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(54) **Title**: DIAGNOSTIC AND THERAPEUTIC METHODS FOR RHEUMATIC HEART DISEASE BASED UPON GROUP A STREPTOCOCCUS MARKERS

(57) **Abstract**: This invention is in the field of identifying patients having rheumatic heart disease (RHD) associated with *Streptococcus pyogenes* (Group A Streptococcus; GAS) infection and identifying patients at risk of developing RHD associated with GAS infection. The invention also provides methods and compositions for preventing and treating RHD associated with GAS infection.

DIAGNOSTIC AND THERAPEUTIC METHODS

TECHNICAL FIELD

This invention is in the field of identifying patients having rheumatic heart disease (RHD) associated with *Streptococcus pyogenes* (Group A Streptococcus; GAS) infection and identifying patients at risk of developing RHD associated with GAS infection. The invention also provides methods and compositions for preventing and treating RHD associated with GAS infection.

BACKGROUND ART

The human pathogen Group A *Streptococcus* (*Streptococcus pyogenes*, GAS) is widely recognized as a major cause of common pharyngitis. Infections with this bacterium can additionally result in severe invasive diseases as well as in non-suppurative autoimmune sequelae. Acute rheumatic fever (ARF) is a multifocal autoimmune disease occurring in 0.1–3% of individuals following untreated GAS infection.

ARF is diagnosed by the updated Jones criteria which were first published in 1944. According to the updated Jones criteria, a diagnosis of ARF can be made when two major criteria (migratory polyarthritis; carditis; subcutaneous nodules; erythema marginatum; Sydenham's chorea), or one major criterion plus two minor criteria (fever; arthralgia; raised erythocyte sedimentation rate or C reactive protein; leukocytosis; ECG showing features of heart block) are present, along with evidence of GAS infection.

The major clinically significant sequela of ARF is rheumatic heart disease (RHD). RHD can lead to serious cardiac involvement, with myocarditis or valvulitis leading to death or valve replacement. Throughout the developing world, RHD remains the leading cause of acquired heart disease in individuals <50 years of age. In the developed world, ARF and RHD are less common due to the availability of antibiotics to treat GAS infections. However, a resurgence of ARF and RHD was reported in several areas of the United States in the mid 1980s and has persisted in the intermountain area surrounding Salt Lake City, UT.

Currently, tests such as ECG and echocardiogram are used to confirm that a patient has developed RHD following diagnosis of ARF. To date, no assays are available for identifying individuals having or at risk of developing RHD as a result of GAS infection.

DISCLOSURE OF THE INVENTION

The invention concerns methods of identifying individuals having or at risk of developing RHD resulting from GAS infection. The invention also concerns protein arrays that can be used in such methods. The invention also provides methods and compositions for preventing and treating RHD associated with GAS infection.

Diagnostic methods

The invention provides a method of diagnosing rheumatic heart disease (RHD) associated with GAS infection in a patient, or of identifying a patient at risk of developing RHD associated with GAS infection, said method comprising the steps of:

a) contacting a biological sample from a patient with at least one GAS antigen under conditions appropriate for binding of any antibodies present in the biological sample to the at least one GAS antigen, and

b) comparing the reactivity of antibodies in the biological sample from the patient to the at least one GAS antigen with the reactivity of antibodies in a control biological sample from a healthy individual to the at least one GAS antigen,

wherein a lower reactivity in the biological sample from the patient compared to the control biological sample from a healthy individual is indicative that the patient is suffering from rheumatic heart disease (RHD) associated with GAS infection or that the patient is at risk of developing RHD associated with GAS infection.

In one aspect, the invention provides a method of diagnosing rheumatic heart disease (RHD) associated with GAS infection in a patient, or of identifying a patient at risk of developing RHD associated with GAS infection, said method comprising the steps of:

a) contacting a biological sample from a patient with at least one GAS antigen selected from the group comprising the amino acid sequences of

SEQ ID NO:1 (GAS5),

SEQ ID NO:2 (GAS5F),

SEQ ID NO:3 (GAS25),

SEQ ID NO:4 (GAS40),

SEQ ID NO:5 (GAS57),

SEQ ID NO:6 (GAS97),

SEQ ID NO:7 (GAS380), and

SEQ ID NO:8 (SpeA),

or functional equivalents thereof, under conditions appropriate for binding of any antibodies present in the biological sample to the at least one GAS antigen or to the functional equivalents thereof;

b) assessing the reactivity of any antibodies in the biological sample from the patient bound to the at least one GAS antigen or to the functional equivalents thereof, and

c) comparing the reactivity in step b) with the reactivity of antibodies in a control biological sample from a healthy individual bound to the at least one GAS antigen or to the functional equivalents thereof,

wherein a lower reactivity in the biological sample from the patient compared to the reactivity in the control biological sample from a healthy individual is indicative that the patient is suffering from rheumatic heart disease (RHD) associated with GAS infection or that the patient is at risk of developing RHD associated with GAS infection.

The term "rheumatic heart disease (RHD)" covers conditions affecting the heart following acute rheumatic fever including damage to the mitral valve and/or the aortic valve, myocarditis and pericarditis.

Analysis of serum samples from patients affected by RHD and from healthy individuals has led to the surprising finding that sera from patients affected by RHD display significantly lower reactivity with certain GAS antigens compared to the reactivity of sera from healthy patients. These findings provide the first evidence that reactivity with GAS antigens can be used to discriminate between sera derived from healthy individuals and sera derived from patients suffering from RHD. Specifically, it has been found that sera derived from RHD patients display a lower reactivity with the eight GAS antigens that are identified in Table 1 below:

Table 1: GAS antigens employed in the diagnostic methods of the invention

SEQ ID NO	Internal GAS ref.	Spy number	gi number
1	GAS5	spy0019	gi-15674263
2	GAS5F	spy0019 (fragment from amino acids 224-398)	gi-15674263
3	GAS25	spy0167	gi-15674372
4	GAS40	spy0269	gi-15674449
5	GAS57	spy0416	gi-15674549
6	GAS97	spy1801	gi-15675636
7	GAS380	spy1813	gi-15675644
8	SpeA	spyM3_1301	gi-21910837

Detection of low reactivity against these eight GAS antigens in patient samples compared with reactivity in control samples from healthy individuals can thus be used to diagnose RHD associated with GAS infection or to identify patients with an increased risk of developing RHD associated with GAS infection. Conversely, detection of antibody reactivity against these eight GAS antigens in a patient sample that is similar to the reactivity present in a control sample from a healthy individual is indicative that the patient is not suffering from RHD and is at lower risk of developing RHD associated with GAS infection.

The methods of the invention may comprise contacting the biological sample from the patient with 1, 2, 3, 4, 5, 6, 7 or all 8 of the GAS antigens recited above, or with functional equivalents thereof.

Where the biological sample from the patient is contacted with 2 of the GAS antigens, the methods may comprise contacting the sample with: SEQ ID NOS:1 and 2; SEQ ID NOS:1 and 3; SEQ ID NOS:1 and 4; SEQ ID NOS:1 and 5; SEQ ID NOS:2 and 3; SEQ

ID NOS:2 and 4; SEQ ID NOS:2 and 5; SEQ ID NOS:3 and 4; SEQ ID NOS:3 and 5; SEQ ID NOS:4 and 5, or functional equivalents thereof. The methods may also comprise contacting the sample with SEQ ID NOS:1 and 6; SEQ ID NOS:1 and 7; SEQ ID NOS:1 and 8; SEQ ID NOS:2 and 6; SEQ ID NOS:2 and 7; SEQ ID NOS:2 and 8; SEQ ID NOS:3 and 6; SEQ ID NOS:3 and 7; SEQ ID NOS:3 and 8; SEQ ID NOS:4 and 6; SEQ ID NOS:4 and 7; SEQ ID NOS:4 and 8; SEQ ID NOS:5 and 6; SEQ ID NOS:5 and 7; SEQ ID NOS:5 and 8; SEQ ID NOS:6 and 7; SEQ ID NOS:6 and 8, or SEQ ID NOS:7 and 8, or functional equivalents thereof

Where the biological sample from the patient is contacted with 3 of the GAS antigens, the methods may comprise contacting the sample with any combination of 3 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8. For example, the methods may comprise contacting the sample with SEQ ID NOS: 1, 2 and 3; SEQ ID NOS: 1, 3 and 4; SEQ ID NOS: 1, 4 and 5; SEQ ID NOS:, 2, 3 and 4; SEQ ID NOS: 2, 4 and 5; SEQ ID NOS: 3, 4 and 5, or functional equivalents thereof.

Where the biological sample from the patient is contacted with 4 of the GAS antigens, the methods may comprise contacting the sample with any combination of 4of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8.For example, the methods may comprise contacting the sample with: SEQ ID NOS: 1, 2, 3 and 4; SEQ ID NOS:2, 3, 4 and 5; SEQ ID NOS: 1, 3, 4 and 5, or functional equivalents thereof.

Where the biological sample from the patient is contacted with 5 of the GAS antigens, the methods may comprise contacting the sample with any combination of 5 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8. For example, the methods may comprise contacting the sample with SEQ ID NOS:1, 2, 3, 4 and 5, or functional equivalents thereof.

Where the biological sample from the patient is contacted with 6 of the GAS antigens, the methods may comprise contacting the sample with any combination of 6 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8.

Where the biological sample from the patient is contacted with 7 of the GAS antigens, the methods may comprise contacting the sample with: SEQ ID NOS: 1, 2, 3, 4, 5, 6, and 7; SEQ ID NOS: 1, 3, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 6 and 8; or functional equivalents thereof.

Alternatively, the biological sample from the patient may be contacted with all 8 of the GAS antigens, i.e. with SEQ ID NOS:1, 2, 3, 4, 5, 6, 7 and 8, or functional equivalents thereof.

The reactivity of antibodies bound to 1, 2, 3, 4, 5, 6, 7 or all 8 of these GAS antigens or functional equivalents thereof in the biological sample from a patient is compared to the reactivity of antibodies binding to these GAS antigens in a control biological sample from a healthy individual. The control biological sample from a healthy individual is contacted with the same combination of GAS antigens as the patient biological sample. Generally, average reactivities of antibodies bound to combinations of these GAS antigens will already have been determined in control biological samples from healthy individuals. Suitable methods for assessment the antibody reactivity are known in the art and are described in more detail below.

Antibody detection:

The methods of the invention described above all comprise the assessment of antibody reactivity, i.e. the detection of antibodies bound to the GAS antigens and of the titres of these antibodies. Methods for detecting antibodies bound to antigens and of determining antibody titres are well known to those of skill in the art and any such methods may be used.

For example, the GAS antigen or antigens (or functional equivalent) may be immobilised at known locations on a surface, such as on the surface of an array as described below. The immobilised antigens may be incubated with the immobilised antigens under conditions that allow the binding of any antibodies present in the sample to the antigens. A suitable incubation period may be around 1 hour. Following washing to remove any unbound antibodies, the detection of antibodies bound to the antigens may be accomplished using an entity that will bind and recognise the bound antibodies.

For example, the step of assessing the reactivity of any antibodies bound to the GAS antigens in any of the methods described above may comprise contacting the biological sample and GAS antigens with a labelled secondary antibody, such as a labelled anti-IgG antibody, under conditions suitable for the binding of the secondary antibody to any antibodies in the biological sample that have bound to the immobilised GAS antigens.

The secondary antibody, such as the anti-IgG antibody, may be labelled with a fluorescent or an enzyme label such that the binding of the secondary antibody, and thus

the presence of antibodies against the GAS antigens in the biological sample, is detected by detecting the label. Where the label is a fluorescent label, comparison of fluorescence intensity may be used to assess relative antibody reactivity and thus determine whether there is a particular patient sample displays a lower antibody reactivity than a control biological sample. The background fluorescence intensity may be expected to be around 5,000. Taking into account standard deviation, a fluorescence intensity of at least 15,000 may be indicative of the presence of an antibody in the sample bound to the GAS antigen. A fluorescence intensity of at least 30,000 may be regarded as indicative of a high reactivity indicative of a high titre of antibodies bound to the GAS antigen in the sample. In some aspects of the invention, a fluorescence intensity of between 15,000 and 30,000 may thus be indicative of a low reactivity likely to be associated with RHD.

The methods described above may be conducted on a protein array, such as the arrays described in more detail below or using standard ELISA or dotblot techniques.

Biological samples:

The biological samples that may be tested in the methods of the invention may be any sample known to contain antibodies against GAS antigens. Examples of suitable samples are saliva samples, blood samples or serum samples. In particular, the sample may be a serum sample.

The biological sample from the patient is from a human patient. The human patient may be an adult, an adolescent between the ages of around 12 to around 18 or from a child under 12. The patient may be displaying clinical symptoms of acute rheumatic disease, including migratory polyarthritis; carditis; subcutaneous nodules; erythema marginatum; Sydenham's chorea, fever; arthralgia; raised erythocyte sedimentation rate or C reactive protein; leukocytosis; or ECG showing features of heart block. The patient may be displaying evidence of current GAS infection. In some cases, the patient may be asymptomatic for current GAS infection and acute rheumatic disease.

The control biological sample may be from a healthy individual from an equivalent geographical location as the biological sample from the patient.

The methods of the invention may be conducted *in vitro*. The methods of the invention may further comprise the step of obtaining the biological sample from the patient.

Protein arrays:

In order to facilitate the screening of biological samples against multiple GAS antigens simultaneously, the GAS antigens employed in the methods of the invention may be displayed on one or more protein arrays. For example, each GAS antigens may be displayed on a separate array or a single array may display multiple GAS antigens simultaneously. According to a further aspect of the invention, protein arrays are provided. These arrays are suitable for use in any of the methods described above.

The invention provides a protein array comprising at least two GAS antigens having an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 or SEQ ID NO:8, or a functional equivalent thereof.

The protein array may comprise 2, 3, 4, 5, 6, 7 or all 8 of these GAS antigens or functional equivalents thereof.

When the array comprises 2 of the GAS antigens, it may comprise antigens comprising the amino acid sequences of: SEQ ID NOS:1 and 2; SEQ ID NOS:1 and 3; SEQ ID NOS:1 and 4; SEQ ID NOS:1 and 5; SEQ ID NOS:2 and 3; SEQ ID NOS:2 and 4; SEQ ID NOS:2 and 5; SEQ ID NOS:3 and 4; SEQ ID NOS:3 and 5; SEQ ID NOS:4 and 5, or functional equivalents thereof. The array may alternatively comprise antigens comprising the amino acid sequences of SEQ ID NOS:1 and 6; SEQ ID NOS:1 and 7; SEQ ID NOS:1 and 8; SEQ ID NOS:2 and 6; SEQ ID NOS:2 and 7; SEQ ID NOS:2 and 8; SEQ ID NOS:3 and 6; SEQ ID NOS:3 and 7; SEQ ID NOS:3 and 8; SEQ ID NOS:4 and 6; SEQ ID NOS:4 and 7; SEQ ID NOS:4 and 8; SEQ ID NOS:5 and 6; SEQ ID NOS:5 and 7; SEQ ID NOS:5 and 8; SEQ ID NOS:6 and 7; SEQ ID NOS:6 and 8, or SEQ ID NOS:7 and 8, or functional equivalents thereof

Where the array comprises 3 GAS antigens, it may comprise any combination of 3 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8. For example, the array may comprise the GAS antigens of: SEQ ID NOS: 1, 2 and 3; SEQ ID NOS: 1, 3 and 4; SEQ ID NOS: 1, 4 and 5; SEQ ID NOS:, 2, 3 and 4; SEQ ID NOS: 2, 4 and 5; SEQ ID NOS: 3, 4 and 5, or functional equivalents thereof.

Where the array comprises 4 GAS antigens, it may comprise any combination of 4 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8. For example, the array may

comprise the GAS antigens of: SEQ ID NOS: 1, 2, 3 and 4; SEQ ID NOS:2, 3, 4 and 5; SEQ ID NOS: 1, 3, 4 and 5, or functional equivalents thereof.

Where the array comprises 5 GAS antigens, it may comprise any combination of 5 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8. For example, the array may comprise the GAS antigens of: SEQ ID NOS:1, 2, 3, 4 and 5, or functional equivalents thereof.

Where the array comprises 6 GAS antigens, it may comprise any combination of 6 of the GAS antigens of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8.

Where the array comprises 7 GAS antigens, it may comprise the GAS antigens of: SEQ ID NOS: 1, 2, 3, 4, 5, 6, and 7; SEQ ID NOS: 1, 3, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 6 and 8; or functional equivalents thereof.

Alternatively, the array may comprise all 8 of the GAS antigens, i.e. SEQ ID NOS:1, 2, 3, 4, 5, 6, 7 and 8, or functional equivalents thereof.

The protein array may comprise additional GAS antigens.

Any type of protein array known in the art may be used in the method of invention. Production of protein arrays is described in Cretich, M., Damin F., et al (Biomolecular Engineering 23, 77-88 (2006)) and Zhu, H & Snyder, M. (Current Opinion in Chemical Biology, 7:55-63 (2003)).

For example, the protein array may be a glass slide to which the antigen or antigens are anchored. In its simplest form, the array may be a glass slide displaying a simple antigen prepared simply by coating glass microscope slides with aminosilane (Ansorge, Faulstich), adding an antigen-containing solution to the slide and drying. Slides coated with aminosilane may be obtained from Telechem and Pierce for coating with the antigen.

Alternatively, the array may display multiple antigens. For example, nitrocellulose-coated slides may be spotted with nanoliters of multiple GAS antigens. Such arrays may display replicates of each GAS antigen. The antigens spots in such arrays may be approximately 150 μm in diameter and contain ~0,35 ng of protein

Other types of protein array include a 3D gel pad and microwell arrays. As will be apparent to the skilled reader, types of protein array that have not yet been conceived but which are devised in the future may well prove to be suitable for use in accordance with the present invention.

The invention further provides a kit comprising a protein array according to the invention and instructions for the use of the array in the diagnosis of patients having or at risk of developing rheumatic heart disease associated with GAS infection.

Methods and compositions for treatment and prevention of RHD

Currently, antibiotic prophylaxis (generally penicillin) is recommended for all patients diagnosed with ARF for a period of at least 5 years following diagnosis to reduce the risk of subsequent GAS infection and the development of RHD. The identification of which patients are at risk of RHD and which are not at risk of RHD allows tailoring of medical treatment for patients who have been diagnosed with ARF.

The invention that provides that, where a patient is identified by the method of the invention as suffering from RHD associated with GAS infection having an increased risk of developing RHD associated with GAS infection, the patient may be treated with antibiotics. Conversely, where a patient is identified by the method of the invention as having a low risk of developing RHD associated with GAS infection, antibiotic treatment may not be necessary.

The realization by the inventors that the sera from healthy individuals display high reactivity with the GAS antigens discussed above suggests that antibodies against these GAS antigens may play a protective role in preventing the development of RHD. The invention therefore provides a composition comprising at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof. The invention also provides a composition comprising at least one antibody that binds specifically to at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof. Theses compositions may be immunogenic compositions, e.g. vaccine compositions.

According to a further aspect, the invention provides a method of treating or preventing RHD associated with GAS infection comprising administering to a patient in need

thereof at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof. The invention further provides at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof for use in treating or preventing RHD associated with GAS infection. The invention also provides the use of at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8 or a functional equivalent thereof, in the manufacture of a medicament for treating or preventing RHD associated with GAS infection. Alternatively, nucleic acid molecules encoding these GAS antigens may be used.

The invention also provides a method of treating or preventing RHD associated with GAS infection comprising administering to a patient in need thereof at least one antibody that binds specifically to at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof. The invention further provides at least one antibody that binds specifically to at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, or a functional equivalent thereof for use in treating or preventing RHD associated with GAS infection. The invention also provides the use of at least one antibody that binds specifically to at least one GAS antigen selected from the group comprising the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8 or a functional equivalent thereof, in the manufacture of a medicament for treating or preventing RHD associated with GAS infection.

Antibodies of the invention will typically bind specifically to the GAS antigen with an affinity of 1μM, 100nM, 10nM, 1nM, 100pM or tighter.The term “antibody” includes intact immunoglobulin molecules, as well as fragments thereof which are capable of binding a polypeptide. These include hybrid (chimeric) antibody molecules [1, 2]; F(ab′)2 and F(ab) fragments and Fv molecules; non-covalent heterodimers [3, 4]; single-chain Fv molecules (sFv) [5]; dimeric and trimeric antibody fragment constructs; minibodies [6, 7]; humanized antibody molecules [8-10]; and any functional fragments obtained from such molecules, as well as antibodies obtained through non-conventional processes such as phage display. In some embodiments, the antibodies are monoclonal antibodies. Methods of obtaining monoclonal antibodies are well known in the art. In some embodiments the antibodies are humanised or fully-human antibodies.

The compositions and methods of treatment of the invention may employ 1, 2, 3, 4, 5, 6, 7, or all 8 of the GAS antigens discussed above, or antibodies that bind specifically to 1, 2, 3, 4, 5, 6, 7, or all 8 of these GAS antigens. Combinations of GAS antigens and antibodies binding specifically to these antigens may be used.

Examples of combinations of GAS antigens that may be used in the compositions and methods of treatment of these aspect of the invention include SEQ ID NOS:1 and 2; SEQ ID NOS:1 and 3; SEQ ID NOS:1 and 4; SEQ ID NOS:1 and 5; SEQ ID NOS:2 and 3; SEQ ID NOS:2 and 4; SEQ ID NOS:2 and 5; SEQ ID NOS:3 and 4; SEQ ID NOS:3 and 5; SEQ ID NOS:4 and 5; SEQ ID NOS:1 and 6; SEQ ID NOS:1 and 7; SEQ ID NOS:1 and 8; SEQ ID NOS:2 and 6; SEQ ID NOS:2 and 7; SEQ ID NOS:2 and 8; SEQ ID NOS:3 and 6; SEQ ID NOS:3 and 7; SEQ ID NOS:3 and 8; SEQ ID NOS:4 and 6; SEQ ID NOS:4 and 7; SEQ ID NOS:4 and 8; SEQ ID NOS:5 and 6; SEQ ID NOS:5 and 7; SEQ ID NOS:5 and 8; SEQ ID NOS:6 and 7; SEQ ID NOS:6 and 8, or SEQ ID NOS:7 and 8; SEQ ID NOS: 1, 2 and 3; SEQ ID NOS: 1, 3 and 4; SEQ ID NOS: 1, 4 and 5; SEQ ID NOS:, 2, 3 and 4; SEQ ID NOS: 2, 4 and 5; SEQ ID NOS: 3, 4 and 5; SEQ ID NOS: 1, 2, 3 and 4; SEQ ID NOS:2, 3, 4 and 5; SEQ ID NOS: 1, 3, 4 and 5; SEQ ID NOS:1, 2, 3, 4 and 5; SEQ ID NOS: 1, 2, 3, 4, 5, 6, and 7; SEQ ID NOS: 1, 3, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 4, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 5, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 6, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 7 and 8; SEQ ID NOS: 1, 2, 3, 4, 5, 6 and 8; or functional equivalents thereof. Antibodies binding to these combinations of GAS antigens may also be used.

The compositions and methods described above may be useful in the treatment and prevention of GAS infection in general, as well as in the treatment and prevention of RHD associated with GAS infection.

Formulation of compositions for treatment and prevention of RHD

As detailed above, compositions of the invention may be useful as vaccines. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

Compositions may thus be pharmaceutically acceptable. They will usually include components in addition to the antigens *e.g.* they typically include one or more pharmaceutical carrier(s) and/or excipient(s).

Compositions will generally be administered to a human in aqueous form. Prior to administration, however, the composition may have been in a non-aqueous form. For instance, although some vaccines are manufactured in aqueous form, then filled and distributed and administered also in aqueous form, other vaccines are lyophilised during manufacture and are reconstituted into an aqueous form at the time of use. Thus a composition of the invention may be dried, such as a lyophilised formulation.

The composition may include preservatives such as thiomersal or 2-phenoxyethanol. It is preferred, however, that the vaccine should be substantially free from (*i.e.* less than 5μg/ml) mercurial material *e.g.* thiomersal-free. Vaccines containing no mercury are more typical. Preservative-free vaccines are particularly favoured.

To improve thermal stability, a composition may include a temperature protective agent. Further details of such agents are provided below.

To control tonicity, it is typical to include a physiological salt, such as a sodium salt. Sodium chloride (NaCl) is generally used, which may be present at between 1 and 20 mg/ml *e.g.* about 10±2mg/ml NaCl. Other salts that may be present include potassium chloride, potassium dihydrogen phosphate, disodium phosphate dehydrate, magnesium chloride, calcium chloride, *etc.*

Compositions will generally have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, more often between 240-360 mOsm/kg, and will more typically fall within the range of 290-310 mOsm/kg.

Compositions may include one or more buffers. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (particularly with an aluminum hydroxide adjuvant); or a citrate buffer. Buffers will typically be included in the 5-20mM range.

The pH of a composition will generally be between 5.0 and 8.1, and more typically between 6.0 and 8.0 *e.g.* 6.5 and 7.5, or between 7.0 and 7.8.

The composition is typically sterile. The composition is also typically non-pyrogenic *e.g.* containing <1 EU (endotoxin unit, a standard measure) per dose, for example <0.1 EU per dose. The composition is often gluten free.

The composition may include material for a single immunisation, or may include material for multiple immunisations (*i.e.* a 'multidose' kit). The inclusion of a

preservative is typical in multidose arrangements. As an alternative (or in addition) to including a preservative in multidose compositions, the compositions may be contained in a container having an aseptic adaptor for removal of material.

Human vaccines are typically administered in a dosage volume of about 0.5ml, although a half dose (*i.e.* about 0.25ml) may be administered to children.

Compositions of the invention may also comprise one or more immunoregulatory agents. Often, one or more of the immunoregulatory agents include one or more adjuvants. The adjuvants may include a TH1 adjuvant and/or a TH2 adjuvant, further discussed below.

Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts (or mixtures thereof). Calcium salts include calcium phosphate (*e.g.* the "CAP" particles disclosed in ref. 11). Aluminum salts include hydroxides, phosphates, sulfates, *etc.*, with the salts taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*). Adsorption to these salts is often employed. The mineral containing compositions may also be formulated as a particle of metal salt [12].

The adjuvants known as aluminum hydroxide and aluminum phosphate may be used. These names are conventional, but are used for convenience only, as neither is a precise description of the actual chemical compound which is present (*e.g.* see chapter 9 of reference 13)). The invention can use any of the "hydroxide" or "phosphate" adjuvants that are in general use as adjuvants. The adjuvants known as "aluminium hydroxide" are typically aluminium oxyhydroxide salts, which are usually at least partially crystalline. The adjuvants known as "aluminium phosphate" are typically aluminium hydroxyphosphates, often also containing a small amount of sulfate (*i.e.* aluminium hydroxyphosphate sulfate). They may be obtained by precipitation, and the reaction conditions and concentrations during precipitation influence the degree of substitution of phosphate for hydroxyl in the salt.

A fibrous morphology (*e.g.* as seen in transmission electron micrographs) is typical for aluminium hydroxide adjuvants. The pI of aluminium hydroxide adjuvants is typically about 11 *i.e.* the adjuvant itself has a positive surface charge at physiological pH. Adsorptive capacities of between 1.8-2.6 mg protein per mg Al^{+++} at pH 7.4 have been reported for aluminium hydroxide adjuvants.

Aluminium phosphate adjuvants generally have a PO_4/Al molar ratio between 0.3 and 1.2, such as between 0.8 and 1.2, typically 0.95±0.1. The aluminium phosphate will generally be amorphous, particularly for hydroxyphosphate salts. A typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. The aluminium phosphate will generally be particulate (*e.g.* plate-like morphology as seen in transmission electron micrographs). Typical diameters of the particles are in the range 0.5-20μm (*e.g.* about 5-10μm) after any antigen adsorption. Adsorptive capacities of between 0.7-1.5 mg protein per mg Al^{+++} at pH 7.4 have been reported for aluminium phosphate adjuvants.

The point of zero charge (PZC) of aluminium phosphate is inversely related to the degree of substitution of phosphate for hydroxyl, and this degree of substitution can vary depending on reaction conditions and concentration of reactants used for preparing the salt by precipitation. PZC is also altered by changing the concentration of free phosphate ions in solution (more phosphate = more acidic PZC) or by adding a buffer such as a histidine buffer (makes PZC more basic). Aluminium phosphates used according to the invention will generally have a PZC of between 4.0 and 7.0, such as between 5.0 and 6.5 *e.g.* about 5.7.

Suspensions of aluminium salts used to prepare compositions of the invention may contain a buffer (*e.g.* a phosphate or a histidine or a Tris buffer), but this is not always necessary. The suspensions are frequently sterile and pyrogen-free. A suspension may include free aqueous phosphate ions *e.g.* present at a concentration between 1.0 and 20 mM, such as between 5 and 15 mM, *e.g.* about 10 mM. The suspensions may also comprise sodium chloride.

The invention can use a mixture of both an aluminium hydroxide and an aluminium phosphate. In this case there may be more aluminium phosphate than hydroxide *e.g.* a weight ratio of at least 2:1 *e.g.* ≥5:1, ≥6:1, ≥7:1, ≥8:1, ≥9:1, *etc.*

The concentration of Al^{+++} in a composition for administration to a mammal is typically less than 10mg/ml *e.g.* ≤5 mg/ml, ≤4 mg/ml, ≤3 mg/ml, ≤2 mg/ml, ≤1 mg/ml, *etc.* A preferred range is between 0.3 and 1mg/ml. A maximum of 0.85mg/dose is preferred.

Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100μg Al^{3+} per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 13; see also ref. 14] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

Various oil-in-water emulsion adjuvants are known, and they typically include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5μm in diameter, and ideally have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

The emulsion can comprise oils such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used *e.g.* obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the practice of this invention. The procedures for separation,

purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti exemplify several of the fish oils which may be used herein. A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoids known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Other preferred oils are the tocopherols (see below). Mixtures of oils can be used.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, such as at least 15, *e.g.* at least 16. The invention can be used with surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Non-ionic surfactants are preferred. Preferred surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

Mixtures of surfactants can be used *e.g.* Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1 %; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1 %, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 %, such as 0.1 to 10 % and in particular 0.1 to 1 % or about 0.5%.

Preferred emulsion adjuvants have an average droplets size of <1μm *e.g.* ≤750nm, ≤500nm, ≤400nm, ≤300nm, ≤250nm, ≤220nm, ≤200nm, or smaller. These droplet sizes can conveniently be achieved by techniques such as microfluidisation.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- A submicron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as 'MF59' [15-17], as described in more detail in Chapter 10 of ref. 18 and chapter 12 of ref. 19. The MF59 emulsion advantageously includes citrate ions *e.g.* 10mM sodium citrate buffer.

- An emulsion of squalene, a tocopherol, and polysorbate 80 (Tween 80). The emulsion may include phosphate buffered saline. It may also include Span 85 (*e.g.* at 1%) and/or lecithin. These emulsions may have from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% Tween 80, and the weight ratio of squalene:tocopherol is typically ≤1 as this provides a more stable emulsion. Squalene and Tween 80 may be present volume ratio of about 5:2 or at a weight ratio of about 11:5. One such emulsion can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL-α-tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets *e.g.* with an average diameter of between 100 and 250nm, often about 180nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d-MPL). Another useful emulsion of this type may comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80 [20].

- An emulsion of squalene, a tocopherol, and a Triton detergent (*e.g.* Triton X-100). The emulsion may also include a 3d-MPL (see below). The emulsion may contain a phosphate buffer.

- An emulsion comprising a polysorbate (*e.g.* polysorbate 80), a Triton detergent (*e.g.* Triton X-100) and a tocopherol (*e.g.* an α-tocopherol succinate). The emulsion may include these three components at a mass ratio of about 75:11:10 (*e.g.* 750μg/ml polysorbate 80, 110μg/ml Triton X-100 and 100μg/ml α-tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion may also include squalene. The emulsion may also include a 3d-MPL (see below). The aqueous phase may contain a phosphate buffer.

- An emulsion of squalane, polysorbate 80 and poloxamer 401 ("Pluronic™ L121"). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion is a useful delivery vehicle for muramyl dipeptides, and has been used with threonyl-MDP in the "SAF-1" adjuvant [21] (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the "AF" adjuvant [22] (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80). Microfluidisation is preferred.

- An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or 'Span 80'). The emulsion is generally thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [23]. The emulsion may also include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. The emulsion may include a TLR4 agonist [24]. Such emulsions may be lyophilized.

- An emulsion of squalene, poloxamer 105 and Abil-Care [25]. The final concentration (weight) of these components in adjuvanted vaccines are 5% squalene, 4% poloxamer 105 (pluronic polyol) and 2% Abil-Care 85 (Bis-PEG/PPG-16/16 PEG/PPG-16/16 dimethicone; caprylic/capric triglyceride).

- An emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. As described in reference 26, preferred phospholipid

components are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin. Submicron droplet sizes are advantageous.

- A submicron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives may be included, such as QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-0100, described in reference 27, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyidioctadecylammonium bromide and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl)propanediamine.

- An emulsion in which a saponin (*e.g.* QuilA or QS21) and a sterol (*e.g.* a cholesterol) are associated as helical micelles [28].

- An emulsion comprising a mineral oil, a non-ionic lipophilic ethoxylated fatty alcohol, and a non-ionic hydrophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [29].

- An emulsion comprising a mineral oil, a non-ionic hydrophilic ethoxylated fatty alcohol, and a non-ionic lipophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [29].

In some embodiments an emulsion may be mixed with antigen extemporaneously, at the time of delivery, and thus the adjuvant and antigen may be kept separately in a packaged or distributed vaccine, ready for final formulation at the time of use. In other embodiments an emulsion is mixed with antigen during manufacture, and thus the composition is packaged in a liquid adjuvanted form,. The antigen will generally be in an aqueous form, such that the vaccine is finally prepared by mixing two liquids. The volume ratio of the two liquids for mixing can vary (*e.g.* between 5:1 and 1:5) but is generally about 1:1. Where concentrations of components are given in the above descriptions of specific emulsions, these concentrations are typically for an undiluted composition, and the concentration after mixing with an antigen solution will thus decrease.

Where a composition includes a tocopherol, any of the α, β, γ, δ, ε or ξ tocopherols can be used, but α-tocopherols are preferred. The tocopherol can take several forms *e.g.* different salts and/or isomers. Salts include organic salts, such as succinate, acetate, nicotinate, *etc.* D-α-tocopherol and DL-α-tocopherol can both be used. Tocopherols are advantageously included in vaccines for use in elderly humans (*e.g.* aged 60 years or older) because vitamin E has been reported to have a positive effect on the immune response in this patient group [30]. They also have antioxidant properties that may help to stabilize the emulsions [31]. A preferred α-tocopherol is DL-α-tocopherol, and the preferred salt of this tocopherol is the succinate. The succinate salt has been found to cooperate with TNF-related ligands *in vivo*.

C. Saponin formulations [chapter 22 of ref. 13]

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterogeneous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. In some cases the saponin is QS21. A method of production of QS21 is disclosed in ref. 32. Saponin formulations may also comprise a sterol, such as cholesterol [33].

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexs (ISCOMs) [chapter 23 of ref. 13]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. In some embodiments, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 33-35. Optionally, the ISCOMS may be devoid of additional detergent [36].

A review of the development of saponin based adjuvants can be found in refs. 37 & 38.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 39-44. Virosomes are discussed further in, for example, ref. 45

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 46. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22μm membrane [46]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [47,48].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 49 & 50.

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 51, 52 and 53 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 54-59.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [60]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 61-63. In some embodiments, the CpG is a CpG-A ODN.

In other embodiments, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 60 & 64-66.

A useful CpG adjuvant is CpG7909, also known as ProMune™ (Coley Pharmaceutical Group, Inc.). Another is CpG1826. As an alternative, or in addition, to using CpG sequences, TpG sequences can be used [67], and these oligonucleotides may be free from unmethylated CpG motifs. The immunostimulatory oligonucleotide may be pyrimidine-rich. For example, it may comprise more than one consecutive thymidine nucleotide (*e.g.* TTTT, as disclosed in ref. 67), and/or it may have a nucleotide composition with >25% thymidine (*e.g.* >35%, >40%, >50%, >60%, >80%, *etc.*). For example, it may comprise more than one consecutive cytosine nucleotide (*e.g.* CCCC, as disclosed in ref. 67), and/or it may have a nucleotide composition with >25% cytosine (*e.g.* >35%, >40%, >50%, >60%, >80%, *etc.*). These oligonucleotides may be free from unmethylated CpG motifs. Immunostimulatory oligonucleotides will typically comprise at least 20 nucleotides. They may comprise fewer than 100 nucleotides.

A particularly useful adjuvant based around immunostimulatory oligonucleotides is known as IC-31™ [68]. Thus an adjuvant used with the invention may comprise a mixture of (i) an oligonucleotide (*e.g.* between 15-40 nucleotides) including at least one (and preferably multiple) CpI motifs (*i.e.* a cytosine linked to an inosine to form a dinucleotide), and (ii) a polycationic polymer, such as an oligopeptide (*e.g.* between 5-20 amino acids) including at least one (and preferably multiple) Lys-Arg-Lys tripeptide sequence(s). The oligonucleotide may be a deoxynucleotide comprising 26-mer

sequence 5'-$(IC)_{13}$-3' (SEQ ID NO: 427). The polycationic polymer may be a peptide comprising 11-mer amino acid sequence KLKLLLLLKLK (SEQ ID NO: [426]). The oligonucleotide and polymer can form complexes *e.g.* as disclosed in references 69 & 70.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. In some embodiments, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 71 and as parenteral adjuvants in ref. 72. The toxin or toxoid is typically in the form of a holotoxin, comprising both A and B subunits. In some embodiments, the A subunit contains a detoxifying mutation; often the B subunit is not mutated. In some embodiments, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivatives thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 73-80. A useful CT mutant is or CT-E29H [81]. Numerical reference for amino acid substitutions is typically based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 82, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [83], *etc.*) [84], interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor. A preferred immunomodulator is IL-12.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [85] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [86].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, in some ebodiments ~200nm to ~30μm in

diameter, *e.g.* ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 13)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 87-89.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [90]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [91] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [92]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Phosphazenes

A phosphazene, such as poly[di(carboxylatophenoxy)phosphazene] ("PCPP") as described, for example, in references 93 and 94, may be used.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

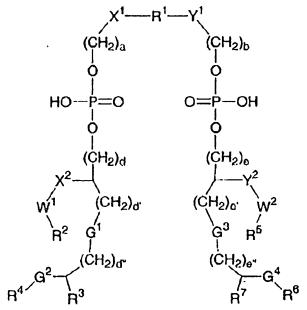
Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquimod ("R-837") [95,96], Resiquimod ("R-848") [97], and their analogs;

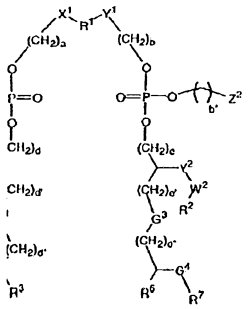
and salts thereof (*e.g.* the hydrochloride salts). Further details about immunostimulatory imidazoquinolines can be found in references 98 to 102.

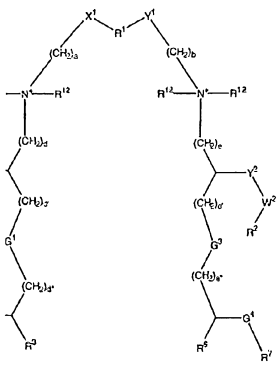
N. Substituted ureas

Substituted ureas useful as adjuvants include compounds of formula I, II or III, or salts thereof:

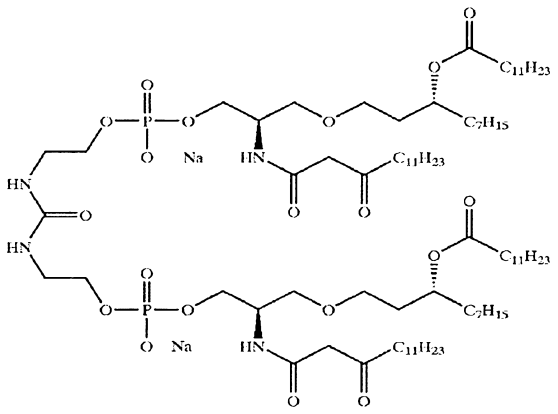
I II III



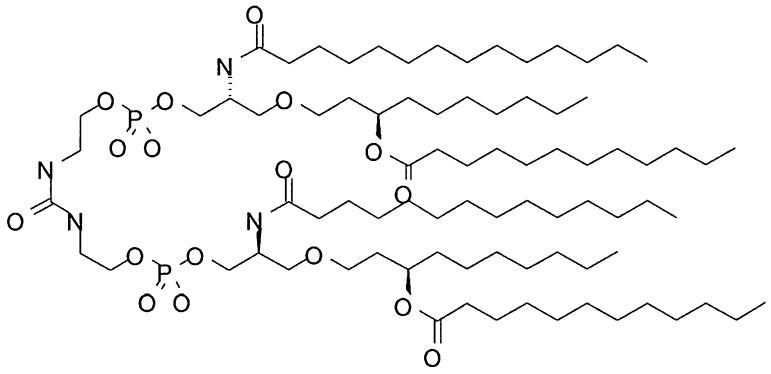




as defined in reference 103, such as 'ER 803058', 'ER 803732', 'ER 804053', ER 804058', 'ER 804059', 'ER 804442', 'ER 804680', 'ER 804764', ER 803022 or 'ER 804057' *e.g.*:



ER804057



ER-803022:

O. Further adjuvants

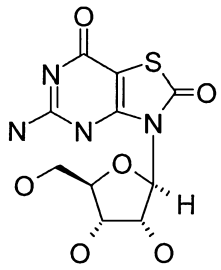
Further adjuvants that may be used with the invention include:

- An aminoalkyl glucosaminide phosphate derivative, such as RC-529 [104,105].

- A thiosemicarbazone compound, such as those disclosed in reference 106. Methods of formulating, manufacturing, and screening for active compounds are also described in reference 106. The thiosemicarbazones are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF-α.

- A tryptanthrin compound, such as those disclosed in reference 107. Methods of formulating, manufacturing, and screening for active compounds are also described in reference 107. The thiosemicarbazones are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF-α.

- A nucleoside analog, such as: (a) Isatorabine (ANA-245; 7-thia-8-oxoguanosine):



and prodrugs thereof; (b) ANA975; (c) ANA-025-1; (d) ANA380; (e) the compounds disclosed in references 108 to 110Loxoribine (7-allyl-8-oxoguanosine) [111].

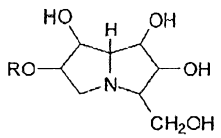
- Compounds disclosed in reference 112, including: Acylpiperazine compounds, Indoledione compounds, Tetrahydraisoquinoline (THIQ) compounds, Benzocyclodione compounds, Aminoazavinyl compounds, Aminobenzimidazole quinolinone (ABIQ) compounds [113,114], Hydrapthalamide compounds, Benzophenone compounds, Isoxazole compounds, Sterol compounds, Quinazilinone compounds, Pyrrole compounds [115], Anthraquinone compounds, Quinoxaline compounds, Triazine compounds, Pyrazalopyrimidine compounds, and Benzazole compounds [116].

- Compounds containing lipids linked to a phosphate-containing acyclic backbone, such as the TLR4 antagonist E5564 [117,118]:

- A polyoxidonium polymer [119,120] or other N-oxidized polyethylene-piperazine derivative.

- Methyl inosine 5'-monophosphate ("MIMP") [121].

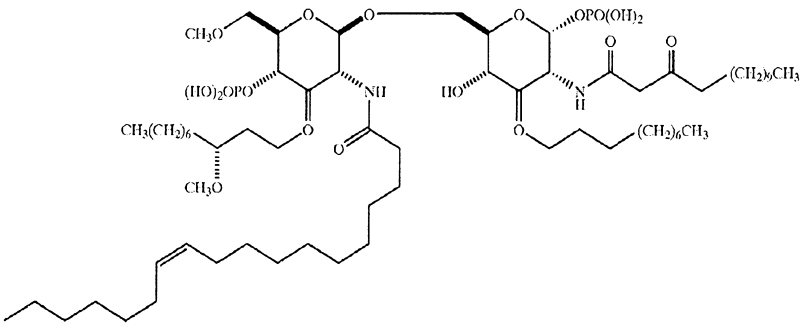
- A polyhydroxlated pyrrolizidine compound [122], such as one having formula:



where R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (*e.g.* cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof. Examples include, but are not limited to: casuarine, casuarine-6-α-D-glucopyranose, 3-*epi*-casuarine, 7-*epi*-casuarine, 3,7-di*epi*-casuarine, *etc.*

- A CD1d ligand, such as an α-glycosylceramide [123-130] (*e.g.* α-galactosylceramide), phytosphingosine-containing α-glycosylceramides, OCH, KRN7000 [(2S,3S,4R)-1-O-(α-D-galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-octadecanetriol], CRONY-101, 3"-O-sulfo-galactosylceramide, *etc.*

- A gamma inulin [131] or derivative thereof, such as algammulin.



Adjuvant combinations

The invention may also comprise combinations of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [132]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [133]; (3) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol; (4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [134]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [135]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 13.

The use of an aluminium hydroxide and/or aluminium phosphate adjuvant is typical, and antigens are generally adsorbed to these salts. Calcium phosphate is another typical adjuvant. Other adjuvant combinations include combinations of Th1 and Th2 adjuvants such as CpG & alum or resiquimod & alum. A combination of aluminium phosphate and 3dMPL may be used.

The compositions of the invention may elicit both a cell mediated immune response as well as a humoral immune response. This immune response may induce long lasting

(*e.g.* neutralising) antibodies and a cell mediated immunity that can quickly respond upon exposure to pnuemococcus.

Two types of T cells, CD4 and CD8 cells, are generally thought necessary to initiate and/or enhance cell mediated immunity and humoral immunity. CD8 T cells can express a CD8 co-receptor and are commonly referred to as Cytotoxic T lymphocytes (CTLs). CD8 T cells are able to recognized or interact with antigens displayed on MHC Class I molecules.

CD4 T cells can express a CD4 co-receptor and are commonly referred to as T helper cells. CD4 T cells are able to recognize antigenic peptides bound to MHC class II molecules. Upon interaction with a MHC class II molecule, the CD4 cells can secrete factors such as cytokines. These secreted cytokines can activate B cells, cytotoxic T cells, macrophages, and other cells that participate in an immune response. Helper T cells or CD4+ cells can be further divided into two functionally distinct subsets: TH1 phenotype and TH2 phenotypes which differ in their cytokine and effector function.

Activated TH1 cells enhance cellular immunity (including an increase in antigen-specific CTL production) and are therefore of particular value in responding to intracellular infections. Activated TH1 cells may secrete one or more of IL-2, IFN-γ, and TNF-β. A TH1 immune response may result in local inflammatory reactions by activating macrophages, NK (natural killer) cells, and CD8 cytotoxic T cells (CTLs). A TH1 immune response may also act to expand the immune response by stimulating growth of B and T cells with IL-12. TH1 stimulated B cells may secrete IgG2a.

Activated TH2 cells enhance antibody production and are therefore of value in responding to extracellular infections. Activated TH2 cells may secrete one or more of IL-4, IL-5, IL-6, and IL-10. A TH2 immune response may result in the production of IgG1, IgE, IgA and memory B cells for future protection.

An enhanced immune response may include one or more of an enhanced TH1 immune response and a TH2 immune response.

A TH1 immune response may include one or more of an increase in CTLs, an increase in one or more of the cytokines associated with a TH1 immune response (such as IL-2, IFN-γ, and TNF-β), an increase in activated macrophages, an increase in NK activity, or an increase in the production of IgG2a. In some embodiments, the enhanced TH1 immune response will include an increase in IgG2a production.

A TH1 immune response may be elicited using a TH1 adjuvant. A TH1 adjuvant will generally elicit increased levels of IgG2a production relative to immunization of the antigen without adjuvant. TH1 adjuvants suitable for use in the invention may include for example saponin formulations, virosomes and virus like particles, non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), immunostimulatory oligonucleotides. Immunostimulatory oligonucleotides, such as oligonucleotides containing a CpG motif, are typical TH1 adjuvants for use in the invention.

A TH2 immune response may include one or more of an increase in one or more of the cytokines associated with a TH2 immune response (such as IL-4, IL-5, IL-6 and IL-10), or an increase in the production of IgG1, IgE, IgA and memory B cells. In some embodiments, the enhanced TH2 immune resonse will include an increase in IgG1 production.

A TH2 immune response may be elicited using a TH2 adjuvant. A TH2 adjuvant will generally elicit increased levels of IgG1 production relative to immunization of the antigen without adjuvant. TH2 adjuvants suitable for use in the invention include, for example, mineral containing compositions, oil-emulsions, and ADP-ribosylating toxins and detoxified derivatives thereof. Mineral containing compositions, such as aluminium salts are typical TH2 adjuvants for use in the invention.

In some embodiments, the invention includes a composition comprising a combination of a TH1 adjuvant and a TH2 adjuvant. Often, such a composition elicits an enhanced TH1 and an enhanced TH2 response, i.e., an increase in the production of both IgG1 and IgG2a production relative to immunization without an adjuvant. Generally, the composition comprising a combination of a TH1 and a TH2 adjuvant elicits an increased TH1 and/or an increased TH2 immune response relative to immunization with a single adjuvant (*i.e.*, relative to immunization with a TH1 adjuvant alone or immunization with a TH2 adjuvant alone).

The immune response may be one or both of a TH1 immune response and a TH2 response. The immune response may provide for one or both of an enhanced TH1 response and an enhanced TH2 response.

The enhanced immune response may be one or both of a systemic and a mucosal immune response. The immune response may provide for one or both of an enhanced systemic and an enhanced mucosal immune response. Typically the mucosal immune

response is a TH2 immune response. Typically the mucosal immune response includes an increase in the production of IgA.

The compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition or a spray-freeze dried composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a mammal. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Where a composition is to be prepared extemporaneously prior to use (*e.g.* where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection.

Compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Where more than one antigen is included in a composition then two antigens may be present at the same dose as each other or at different doses.

As mentioned above, a composition may include a temperature protective agent, and this component may be particularly useful in adjuvanted compositions (particularly those

containing a mineral adjuvant, such as an aluminium salt). As described in reference 136, a liquid temperature protective agent may be added to an aqueous vaccine composition to lower its freezing point *e.g.* to reduce the freezing point to below 0°C. Thus the composition can be stored below 0°C, but above its freezing point, to inhibit thermal breakdown. The temperature protective agent also permits freezing of the composition while protecting mineral salt adjuvants against agglomeration or sedimentation after freezing and thawing, and may also protect the composition at elevated temperatures *e.g.* above 40°C. A starting aqueous vaccine and the liquid temperature protective agent may be mixed such that the liquid temperature protective agent forms from 1-80% by volume of the final mixture. Suitable temperature protective agents should be safe for human administration, readily miscible/soluble in water, and should not damage other components (*e.g.* antigen and adjuvant) in the composition. Examples include glycerin, propylene glycol, and/or polyethylene glycol (PEG). Suitable PEGs may have an average molecular weight ranging from 200-20,000 Da. In one embodiment, the polyethylene glycol can have an average molecular weight of about 300 Da ('PEG-300').

The invention provides a composition comprising: (i) one or more antigen(s); and (ii) a temperature protective agent. This composition may be formed by mixing (i) an aqueous composition comprising one or more antigen(s), with (ii) a temperature protective agent. The mixture may then be stored *e.g.* below 0°C, from 0-20°C, from 20-35°C, from 35-55°C, or higher. It may be stored in liquid or frozen form. The mixture may be lyophilised. The composition may alternatively be formed by mixing (i) a dried composition comprising one or more antigen(s), with (ii) a liquid composition comprising the temperature protective agent. Thus component (ii) can be used to reconstitute component (i).

Functional equivalents:

The SEQ ID NOS used to identify the GAS antigens that may be used in the methods, protein arrays and medical uses of the invention described above are full length sequences for these GAS antigens.

The methods, protein arrays and medical uses of the invention are not limited to the use of these full-length GAS antigens but also encompass any "functional equivalent" of any of these GAS antigens.

The term "functional equivalent" as used herein is intended to encompass variants of the GAS antigens having the full-length sequences shown in the sequence listing that retain the ability to interact with antibodies against the full-length GAS antigen present in the biological and that may thus be used in place of the full-length GAS antigens.

The term "functional equivalent" thus encompasses fragments of the full-length GAS antigens having the sequences shown in the sequence listing. Such fragments may retain the ability to bind to antibodies that bind to the full-length GAS antigens. The functional equivalents of the invention may bind to antibodies generated against the full-length GAS antigen with an affinity of at least 10^{-7}M.

Fragments include at least *n* consecutive amino acids of the full-length GAS antigen sequences, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Fragments may comprise an epitope from the full-length GAS antigen sequence. Further fragments may lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of the full-length sequence. For example, fragments that may be employed in the methods and arrays of the invention include fragments that are lacking the leader sequences and/or the transmembrane sequences present in the full-length GAS antigens.

Further examples of fragments that may be used in the methods and arrays of the invention include N-terminal fragments. Examples of such fragments include the amino acid sequence shown in SEQ ID NO:9 (which is an N-terminal fragment of the sequence in SEQ ID NO:5) and the amino acid sequence shown in SEQ ID NO:10 (which is an N-terminal fragment of SEQ ID NO:4).

The term "functional equivalent" also includes variants of the full-length GAS proteins having amino acid substitutions and fragments of such variants. Variants may have 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to the full-length GAS antigen sequences provided herein. Variants may contain conservative amino acid substitutions compared to the GAS antigen sequence given in the sequence listing. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; or among the aromatic residues Phe and Tyr.

The term "functional equivalent" additionally encompasses longer variants of the GAS antigens including fusion proteins that include an additionally entity that has been chemically or genetically linked to the GAS antigen. For example, the GAS antigen may be attached a label that facilitates its localisation on a protein array or facilitates detecting it when it is bound to an antibody. Examples of such labels include an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme. Alternatively, the GAS antigen may be fused to a domain that facilitated its initial purification, such as a histidine or GST domain.

The term "functional equivalent" also includes mimetics of the GAS antigens, variants and fragments described above, which are structurally similar to the GAS antigens and retain the ability to bind to antibodies against the full-length GAS antigens.

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

Unless specifically stated, a process comprising a step of mixing two or more components does not require any specific order of mixing. Thus components can be mixed in any order. Where there are three components then two components can be combined with each other, and then the combination may be combined with the third component, *etc.*

Identity between polypeptide sequences is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Age distribution of patients with rheumatic heart disease (RHD) and Yemeni healthy blood donors (YHD) from whom sera was collected. Age-matched sera samples selected for the study shown.

Figure 2. Protein micro array set-up and validation. A, SDS-PAGE analysis of purified recombinant GAS proteins stained with Coomassie. Molecular weight markers in lane 1. B, Representative image of a chip after incubation with a human serum and with Cy3-labelled anti-human IgG and Cy5-labelled anti-human IgM. Replicates of tested antigens and of negative and positive IgG and IgM controls are highlighted. C, graphic representation of the control human IgG curve. The chip image of different IgG concentration revealed by incubation with anti-human IgG-Cy3 is shown below the graph. D, Sigmoid-derived data normalization method. Data were normalized using the sigmoid control curve (black) adjusted to a reference sigmoid curve (red; id, ideal sigmoid curve; P and P', intersection points of not normalized, Val, and normalized, N(Val), MFI values on the experimental and reference sigmoid curves; HL, values correspond to normalized MFI values of 30,000; LL are normalized MFI values of 15,000.

Figure 3. Percentage of Yeminite and Italian healthy donor sera with high responses (MFI>30000) to GAS antigens. Antigens are represented in decreasing order responses.

Figure 4. Comparison of the immuno reactivity of the 40 YHD (dark grey in Figure 4) and 43 RHD (light grey in Figure 4) age matched selected human sera. Normalized FI (MFI) values were subjected to unsupervised bi-dimensional hierarchical clustering using dedicated software (TIGR Multiexperiment Viewer (MeV) software (http://www.tigr.org/software/tm4/mev.html) to define the antigen recognition patterns of the two groups of sera, resulting in the identification of two major groups of highly recognized antigens (1 and 2 in Figure 4A, also shown in Figure 4B). This clustering analysis distributed the sera from higher reactivity (on the left) to lower reactivity (on the right). Two main groups of sera could be distinguished, a highly reactive group which comprised mainly sera from healthy donors (A in left of Figure 4) and second group displaying lower reactivity and including mainly sera from RHD patients (group B in Figure 4).

Figure 5. Application of K-mean cluster analysis to classify the GAS antigens present in the chip in 10 clusters (KA1 to KA10) eliciting similar recognition patterns. Four of the antigen clusters (numbers KA1, KA5, KA9, KA10) identified contained antigens with higher fluorescence values than the remaining clusters. In cluster KA 1, the most reactive sera comprised a large number of YHD, suggesting the presence on the chip of a group of antigens, the reactivity of which allows discrimination between sera derived from healthy donors and sera from RHD patients.

Figure 6. Identification of antigen clusters that enable discrimination between healthy and RHD patients. Sera from the KA1 cluster in Figure 6 were further classified on the basis of their recognition profiles to different groups of antigens using a monodimensional hierarchical clustering analysis, allowing the definition of two sera clusters HS 1 (violet box) and HS2 (blue box). The numbers of healthy sera and patient sera present or absent in each of the two clusters is reported in Figure 6B. Most of the YHD can be found in the high reactivity, blue, HS2 cluster, while the majority of RHD sera are found in the low reactivity, violet HS1 cluster. The ability to distinguish between the two sera populations using this type of test was defined in terms of specificity and sensitivity (Figure 6C). For this particular group of antigens, specificity and sensitivity values of 0.73 and 0.69 were obtained. Figure 6C also shows the ideal theoretical example of maximum specificity and sensitivity (values of 1).

Figure 7. The analysis described in Figure 6 was applied to other antigen clusters: KA5 (B), KA9 (C), KA10 (D), KA5+M9 (E), GAS5+GAS5F+GAS25+GAS40 (F), GAS5+GAS5F+GAS25+GAS40+GAS57 (G), GAS5+GAS25+GAS40+GAS57 (H), GAS5F+GAS25+GAS40+GAS57 (I). The specificity and sensitivity values are shown for each cluster.

MODES FOR CARRYING OUT THE INVENTION

Introduction

We have developed a protein microarray containing 130 recombinant GAS protein antigens. The chip was instrumental for the selection of antigens eliciting high antibody responses in pharyngitis patients and also allowed to unveil high responses against GAS antigens in sera from patients with Tic disease, strongly supporting that GAS antigen-dependent induction of autoantibodies in susceptible individuals may be involved in the

occurrence of tic disorders (Bombaci M, et al. 2009 PLoS ONE 4, 7: e6332. doi:10.1371).

Here we used the protein microarray to analyze the immune response against 130 recombinant GAS proteins in RHD patients and healthy donors, with the aim of identifying antigen recognition patterns allowing us to discriminate between the two populations. This approach led to the identification of a cluster of antigens highly recognized by healthy donors, but not by RHD patients, which can set the basis for a diagnosis test.

Materials and Methods

Human sera

Rheumatic heart disease patient sera were collected from 60 male or female 11-40 years old patients from a Middle-East country (Yemen) presenting clinical symptoms of RHD.

Anti-GAS titers are known vary according to a number of factors, including age and geographic origin. In fact, anti GAS antibody titers in healthy people are low in early childhood, rise to a peak in children aged 5 to 15 years, decrease in late adolescence and early adulthood, and then flatten off after that. For this reason, comparison between rheumatic heart disease (RHD) sera and control Yemeni healthy donor (YHD) groups was performed using groups of the same age range (17-40 year old), thus excluding 11 to 16 year old RHD patient sera, for which control sera were not available. The final number of sera used in the comparison was 40 YHD and 43 RHD.

Figure 1 shows the distribution analysis of the two populations available and those selected for the study.

In addition, a collection of 20 sera from healthy Italian human donors (IHD) was used for an additional comparison to healthy Yemenites, taking into consideration the higher use of antibiotic prophylaxis of GAS infections in the former western population.

All serum samples were residuals obtained during routine medical controls for RHD diagnosis or bloodlettings and were made available by the Department of Child and Adolescent Neuropsychiatry, University La Sapienza, Rome.

GAS protein microarray

A protein array was generated by depositing on a nitrocellulose chip 130 recombinant proteins mainly selected from the GAS SF370 M1 genome (see Figure 2 for details on chip set up).

The chips were incubated with the different sera and reactivity was evaluated by detecting total IgG bound to each deposited protein whit fluorescently labeled anti-human IgG and measuring the resulting Fluorescence Intensity (FI) values. For each slide, protein MFI values were normalized to a sigmoid adjusted standard IgG curve used as reference (Figure 2).

Antigen recognition by tested sera was considered positive when MFI values were equal to or higher than 15,000, corresponding to the background value plus 2 standard deviations. MFI values equal to or above 30,000 were considered as high responses. The array was probed with 120 sera from patients (20 sera of Italian healthy donors (IHD), 40 of Yemenite healthy donors (YHD) and 60 of RHD Yemenite patients (RHD)).

Results

GAS antigens recognized by sera from healthy donors: antibody responses are higher in Yemenites than in Italians

Anti GAS antibody responses in populations belonging to the two different geographical areas were investigated using 40 sera from Yemen healthy blood donors and 20 healthy donors from Italy. Figure 3 reports the percentage of healthy donor sera with high responses to GAS antigens. As shown, background antistreptococcal responses are much higher in Yemenite than in Italian samples, both in terms of number of highly recognized antigens and as number of highly positive sera.

Differential immuno reactivity of GAS antigens between healthy and RHD Yemenite patients

We compared the immuno reactivity of the 40 YHD (dark grey in Figure 4) and 43 RHD (light grey in Figure 4) age matched selected human sera. To define the antigen recognition patterns of the two groups of sera, normalized FI (MFI) values were subjected to unsupervised bi-dimensional hierarchical clustering using dedicated software (TIGR Multiexperiment Viewer (MeV) software (http://www.tigr.org/software/tm4/mev.html).

The clustered view of the antibody recognition profiles identified two major groups of highly recognized antigens (1 and 2 in Figure 4). Group 1 included GAS5F (putative secreted protein), GAS25 (streptolysin O precursor), GAS40 (putative surface exclusion protein), M1, GAS179 (putative esterase), GAS97 (immunogenic secreted protein precursor homolog), GAS193 (uimmunogenic secreted protein precursor). Group 2 included 5 different M proteins (M12, M23, M2, M3 and M9), GAS57 (putative cell envelope proteinase), GAS380 (hypothetical protein) and SpeI.

Furthermore, as shown in Figure 4, this clustering analysis distributed the sera from higher reactivity (on the left) to lower reactivity (on the right). Indeed, two main groups of sera could be distinguished, a highly reactive group which comprised mainly sera from healthy donors (A in left of Figure 4) and second group displaying lower reactivity and including mainly sera from RHD patients (group B in Figure 4).

We then applied the K-mean cluster analysis in order to classify the GAS antigens present in the chip in 10 clusters (KA1 to KA10 in Figure 5) eliciting similar recognition patterns. k-mean clustering is a statistical method aimed at partitioning n observations into k clusters in which each observation belongs to the cluster with the nearest mean. It is similar to the expectation-maximization algorithm for mixtures of Gaussians in that they both attempt to find the centers of natural clusters in the data.

As shown in Figure 5, four of the antigen clusters (numbers KA 1, KA 5, KA 9, KA 10) identified contained antigens with higher fluorescence values than the remaining clusters. Interestingly, even though the clustering was monodimensional, i.e. was intended for antigen classification and not for classification of the sera, we observed in that in cluster KA 1, the most reactive sera comprised a large number of YHD. This observation suggested the presence in the chip of a group of antigens, the reactivity of which allows discrimination between sera derived from healthy donors and sera from RHD patients.

We then tried to define in a more precise manner the group of antigens allowing us to discriminate between healthy and cardiopathic patients. For this purpose, sera were further classified on the basis of their recognition profiles to different groups of antigens using a monodimensional hierarchical clustering analysis.

This type of analysis was first applied to the group of antigens included in cluster KA1 of Figure 5, allowing the definition of two sera clusters at the first hierarchical level, HS

1 and HS2, corresponding to violet (HS1) and blue (HS2) boxes in Figure 6A. The numbers of healthy sera and patient sera present or absent in each of the two clusters is reported in Figure 6B. As shown, most of the YHD can be found on the high reactivity, blue, HS2 cluster, while the majority of RHD sera are found in the low reactivity, violet HS1 cluster. The ability to distinguish between the two sera populations using this type of test was defined in terms of specificity and sensitivity. Figure 6C shows the ideal theoretical example of maximum specificity and sensitivity (values of 1). As shown, for this particular group of antigens we obtained specificity and sensitivity values of 0.73 and 0.69.

The same type of analysis was applied to the antigens in clusters KA5, KA9, KA10, and KA5+M9. The same type of analysis was also applied to other groups of antigens, including GAS5+GAS5F+GAS25+GAS40, GAS5+GAS5F+GAS25+GAS40+GAS57, GAS5+GAS25+GAS40+GAS57, GAS5F+GAS25+GAS40+GAS57. The results are summarized in Figure 7 A-I.

As shown, the cluster yielding highest specificity and sensitivity values comprised antigens in cluster KA1 (GAS5, GAS40, GAS5F, GAS57, GAS97, GAS380 and SpeA), and antigens GAS5, GAS25, GAS40 and GAS57, while lowest values were obtained for the cluster including GAS M variants.

DISCUSSION

We believe that this type of analysis using antigens GAS5, GAS25, GAS40 and GAS57 could set the basis for a more precise diagnosis of RHD and allow prediction of whether a patient with ARF is likely to develop RHD, thus guiding medical professionals to decide on the best prophylactic therapy for ARF patients.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treating or preventing rheumatic heart disease (RHD) associated with Group A Streptococcus (GAS) infection, said method comprising administering to a patient in need thereof at least one GAS antigen comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6, 7 or 8, and functional equivalent(s) thereof.

2. An isolated Group A Streptococcus (GAS) antigen comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, and functional equivalent(s) thereof for use in treatment or prevention of rheumatic heart disease (RHD) associated with GAS infection.

3. A method of diagnosing rheumatic heart disease (RHD) associated with Group A Streptococcus (GAS) infection in a patient, or a method of identifying a patient at risk of developing RHD associated with GAS infection, said method comprising:

a) contacting a biological sample from a patient with at least one GAS antigen under conditions appropriate for binding of any antibodies present in the biological sample to the at least one GAS antigen, and

b) comparing the reactivity of antibodies in the biological sample from the patient to the at least one GAS antigen with the reactivity of antibodies in a control biological sample from a healthy individual to the at least one GAS antigen,

wherein a lower reactivity in the biological sample from the patient compared to the control biological sample from a healthy individual is indicative that the patient is suffering from RHD associated with GAS infection or that the patient is at risk of developing RHD associated with GAS infection.

4. The method according to claim 3 comprising the steps of:

a) contacting a biological sample from a patient with at least one GAS antigen comprising an amino acid sequence selected from the group consisting of:

SEQ ID NO:1 (GAS5) ; SEQ ID NO:2 (GAS5F) ; SEQ ID NO:3 (GAS25) ; SEQ ID NO:4 (GAS40) ; SEQ ID NO:5 (GAS57) ; SEQ ID NO:6 (GAS97) ; SEQ ID NO:7 (GAS380) ; and SEQ ID NO:8 (SpeA),

or functional equivalent(s) thereof, under conditions appropriate for binding of any antibodies present in the biological sample to the at least one GAS antigen or to functional equivalent(s) thereof; and

b) assessing the reactivity of any antibodies in the biological sample from the patient bound to the at least one GAS antigen or to the functional equivalent(s) thereof ; and

c) comparing the reactivity at b) with the reactivity of antibodies in a control biological sample from a healthy individual bound to the at least one GAS antigen or to the functional equivalent(s) thereof.

wherein a lower reactivity in the biological sample from the patient compared to the reactivity in the control biological sample from a healthy individual is indicative that the patient is suffering from RHD associated with GAS infection or that the patient is at risk of developing RHD associated with GAS infection.

5. The method of claim 4, wherein a) comprises contacting the sample with 1, 2, 3, 4, 5, 6, 7, or 8 of the GAS antigens or functional equivalent(s) thereof .

6. The method of claim 4 or 5, wherein a) comprises contacting the sample with 3 GAS antigens selected from the group consisting of:

(i) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 2 and 3 or functional equivalent(s) thereof;

(ii) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 3 and 4 or functional equivalent(s) thereof;

(iii) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 4 and 5 or functional equivalent(s) thereof;

(iv) GAS antigens comprising the amino acid sequences of SEQ ID NOS:, 2, 3 and 4 or functional equivalent(s) thereof;

(v) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 2, 4 and 5 or functional equivalent(s) thereof;

(vi) or GAS antigens comprising the amino acid sequences of SEQ ID NOS: 3, 4 and 5 or functional equivalents thereof.

7. The method of claim 4 or 5, wherein a) comprises contacting the sample with 4 GAS antigens selected from the group consisting of:

(i) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 2, 3 and 4 or functional equivalent(s) thereof;

(ii) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 2, 3, 4 and 5 or functional equivalent(s) thereof;

(iii) GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 3, 4 and 5 or functional equivalents thereof.

8. The method of claim 4 or 5, wherein a) comprises contacting the sample with 5 GAS antigens selected from the group consisting of GAS antigens comprising the amino acid sequences of SEQ ID NOS: 1, 2, 3, 4 and 5.

9. The method according to any one of claims 3 to 8 wherein the biological sample is a serum sample.

10. The method according to any one of claims 3 to 9, wherein the biological sample is from an adolescent or from a child.

11. The method according to any one of claims 3 to 10, wherein the GAS antigens are displayed on one or more protein arrays.

12. A protein array comprising at least two Group A Streptococcus (GAS) antigens wherein one of the at least two GAS antigens comprises an amino acid sequence set forth in SEQ ID NO:2 and one of the at least two GAS antigens comprises an amino acid sequence set forth in SEQ ID NO:8.

13. A kit comprising the protein array according to claim 12 and instructions for the use of the array in the diagnosis of patients having or at risk of developing rheumatic heart disease associated with GAS infection.

14. The protein array according to claim 12 when used in the method according to any use of claims 3 to 11.

15. The method according to claim 1 or claims 3 to 11 substantially as hereinbefore described with reference to the accompanying drawings, sequence listing and/or examples.

16. The isolated GAS antigen according to claim 2 substantially as hereinbefore described with reference to the accompanying drawings, sequence listing and/or examples.

17. The protein array according to claim 12 or 14 substantially as hereinbefore described with reference to the accompanying drawings, sequence listing and/or examples.

18. The kit according to claim 13 substantially as hereinbefore described with reference to the accompanying drawings, sequence listing and/or examples.

FIG. 1

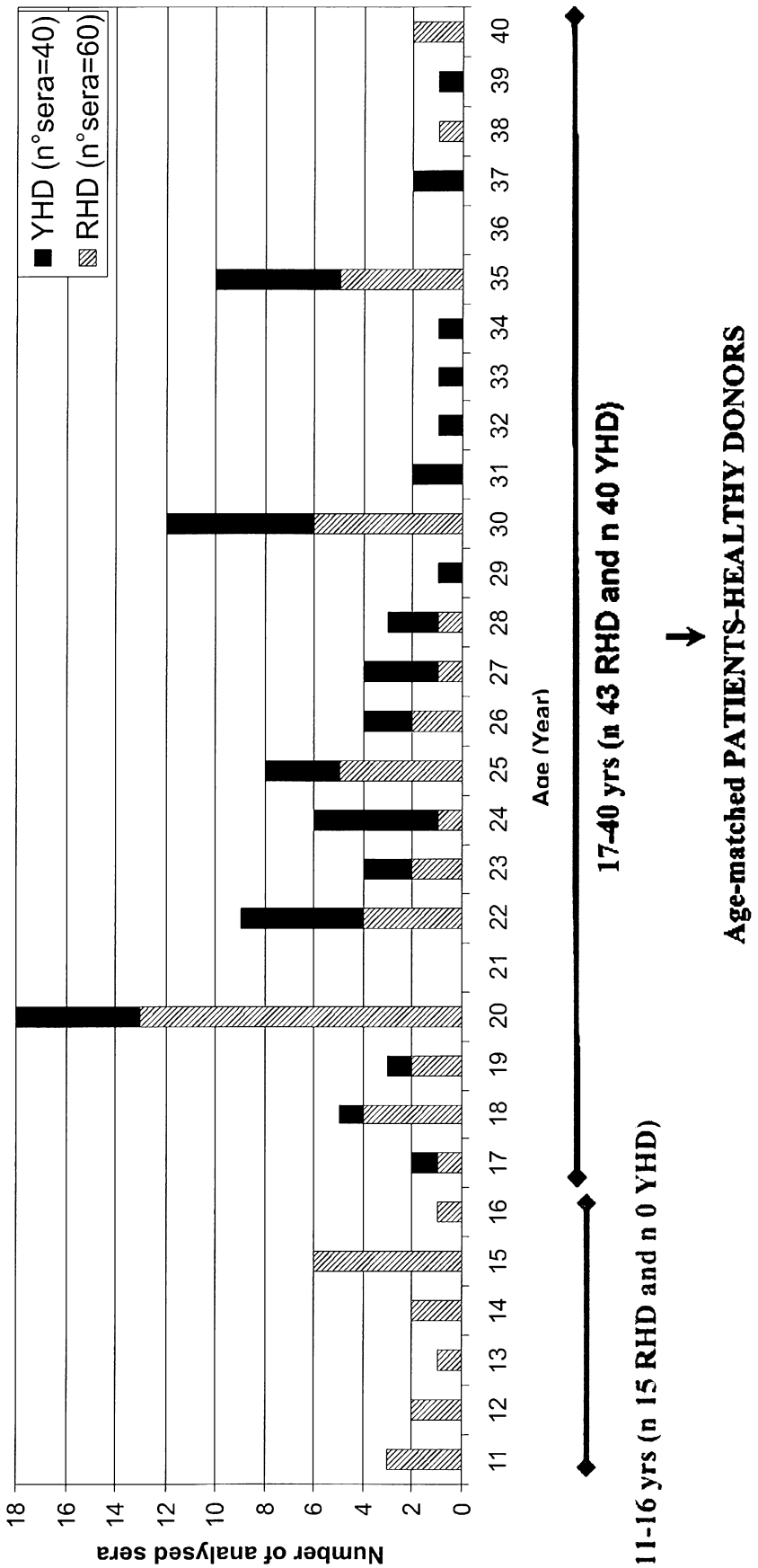

Age distribution
YHD (n°sera=40)
RHD (n°sera=60)
Number of analysed sera
0
2
4
6
8
10
12
14
16
18
Age (Year)
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
11-16 yrs (n 15 RHD and n 0 YHD)
17-40 yrs (n 43 RHD and n 40 YHD)
Age-matched PATIENTS-HEALTHY DONORS

FIG. 2A

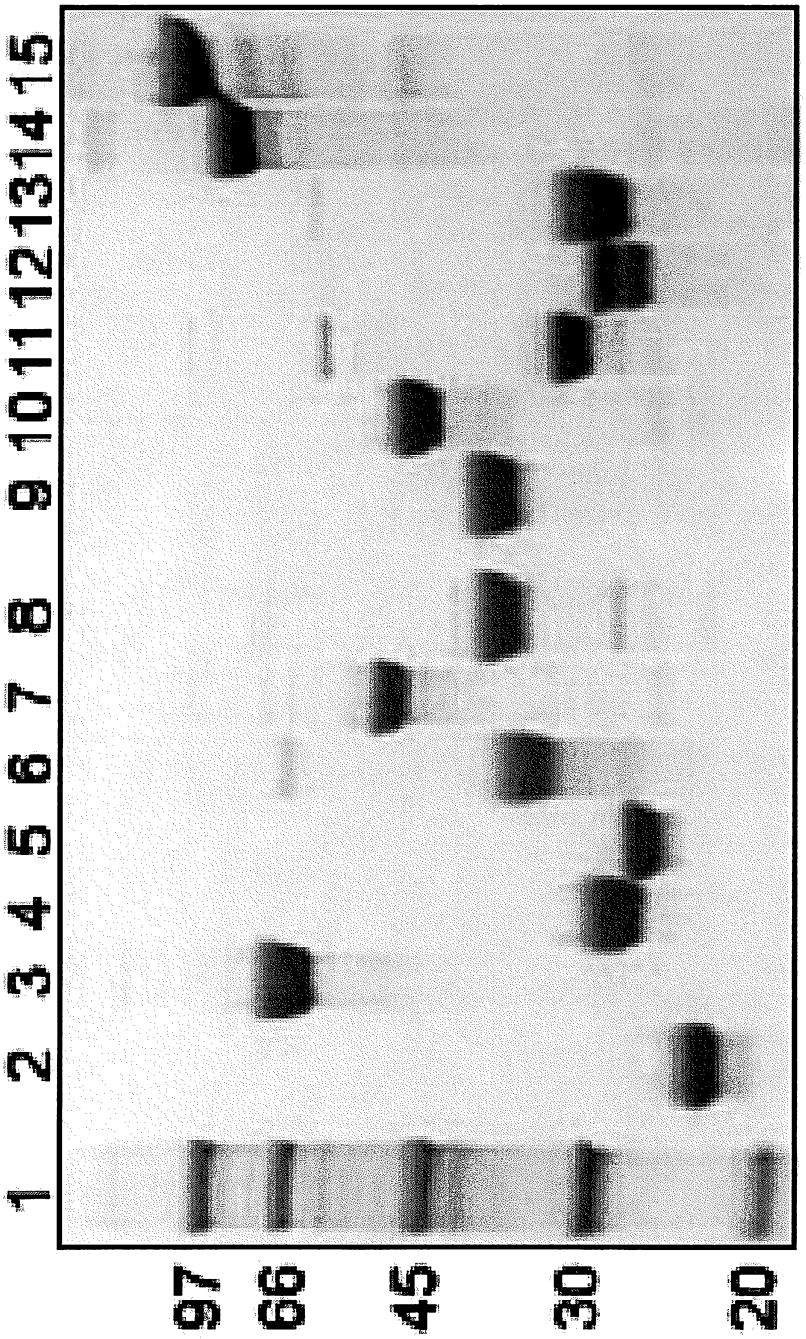


FIG. 2B

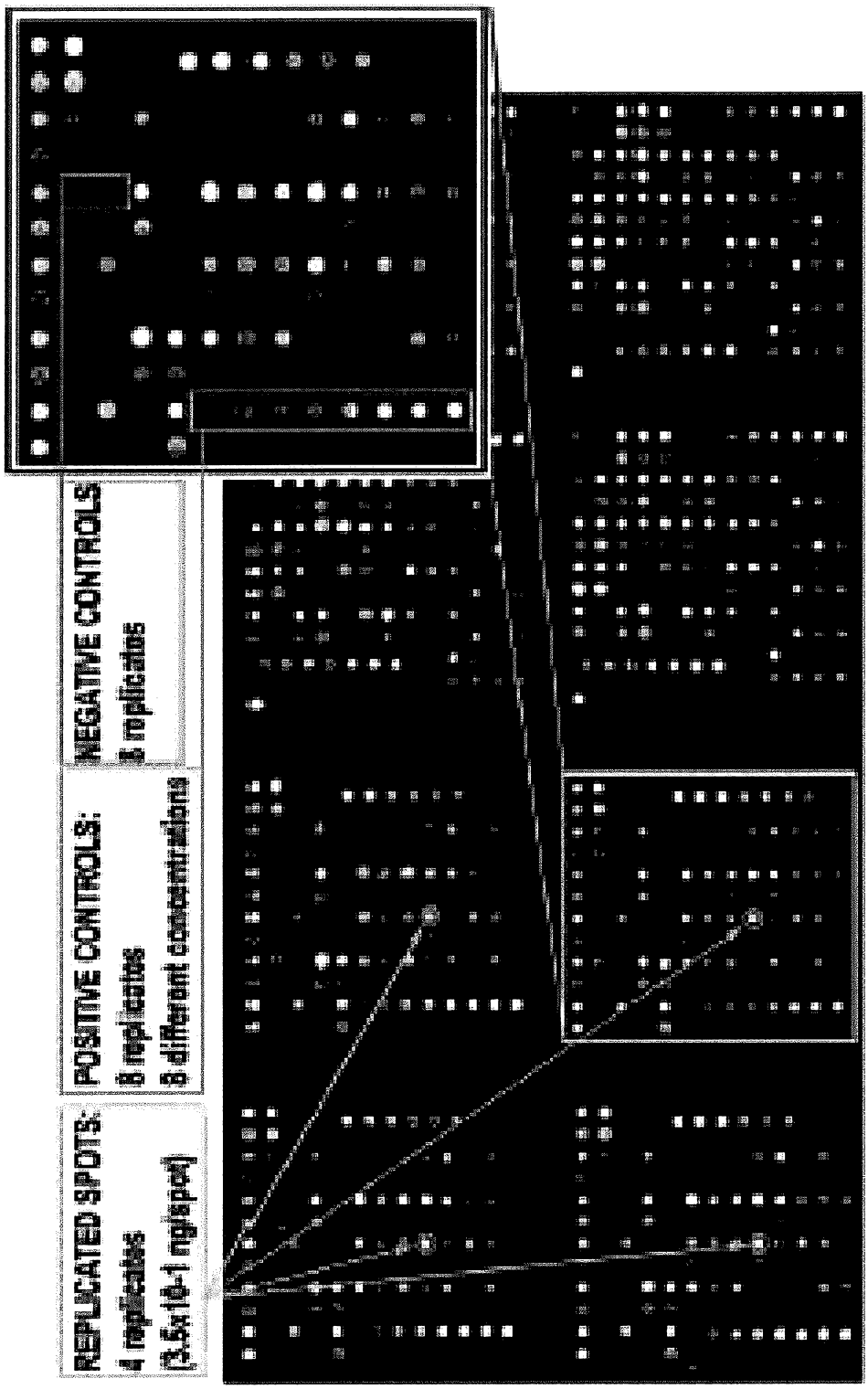

NEGATIVE CONTROLS
6 replicates
POSITIVE CONTROLS:
8 replicates
8 different concentrations
REPLICATED SPOTS:
4 replicates

FIG. 2C

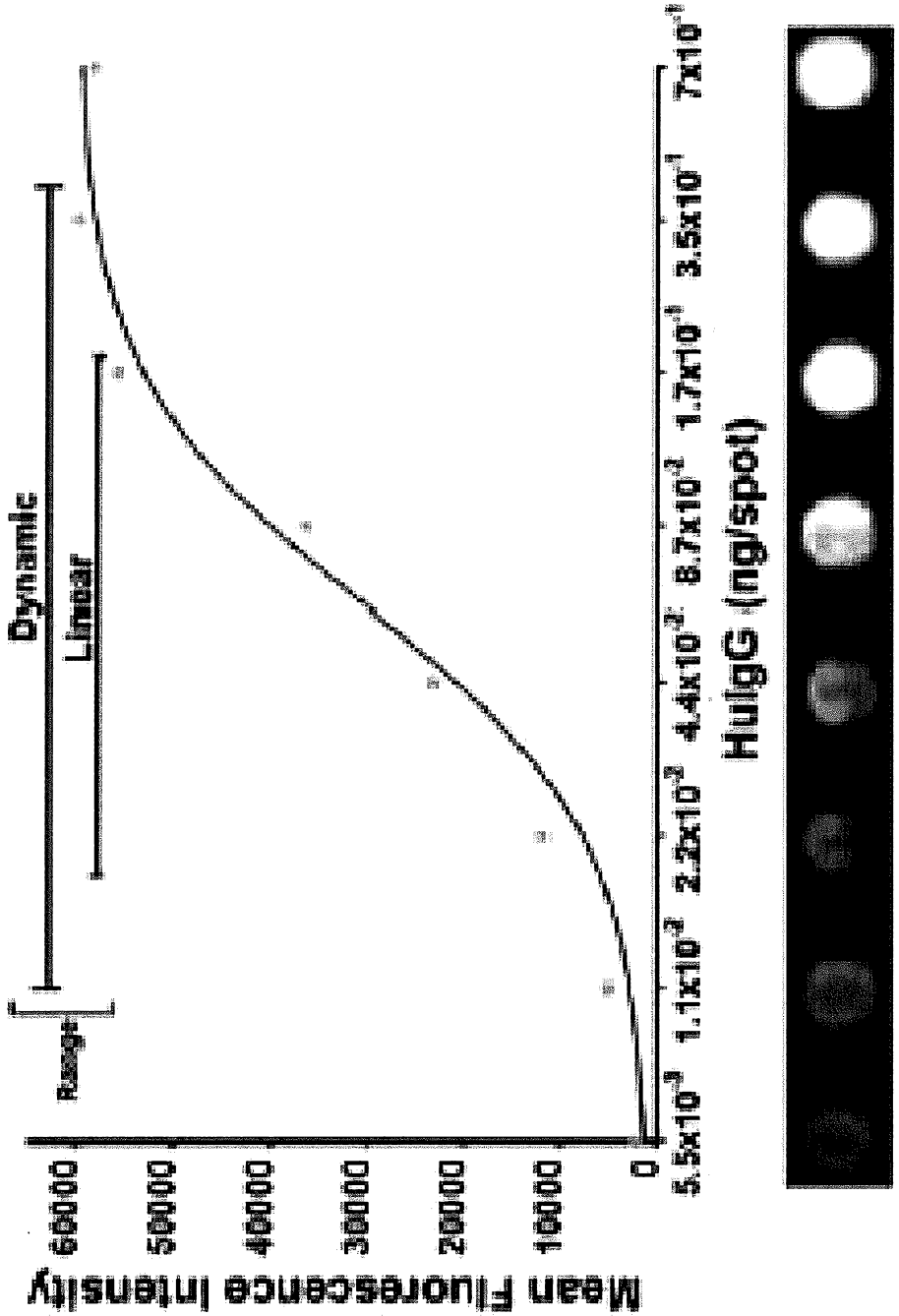

Dynamic
Linear
Range
Mean Fluorescence Intensity
60000
50000
40000
30000
20000
10000
0
5.5x10⁻³
1.1x10⁻²
2.2x10⁻²
4.4x10⁻²
8.7x10⁻²
1.7x10⁻¹
3.5x10⁻¹
7x10⁻¹
HuIgG (ng/spot)

FIG. 2D

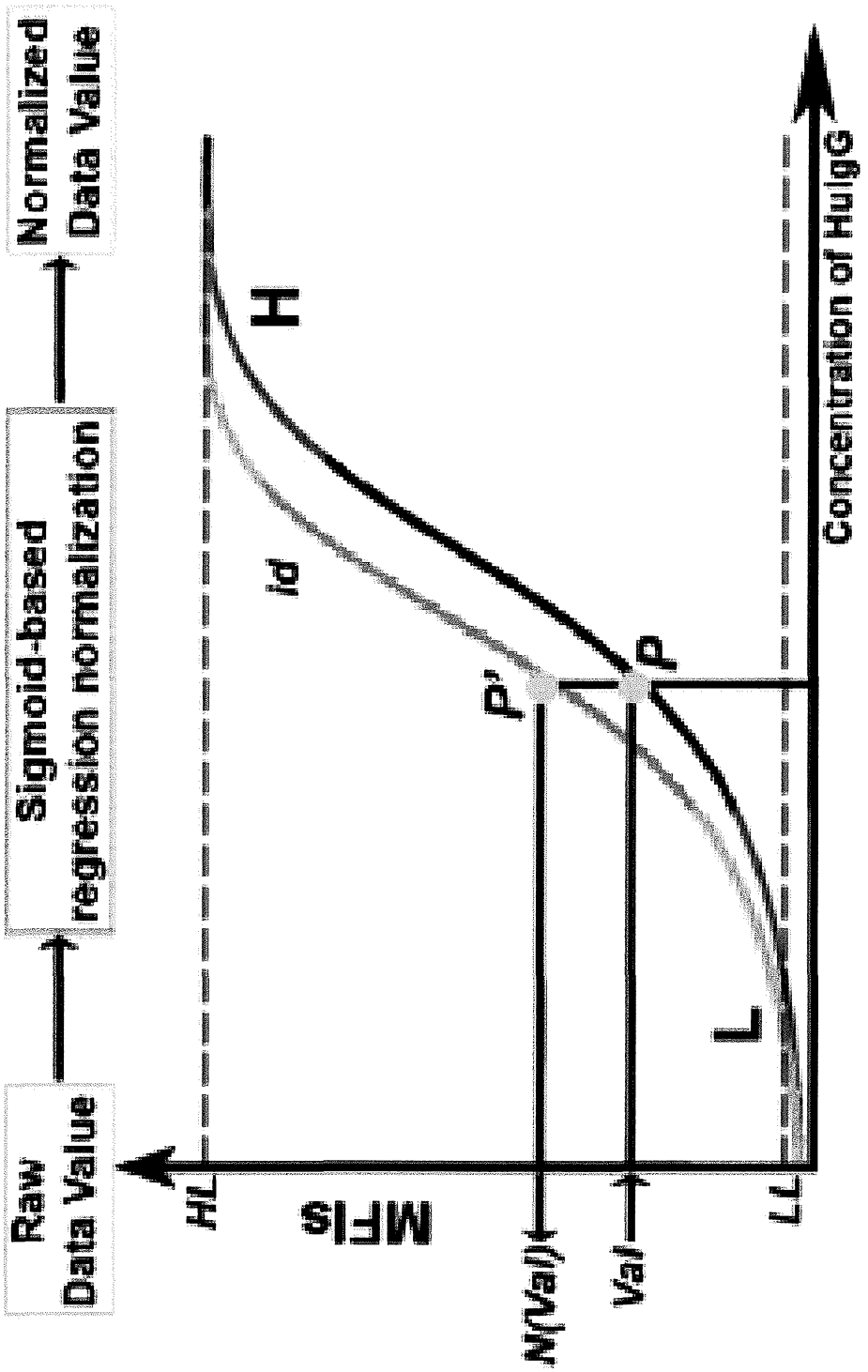

Raw Data Value
Sigmoid-based regression normalization
Normalized Data Value
MFIs
HL
LL
H
L
Id
P'
P
N(Val)
Val
Concentration of HulgG

FIG. 3

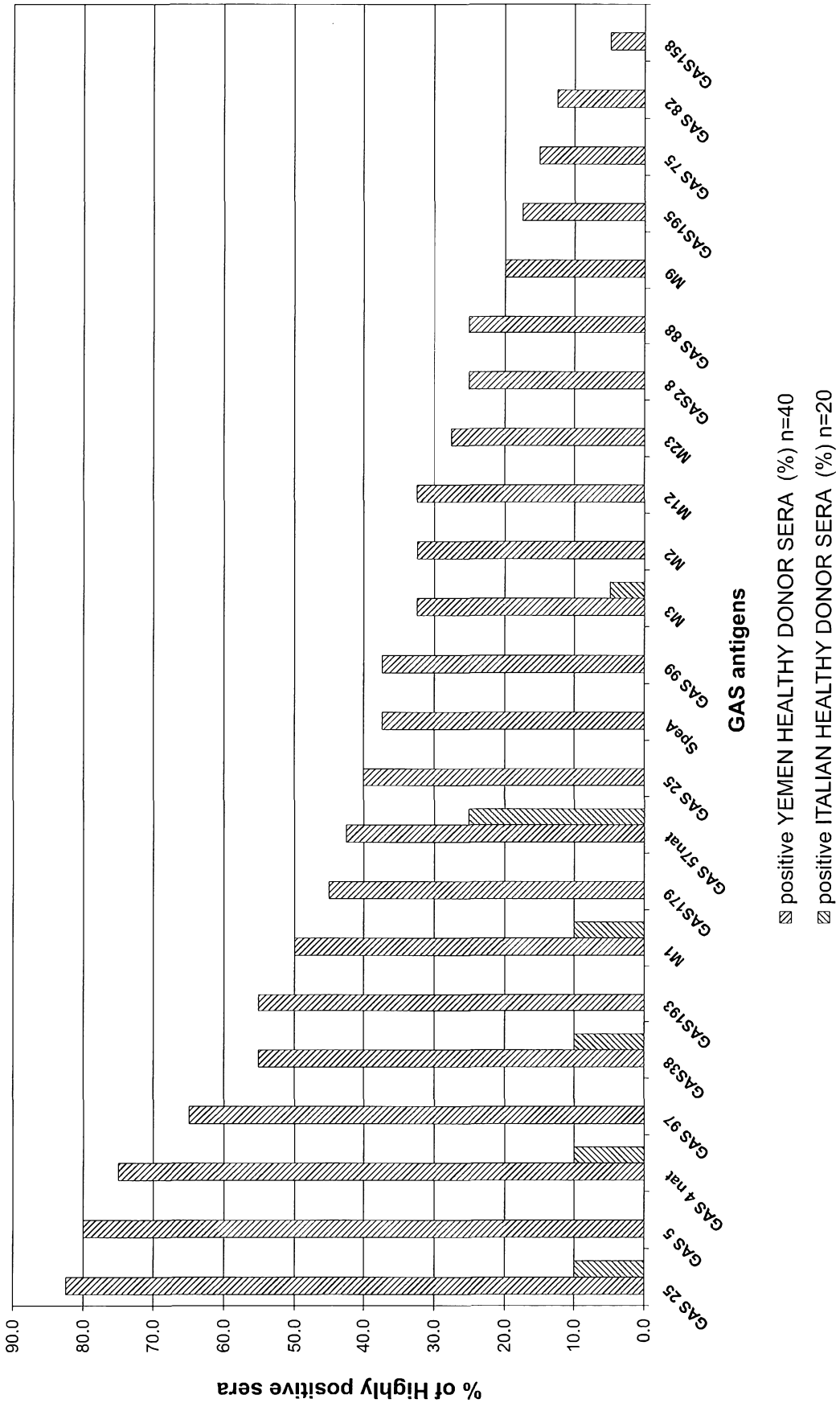
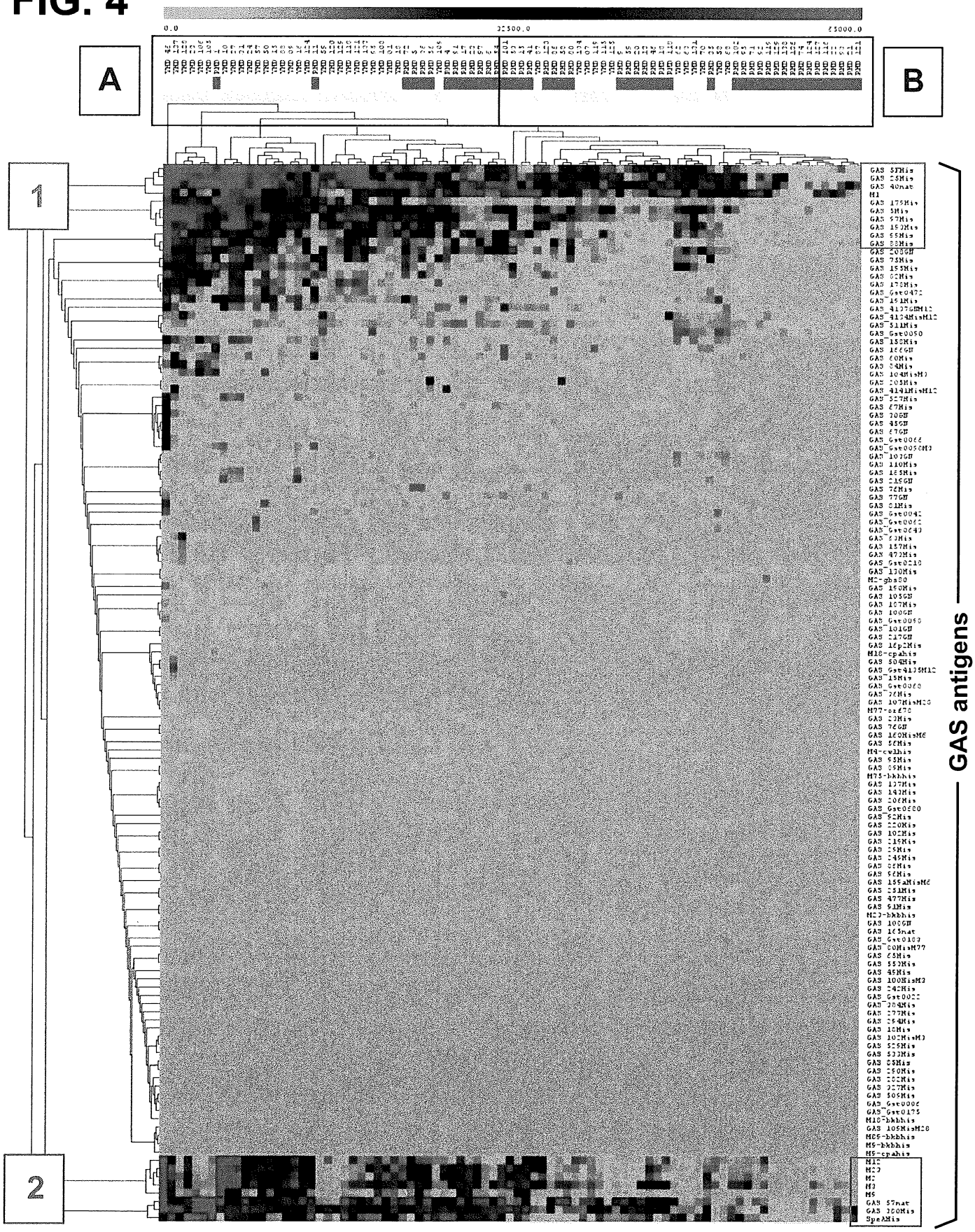

MFI>30000
% of Highly positive sera
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
GAS 25
GAS 5
GAS 4 nat
GAS 97
GAS38
GAS193
M1
GAS179
GAS 57nat
GAS 25
SpeA
GAS 99
M3
M2
M12
M23
GAS 2 8
GAS 88
M9
GAS195
GAS 75
GAS 82
GAS158
GAS antigens
positive YEMEN HEALTHY DONOR SERA (%) n=40
positive ITALIAN HEALTHY DONOR SERA (%) n=20

FIG. 4



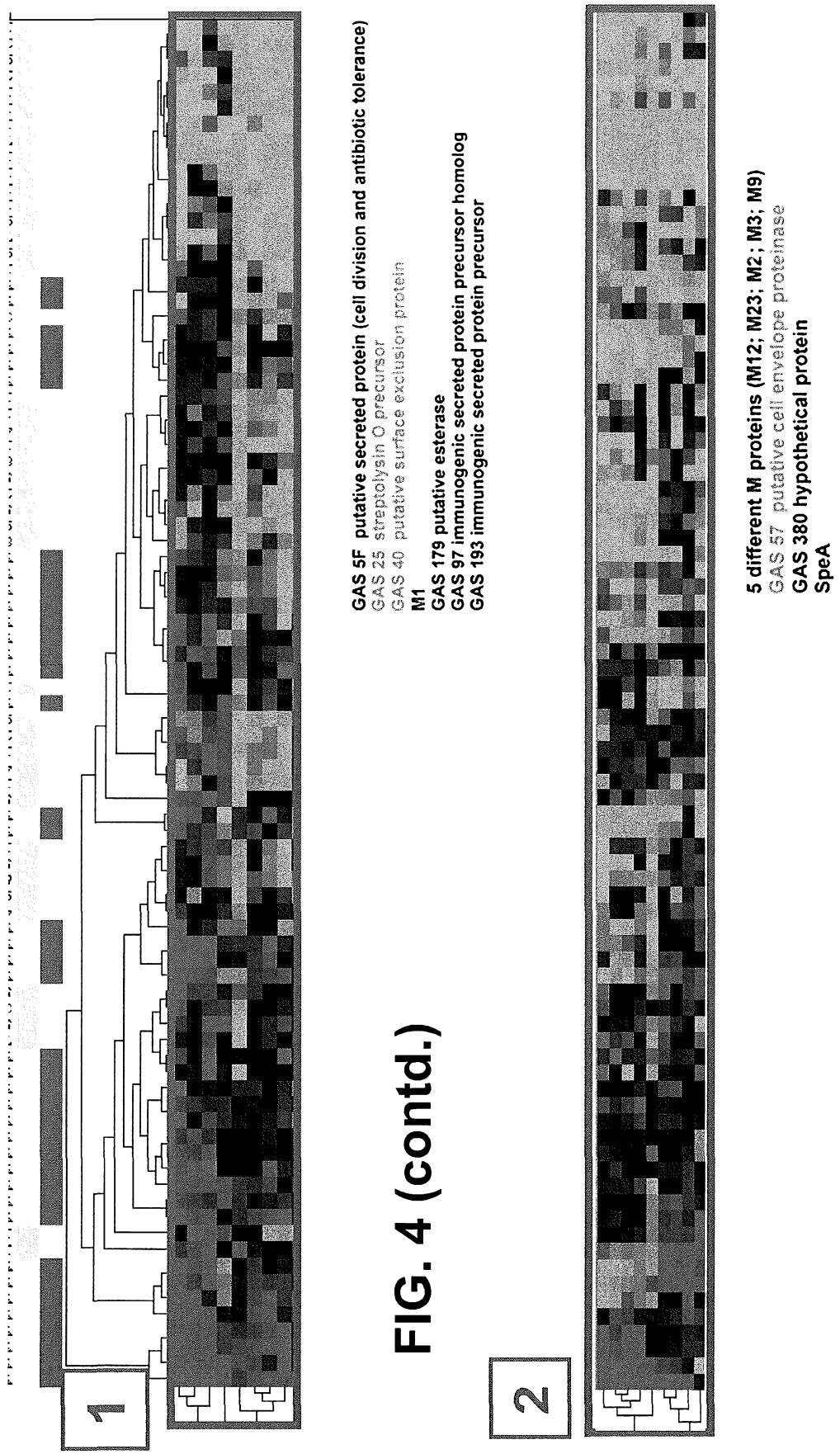
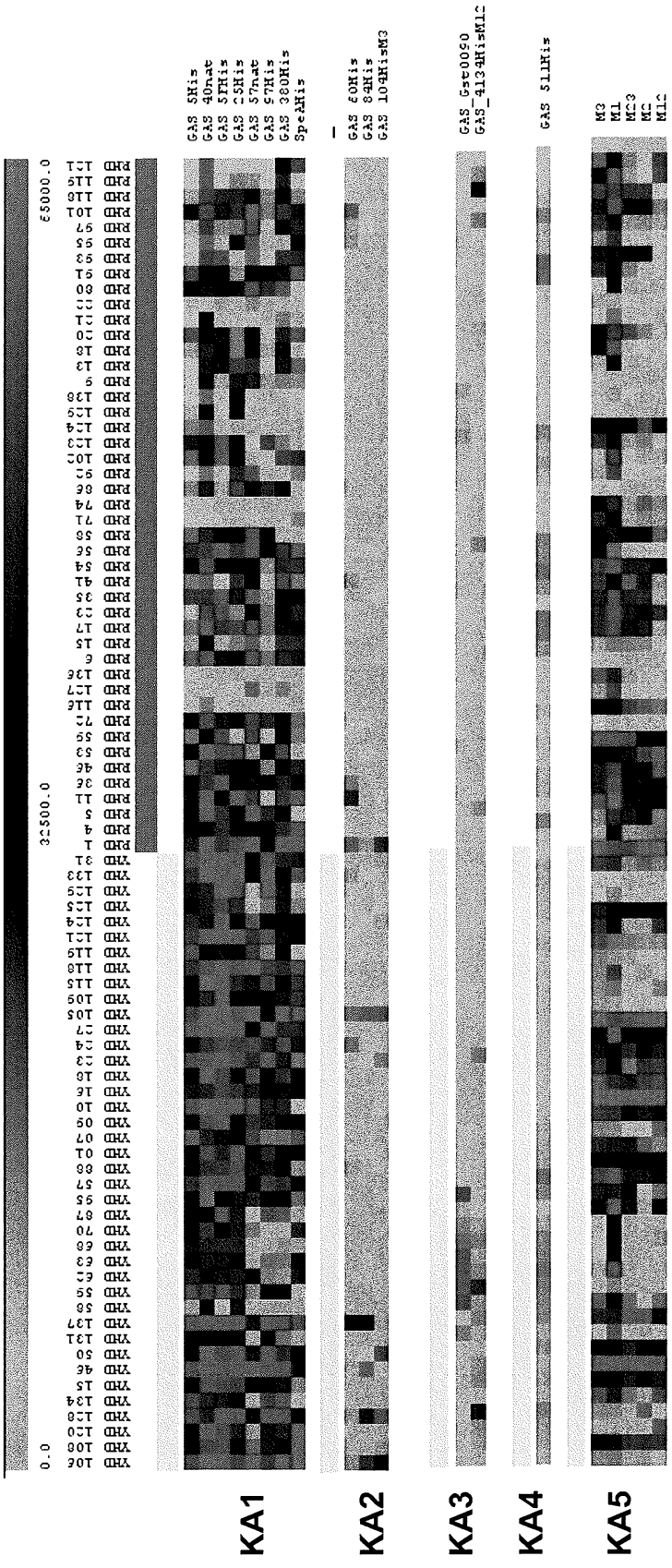

1
GAS 5F putative secreted protein (cell division and antibiotic tolerance)
GAS 25 streptolysin O precursor
GAS 40 putative surface exclusion protein
M1
GAS 179 putative esterase
GAS 97 immunogenic secreted protein precursor homolog
GAS 193 immunogenic secreted protein precursor
2
5 different M proteins (M12; M23; M2 ; M3; M9)
GAS 57 putative cell envelope proteinase
GAS 380 hypothetical protein
SpeA

FIG. 4 (contd.)

FIG. 5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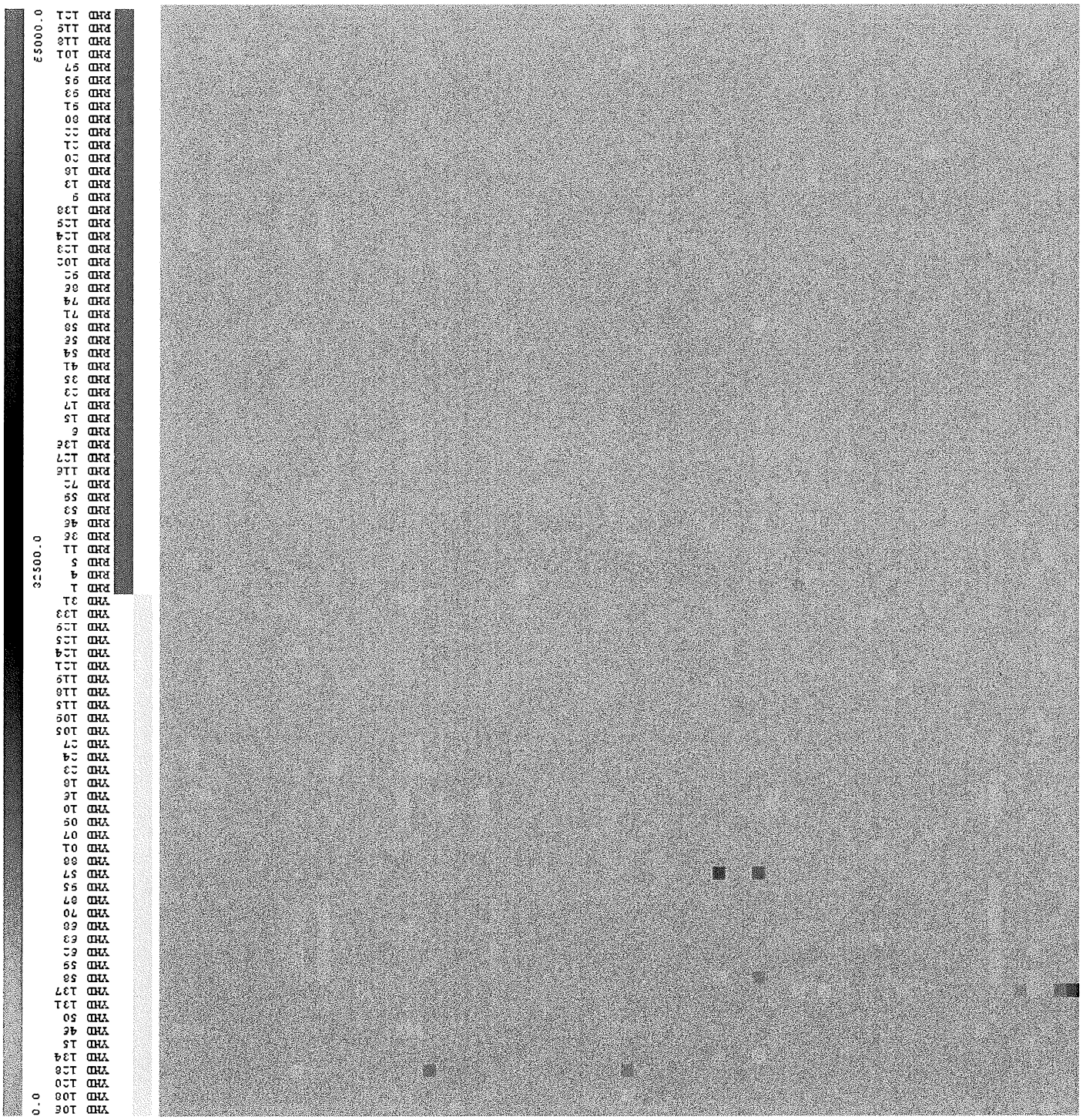
KA2
KA3
KA4
KA5
GAS 5His
GAS 40nat
GAS 51His
GAS 25His
GAS 57nat
GAS 97His
GAS 380His
SpeAHis
GAS 60His
GAS 84His
GAS 109HisM3
GAS_Gat0090
GAS_4134HisM1C
GAS 51lHis
M3
M1
MC3
MC
M1C

FIG. 5 (contd.)



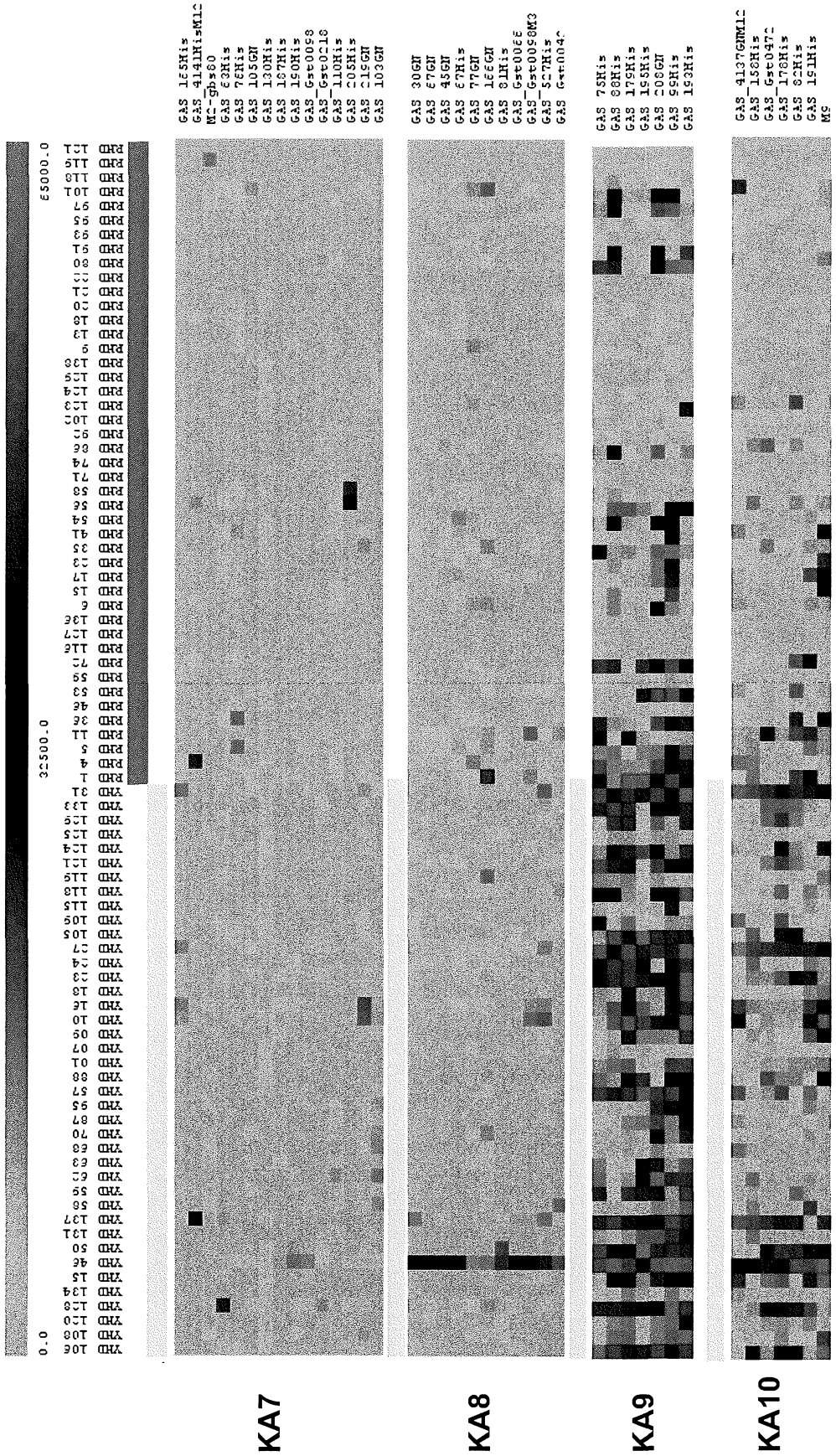


FIG. 5 (contd.)

FIG. 6A

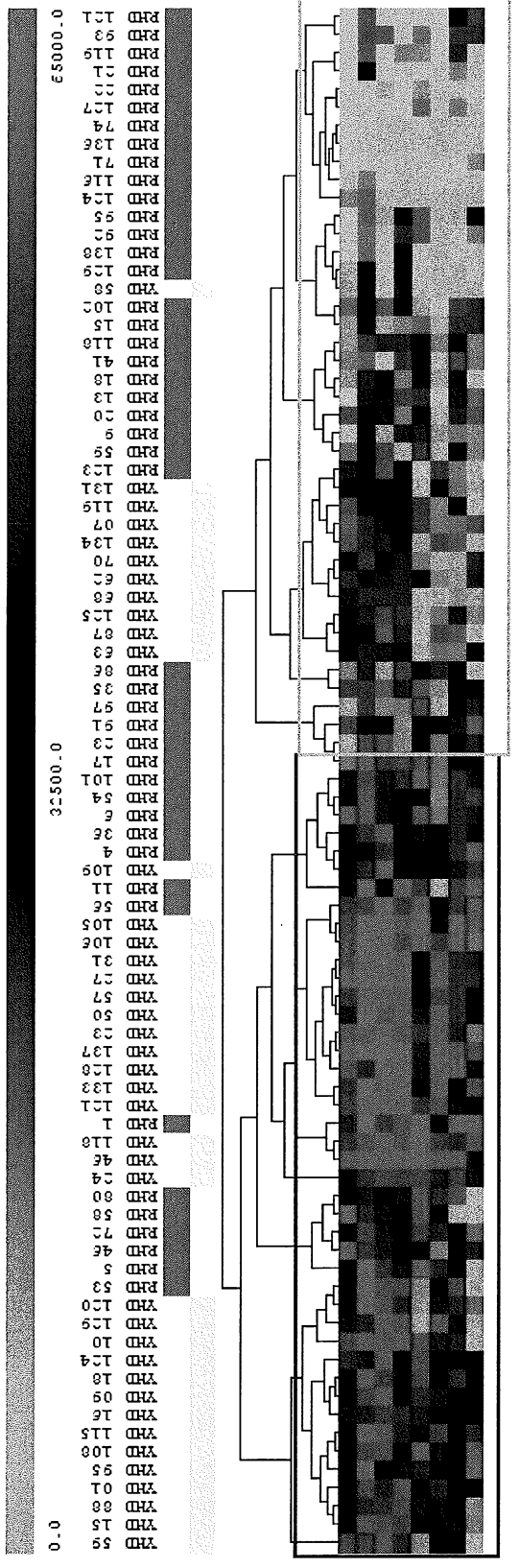


FIG. 6B KA 1

Cluster	Actual condition: RHD patient	Actual condition: Healthy
HS1	31	11
HS2	14	29

Test: specificity	Test: sensitivity
0.73	0.69

FIG. 6C Ideal example

Cluster	Actual condition: RHD patient	Actual condition: Healthy
HS1	43	0
HS2	0	40

Test: specificity	Test: sensitivity
1.00	1.00

FIG. 7

A KA 1

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	31	11	**0.73**	0.69
HS2	14	29		

B KA 5

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	43	35	**0.13**	1.00
HS2	0	5		

C KA 9

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	43	33	**0.18**	1.00
HS2	0	7		

D KA 10

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	43	39	**0.03**	1.00
HS2	0	1		

E KA 5 + M9

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	43	35	**0.13**	1.00
HS2	0	5		

F GAS5 GAS5 GAS25 GAS40

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	35	4	**0.90**	0.78
HS2	10	36		

G GAS5 GAS5F GAS25 GAS40 GAS57

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	38	7	**0.82**	0.83
HS2	8	32		

H **GAS5 GAS25 GAS40 GAS57**

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	18	5	**0.88**	0.42
HS2	25	35		

I GAS5F GAS25 GAS40 GAS57

Cluster	Actual condition: RHD patient	Actual condition: Healthy	Test: specificity	Test: sensitivity
HS1	34	4	**0.90**	0.79
HS2	9	36		

FIG. 7 (contd.)

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SEQUENCE LISTING

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<120> DIAGNOSTIC AND THERAPEUTIC METHODS FOR RHEUMATIC HEART DISEASE BASED UPON GROUP A STREPTOCOCCUS MARKERS

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<151> 2009-10-20

<160> 10

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<213> Streptococcus pyogenes

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Gly Ala Ala Thr Thr Val Gly Ala Glu Asp Leu Ser Thr Lys Ile Ala
            20                  25                  30

Lys Gln Asp Ser Ile Ile Ser Asn Leu Thr Thr Glu Gln Lys Ala Ala
        35                  40                  45

Gln Asn Gln Val Ser Ala Leu Gln Ala Gln Val Ser Ser Leu Gln Ser
    50                  55                  60

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

Lys Arg Phe Glu Gln Glu Ile Lys Ala Leu Thr Ser Gln Ile Val Ala
                85                  90                  95

Arg Asn Glu Lys Leu Lys Asn Gln Ala Arg Ser Ala Tyr Lys Asn Asn
            100                 105                 110

Glu Thr Ser Gly Tyr Ile Asn Ala Leu Leu Asn Ser Lys Ser Ile Ser
        115                 120                 125

Asp Val Val Asn Arg Leu Val Ala Ile Asn Arg Ala Val Ser Ala Asn
    130                 135                 140

Ala Lys Leu Leu Glu Gln Gln Lys Ala Asp Lys Val Ser Leu Glu Glu
145                 150                 155                 160
```

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```
Lys Gln Ala Ala Asn Gln Thr Ala Ile Asn Thr Ile Ala Ala Asn Met
                165                 170                 175

Ala Met Ala Glu Glu Asn Gln Asn Thr Leu Arg Thr Gln Gln Ala Asn
            180                 185                 190

Leu Val Ala Ala Thr Ala Asn Leu Ala Leu Gln Leu Ala Ser Ala Thr
        195                 200                 205

Glu Asp Lys Ala Asn Leu Val Ala Gln Lys Glu Ala Ala Glu Lys Ala
    210                 215                 220

Ala Ala Glu Ala Leu Ala Gln Glu Gln Ala Ala Lys Val Lys Ala Gln
225                 230                 235                 240

Glu Gln Ala Ala Gln Gln Ala Ala Ser Val Glu Ala Ala Lys Ser Ala
                245                 250                 255

Ile Thr Pro Ala Pro Gln Ala Thr Pro Ala Ala Gln Ser Ser Asn Ala
            260                 265                 270

Ile Glu Pro Ala Ala Leu Thr Ala Pro Ala Ala Pro Ser Ala Gly Pro
        275                 280                 285

Gln Thr Ser Tyr Asp Ser Ser Asn Thr Tyr Pro Val Gly Gln Cys Thr
    290                 295                 300

Trp Gly Ala Lys Ser Leu Ala Pro Trp Ala Gly Asn Asn Trp Gly Asn
305                 310                 315                 320

Gly Gly Gln Trp Ala Tyr Ser Ala Gln Ala Ala Gly Tyr Arg Thr Gly
                325                 330                 335

Ser Thr Pro Met Val Gly Ala Ile Ala Val Trp Asn Asp Gly Gly Tyr
            340                 345                 350

Gly His Val Ala Val Val Val Glu Val Gln Ser Ala Ser Ser Ile Arg
        355                 360                 365

Val Met Glu Ser Asn Tyr Ser Gly Arg Gln Tyr Ile Ala Asp His Arg
    370                 375                 380

Gly Trp Phe Asn Pro Thr Gly Val Thr Phe Ile Tyr Pro His
385                 390                 395

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1               5                   10                  15

Gln Glu Gln Ala Ala Gln Gln Ala Ala Ser Val Glu Ala Ala Lys Ser
```

20 25 30

Ala Ile Thr Pro Ala Pro Gln Ala Thr Pro Ala Ala Gln Ser Ser Asn
35 40 45

Ala Ile Glu Pro Ala Ala Leu Thr Ala Pro Ala Ala Pro Ser Ala Gly
50 55 60

Pro Gln Thr Ser Tyr Asp Ser Ser Asn Thr Tyr Pro Val Gly Gln Cys
65 70 75 80

Thr Trp Gly Ala Lys Ser Leu Ala Pro Trp Ala Gly Asn Asn Trp Gly
85 90 95

Asn Gly Gly Gln Trp Ala Tyr Ser Ala Gln Ala Ala Gly Tyr Arg Thr
100 105 110

Gly Ser Thr Pro Met Val Gly Ala Ile Ala Val Trp Asn Asp Gly Gly
115 120 125

Tyr Gly His Val Ala Val Val Val Glu Val Gln Ser Ala Ser Ser Ile
130 135 140

Arg Val Met Glu Ser Asn Tyr Ser Gly Arg Gln Tyr Ile Ala Asp His
145 150 155 160

Arg Gly Trp Phe Asn Pro Thr Gly Val Thr Phe Ile Tyr Pro His
165 170 175

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<211> 571
<212> PRT
<213> Streptococcus pyogenes

<400> 3
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
1 5 10 15

Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
20 25 30

Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
35 40 45

Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
50 55 60

Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65 70 75 80

Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
85 90 95

Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
100 105 110

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```
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125

Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140

Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160

Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175

Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190

Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205

Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220

Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240

Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255

Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270

Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285

Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300

Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320

Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335

Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350

Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365

Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380

Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
```

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His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
405 410 415

Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
420 425 430

Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
435 440 445

Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
450 455 460

Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465 470 475 480

Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
485 490 495

Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
500 505 510

Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
515 520 525

Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
530 535 540

Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545 550 555 560

Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
565 570

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20 25 30

Gln Val Lys Ala Asp Asp Arg Ala Ser Gly Glu Thr Lys Ala Ser Asn
35 40 45

Thr His Asp Asp Ser Leu Pro Lys Pro Glu Thr Ile Gln Glu Ala Lys
50 55 60

Ala Thr Ile Asp Ala Val Glu Lys Thr Leu Ser Gln Gln Lys Ala Glu
65 70 75 80

Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys Thr Thr Ala Glu Ile Asn

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```
                85                  90                  95

His Leu Lys Glu Gln Gln Asp Asn Glu Gln Lys Ala Leu Thr Ser Ala
            100                 105                 110

Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser Ser Glu Glu Thr Leu Leu
        115                 120                 125

Ala Gln Gly Ala Glu His Gln Arg Glu Leu Thr Ala Thr Glu Thr Glu
    130                 135                 140

Leu His Asn Ala Gln Ala Asp Gln His Ser Lys Glu Thr Ala Leu Ser
145                 150                 155                 160

Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr Thr Arg Ala Gln Asp Leu
                165                 170                 175

Val Glu Gln Val Lys Thr Ser Glu Gln Asn Ile Ala Lys Leu Asn Ala
            180                 185                 190

Met Ile Ser Asn Pro Asp Ala Ile Thr Lys Ala Ala Gln Thr Ala Asn
        195                 200                 205

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

Leu Glu Asn Gln Lys Ala Lys Val Lys Lys Gln Leu Thr Glu Glu Leu
225                 230                 235                 240

Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys Glu Ala Glu Leu Ser Arg
                245                 250                 255

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

Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu Glu Glu Leu Lys Lys Leu
        275                 280                 285

Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser Tyr Asn Asn Tyr Tyr Lys
    290                 295                 300

Glu His Ala Asp Gln Ile Ile Ala Lys Ala Ser Pro Gly Asn Gln Leu
305                 310                 315                 320

Asn Gln Tyr Gln Asp Ile Pro Ala Asp Arg Asn Arg Phe Val Asp Pro
                325                 330                 335

Asp Asn Leu Thr Pro Glu Val Gln Asn Glu Leu Ala Gln Phe Ala Ala
            340                 345                 350

His Met Ile Asn Ser Val Arg Arg Gln Leu Gly Leu Pro Pro Val Thr
        355                 360                 365

Val Thr Ala Gly Ser Gln Glu Phe Ala Arg Leu Leu Ser Thr Ser Tyr
    370                 375                 380
```

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Lys Lys Thr His Gly Asn Thr Arg Pro Ser Phe Val Tyr Gly Gln Pro
385 390 395 400

Gly Val Ser Gly His Tyr Gly Val Gly Pro His Asp Lys Thr Ile Ile
405 410 415

Glu Asp Ser Ala Gly Ala Ser Gly Leu Ile Arg Asn Asp Asp Asn Met
420 425 430

Tyr Glu Asn Ile Gly Ala Phe Asn Asp Val His Thr Val Asn Gly Ile
435 440 445

Lys Arg Gly Ile Tyr Asp Ser Ile Lys Tyr Met Leu Phe Thr Asp His
450 455 460

Leu His Gly Asn Thr Tyr Gly His Ala Ile Asn Phe Leu Arg Val Asp
465 470 475 480

Lys His Asn Pro Asn Ala Pro Val Tyr Leu Gly Phe Ser Thr Ser Asn
485 490 495

Val Gly Ser Leu Asn Glu His Phe Val Met Phe Pro Glu Ser Asn Ile
500 505 510

Ala Asn His Gln Arg Phe Asn Lys Thr Pro Ile Lys Ala Val Gly Ser
515 520 525

Thr Lys Asp Tyr Ala Gln Arg Val Gly Thr Val Ser Asp Thr Ile Ala
530 535 540

Ala Ile Lys Gly Lys Val Ser Ser Leu Glu Asn Arg Leu Ser Ala Ile
545 550 555 560

His Gln Glu Ala Asp Ile Met Ala Ala Gln Ala Lys Val Ser Gln Leu
565 570 575

Gln Gly Lys Leu Ala Ser Thr Leu Lys Gln Ser Asp Ser Leu Asn Leu
580 585 590

Gln Val Arg Gln Leu Asn Asp Thr Lys Gly Ser Leu Arg Thr Glu Leu
595 600 605

Leu Ala Ala Lys Ala Lys Gln Ala Gln Leu Glu Ala Thr Arg Asp Gln
610 615 620

Ser Leu Ala Lys Leu Ala Ser Leu Lys Ala Ala Leu His Gln Thr Glu
625 630 635 640

Ala Leu Ala Glu Gln Ala Ala Ala Arg Val Thr Ala Leu Val Ala Lys
645 650 655

Lys Ala His Leu Gln Tyr Leu Arg Asp Phe Lys Leu Asn Pro Asn Arg
660 665 670

Leu Gln Val Ile Arg Glu Arg Ile Asp Asn Thr Lys Gln Asp Leu Ala
675 680 685

Lys Thr Thr Ser Ser Leu Leu Asn Ala Gln Glu Ala Leu Ala Ala Leu
690 695 700

Gln Ala Lys Gln Ser Ser Leu Glu Ala Thr Ile Ala Thr Thr Glu His
705 710 715 720

Gln Leu Thr Leu Leu Lys Thr Leu Ala Asn Glu Lys Glu Tyr Arg His
725 730 735

Leu Asp Glu Asp Ile Ala Thr Val Pro Asp Leu Gln Val Ala Pro Pro
740 745 750

Leu Thr Gly Val Lys Pro Leu Ser Tyr Ser Lys Ile Asp Thr Thr Pro
755 760 765

Leu Val Gln Glu Met Val Lys Glu Thr Lys Gln Leu Leu Glu Ala Ser
770 775 780

Ala Arg Leu Ala Ala Glu Asn Thr Ser Leu Val Ala Glu Ala Leu Val
785 790 795 800

Gly Gln Thr Ser Glu Met Val Ala Ser Asn Ala Ile Val Ser Lys Ile
805 810 815

Thr Ser Ser Ile Thr Gln Pro Ser Ser Lys Thr Ser Tyr Gly Ser Gly
820 825 830

Ser Ser Thr Thr Ser Asn Leu Ile Ser Asp Val Asp Glu Ser Thr Gln
835 840 845

Arg Ala Leu Lys Ala Gly Val Val Met Leu Ala Ala Val Gly Leu Thr
850 855 860

Gly Phe Arg Phe Arg Lys Glu Ser Lys
865 870

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Phe Ser Val Leu Ile Gly Ser Val Phe Leu Val Met Thr Thr Thr Val
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Ala Ala Asp Glu Leu Ser Thr Met Ser Glu Pro Thr Ile Thr Asn His
35 40 45

Ala Gln Gln Gln Ala Gln His Leu Thr Asn Thr Glu Leu Ser Ser Ala

50 55 60

Glu Ser Lys Ser Gln Asp Thr Ser Gln Ile Thr Leu Lys Thr Asn Arg
65 70 75 80

Glu Lys Glu Gln Ser Gln Asp Leu Val Ser Glu Pro Thr Thr Thr Glu
85 90 95

Leu Ala Asp Thr Asp Ala Ala Ser Met Ala Asn Thr Gly Ser Asp Ala
100 105 110

Thr Gln Lys Ser Ala Ser Leu Pro Pro Val Asn Thr Asp Val His Asp
115 120 125

Trp Val Lys Thr Lys Gly Ala Trp Asp Lys Gly Tyr Lys Gly Gln Gly
130 135 140

Lys Val Val Ala Val Ile Asp Thr Gly Ile Asp Pro Ala His Gln Ser
145 150 155 160

Met Arg Ile Ser Asp Val Ser Thr Ala Lys Val Lys Ser Lys Glu Asp
165 170 175

Met Leu Ala Arg Gln Lys Ala Ala Gly Ile Asn Tyr Gly Ser Trp Ile
180 185 190

Asn Asp Lys Val Val Phe Ala His Asn Tyr Val Glu Asn Ser Asp Asn
195 200 205

Ile Lys Glu Asn Gln Phe Glu Asp Phe Asp Glu Asp Trp Glu Asn Phe
210 215 220

Glu Phe Asp Ala Glu Ala Glu Pro Lys Ala Ile Lys Lys His Lys Ile
225 230 235 240

Tyr Arg Pro Gln Ser Thr Gln Ala Pro Lys Glu Thr Val Ile Lys Thr
245 250 255

Glu Glu Thr Asp Gly Ser His Asp Ile Asp Trp Thr Gln Thr Asp Asp
260 265 270

Asp Thr Lys Tyr Glu Ser His Gly Met His Val Thr Gly Ile Val Ala
275 280 285

Gly Asn Ser Lys Glu Ala Ala Ala Thr Gly Glu Arg Phe Leu Gly Ile
290 295 300

Ala Pro Glu Ala Gln Val Met Phe Met Arg Val Phe Ala Asn Asp Ile
305 310 315 320

Met Gly Ser Ala Glu Ser Leu Phe Ile Lys Ala Ile Glu Asp Ala Val
325 330 335

Ala Leu Gly Ala Asp Val Ile Asn Leu Ser Leu Gly Thr Ala Asn Gly
340 345 350

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Ala Gln Leu Ser Gly Ser Lys Pro Leu Met Glu Ala Ile Glu Lys Ala
        355                 360                 365

Lys Lys Ala Gly Val Ser Val Val Val Ala Ala Gly Asn Glu Arg Val
    370                 375                 380

Tyr Gly Ser Asp His Asp Asp Pro Leu Ala Thr Asn Pro Asp Tyr Gly
385                 390                 395                 400

Leu Val Gly Ser Pro Ser Thr Gly Arg Thr Pro Thr Ser Val Ala Ala
                405                 410                 415

Ile Asn Ser Lys Trp Val Ile Gln Arg Leu Met Thr Val Lys Glu Leu
            420                 425                 430

Glu Asn Arg Ala Asp Leu Asn His Gly Lys Ala Ile Tyr Ser Glu Ser
        435                 440                 445

Val Asp Phe Lys Asp Ile Lys Asp Ser Leu Gly Tyr Asp Lys Ser His
    450                 455                 460

Gln Phe Ala Tyr Val Lys Glu Ser Thr Asp Ala Gly Tyr Asn Ala Gln
465                 470                 475                 480

Asp Val Lys Gly Lys Ile Ala Leu Ile Glu Arg Asp Pro Asn Lys Thr
                485                 490                 495

Tyr Asp Glu Met Ile Ala Leu Ala Lys Lys His Gly Ala Leu Gly Val
            500                 505                 510

Leu Ile Phe Asn Asn Lys Pro Gly Gln Ser Asn Arg Ser Met Arg Leu
        515                 520                 525

Thr Ala Asn Gly Met Gly Ile Pro Ser Ala Phe Ile Ser His Glu Phe
    530                 535                 540

Gly Lys Ala Met Ser Gln Leu Asn Gly Asn Gly Thr Gly Ser Leu Glu
545                 550                 555                 560

Phe Asp Ser Val Val Ser Lys Ala Pro Ser Gln Lys Gly Asn Glu Met
                565                 570                 575

Asn His Phe Ser Asn Trp Gly Leu Thr Ser Asp Gly Tyr Leu Lys Pro
            580                 585                 590

Asp Ile Thr Ala Pro Gly Gly Asp Ile Tyr Ser Thr Tyr Asn Asp Asn
        595                 600                 605

His Tyr Gly Ser Gln Thr Gly Thr Ser Met Ala Ser Pro Gln Ile Ala
    610                 615                 620

Gly Ala Ser Leu Leu Val Lys Gln Tyr Leu Glu Lys Thr Gln Pro Asn
625                 630                 635                 640
```

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Leu Pro Lys Glu Lys Ile Ala Asp Ile Val Lys Asn Leu Leu Met Ser
645 650 655

Asn Ala Gln Ile His Val Asn Pro Glu Thr Lys Thr Thr Thr Ser Pro
660 665 670

Arg Gln Gln Gly Ala Gly Leu Leu Asn Ile Asp Gly Ala Val Thr Ser
675 680 685

Gly Leu Tyr Val Thr Gly Lys Asp Asn Tyr Gly Ser Ile Ser Leu Gly
690 695 700

Asn Ile Thr Asp Thr Met Thr Phe Asp Val Thr Val His Asn Leu Ser
705 710 715 720

Asn Lys Asp Lys Thr Leu Arg Tyr Asp Thr Glu Leu Leu Thr Asp His
725 730 735

Val Asp Pro Gln Lys Gly Arg Phe Thr Leu Thr Ser His Ser Leu Lys
740 745 750

Thr Tyr Gln Gly Gly Glu Val Thr Val Pro Ala Asn Gly Lys Val Thr
755 760 765

Val Arg Val Thr Met Asp Val Ser Gln Phe Thr Lys Glu Leu Thr Lys
770 775 780

Gln Met Pro Asn Gly Tyr Tyr Leu Glu Gly Phe Val Arg Phe Arg Asp
785 790 795 800

Ser Gln Asp Asp Gln Leu Asn Arg Val Asn Ile Pro Phe Val Gly Phe
805 810 815

Lys Gly Gln Phe Glu Asn Leu Ala Val Ala Glu Glu Ser Ile Tyr Arg
820 825 830

Leu Lys Ser Gln Gly Lys Thr Gly Phe Tyr Phe Asp Glu Ser Gly Pro
835 840 845

Lys Asp Asp Ile Tyr Val Gly Lys His Phe Thr Gly Leu Val Thr Leu
850 855 860

Gly Ser Glu Thr Asn Val Ser Thr Lys Thr Ile Ser Asp Asn Gly Leu
865 870 875 880

His Thr Leu Gly Thr Phe Lys Asn Ala Asp Gly Lys Phe Ile Leu Glu
885 890 895

Lys Asn Ala Gln Gly Asn Pro Val Leu Ala Ile Ser Pro Asn Gly Asp
900 905 910

Asn Asn Gln Asp Phe Ala Ala Phe Lys Gly Val Phe Leu Arg Lys Tyr
915 920 925

Gln Gly Leu Lys Ala Ser Val Tyr His Ala Ser Asp Lys Glu His Lys

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```
    930                 935                 940

Asn Pro Leu Trp Val Ser Pro Glu Ser Phe Lys Gly Asp Lys Asn Phe
945                 950                 955                 960

Asn Ser Asp Ile Arg Phe Ala Lys Ser Thr Thr Leu Leu Gly Thr Ala
                965                 970                 975

Phe Ser Gly Lys Ser Leu Thr Gly Ala Glu Leu Pro Asp Gly His Tyr
            980                 985                 990

His Tyr Val Val Ser Tyr Tyr Pro Asp Val Val Gly Ala Lys Arg Gln
        995                 1000                1005

Glu Met Thr Phe Asp Met Ile Leu Asp Arg Gln Lys Pro Val Leu Ser
    1010                1015                1020

Gln Ala Thr Phe Asp Pro Glu Thr Asn Arg Phe Lys Pro Glu Pro Leu
1025                1030                1035                1040

Lys Asp Arg Gly Leu Ala Gly Val Arg Lys Asp Ser Val Phe Tyr Leu
                1045                1050                1055

Glu Arg Lys Asp Asn Lys Pro Tyr Thr Val Thr Ile Asn Asp Ser Tyr
            1060                1065                1070

Lys Tyr Val Ser Val Glu Asp Asn Lys Thr Phe Val Glu Arg Gln Ala
        1075                1080                1085

Asp Gly Ser Phe Ile Leu Pro Leu Asp Lys Ala Lys Leu Gly Asp Phe
    1090                1095                1100

Tyr Tyr Met Val Glu Asp Phe Ala Gly Asn Val Ala Ile Ala Lys Leu
1105                1110                1115                1120

Gly Asp His Leu Pro Gln Thr Leu Gly Lys Thr Pro Ile Lys Leu Lys
                1125                1130                1135

Leu Thr Asp Gly Asn Tyr Gln Thr Lys Glu Thr Leu Lys Asp Asn Leu
            1140                1145                1150

Glu Met Thr Gln Ser Asp Thr Gly Leu Val Thr Asn Gln Ala Gln Leu
        1155                1160                1165

Ala Val Val His Arg Asn Gln Pro Gln Ser Gln Leu Thr Lys Met Asn
    1170                1175                1180

Gln Asp Phe Phe Ile Ser Pro Asn Glu Asp Gly Asn Lys Asp Phe Val
1185                1190                1195                1200

Ala Phe Lys Gly Leu Lys Asn Asn Val Tyr Asn Asp Leu Thr Val Asn
                1205                1210                1215

Val Tyr Ala Lys Asp Asp His Gln Lys Gln Thr Pro Ile Trp Ser Ser
            1220                1225                1230
```

Gln Ala Gly Ala Ser Val Ser Ala Ile Glu Ser Thr Ala Trp Tyr Gly
1235 1240 1245

Ile Thr Ala Arg Gly Ser Lys Val Met Pro Gly Asp Tyr Gln Tyr Val
1250 1255 1260

Val Thr Tyr Arg Asp Glu His Gly Lys Glu His Gln Lys Gln Tyr Thr
1265 1270 1275 1280

Ile Ser Val Asn Asp Lys Lys Pro Met Ile Thr Gln Gly Arg Phe Asp
1285 1290 1295

Thr Ile Asn Gly Val Asp His Phe Thr Pro Asp Lys Thr Lys Ala Leu
1300 1305 1310

Asp Ser Ser Gly Ile Val Arg Glu Glu Val Phe Tyr Leu Ala Lys Lys
1315 1320 1325

Asn Gly Arg Lys Phe Asp Val Thr Glu Gly Lys Asp Gly Ile Thr Val
1330 1335 1340

Ser Asp Asn Lys Val Tyr Ile Pro Lys Asn Pro Asp Gly Ser Tyr Thr
1345 1350 1355 1360

Ile Ser Lys Arg Asp Gly Val Thr Leu Ser Asp Tyr Tyr Tyr Leu Val
1365 1370 1375

Glu Asp Arg Ala Gly Asn Val Ser Phe Ala Thr Leu Arg Asp Leu Lys
1380 1385 1390

Ala Val Gly Lys Asp Lys Ala Val Val Asn Phe Gly Leu Asp Leu Pro
1395 1400 1405

Val Pro Glu Asp Lys Gln Ile Val Asn Phe Thr Tyr Leu Val Arg Asp
1410 1415 1420

Ala Asp Gly Lys Pro Ile Glu Asn Leu Glu Tyr Tyr Asn Asn Ser Gly
1425 1430 1435 1440

Asn Ser Leu Ile Leu Pro Tyr Gly Lys Tyr Thr Val Glu Leu Leu Thr
1445 1450 1455

Tyr Asp Thr Asn Ala Ala Lys Leu Glu Ser Asp Lys Ile Val Ser Phe
1460 1465 1470

Thr Leu Ser Ala Asp Asn Asn Phe Gln Gln Val Thr Phe Lys Ile Thr
1475 1480 1485

Met Leu Ala Thr Ser Gln Ile Thr Ala His Phe Asp His Leu Leu Pro
1490 1495 1500

Glu Gly Ser Arg Val Ser Leu Lys Thr Ala Gln Asp Gln Leu Ile Pro
1505 1510 1515 1520

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Leu Glu Gln Ser Leu Tyr Val Pro Lys Ala Tyr Gly Lys Thr Val Gln
1525 1530 1535

Glu Gly Thr Tyr Glu Val Val Val Ser Leu Pro Lys Gly Tyr Arg Ile
1540 1545 1550

Glu Gly Asn Thr Lys Val Asn Thr Leu Pro Asn Glu Val His Glu Leu
1555 1560 1565

Ser Leu Arg Leu Val Lys Val Gly Asp Ala Ser Asp Ser Thr Gly Asp
1570 1575 1580

His Lys Val Met Ser Lys Asn Asn Ser Gln Ala Leu Thr Ala Ser Ala
1585 1590 1595 1600

Thr Pro Thr Lys Ser Thr Thr Ser Ala Thr Ala Lys Ala Leu Pro Ser
1605 1610 1615

Thr Gly Glu Lys Met Gly Leu Lys Leu Arg Ile Val Gly Leu Val Leu
1620 1625 1630

Leu Gly Leu Thr Cys Val Phe Ser Arg Lys Lys Ser Thr Lys Asp
1635 1640 1645

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<211> 503
<212> PRT
<213> Streptococcus pyogenes

<400> 6
Met Asn Lys Asn Lys Leu Leu Arg Val Ala Met Leu Leu Ser Leu Leu
1 5 10 15

Ala Pro Thr Ala Glu Ser Met Thr Val Leu Ala Gln Asp Val Met Leu
20 25 30

Glu Thr His Lys Ala Thr Thr Asn Glu Thr Ser Asp Ser Ser Ser Lys
35 40 45

Glu Glu Asn Asn Lys Asn Ala Ala Pro Thr Thr Ser Asp Lys Thr Asp
50 55 60

Gln Gly Pro Leu Asp Ala Ser Ala Glu Thr Asn Ser Asn Ser Leu Val
65 70 75 80

Asn Ala Asp Asp Lys Lys Arg Ser Asp Ser Ser Gln Ser Ala Ile Gly
85 90 95

Ser Ser Asp Asn Lys Ala Glu Ala Glu Asn Gln Val Asp Asp Lys Ser
100 105 110

Thr Asp His Ser Lys Ser Thr Asp His Ser Lys Pro Thr Asp Gln Pro
115 120 125

Lys Pro Ser Pro Ser Lys Val Asp Thr Ala Pro Ala Ser Ser Leu Ser

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    130                 135                 140

Lys Gln Leu Pro Glu Ala Arg Thr Pro Ile Gln Ser Leu Ser Pro Tyr
145                 150                 155                 160

Val Ser Asp Leu Asp Leu Ser Glu Ile Asp Ile Pro Ser Val Asn Thr
                165                 170                 175

Tyr Ala Ala Tyr Val Glu His Trp Ser Gly Lys Asn Ala Tyr Thr His
            180                 185                 190

His Leu Leu Ser Arg Arg Tyr Gly Ile Lys Ala Asp Gln Ile Asp Ser
        195                 200                 205

Tyr Leu Lys Ser Thr Gly Ile Ala Tyr Asp Ser Thr Arg Ile Asn Gly
    210                 215                 220

Glu Lys Leu Leu Gln Trp Glu Lys Lys Ser Gly Leu Asp Val Arg Ala
225                 230                 235                 240

Ile Val Ala Ile Ala Met Ser Glu Ser Ser Leu Gly Thr Gln Gly Ile
                245                 250                 255

Ala Thr Leu Leu Gly Ala Asn Met Phe Gly Tyr Ala Ala Phe Asp Leu
            260                 265                 270

Asp Pro Thr Gln Ala Ser Lys Phe Asn Asp Asp Ser Ala Ile Val Lys
        275                 280                 285

Met Thr Gln Asp Thr Ile Ile Lys Asn Lys Asn Ser Asn Phe Ala Leu
    290                 295                 300

Gln Asp Leu Lys Ala Ala Lys Phe Ser Arg Gly Gln Leu Asn Phe Ala
305                 310                 315                 320

Ser Asp Gly Gly Val Tyr Phe Thr Asp Thr Thr Gly Ser Gly Lys Arg
                325                 330                 335

Arg Ala Gln Ile Met Glu Asp Leu Asp Lys Trp Ile Asp Asp His Gly
            340                 345                 350

Gly Thr Pro Ala Ile Pro Ala Glu Leu Lys Val Gln Ser Ser Ala Ser
        355                 360                 365

Phe Ala Ser Val Pro Ala Gly Tyr Lys Leu Ser Lys Ser Tyr Asp Val
    370                 375                 380

Leu Gly Tyr Gln Ala Ser Ser Tyr Ala Trp Gly Gln Cys Thr Trp Tyr
385                 390                 395                 400

Val Tyr Asn Arg Ala Lys Glu Leu Gly Tyr Gln Phe Asp Pro Phe Met
                405                 410                 415

Gly Asn Gly Gly Asp Trp Lys Tyr Lys Val Gly Tyr Ala Leu Ser Lys
            420                 425                 430
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Thr Pro Lys Val Gly Tyr Ala Ile Ser Phe Ala Pro Gly Gln Ala Gly
        435                 440                 445

Ala Asp Gly Thr Tyr Gly His Val Ser Ile Val Glu Asp Val Arg Lys
    450                 455                 460

Asp Gly Ser Ile Leu Ile Ser Glu Ser Asn Cys Ile Gly Leu Gly Lys
465                 470                 475                 480

Ile Ser Tyr Arg Thr Phe Thr Ala Gln Gln Ala Glu Gln Leu Thr Tyr
                485                 490                 495

Val Ile Gly Lys Ser Lys Asn
            500

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<212>   PRT
<213>   Streptococcus pyogenes

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Met Asp Lys His Leu Leu Val Lys Arg Thr Leu Gly Cys Val Cys Ala
1               5                   10                  15

Ala Thr Leu Met Gly Ala Ala Leu Ala Thr His His Asp Ser Leu Asn
            20                  25                  30

Thr Val Lys Ala Glu Glu Lys Thr Val Gln Val Gln Lys Gly Leu Pro
        35                  40                  45

Ser Ile Asp Ser Leu His Tyr Leu Ser Glu Asn Ser Lys Lys Glu Phe
    50                  55                  60

Lys Glu Glu Leu Ser Lys Ala Gly Gln Glu Ser Gln Lys Val Lys Glu
65                  70                  75                  80

Ile Leu Ala Lys Ala Gln Gln Ala Asp Lys Gln Ala Gln Glu Leu Ala
                85                  90                  95

Lys Met Lys Ile Pro Glu Lys Ile Pro Met Lys Pro Leu His Gly Ser
            100                 105                 110

Leu Tyr Gly Gly Tyr Phe Arg Thr Trp His Asp Lys Thr Ser Asp Pro
        115                 120                 125

Thr Glu Lys Asp Lys Val Asn Ser Met Gly Glu Leu Pro Lys Glu Val
    130                 135                 140

Asp Leu Ala Phe Ile Phe His Asp Trp Thr Lys Asp Tyr Ser Leu Phe
145                 150                 155                 160

Trp Lys Glu Leu Ala Thr Lys His Val Pro Lys Leu Asn Lys Gln Gly
                165                 170                 175
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Thr Arg Val Ile Arg Thr Ile Pro Trp Arg Phe Leu Ala Gly Gly Asp
            180                 185                 190

Asn Ser Gly Ile Ala Glu Asp Thr Ser Lys Tyr Pro Asn Thr Pro Glu
        195                 200                 205

Gly Asn Lys Ala Leu Ala Lys Ala Ile Val Asp Glu Tyr Val Tyr Lys
    210                 215                 220

Tyr Asn Leu Asp Gly Leu Asp Val Asp Val Glu His Asp Ser Ile Pro
225                 230                 235                 240

Lys Val Asp Lys Lys Glu Asp Thr Ala Gly Val Glu Arg Ser Ile Gln
                245                 250                 255

Val Phe Glu Glu Ile Gly Lys Leu Ile Gly Pro Lys Gly Val Asp Lys
            260                 265                 270

Ser Arg Leu Phe Ile Met Asp Ser Thr Tyr Met Ala Asp Lys Asn Pro
        275                 280                 285

Leu Ile Glu Arg Gly Ala Pro Tyr Ile Asn Leu Leu Leu Val Gln Val
    290                 295                 300

Tyr Gly Ser Gln Gly Glu Lys Gly Gly Trp Glu Pro Val Ser Asn Arg
305                 310                 315                 320

Pro Glu Lys Thr Met Glu Glu Arg Trp Gln Gly Tyr Ser Lys Tyr Ile
                325                 330                 335

Arg Pro Glu Gln Tyr Met Ile Gly Phe Ser Phe Tyr Glu Glu Asn Ala
            340                 345                 350

Gln Glu Gly Asn Leu Trp Tyr Asp Ile Asn Ser Arg Lys Asp Glu Asp
        355                 360                 365

Lys Ala Asn Gly Ile Asn Thr Asp Ile Thr Gly Thr Arg Ala Glu Arg
    370                 375                 380

Tyr Ala Arg Trp Gln Pro Lys Thr Gly Gly Val Lys Gly Gly Ile Phe
385                 390                 395                 400

Ser Tyr Ala Ile Asp Arg Asp Gly Val Ala His Gln Pro Lys Lys Tyr
                405                 410                 415

Ala Lys Gln Lys Glu Phe Lys Asp Ala Thr Asp Asn Ile Phe His Ser
            420                 425                 430

Asp Tyr Ser Val Ser Lys Ala Leu Lys Thr Val Met Leu Lys Asp Lys
        435                 440                 445

Ser Tyr Asp Leu Ile Asp Glu Lys Asp Phe Pro Asp Lys Ala Leu Arg
    450                 455                 460

Glu Ala Val Met Ala Gln Val Gly Thr Arg Lys Gly Asp Leu Glu Arg
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465 470 475 480

Phe Asn Gly Thr Leu Arg Leu Asp Asn Pro Ala Ile Gln Ser Leu Glu
485 490 495

Gly Leu Asn Lys Phe Lys Lys Leu Ala Gln Leu Asp Leu Ile Gly Leu
500 505 510

Ser Arg Ile Thr Lys Leu Asp Arg Ser Val Leu Pro Ala Asn Met Lys
515 520 525

Pro Gly Lys Asp Thr Leu Glu Thr Val Leu Glu Thr Tyr Lys Lys Asp
530 535 540

Asn Lys Glu Glu Pro Ala Thr Ile Pro Pro Val Ser Leu Lys Val Ser
545 550 555 560

Gly Leu Thr Gly Leu Lys Glu Leu Asp Leu Ser Gly Phe Asp Arg Glu
565 570 575

Thr Leu Ala Gly Leu Asp Ala Ala Thr Leu Thr Ser Leu Glu Lys Val
580 585 590

Asp Ile Ser Gly Asn Lys Leu Asp Leu Ala Pro Gly Thr Glu Asn Arg
595 600 605

Gln Ile Phe Asp Thr Met Leu Ser Thr Ile Ser Asn His Val Gly Ser
610 615 620

Asn Glu Gln Thr Val Lys Phe Asp Lys Gln Lys Pro Thr Gly His Tyr
625 630 635 640

Pro Asp Thr Tyr Gly Lys Thr Ser Leu Arg Leu Pro Val Ala Asn Glu
645 650 655

Lys Val Asp Leu Gln Ser Gln Leu Leu Phe Gly Thr Val Thr Asn Gln
660 665 670

Gly Thr Leu Ile Asn Ser Glu Ala Asp Tyr Lys Ala Tyr Gln Asn His
675 680 685

Lys Ile Ala Gly Arg Ser Phe Val Asp Ser Asn Tyr His Tyr Asn Asn
690 695 700

Phe Lys Val Ser Tyr Glu Asn Tyr Thr Val Lys Val Thr Asp Ser Thr
705 710 715 720

Leu Gly Thr Thr Thr Asp Lys Thr Leu Ala Thr Asp Lys Glu Glu Thr
725 730 735

Tyr Lys Val Asp Phe Phe Ser Pro Ala Asp Lys Thr Lys Ala Val His
740 745 750

Thr Ala Lys Val Ile Val Gly Asp Glu Lys Thr Met Met Val Asn Leu
755 760 765

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Ala Glu Gly Ala Thr Val Ile Gly Gly Ser Ala Asp Pro Val Asn Ala
    770                 775                 780

Arg Lys Val Phe Asp Gly Gln Leu Gly Ser Glu Thr Asp Asn Ile Ser
785                 790                 795                 800

Leu Gly Trp Asp Ser Lys Gln Ser Ile Ile Phe Lys Leu Lys Glu Asp
                805                 810                 815

Gly Leu Ile Lys His Trp Arg Phe Phe Asn Asp Ser Ala Arg Asn Pro
            820                 825                 830

Glu Thr Thr Asn Lys Pro Ile Gln Glu Ala Ser Leu Gln Ile Phe Asn
        835                 840                 845

Ile Lys Asp Tyr Asn Leu Asp Asn Leu Leu Glu Asn Pro Asn Lys Phe
    850                 855                 860

Asp Asp Glu Lys Tyr Trp Ile Thr Val Asp Thr Tyr Ser Ala Gln Gly
865                 870                 875                 880

Glu Arg Ala Thr Ala Phe Ser Asn Thr Leu Asn Asn Ile Thr Ser Lys
                885                 890                 895

Tyr Trp Arg Val Val Phe Asp Thr Lys Gly Asp Arg Tyr Ser Ser Pro
            900                 905                 910

Val Val Pro Glu Leu Gln Ile Leu Gly Tyr Pro Leu Pro Asn Ala Asp
        915                 920                 925

Thr Ile Met Lys Thr Val Thr Thr Ala Lys Glu Leu Ser Gln Gln Lys
    930                 935                 940

Asp Lys Phe Ser Gln Lys Met Leu Asp Glu Leu Lys Ile Lys Glu Met
945                 950                 955                 960

Ala Leu Glu Thr Ser Leu Asn Ser Lys Ile Phe Asp Val Thr Ala Ile
                965                 970                 975

Asn Ala Asn Ala Gly Val Leu Lys Asp Cys Ile Glu Lys Arg Gln Leu
            980                 985                 990

Leu Lys Lys
        995

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<212>   PRT
<213>   Streptococcus pyogenes

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Gln Gln Asp Pro Asp Pro Ser Gln Leu His Arg Ser Ser Leu Val Lys
1               5                   10                  15
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Asn Leu Gln Asn Ile Tyr Phe Leu Tyr Glu Gly Asp Pro Val Thr His
20 25 30

Glu Asn Val Lys Ser Val Asp Gln Leu Leu Ser His Asp Leu Ile Tyr
35 40 45

Asn Val Ser Gly Pro Asn Tyr Asp Lys Leu Lys Thr Glu Leu Lys Asn
50 55 60

Gln Glu Met Ala Thr Leu Phe Lys Asp Lys Asn Val Asp Ile Tyr Gly
65 70 75 80

Val Glu Tyr Tyr His Leu Cys Tyr Leu Cys Glu Asn Ala Glu Arg Ser
85 90 95

Ala Cys Ile Tyr Gly Gly Val Thr Asn His Glu Gly Asn His Leu Glu
100 105 110

Ile Pro Lys Lys Ile Val Val Lys Val Ser Ile Asp Gly Ile Gln Ser
115 120 125

Leu Ser Phe Asp Ile Glu Thr Asn Lys Lys Met Val Thr Ala Gln Glu
130 135 140

Leu Asp Tyr Lys Val Arg Lys Tyr Leu Thr Asp Asn Lys Gln Leu Tyr
145 150 155 160

Thr Asn Gly Pro Ser Lys Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Pro
165 170 175

Lys Asn Lys Glu Ser Phe Trp Phe Asp Phe Phe Pro Glu Pro Glu Phe
180 185 190

Thr Gln Ser Lys Tyr Leu Met Ile Tyr Lys Asp Asn Glu Thr Leu Asp
195 200 205

Ser Asn Thr Ser Gln Ile Glu Val Tyr Leu Thr Thr Lys
210 215 220

<210> 9
<211> 865
<212> PRT
<213> Streptococcus pyogenes

<400> 9
Ala Asp Glu Leu Ser Thr Met Ser Glu Pro Thr Ile Thr Asn His Ala
1 5 10 15

Gln Gln Gln Ala Gln His Leu Thr Asn Thr Glu Leu Ser Ser Ala Glu
20 25 30

Ser Lys Ser Gln Asp Thr Ser Gln Ile Thr Leu Lys Thr Asn Arg Glu
35 40 45

Lys Glu Gln Ser Gln Asp Leu Val Ser Glu Pro Thr Thr Thr Glu Leu

50 55 60

Ala Asp Thr Asp Ala Ala Ser Met Ala Asn Thr Gly Ser Asp Ala Thr
65 70 75 80

Gln Lys Ser Ala Ser Leu Pro Pro Val Asn Thr Asp Val His Asp Trp
85 90 95

Val Lys Thr Lys Gly Ala Trp Asp Lys Gly Tyr Lys Gly Gln Gly Lys
100 105 110

Val Val Ala Val Ile Asp Thr Gly Ile Asp Pro Ala His Gln Ser Met
115 120 125

Arg Ile Ser Asp Val Ser Thr Ala Lys Val Lys Ser Lys Glu Asp Met
130 135 140

Leu Ala Arg Gln Lys Ala Ala Gly Ile Asn Tyr Gly Ser Trp Ile Asn
145 150 155 160

Asp Lys Val Val Phe Ala His Asn Tyr Val Glu Asn Ser Asp Asn Ile
165 170 175

Lys Glu Asn Gln Phe Glu Asp Phe Asp Glu Asp Trp Glu Asn Phe Glu
180 185 190

Phe Asp Ala Glu Ala Glu Pro Lys Ala Ile Lys Lys His Lys Ile Tyr
195 200 205

Arg Pro Gln Ser Thr Gln Ala Pro Lys Glu Thr Val Ile Lys Thr Glu
210 215 220

Glu Thr Asp Gly Ser His Asp Ile Asp Trp Thr Gln Thr Asp Asp Asp
225 230 235 240

Thr Lys Tyr Glu Ser His Gly Met His Val Thr Gly Ile Val Ala Gly
245 250 255

Asn Ser Lys Glu Ala Ala Ala Thr Gly Glu Arg Phe Leu Gly Ile Ala
260 265 270

Pro Glu Ala Gln Val Met Phe Met Arg Val Phe Ala Asn Asp Ile Met
275 280 285

Gly Ser Ala Glu Ser Leu Phe Ile Lys Ala Ile Glu Asp Ala Val Ala
290 295 300

Leu Gly Ala Asp Val Ile Asn Leu Ser Leu Gly Thr Ala Asn Gly Ala
305 310 315 320

Gln Leu Ser Gly Ser Lys Pro Leu Met Glu Ala Ile Glu Lys Ala Lys
325 330 335

Lys Ala Gly Val Ser Val Val Val Ala Ala Gly Asn Glu Arg Val Tyr
340 345 350

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Gly Ser Asp His Asp Asp Pro Leu Ala Thr Asn Pro Asp Tyr Gly Leu
        355                 360                 365

Val Gly Ser Pro Ser Thr Gly Arg Thr Pro Thr Ser Val Ala Ala Ile
    370                 375                 380

Asn Ser Lys Trp Val Ile Gln Arg Leu Met Thr Val Lys Glu Leu Glu
385                 390                 395                 400

Asn Arg Ala Asp Leu Asn His Gly Lys Ala Ile Tyr Ser Glu Ser Val
                405                 410                 415

Asp Phe Lys Asp Ile Lys Asp Ser Leu Gly Tyr Asp Lys Ser His Gln
            420                 425                 430

Phe Ala Tyr Val Lys Glu Ser Thr Asp Ala Gly Tyr Asn Ala Gln Asp
        435                 440                 445

Val Lys Gly Lys Ile Ala Leu Ile Glu Arg Asp Pro Asn Lys Thr Tyr
    450                 455                 460

Asp Glu Met Ile Ala Leu Ala Lys Lys His Gly Ala Leu Gly Val Leu
465                 470                 475                 480

Ile Phe Asn Asn Lys Pro Gly Gln Ser Asn Arg Ser Met Arg Leu Thr
                485                 490                 495

Ala Asn Gly Met Gly Ile Pro Ser Ala Phe Ile Ser His Glu Phe Gly
            500                 505                 510

Lys Ala Met Ser Gln Leu Asn Gly Asn Gly Thr Gly Ser Leu Glu Phe
        515                 520                 525

Asp Ser Val Val Ser Lys Ala Pro Ser Gln Lys Gly Asn Glu Met Asn
    530                 535                 540

His Phe Ser Asn Trp Gly Leu Thr Ser Asp Gly Tyr Leu Lys Pro Asp
545                 550                 555                 560

Ile Thr Ala Pro Gly Gly Asp Ile Tyr Ser Thr Tyr Asn Asp Asn His
                565                 570                 575

Tyr Gly Ser Gln Thr Gly Thr Ser Met Ala Ser Pro Gln Ile Ala Gly
            580                 585                 590

Ala Ser Leu Leu Val Lys Gln Tyr Leu Glu Lys Thr Gln Pro Asn Leu
        595                 600                 605

Pro Lys Glu Lys Ile Ala Asp Ile Val Lys Asn Leu Leu Met Ser Asn
    610                 615                 620

Ala Gln Ile His Val Asn Pro Glu Thr Lys Thr Thr Thr Ser Pro Arg
625                 630                 635                 640
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Gln Gln Gly Ala Gly Leu Leu Asn Ile Asp Gly Ala Val Thr Ser Gly
645 650 655

Leu Tyr Val Thr Gly Lys Asp Asn Tyr Gly Ser Ile Ser Leu Gly Asn
660 665 670

Ile Thr Asp Thr Met Thr Phe Asp Val Thr Val His Asn Leu Ser Asn
675 680 685

Lys Asp Lys Thr Leu Arg Tyr Asp Thr Glu Leu Leu Thr Asp His Val
690 695 700

Asp Pro Gln Lys Gly Arg Phe Thr Leu Thr Ser His Ser Leu Lys Thr
705 710 715 720

Tyr Gln Gly Gly Glu Val Thr Val Pro Ala Asn Gly Lys Val Thr Val
725 730 735

Arg Val Thr Met Asp Val Ser Gln Phe Thr Lys Glu Leu Thr Lys Gln
740 745 750

Met Pro Asn Gly Tyr Tyr Leu Glu Gly Phe Val Arg Phe Arg Asp Ser
755 760 765

Gln Asp Asp Gln Leu Asn Arg Val Asn Ile Pro Phe Val Gly Phe Lys
770 775 780

Gly Gln Phe Glu Asn Leu Ala Val Ala Glu Glu Ser Ile Tyr Arg Leu
785 790 795 800

Lys Ser Gln Gly Lys Thr Gly Phe Tyr Phe Asp Glu Ser Gly Pro Lys
805 810 815

Asp Asp Ile Tyr Val Gly Lys His Phe Thr Gly Leu Val Thr Leu Gly
820 825 830

Ser Glu Thr Asn Val Ser Thr Lys Thr Ile Ser Asp Asn Gly Leu His
835 840 845

Thr Leu Gly Thr Phe Lys Asn Ala Asp Gly Lys Phe Ile Leu Glu Lys
850 855 860

Asn
865

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<212> PRT
<213> Streptococcus pyogenes

<400> 10
Ser Val Gly Val Ser His Gln Val Lys Ala Asp Asp Arg Ala Ser Gly
1 5 10 15

Glu Thr Lys Ala Ser Asn Thr His Asp Asp Ser Leu Pro Lys Pro Glu

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            20                  25                  30

Thr Ile Gln Glu Ala Lys Ala Thr Ile Asp Ala Val Glu Lys Thr Leu
        35                  40                  45

Ser Gln Gln Lys Ala Glu Leu Thr Glu Leu Ala Thr Ala Leu Thr Lys
    50                  55                  60

Thr Thr Ala Glu Ile Asn His Leu Lys Glu Gln Gln Asp Asn Glu Gln
65                  70                  75                  80

Lys Ala Leu Thr Ser Ala Gln Glu Ile Tyr Thr Asn Thr Leu Ala Ser
                85                  90                  95

Ser Glu Glu Thr Leu Leu Ala Gln Gly Ala Glu His Gln Arg Glu Leu
            100                 105                 110

Thr Ala Thr Glu Thr Glu Leu His Asn Ala Gln Ala Asp Gln His Ser
        115                 120                 125

Lys Glu Thr Ala Leu Ser Glu Gln Lys Ala Ser Ile Ser Ala Glu Thr
    130                 135                 140

Thr Arg Ala Gln Asp Leu Val Glu Gln Val Lys Thr Ser Glu Gln Asn
145                 150                 155                 160

Ile Ala Lys Leu Asn Ala Met Ile Ser Asn Pro Asp Ala Ile Thr Lys
                165                 170                 175

Ala Ala Gln Thr Ala Asn Asp Asn Thr Lys Ala Leu Ser Ser Glu Leu
            180                 185                 190

Glu Lys Ala Lys Ala Asp Leu Glu Asn Gln Lys Ala Lys Val Lys Lys
        195                 200                 205

Gln Leu Thr Glu Glu Leu Ala Ala Gln Lys Ala Ala Leu Ala Glu Lys
    210                 215                 220

Glu Ala Glu Leu Ser Arg Leu Lys Ser Ser Ala Pro Ser Thr Gln Asp
225                 230                 235                 240

Ser Ile Val Gly Asn Asn Thr Met Lys Ala Pro Gln Gly Tyr Pro Leu
                245                 250                 255

Glu Glu Leu Lys Lys Leu Glu Ala Ser Gly Tyr Ile Gly Ser Ala Ser
            260                 265                 270

Tyr Asn Asn Tyr Tyr Lys Glu His Ala Asp Gln Ile Ile Ala Lys Ala
        275                 280                 285

Ser Pro Gly Asn Gln Leu Asn Gln Tyr Gln
    290                 295
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