 835 840 845
 His Asp Tyr Asn Lys Val Glu Asn Glu Arg Asp Ile Leu Ala Asp Ser
 850 855 860
 Leu Lys Arg Phe Tyr Ser Val Thr Thr His Ala Val Thr Leu His Lys
 865 870 875 880
 Val Lys Val Asn Tyr Leu Asn Glu Trp Ile Val Ile Glu
 885 890

<210> SEQ ID NO 16
 <211> LENGTH: 66
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 16

Met Thr Thr Arg Glu Asn Ile Cys Ser Thr Ser Phe Ser Glu Lys Glu
 1 5 10 15
 Asp His Leu Thr Gly Lys Val Ala Asn Asn Pro Val Thr Val Leu Gly
 20 25 30
 Ser Gly Val Ala Cys Gly Lys Leu Leu Thr Ala Ser Leu Arg Arg Gln
 35 40 45
 Thr Asp Ile Val Arg Lys Gly Val Asp Ser Lys Asp Arg Val Glu Arg
 50 55 60
 Leu Trp
 65

<210> SEQ ID NO 17
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 17

Ile Lys Phe Ser Lys Leu Glu Arg Arg Gln Arg Val Ser Leu Arg Leu
 1 5 10 15
 Lys Gln Ala Ala Tyr Lys Ser Val Ile Val Val Tyr Cys Glu Leu Ser
 20 25 30
 Ser Ser Leu Gly Phe Ile Asn Gly Leu Leu Asn Gly Tyr Lys Tyr Arg
 35 40 45
 Trp Met Thr Thr Ala Val Ile Asn
 50 55

<210> SEQ ID NO 18
 <211> LENGTH: 119
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 18

Met Phe Lys Gln His Ala Gln Tyr Val Gln Ser Ser Glu Ile Ile Phe
 1 5 10 15

-continued

Asp Lys Phe Ile Asn Phe Leu Ser Ser Ser Ile Trp Asn Ile Pro Ile
 20 25 30
 Ala Thr Phe Leu Glu Gln Tyr Ser Ile Val Phe Asp Asp Lys Gln Asn
 35 40 45
 Asp Leu Leu Leu Tyr Gln Lys Ile His Asp Glu Phe Lys Ser Met Val
 50 55 60
 Asp Thr Leu Met Asp Gly Phe Cys Gly Asp Leu Gln Ile Lys Ala Arg
 65 70 75 80
 Glu Leu Val Thr Ala Leu Lys Gln His Asp Asn Ser Asn Lys Leu Ser
 85 90 95
 Thr Lys Asn Arg Val Ala Leu Phe Phe Ser Ser Ile Asn Asn Leu Val
 100 105 110
 Tyr Leu Ile Asn Leu Lys Leu
 115

<210> SEQ ID NO 19
 <211> LENGTH: 106
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 19

Met Thr Leu Asp Cys Cys Arg Leu Asn Phe Gly Asp Glu Ala Leu Val
 1 5 10 15
 Leu Ala Thr Asn Ala Leu Met Ala Gln Lys Ile Asn Ala Gly Glu Arg
 20 25 30
 Ser Ser Ile Thr Arg Gly Arg Thr Glu Asn Leu Arg Arg Arg Lys Val
 35 40 45
 Val Ile Phe His Leu Gly Asp Val Ser Ser Leu Val Glu Leu Tyr Pro
 50 55 60
 His Thr Ser Ile Leu Ala Asp Thr Tyr Leu Phe Asp Asn Ile Asn Ile
 65 70 75 80
 Gly His Ile Asp Gln Tyr Ile Phe Arg Ile Ala Arg Arg Gln Arg Trp
 85 90 95
 Trp Lys Arg Ser Arg Asn Pro Asp Ile Asp
 100 105

<210> SEQ ID NO 20
 <211> LENGTH: 93
 <212> TYPE: PRT
 <213> ORGANISM: Loa loa

<400> SEQUENCE: 20

Met Val Gln Pro Leu Gly Phe Gly Cys Glu Leu Asn Tyr Gly Gly Val
 1 5 10 15
 Trp Gly Gln Thr Gly His Cys Pro Phe Glu Ser Arg Gly Tyr Pro Lys
 20 25 30
 Ala Val Gln Phe Ala Thr Met Lys Asn Val Phe Gly Gly Gln Glu Asp
 35 40 45
 Glu Leu Pro Ala His Pro Gln Asp Pro Ile Leu Ile His Gln Lys Thr
 50 55 60
 Phe Glu Asn Thr Thr Ile Lys Lys Cys Gln Ala Ile Glu Phe Pro Ile
 65 70 75 80
 Gln Leu Asn Ala Thr Thr Val Asp Lys Thr Ser Arg Ile
 85 90

The invention claimed is:

1. A method of detecting the presence of *Loa loa* antigens in a biological sample from a subject, 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, 4, 14, and 15, wherein the presence of at least one of the antigens in the biological sample is indicative of the presence of *Loa loa* in the subject.

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, 4, 14, and 15 in the biological sample, wherein the presence of at least two of the antigens in the biological sample is indicative of the presence of *Loa loa* in the subject.

3. The method according to claim 1, 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, 4, 14, and 15, 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 subject.

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, 4, 14, and 15, 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 subject.

6. The method according to claim 1, 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 subject.

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 from a subject, 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, 4, 14, and 15, wherein the presence of at least one of the antibodies in the biological sample is indicative of the presence of *Loa loa* in the subject.

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, 4, 14, and 15, wherein the presence of at least two of the antibodies is indicative of the presence of *Loa loa* in the subject.

10. The method according to claim 1, 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 claim 1, wherein the biological sample is a human biological sample.

13. The method of claim 1, wherein the biological sample is whole blood, serum, or plasma.

14. The method of claim 1, wherein the biological sample is urine.

15. The method of claim 1, 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 NOs: 1, 4, 14, and 15 and a physiologically-acceptable carrier, and further comprising an adjuvant.

17. 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 NOs: 1, 4, 14, and 15, the method comprising administering the composition of claim 16 to an animal under conditions sufficient for the animal to develop an immune response to the antigen.

18. The method of claim 17, comprising harvesting serum from the animal after the animal has developed the immune response, wherein the serum comprises the antibody.

19. The method of claim 17, 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.

20. 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 NOs: 1, 4, 14, and 15;
- (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).

21. The method according to claim 1, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 14.

22. The method according to claim 17, wherein the antigen has an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 14.

23. The composition according to claim 16, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 14.

24. The test kit according to claim 20, wherein each antigen has a different amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 14.

25. The method according to claim 1, wherein the antigen has the amino acid sequence SEQ ID NO: 1.

26. The method according to claim 1, wherein the antigen has the amino acid sequence SEQ ID NO: 4.

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27. The method according to claim **1**, wherein the antigen has the amino acid sequence SEQ ID NO: 14.

28. The method according to claim **1**, wherein the antigen has the amino acid sequence SEQ ID NO: 15.

* * * * *

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专利名称(译)	成分和检测方法罗阿罗阿		
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申请号	US15/569507	申请日	2016-04-28
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当前申请(专利权)人(译)	美利坚合众国为代表局局长，卫生与公众服务		
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摘要(译)

公开了使用一种或多种抗原检测生物样品中罗阿罗阿 的存在的方法，每种抗原具有选自SEQ ID NO：1-20的不同氨基酸序列。还公开了相关的组合物，特异性结合伴侣和测试试剂盒。

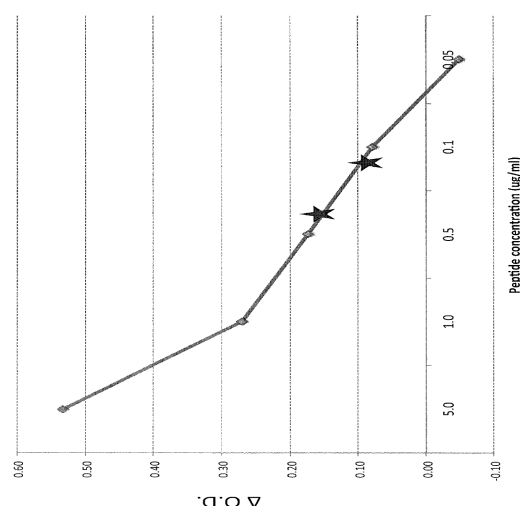


FIG. 1