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(54) Title: COMPOSITIONS AND METHODS FOR DETECTING *TREPONEMA PALLIDUM*

(57) Abstract: Methods for the specific and highly sensitive detection of *Treponema pallidum* infection comprising the use of specific antigenic proteins and peptides unique to *Treponema pallidum* are provided. In particular, detection assays based recognition of acidic repeat protein are provided. The methods of the present invention are particularly useful for detection of primary syphilis at early stages of infection. In addition, the methods and compositions of the present invention are directed to the differential detection of specific *Treponema* infections enabling the identification of causative agents for specific *Treponema* disease states: syphilis *Treponema pallidum subspecies pallidum*, yaws *Treponema pallidum subspecies pertenue*, and bejel *Treponema pallidum* subspecies *endemicum*.

COMPOSITIONS AND METHODS FOR DETECTING *TREPONEMA PALLIDUM*

This invention was made in the Centers for Disease Control and Prevention. Therefore, the United States Government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to the fields of microbiology and immunology and more specifically relates to compositions and methods for diagnosing diseases caused by *Treponema pallidum* such as syphilis. In particular, the invention pertains to the detection of specific antigenic proteins and peptides that are unique to *Treponema pallidum.*

BACKGROUND OF THE INVENTION

Treponema pallidum (*T. pallidum*) is the microaerophilic spirochete that causes syphilis, a systemic venereal disease with multiple clinical presentations. Other closely related treponemas cause pinta (*Treponema carateum*), yaws (*Treponema pallidum* subspecies *pertenue*), and bejel (*Treponema pallidum* subspecies *endemicum*).

In 1996 over 11,000 cases of primary and secondary syphilis in the United States were reported to the U.S. Centers for Disease Control and Prevention. The initial infection causes an ulcer at the site of infection; however, the bacteria move throughout the body, damaging many organs over time. Although treatment with penicillin in the early stages may be successful, the early symptoms of syphilis can be very mild, and many people do not seek treatment when they first become infected. This delay in

seeking treatment is harmful because the damage to the organs in late syphilis cannot be reversed. Also of increasing concern is the risk of transmitting and acquiring the human immunodeficiency virus (HIV) that causes AIDS via open ulcers caused by syphilis.

Medical experts describe the course of the syphilis disease by dividing it into stages: primary, secondary, latent, and tertiary (late). An infected person who has not been treated may infect others during the first two stages, which usually last one to two years. The bacteria spread from the initial ulcer of an infected person to the skin or mucous membranes of the genital area, the mouth, or the anus of a sexual partner. The bacteria can also pass through broken skin on other parts of the body. In its late stages, untreated syphilis, although not contagious, can cause serious heart abnormalities, mental disorders, blindness, other neurologic problems, and even death.

The first symptom of primary syphilis is an ulcer called a chancre. The chancre can appear within 10 days to three months after exposure, but it generally appears within two to six weeks. The chancre is usually found on the part of the body exposed to the partner's ulcer, such as the penis, the vulva, or the vagina. A chancre also can develop on the cervix, tongue, lips, or other parts of the body. Because the chancre may be painless and may occur inside the body, it may go unnoticed. Although the chancre disappears within a few weeks whether or not a person is treated, if the infection is not treated during the primary stage, about one-third of those infected will progress to the chronic stages of syphilis.

Secondary syphilis is often marked by a skin rash that is characterized by brown sores about the size of a penny. The rash appears anywhere from three to six weeks after the chancre appears. While the rash may cover the whole body, the palms of the hands and soles of the feet are the most common sites of presentation. Because active bacteria are present in these sores, any physical contact, sexual or nonsexual, with the broken skin of an infected person may spread the infection at this stage. The rash usually heals within several weeks or months. Other symptoms may also occur such as mild fever, fatigue, headache, sore throat, patchy hair loss, and swollen lymph glands throughout the body. These symptoms may be very mild and, like the chancre of primary syphilis, will disappear without treatment.

The signs of secondary syphilis may come and go over the next one to two years. If untreated, syphilis may lapse into a latent stage during which the disease is no longer contagious and no symptoms are present. Although many individuals who are not treated will suffer no further consequences of the disease, approximately one-third of those who have secondary syphilis develop the complications of late, or tertiary, syphilis.

In the tertiary stage of syphilis, bacteria damage the heart, eyes, brain, nervous system, bones, joints, or almost any other part of the body. This stage can last for years, or even decades. Late syphilis can result in mental illness, blindness, other neurologic problems, heart disease, and even death.

During the early stages of infection, syphilis bacteria also frequently invade the nervous system, and approximately three to seven percent of persons with untreated syphilis develop neurosyphilis. However, development of neurosyphilis can take up to twenty years and some persons with neurosyphilis never develop any symptoms. Those who do present symptoms may experience headaches, stiff necks, and fever, which result from an inflammation of the lining of the brain. Seizures and symptoms of stroke such as numbness, weakness, or visual problems may also afflict those patients with neurosyphilis. Although neurosyphilis can be treated, treatment may be more difficult and its course may be different in persons infected with HIV.

The effects of syphilis in pregnant women are particularly compelling because of the consequential effects on the unborn child. It is likely that an untreated pregnant woman with active syphilis will pass the infection to her unborn child. About 25 percent of these pregnancies result in stillbirth or neonatal death. Between 40 to 70 percent of such pregnancies will yield a syphilis-infected infant. Some infants with congenital syphilis may have symptoms at birth, but most develop symptoms between two and three weeks post partum. These symptoms may include skin sores, rashes, fever, swollen liver and spleen, jaundice, anemia, and various deformities. Care must be taken in handling an infant with congenital syphilis because the moist sores are infectious. Rarely, the symptoms of syphilis go undetected in infants. As infected infants become older children and

teenagers, they may develop the symptoms of late-stage syphilis including bone, tooth, eye, ear, and brain damage.

Due to the sometimes serious and life threatening effects of syphilis infection, and the risk of transmitting or contracting HIV, specific and early diagnosis of the infection is essential. Syphilis, however, has sometimes been called "the great imitator" because its early symptoms are similar to those of many other diseases. Therefore, a doctor usually does not rely upon a recognition of the signs and symptoms of syphilis, but performs both microscopic identification of syphilis bacteria and blood tests.

To diagnose syphilis by a microscopic identification of the bacterium, the physician may take a scraping from the surface of the ulcer or chancre and examine it under a special "dark-field" microscope to detect the organism. However, dark-field microscopy requires considerable skill and is prone to misinterpretation. For these reasons, most cases of syphilis are diagnosed serologically. The blood tests most often used to detect evidence of syphilis are the VDRL (Venereal Disease Research Laboratory) test and the RPR (rapid plasma reagent) test. These non-treponemal tests employ natural lipids, cardiolipin and lecithin, to detect antibodies against non-specific antigens during an active syphilitic infection.

However, one of the complaints about the non-treponemal tests is their lack of specificity in comparison to the treponemal tests. Due to the occurrence of false positives and false negatives when using non-treponemal tests, more than one blood test is usually required. The rate of false positives and the need for multiple blood tests is increased in those individuals with autoimmune disorders, certain viral infections, and other conditions involving substantial tissue destruction or liver involvement. Although treponemal-based tests such as the fluorescent treponemal antibody-absorption (FTA-ABS) and the *T. pallidum* hemagglutination assay (TPHA) may be used to confirm a positive test result, treponemal-based tests are more expensive and more difficult to use than non-treponemal tests. Treponemal tests also cannot be used as tests for cure after treatment because they remain positive even after eradication of the infection.

Some treponemal tests currently in use depend upon the detection of proteins anchored in the *T. pallidum* cytoplasmic membrane. Detection of such proteins is particularly difficult because of the unusual

structure of the *T. pallidum* membrane which consists predominantly of lipids that tend to "shield" these proteins from detection. This shielding effect often delays the host's immune response frequently resulting in false negative serological results.

Currently available treponemal tests depend mainly on the detection of antibodies to cytoplasmic membrane anchored lipoproteins. Response to these proteins is typically delayed because of their lack of surface exposure since the outer membrane consists mainly of lipids and is protein poor. The tests often yield confusing and inaccurate results because these lipoproteins are highly antigenic and may be responsible for the long lasting response in treponemal tests. Because of this latter property, treponemal tests cannot differentiate a current versus a past infection.

Syphilis usually is treated with penicillin, administered by injection. Other antibiotics are used for treating patients allergic to penicillin. A patient typically loses the ability to transmit syphilis within 24 hours from initiating therapy. Some infected individuals, however, do not respond to the usual doses of penicillin. Therefore, it is important that patients undergoing treatment for syphilis are monitored through periodic blood tests to ensure that the infectious agent has been completely destroyed. Persons with neurosyphilis may need to be re-tested for up to two years after treatment.

In all stages of syphilis, proper treatment may cure the disease, but in late syphilis, damage already done to body organs cannot be reversed. Screening and treatment of infected individuals, or secondary prevention, is one of the few options available for preventing the advanced stages of syphilis disease. Testing and treatment early in pregnancy is the best way to prevent syphilis in infants and should be a routine part of prenatal care. A vital component in the successful treatment and prevention of syphilis is early and accurate detection of *T. pallidum* infection.

Diseases Associated with Other Treponemal Infections

Pinta, caused by *Treponema carateum,* has become very rare, and is limited to the warm arid tropical Americas (in particular, Mexico, Central America, and Colombia). The disease manifests in the form of primary and secondary lesions. The primary lesions, which may persist for several years, are coalescing pruritic papules on the extremities,

face, neck, chest, or abdomen. The secondary lesions are disseminated small, scaly papules, called pintids. These may become dyschromic (i.e., change from the normal color of the skin). Late lesions are achromic (without pigment).

Bejel, caused by *Treponema pallidum* subspecies *endemicum*, is known by many names in local languages as a form of syphilis which is not sexually transmitted and occurs in children. Transmission can be by direct contact, and also (in contradistinction to all the other treponemal diseases) via fomites, as in sharing drinking vessels and eating utensils. Except for the fact that the primary lesion, which is probably in the oral mucosa, is rarely observed, the disease is virtually identical to syphilis, with gummas, condylomata lata, and periostitis.

Yaws, caused by *Treponema pallidum* subspecies *pertenue*, occurs in warm, humid tropics. Yaws disease also predominantly manifests in the form of lesions. The primary lesion is a papillomatous skin lesion that heals spontaneously, only to be followed by the secondary lesions, which are large papillomatous nodules that are widely distributed over the skin surface. The late stage of the disease is characterized by gummas of various bones and the nasopharynx as well as destruction lesions of the skin, lymph nodes, and bones. The skin over the gummas may ulcerate. The disease is present in primitive tropical areas in parts of South America, Central Africa, and Southeast Asia and is spread by direct contact with infected skin.

Though some treatments for treponemal infection are available, control of treponemal diseases is managed by eliminating person to person spread. Accordingly, early detection of treponemal infection is vital for reducing widespread dissemination of related diseases.

What is needed are accurate and improved methods and compositions for the effective, accurate early diagnosis of *T. pallidum* infection; and methods for monitoring *T. pallidum* therapy.

SUMMARY OF THE INVENTION

Efficient and sensitive methods and compositions for the detection of *Treponema* infection are provided. In particular, methods and compositions for the detection of *Treponema pallidum* (*T. pallidum*) are provided. In accordance with the methods, a sample is analyzed for the

presence of protein products of particular genes such as the acidic repeat protein *(arp)* gene. Specifically, methods for detecting *T. pallidum* based on the detection of certain peptides, and/or secreted acidic repeat protein gene products and antibodies against these protein/peptides in infected individuals are provided.

In addition, methods are provided wherein samples are combined with antibodies specific for *T. pallidum* antigens, such as immunogenic proteins, under conditions to form an antibody-antigen complex. More particularly, methods are provided wherein samples are combined with proteins or peptides of the *arp* gene. Detection of antibodies indicates the presence of *T. pallidum* in a patient.

In a preferred embodiment of the present invention, assays comprising methods for the detection of various gene products of the antigenic sequences are provided.

In another preferred embodiment of the present invention, methods specific for the detection of the *arp* gene, acidic repeat protein, are provided.

In an additional embodiment of the present invention, methods and compositions are provided for the differential diagnosis of treponemal infection. In particular, methods that enable the specific identification of *Treponema pallidum* subspecies *pallidum*, *Treponema pallidum* subspecies *pertenue*, and *Treponema pallidum* subspecies *endemicum* are provided.

Accordingly, it is an object of the present invention to provide a sensitive assay for the detection of *T. pallidum*.

It is another object of the present invention to provide an assay capable of detecting proteins comprising antigenic gene products of *T. pallidum*.

Yet another object of the present invention is to provide a method for early detection of primary syphilis.

Another object of the present invention is to provide methods and compositions for differential diagnosis of syphilis, yaws and bejel.

Yet another object of the present invention is to provide an antibody specific for *T. pallidum*.

It is a further object of the present invention to provide a kit for automated point-of-use analysis for detecting *T. pallidum* in biological samples.

Another object of the present invention is to provide a method for early detection of *T. pallidum* that is independent of antigenic proteins wholly contained in the cytoplasmic membrane of the infectious agent.

Yet another object of the present invention is to provide a method for treating *T. pallidum* infection comprising the use of antibodies raised against antigenic gene products of *T. pallidum.*

An additional object of the present invention is to provide an immunoassay for the detection of antigenic gene products of *T. pallidum.*

Another object of the present invention is to provide a method for detecting acidic repeat protein.

Yet another object of the present invention is to provide an immunoassay for the detection of syphilis, yaws or bejel using acidic repeat protein and/or peptides derived thereof.

Another object of the present invention is to provide a solid phase particle that may be used in rapid-flow cytometry-type diagnosis of *T. pallidum.*

Yet another object of the present invention is to provide a solid phase particle that may be used in agglutination-type assay for a rapid diagnosis of *T. pallidum* infection.

Yet another object of the present invention is to provide a method for detecting *T. pallidum* comprising enzymatic amplification (ELISA).

It is another object of the present invention to provide an assay capable of detecting antibodies to *T. pallidum.*

Yet another object of the present invention is to provide a kit for automated point-of-use analysis for detecting anti-*T. pallidum* antibodies in biological samples.

Another object of the present invention is to provide an immunoassay for the detection of antibodies against *T. pallidum.*

Another object of the present invention is to provide a method for the detection of antibodies to acidic repeat protein.

Yet, another object of the present invention is to provide an immunoassay for the detection of antibodies to acidic repeat protein in people infected with syphilis, yaws, or bejel using acidic repeat protein and/or peptides derived therefrom.

Another object of the present invention is to provide a solid phase particle that may be used in rapid-flow cytometry type of diagnosis of *T. pallidum* infection using the arp protein or peptides.

Yet another object of the present invention is to provide a method for detecting anti-*T. pallidum* antibodies comprising enzymatic amplification (ELISA).

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of a Western Blot gel showing the ability of syphilitic rabbit sera to recognize the recombinant *arp* protein.

Figure 2 shows the structure of an acidic repeat protein showing the potential membrane-spanning domain, the potential location of the signal peptidase I cutting site, the hydrophilicity plot of the protein and the potential antigenic index of the protein.

Figure 3 provides a graph showing the reaction of various peptides isolated from different regions of the acidic repeat protein (solid square represents SEQ ID NO: 9, open circle represents SEQ ID NO: 10, solid circle represents SEQ ID NO: 13, and open triangle represents SEQ ID NO: 14) with syphilitic human sera.

Figure 4 is a graph showing the results of ELISA to detect the presence of anti-arp antibodies in humans.

Figure 5 provides the nucleotide sequence for *Treponema pallidum.*

Figure 6 provides the complete amino acid sequence listing for *T. pallidum* subspecies *pallidum* (SEQ ID NO: 2) and also indicates the various types of repeats observed in the sequence.

Figure 7 provides the nucleotide sequence for *T. pallidum ssp. Pertenue* (CDC-2).

Figure 8 provides the complete amino acid sequence listing for *T. pallidum* subspecies *pertenue*, CDC-2 strain, (SEQ ID NO: 4) and also indicates the various types of repeats observed in the sequence.

Figure 9 provides the nucleotide sequence for *T. pallidum ssp. endemicum* (Bosnia).

Figure 10 provides the complete amino acid sequence listing for *T. pallidum* subspecies *endemicum*, Bosnia strain, (SEQ ID NO: 6) and also indicates the various types of repeats observed in the sequence.

Figure 11 provides the protein sequences for the preferred arp proteins of the present invention.

Figure 12 depicts two graphs indicating that current syphilis infection (primary syphilis) can be separated into three stages based on serological responses toward arp peptides.

Figure 13 is a representative graph showing the results of flowcytometric analyses of human syphilitic sera using arp peptides.

DETAILED DESCRIPTION

The present invention may be understood more readily by reference to the following detailed description of specific embodiments included herein. Although the present invention has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention. The entire text of the references mentioned herein are hereby incorporated in their entireties by reference.

Definitions

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

The terms "detecting" or "detected" as used herein mean using known techniques for detection of biologic molecules such as immunochemical or histological methods and refer to qualitatively or quantitatively determining the presence or concentration of the biomolecule under investigation.

By "isolated" is meant a biological molecule free from at least some of the components with which it naturally occurs.

As used herein, the term "soluble" means partially or completely dissolved in an aqueous solution.

Peptides and Proteins for Use in Detection of T. pallidum

The methods of the present invention comprise the use of previously unidentified antigenic proteins that are utilized in detection assays for diagnosing diseases caused by *T. pallidum* infection, primarily syphilis. Although a large number of protein products from *T. pallidum* have been previously utilized in diagnosis of syphilis, specific proteins particularly useful for accurate, early diagnosis of syphilis, or differential diagnosis of syphilis, yaws and bejel, were heretofore unidentified.

Proteins specifically utilized in prior art assays include a 47kD lipoprotein, a 17kD lipoprotein and a 15kD lipoprotein, most of which appeared to be anchored in the cytoplasmic membrane usually by lipid modification of the protein and anchored through the resulting amino terminal lipid moieties. Although all of these proteins are present in large amounts in *T. pallidum*, and although they are highly antigenic, a serious drawback in their use for diagnosis is that they comprise major proteins responded to in the whole treponeme, and thus do not give a positive diagnosis any faster than using whole treponemal cells.

Though the inventors do not wish to be bound by the following theory, it is believed that the unusual outer membrane structure of *T. pallidum* causes a significant delay in host response to syphilis infection and therefore early cases of primary syphilis often show negative treponemal serology. The outer membrane, or envelope, of *T. pallidum* appears to be composed mainly of lipids with only a very small number of proteins. Furthermore, it is believed that proteins anchored in the cytoplasmic membranes are shielded from the host immune system, resulting, therefore, in a delayed or diminished immune response. Consequently, detection assays based on membrane-anchored proteins often show a delay in serological reactivity, with some primary syphilis patients producing false negative results.

In contrast to the proteins previously utilized in *T. pallidum* detection assays, the proteins and peptides of the present invention enable accurate diagnosis of *T. pallidum* infection at early stages. Though the inventors do not wish to be bound by the following theory, detection of

secreted proteins according to the methods of the present invention, overcomes previous problems associated with the structure of the *T. pallidum* outer membrane, and is therefore particularly advantageous over prior assays that rely upon cloned, membrane-shielded antigens. Furthermore, secreted antigenic proteins are more likely to generate a detectable immune response as compared to membrane-shielded antigens, thereby facilitating diagnosis by recognition of corresponding antibodies. In addition, the repeated nature of the proteins make them extremely antigenic and, thus, suitable for early detection of syphilis.

Early detection is crucial for treatment as it can prevent subsequent deterioration to secondary and tertiary forms of syphilis which are marked by more severe and harder to treat symptoms. Therefore, the methods of the present invention address the need for early detection of primary syphilis which until now has been a serious problem area in syphilis serology.

The Nichols strain of *T. pallidum* is the type strain of *T. pallidum ssp. pallidum.* As described herein, by the inventors, this strain contains unique repetitive sequences that are each 60 base pairs long, resulting in a protein that contains fourteen repeats, each composed of 20 amino acids within the body of the protein (see Figure 6). The repeat region contains 6 codons for glutamic acid and it is estimated that the protein product has a pI of approximately 4.3, hence the name acidic repeat protein (or *arp).* There is some minor variation in the 20 amino acid repeats, but the repeats are at least 90% conserved up until the last two repeats in the Nichols strain (rare substitutions are generally conservative). The nucleotide sequence of the acidic repeat protein is provided in the Sequence Listing as SEQ ID NO: 1 (see also Figure 5), and the amino acid sequence is provided in SEQ ID NO: 2 (see also Figure 6).

Though the inventors do not wish to be bound by the following theory, it is believed that the *arp* gene product, the acidic repeat protein, comprises a protein that exists in a membrane-anchored form or a secreted form. The structural characteristics of the acidic repeat protein are shown in Figure 2, which gives a hydrophobicity profile of the protein as well as showing the sequence of one of the repeat elements from the Nichols strain of *T. pallidum.* The protein has a slightly basic amino terminus followed by a hydrophobic stretch of amino acids that may constitute a

membrane-spanning domain for the membrane-anchored form. A run of four alanines occurs shortly after the end of the potential membrane-spanning domain and is a potential site for signal peptidase I cleavage. In the Nichols strain of *T. pallidum*, the majority of the remainder of the protein is taken up by the repeat sequences which constitute approximately two-thirds of the total reading frame in this strain.

Active portions of immunogenic regions of the acidic repeat protein can be identified by isolating or synthesizing truncated peptides from the acidic repeat protein and then testing the peptides for immunogenic activity using techniques and methods known to those skilled in the art. The present invention is particularly directed to active portions of the immunogenic domain of acidic repeat protein.

For example, a preferred active portion of the acidic repeat protein comprises approximately amino acid 128 to 407 of the protein as set forth in SEQ ID NO: 1, more preferably amino acid 168 to 187 also as set forth in SEQ ID NO: 1, and most preferably the peptide having the amino acid sequence set forth in SEQ ID NO: 15.

In one embodiment of the present invention, a preferred protein or peptide for use in accordance with the methods of the present invention comprises the acidic repeat protein encoded by the nucleotide sequence set forth in SEQ ID NO: 1, or an immunogenic fragment thereof.

In another embodiment of the present invention, a preferred protein or peptide for use in accordance with the methods of the present invention comprises an immunogenic fragment of the acidic repeat protein, having the amino acid sequence set forth in SEQ ID NO: 15.

In an alternative embodiment of the present invention, a preferred protein or peptide for use in accordance with the methods of the present invention comprises an immunogenic fragment of the acidic repeat protein, arp 3 peptide, having the amino acid sequence set forth in SEQ ID NO: 9.

In another embodiment of the present invention a preferred peptide for use in accordance with the methods of the present invention comprises an active fragment of the acidic repeat protein having the amino acid sequence set forth in SEQ ID NO: 13.

In yet another embodiment of the present invention preferred peptides for use in accordance with the methods of the present invention

comprise an active fragment of the acidic repeat protein having the amino acid sequence set forth in any of SEQ ID NOS: 7-18.

One of skill in the art will recognize that, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid.

In accordance with one embodiment of the method of the present invention, a sample is combined with antibodies specific for a protein or peptide product of the repeat gene sequence under conditions to form an antibody-antigen complex. Detection of the complex using antigen capture methods indicates the presence of *T. pallidum* in the patient. Alternatively, detection of the antigen-antibody complex using antigen as the probe is indicative of the presence of previous or present infection with *T. pallidum*. Preferably the protein product of the repeat gene sequence is the acidic repeat protein or an antigenic peptide fragment thereof.

Peptides or Protein Fragments

The acidic repeat protein is isolated from *T. pallidum* organisms, or synthesized by chemical or biological methods, such as cell culture, recombinant gene expression, and peptide synthesis as described in the Examples. Recombinant techniques include gene amplification from DNA sources using the polymerase chain reaction (PCR), and gene amplification from RNA sources using reverse transcriptase/PCR. The amino acid sequence of acidic repeat protein is set forth in SEQ ID NO: 2. Peptides and protein fragments of acidic repeat protein preferably have an amino acid sequence within the amino acid sequence set forth in SEQ ID NO: 2.

Acidic repeat protein can be produced according to the methods described above and tested for immunogenic or antigenic activity using techniques and methods known to those skilled in the art. For example, full length recombinant acidic repeat protein can be produced using the baculovirus gene expression system or using *E. coli* transformed with the expression vector plasmid containing a complete *arp* gene. Full length proteins can be cleaved into individual domains or digested using various

methods such as, for example, the method described by Enjyoji *et al.* (*Biochemistry* 34:5725-5735 (1995)). In accordance with the method of Enjyoji *et al.*, recombinant acidic repeat protein may be treated with a digestion enzyme, such as human neutrophil elastase, and the digest purified using a heparin column in order to obtain fragments that may then be tested for immunogenicity.

Alternatively, fragments are prepared by digesting the entire protein, or large fragments thereof exhibiting immunogenic activity, to remove one amino acid at a time. Each progressively shorter fragment is then tested for immunogenic activity. Similarly, fragments of various lengths may be synthesized and tested for immunogenic activity. By increasing or decreasing the length of a fragment, one skilled in the art may determine the exact number, identity, and sequence of amino acids within the protein that are required for immunogenic activity using routine digestion, synthesis, and screening procedures known to those skilled in the art.

The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeable and are defined to mean a biomolecule composed of two or more amino acids linked by a peptide bond.

The term "peptides" is defined to mean chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. The terminal amino acid at one end of the chain (i.e., the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (i.e., the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free alpha-amino group on the amino acid at the amino terminus of the peptide, or to the alpha-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminus and increasing in the direction

toward the carboxy terminus of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminus of the peptide than the preceding amino acid.

The term "residue" is used herein to refer to an amino acid that is incorporated into a peptide by an amide bond. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that function in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

Furthermore, one of skill will recognize that, as mentioned above, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides described herein do not contain materials normally associated with their *in situ* environment. Typically, the isolated, immunogenic peptides described

herein are at least about 80% pure, usually at least about 90%, and preferably at least about 95% as measured by band intensity on a silver stained gel.

Protein purity or homogeneity may be indicated by a number of methods well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

When the immunogenic peptides are relatively short in length (i.e., less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the immunogenic peptides described herein. Techniques for solid phase synthesis are known to those skilled in the art.

Alternatively, the immunogenic peptides described herein are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or polypeptide and, if required, renaturing the peptide. Techniques sufficient to guide one of skill through such procedures are found in the literature.

Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 to 95% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the immunogenic peptides may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the immunogenic peptide and then to cause the peptide to re-fold into a biologically and biochemically active conformation.

Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

Antigenicity of the purified protein may be confirmed, for example, by demonstrating reaction with *T. pallidum* immune serum, or with anti-arp sera produced in a laboratory animal.

Though the inventors do not wish to be bound to the following theory, the present invention is particularly desirable because recognition of the acidic repeat protein, by, for example immunoassays, provides utility for the protein in diagnosis of syphilis, determination of the state of immunity of the patient, and an assessment of the progress of the disease.

Another highly advantageous aspect of the present invention is the ability to produce desired proteins in large quantities from cloned genes. As described above, the proteins may then be used in diagnostic assays for syphilis detection through antibody recognition, antigen capture, or for the development of vaccines for treatment of syphilis.

Anti-T. pallidum Antigen Antibodies

The terms "antibody" and "antibodies" as used herein include monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, and humanized antibodies as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

The term "antigen" refers to an entity or fragment thereof which can induce an immune response in a mammal. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

The antibody provided herein is a monoclonal or polyclonal antibody having binding specificity for a *T. pallidum* antigen comprising a protein or peptide representative of an immunogenic region. A preferred gene target comprises the arp gene or a member of the *arp* gene family. The preferred antibody is a monoclonal antibody, due to its higher specificity for the antigen. The antibody is specific for the *arp* protein and exhibits minimal or no crossreactivity with other *T. pallidum* proteins or peptides. Preferably, the antibody is specific for the secreted protein encoded by the *arp* gene, the acidic repeat protein or an antigenic peptide fragment thereof.

The preferred monoclonal antibody is prepared by immunizing an animal, such as a mouse, rat, or rabbit, with a whole gene product protein, such as the acidic repeat protein or peptides thereof. Spleen cells are harvested from the immunized animals and hybridomas generated by fusing sensitized spleen cells with a myeloma cell line, such as murine SP2/O myeloma cells (ATCC, Manassas, VA). The cells are induced to fuse by the addition of polyethylene glycol. Hybridomas are chemically selected by plating the cells in a selection medium containing hypoxanthine, aminopterin and thymidine (HAT).

Hybridomas are subsequently screened for the ability to produce monoclonal antibodies against *T. pallidum* immunogenic proteins. Immunogenic proteins used for screening purposes are obtained from analyzed specimens. Alternatively, such proteins may comprise recombinant peptides made according to methods known to those skilled in the art. Hybridomas producing antibodies that bind to the immunogenic protein preparations are cloned, expanded and stored frozen for future production. The preferred hybridoma produces a monoclonal antibody having the IgG isotype.

The preferred polyclonal antibody is prepared by immunizing animals, such as mice or rabbits, with the immunogenic proteins or peptides described above. Blood is subsequently collected from the animals, and antibodies in the sera screened for binding reactivity against the immunogenic proteins, preferably the antigens that are reactive with the monoclonal antibody described above.

Either the monoclonal antibody or the polyclonal antibody, or both may be labeled directly with a detectable label for identification *T. pallidum* in a biological sample as described below. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including colored particles, such as colloidal gold and latex beads. The antibodies may also be bound to a solid phase to facilitate separation of antibody-antigen complexes from non-reacted components in an immunoassay. Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes, magnetic, plastic or glass beads and slides. Methods for coupling antibodies to solid phases are well known to those skilled in the art.

Alternatively, the antibody may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibody may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

In a preferred embodiment, the antibody is labeled indirectly by reactivity with a second antibody that has been labeled with a detectable label. The second antibody is preferably one that binds to antibodies of the animal from which the monoclonal antibody is derived. In other words, if the monoclonal antibody is a mouse antibody, then the labeled, second antibody is an anti-mouse antibody. For the monoclonal antibody to be used in the assay described below, this label is preferably an antibody-coated bead, particularly a magnetic bead. For the polyclonal antibody to be employed in the immunoassay described herein, the label is preferably a detectable molecule such as a radioactive, fluorescent or an electrochemiluminescent substance.

T. pallidum Immunoassay

A highly sensitive *T. pallidum* immunoassay employing one or more of the recombinant or isolated proteins or peptides for the detection of *T. pallidum* antibodies described above is provided. The immunoassay is useful for detecting the presence of *T. pallidum* infection in a variety of samples, particularly biological samples, such as human or animal biological fluids. The sample may be obtained from any source in which the *T. pallidum* organism may exist.

In a first preferred embodiment, the immunoassay is designed using the antigenic protein or peptide to detect the presence of *T. pallidum* antibodies. This is achieved by coating the solid phase with the protein or peptides. Subsequently, the biological sample is incubated with the coated surface to allow the binding of antibodies to the protein/peptides.

An exemplary mechanism is incubating the biological sample and the coated surface at a temperature above room temperature, preferably at a temperature of approximately 20°C to 45°C for approximately 10 to 150 minutes. More preferably, the biological sample and coated surface are incubate at a temperature of approximately 37°C for a period of about 60 minutes in the dark. The results of this immunoassay provide a direct indication of *T. pallidum* infection.

It will be understood by those skilled in the art that one or more of the antigens (arp peptides or protein) described above may be employed in any heterogenous or homogeneous (competitive) immunoassay for the detection of *T. pallidum* infection. As mentioned above, for use in the immunoassay provided herein, the peptides are coated to the solid phase, the solid phase may comprise any article suitable for such use. Suitable articles are well-known to those skilled in the art, and include, but are not limited to, latex particles, filter paper, and glass beads. The preferred solid phase is a commercially available ELISA microtiter plate, such as Immunlon 2HB™ plate available from Dynex Technologies (Chantilly, Virginia).

In accordance with the preferred method, the antigen bound to a solid phase and antibody containing fluid are reacted together for a sufficient amount of time under conditions that promote the binding of antibody to the antigen. It will be understood by those skilled in the art that the immunoassay reagents and samples may be reacted in different combinations and orders.

A physical means is employed to separate reagents bound to the solid phase from unbound reagents such as filtration of particles, decantation of reaction solutions from coated tubes or wells, magnetic separation, capillary action, and other means known to those skilled in the art. It will be understood that a separate washing of the solid phase may be included in the method.

The antigen-antibody complex formed in the immunoassay are detected using methods known to those skilled in the art. The complexes are exposed to anti-human immunoglobulin antibodies which have been labeled with a detectable marker. Such markers include chemiluminescent, labels, such as horseradish peroxidase; electrochemiluminescent labels, such as FITC; and enzymatic labels, such as alkaline phosphatase, β-galactosidase, and horseradish peroxidase.

Preferably, the detecting antibody is modified by the addition of a peroxidase label.

The labeled complex is then detected using a detection technique or instrument specific for detection of the label employed. Preferably, the complexes are analyszed with an ELISA reader such as the Ceres 900 HDL (BioTek Instrument, Inc., Winooski, Vermont) for detection of peroxidase. Alternatively, a Becton-Dickinson FACS sorter (Franklin Lakes, New Jersey) may be used for detection of the FITC label. Soluble antigen or antibodies may also be incubated with magnetic beads coated with non-specific antibodies in an identical assay format to determine the background values of samples analyzed in the assay.

In a second preferred embodiment, the immunoassay is designed using the anti-*arp* monoclonal (or polyclonal) antibodies to detect the presence of arp peptides and/or proteins from T. *pallidum* in biological fluid. This is achieved by incubating a biological sample to allow binding of the protein or peptide with an antibody. An exemplary mechanism is incubation at a temperature above room temperature, preferably approximately 20-45°C for approximately 10 to 150 minutes, more preferably approximately 37°C for 60 minutes in the dark. The results of this immunoassay provide a direct indication of the presence of *T. pallidum* infection.

It will be understood by those skilled in the art that one or more of the antibodies described above may be employed in any heterogeneous or homogeneous, competitive immunoassay for the detection of *T. pallidum* infection. As mentioned above, for use in the immunoassay provided herein, the antibody is labeled with a detectable label or coupled to a solid phase. Preferably, both a monoclonal antibody and a polyclonal antibody are used in the assay, with the monoclonal antibody coupled to a solid phase and the polyclonal antibody labeled with a detectable label. The solid phase may comprise any particle suitable for such use well-known to those skilled in the art, including but not limited to latex particles, filter paper, and glass beads. The preferred solid phase is a commercially available ELISA microtiter plate, such as Immunolon 2HB™ plate available from Dynex Technologies (Chantilly, Virginia).

In accordance with the preferred method, the sample and the antibody bound to a solid phase are reacted together for a sufficient amount

of time under conditions that promote the binding of antibody to the immunogenic protein in the sample. The immunogenic protein preferably comprises acidic repeat protein. It will be understood by those skilled in the art that the immunoassay reagents and sample may be reacted in different combinations and orders. A physical means is employed to separate reagents bound to the solid phase from unbound reagents such as filtration of particles, decantation of reaction solutions from coated tubes or wells, magnetic separation, capillary action, and other means known to those skilled in the art. It will also be understood that a separate washing of the solid phase may be included in the method.

The antibody-antigen complexes formed in the immunoassay are detected using immunoassay methods known to those skilled in the art, including sandwich immunoassays and competitive immunoassays. The antibody-antigen complexes are exposed to antibodies similar to those used to capture the antigen, but which have been labeled with a detectable label. Suitable labels include: chemiluminescent labels, such as horseradish peroxidase; electrochemiluminescent labels, such as ruthenium and aequorin; bioluminescent labels, such as luciferase; fluorescent labels such as FITC; and enzymatic labels such as alkaline phosphatase, ß-galactosidase, and horseradish peroxidase. Preferably, the label is detected by electrochemiluminescence. Most preferably, the detecting antibody is modified by the addition of a peroxidase label.

The labeled complex is then detected using a detection technique or instrument specific for detection of the label employed. Preferably, the complexes are analyzed with an ELISA reader such as the Ceres 900 HDL (BioTek Instrument, Inc., Winooski, Vermont) for detection of the peroxidase. Alternatively, a Becton-Dickinson FACS sorter (Franklin Lakes, New Jersey) may be used for detection of the FITC label. Soluble antigen or antigens may also be incubated with magnetic beads coated with non-specific or specific antibodies in an identical assay format to determine the background values of samples analyzed in the assay.

Assay Characteristics

The immunoassay provided herein allows for the detection of *T. pallidum* in a sample, thereby permitting a realistic indication of the consequences of infection with regard to manifestation of disease.

The detection assay described herein is effective because it is based upon the detection of immunogenic or antigenic proteins representative of specific gene sequences or antibodies to those proteins. Unlike prior art methods, the detection assays of the present invention are unconcerned with membrane-bound antigenic proteins typically associated with *T. pallidum*, and therefore, since detection involves recognition of secreted proteins, results are not hampered by proteins that are anchored or shielded by the cytoplasmic membrane. Detection based upon secreted proteins is preferred because they are more likely to elicit an early immune response as compared to membrane-anchored proteins.

The assay is also valuable for epidemiological reasons as it may be used to identify level of infections in patients. For example, high levels of acidic repeat protein may be correlated with progressed stages of disease. This is especially important because diagnosis of disease at early stages can lead to effective treatment early on, preventing deterioration into more serious conditions later on. Unlike the assay described herein, presently available assays for *T. pallidum* are generally considered inaccurate and inefficient because they require significant processing time and rely upon the detection of antigenic markers that are typically membrane-bound proteins.

Unlike assays currently used in the art, the presently described method detects *T. pallidum* by recognition of secreted antigenic proteins or antibodies to those proteins. The advantage of this type of recognition is that the assay is neither dependent upon recognizing the parasite in particulate form or upon detecting the presence of membrane-bound proteins that are usually shielded from the host immune system. Detection based on the presence of secreted protein antigens both increases the sensitivity of the method, and reduces time periods for accurate diagnosis, thereby enabling detection of primary syphilis.

Differential Diagnosis of T. pallidum *Infection*

In addition to providing the nucleotide and amino acid sequences for *T. pallidum* subspecies *pallidum* (SEQ ID NOS: 1 and 2, respectively), the present invention also provides previously unidentified nucleotide and amino acid sequences corresponding to *T. pallidum* subspecies *pertenue* (SEQ ID NOS: 3 and 4, respectively, and Figure 6),

and *T. pallidum* subspecies *endemicum* (SEQ ID NOS: 5 and 6, respectively, and Figure 7). Accordingly, one skilled in the art may employ the techniques taught by the present invention for the differential diagnosis of *T. pallidum* infection and thereby identify the causative agent of disease as *T. pallidum* subspecies *pallidum, T. pallidum* subspecies *pertenue*, or *T. pallidum* subspecies *endemicum*. This discovery is particularly valuable for the early detection and identification of infection as it facilitates the control of further dissemination of disease. In addition, specific identification of each of the *Treponema* subspecies enables the development of specific antibodies that may be utilized in therapeutic treatments. An additional advantage of specifically identifying particular subspecies is that the manifestation of particular disease, either syphilis, yaws or bejel, may be anticipated allowing for appropriate measures to be taken to either prevent, or at least diminish, the various symptoms.

Though the inventors do not wish to be bound by the following theory, it is believed that the antibody titers against the *arp* protein will decline when the organisms have been eliminated. This suggests that assays utilizing *arp* peptides/proteins for immunodetection of anti-treponemal antibodies can be used to differentiate between current infections vs. past infections.

The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention.

EXAMPLE 1

Characteristics of the Acidic Repeat Protein

The genes coding for the acidic repeat proteins from *T. pallidum* (Nichols strain, CDC-2 strain and Bosnia strain) were cloned. The nucleotide sequences are set forth in SEQ ID NOS: 1, 3 and 5. (Genebank Accession No. AF015824)

The arp protein of the Nichols strain is characterized by a transmembrane domain, a signal peptidase I cutting site, and 14 almost identical repeats (see Figure 2). The top portion of Figure 2 represents the hydrophobicity plot of the protein according to its primary sequence. Most of the protein is hydrophilic, and therefore, though the inventors do not wish to be bound by the following theory, it is believed that this property corresponds to the protein's antigenic index (lower part of the Figure 2). At the N terminal end, there is a stretch of hydrophobic amino acids (aa27 to aa43) which constitute the dip in the hydrophobicity plot. This region is the potential membrane-spanning domain. Immediately after the membrane-spanning domain there is a potential signal peptidase I cutting site. The most significant feature of the arp protein is the 14 almost identical repeats, each about 20 amino acids in length. The repeats are extremely high in glutamic acid accounting for the low predicted pI 4.3. The repeats were classified into 4 types according to their similarities. Type II repeats made up 42% of the total repeats (6 out of 14) and were the predominant type. It is predicted that most of the *T. pallidum* species will have this type of repeats. The inventors of the present invention have discovered that peptides made from this repeat region are the most useful in serodiagnosis. This is demonstrated below.

EXAMPLE 2

Potential Usages of arp Protein in Diagnosis of Syphilis

The following studies were directed to further characterize the arp protein with emphasis on the region of immunogenic peptides. The newly identified immunogenic peptides serve as targets for constructing immuno diagnostic kits having improved and superior sensitivity.

Initially, after discovering the arp protein's hydrophobicity plot and its antigenic index as predicted from its protein sequence, the inventors hypothesized that that certain regions in the arp protein may be immunogenic. Peptide fragments from the repeat region of the protein were prepared and used to immunize rabbits. It was discovered that sera from peptide-immunized rabbits recognized the expressed recombinant protein from an *arp* gene-containing plasmid. In addition, sera from treponemal infected rabbits also recognized this recombinant protein. (Western blot analyses shown in Fig. 1: Lane 1 = total *T. pallidum* protein identified by

anti-*T. pallidum* serum; Lane 2 = anti-peptide [1,2,3] sera failed to identify arp in total *T. pallidum* protein extracts; Lane 3 = recombinant arp protein identified by anti-arp peptide serum; Lane 4 = arp protein identified by anti-*T. pallidum* serum; Lane 5 = pre-bled (bleeding right before injection of the antigen) control).

EXAMPLE 3

Immune Response Toward Peptides of

T. pallidum *Repeat Protein*

Peptides designed from different regions of the arp protein were used in this experiment (see Table 1). Syphilitic human sera were used in an ELISA assay to determine the reactivity toward these peptide fragments. The syphilitic sera were either rapid plasma reagent (RPR) positive or negative (RPR+ or RPR-) according to commercial RPR test kits. It was discovered that most of the RPR+ sera reacted with arp peptides 3, 7 and 9 vigorously, whereas none of the RPR- sera reacted with any of the peptides. Reactivity was detected at 1:100 dilution (most commercial ELISA kits use 1:20 dilution for detection).

Other peptides (peptide 1-12, excluding 3, 7 and 9) were derived either from the N or C terminal ends of arp protein or from type I, III or IV repeats. Though the inventors do not wish to be bound by the following statement, analyses based on reactivities of these syphilitic sera to peptides indicates that one of the immunogenic region is confined to amino acids DVPK.

The results of this study are graphically provided in Figure 3.

TABLE 1

Peptide #	Amino Acid Sequence	SEQ ID NO:
arp 1	LVSPLREVEDAPKVVEPAS	SEQ ID NO: 7
arp 2	SREVEDAPKVVEPASEREGG	SEQ ID NO: 8
arp 3	PKVVEPASEREGGEREVEDA	SEQ ID NO: 9
arp 4	PKNTAVEISNLEKNAKAQAVV	SEQ ID NO: 10
arp 5	GHAGIPGLLVSLAPAAAAQLGIGVY	SEQ ID NO: 11
arp 6	VPARPAQRDPLSSPPAGHTVPEYRD	SEQ ID NO: 12
arp 7	VVEPASEREGGEREVEDVPKV	SEQ ID NO: 13
arp 8	VVEPASGHEGGEREVASQHTKQPSHS	SEQ ID NO: 14
arp 9	EVEDVPKVVEPASEREGGER	SEQ ID NO: 15
arp 10	EVENVPKVVEPASEREGGER	SEQ ID NO: 16
arp 11	EVEDAPKVVEPASEREGGER	SEQ ID NO: 17
arp 12	EVEDVPGVVEPASGHEGGER	SEQ ID NO: 18

EXAMPLE 4

Sequence Comparisons between the arp proteins of T. pallidum *subspecies*

The *arp* genes of two type strains, CDC-2 and Bosnia, from each of the *T. pallidum* subspecies, *T. pallidum* ssp. *pertenue* and *T. pallidum* ssp. *endemicum*, were cloned and tested. The gene sequences showed

significant homology with the Nichols strain of *T. pallidum* ssp. *pallidum*. The 5' end and 3' end of the genes of the three subspecies are completely identical, while the repeat regions showed some variations. The interesting observation was that the translated arp protein of the two subspecies showed a single type of repeats, type II, which is the predominant type in the Nichols strain. This finding confirms that those peptides synthesized in regions with the predominant type of repeat (type II) are immunogenic (as shown in Figure 4). The other repeats (types I, III, and IV) are also immunogenic.

Modifications and variations of the present method will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

EXAMPLE 5

ELISA assay using arp peptide classified syphilitic infection in two different stages

Peptide arp #9 (Seq. ID 15) was used in this experiment (Figure 8). Sera from patients with current syphilitic infection were tested in an ELISA assay. All patients in this study had positive PCR reaction in their ulcer specimens. It was found that patients can be classified into early infection (IgM positive), intermittent infection (both IgM and IgG positive) and late infection (IgG positive only).

EXAMPLE 6

Rapid flowmetric analyses of syphilitic infection

Flow cytometer has been routinely used in immunologic laboratories. The Luminex™ company has developed a system for which diagnosis of multiple diseases and disease markers can be easily multiplexed. Current tests that have been developed or are under development include human cytokines (IL-2, 3, 4, 6, etc.) and viral and bacterial infections (HIV, hepatitis, etc.). Arp #9 peptides were coupled to biotin molecule. This biotinylated peptide is further bound to strepavidin beads which are available from Luminex™. Two sera were tested in this system. It was clear that the RPR+ sera reacted strongly in the assay, whereas RPR- normal sera has very low background level of fluorescent

response (Figure 9). This result demonstrated the possibility of multiplexing our arp peptide beads with other clinical tests using the Luminex system.

We claim:

1. A method of detecting the presence of *Treponema pallidum*, anti-treponemal antibodies, or both in a biological sample, said method comprising:

(a) contacting the acidic repeat protein or one or more isolated, immunogenic *Treponema pallidum* peptides of the acidic repeat protein with an antibody-containing biological sample, and

(b) detecting the formation of a complex between the immunogenic protein or peptide and the antibody wherein the presence of the complex indicates the presence of *Treponema pallidum.*

2. The method of Claim 1, wherein the immunogenic peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6-18, and conservative variations thereof.

3. The method of Claim 1, wherein the immunogenic peptide is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 1, 3, and 5.

4. The method of Claim 1, wherein the immunogenic peptide comprises an amino acid sequence having the sequence of SEQ ID NO: 15.

5. The method of Claim 1, wherein the *Treponema pallidum* is selected from the group consisting of *Treponema pallidum* subspecies *pallidum*, *Treponema pallidum* subspecies *pertenue*, and *Treponema pallidum* subspecies *endemicum.*

6. The method of Claim 1, wherein detecting the presence of the complex indicates the presence of a disease selected from the group consisting of syphilis, yaws, and bejel.

7. The method of Claim 1, wherein the immunogenic peptide comprises an amino acid sequence having the sequence of SEQ ID NO: 2.

8. The method of Claim 1, wherein the immunogenic peptide comprises an amino acid sequence comprising SEQ ID NO: 4, and wherein the presence of the complex indicates the presence of yaws.

9. The method of Claim 1, wherein the immunogenic peptide comprises an amino acid sequence comprising SEQ ID NO: 6, and wherein the presence of the complex indicates the presence of bejel.

10. The method of Claim 1, wherein the peptide is bound to a solid phase.

11. The method of Claim 1, wherein the peptide is labeled.

12. The method of Claim 11, wherein the label is selected from the group consisting of an electrochemiluminescent label, a chemiluminescent label, an enzymatic label, a bioluminescent label, and a fluorescent label.

13. The method of Claim 1, further comprising incubating the peptide-antibody complex with a second antibody specific for the peptide, wherein the second antibody is labeled with a detectable label and binds to the peptide-antibody complex.

14. The method of Claim 1, wherein the biological sample comprises wounds, blood, tissues, saliva, semen, vaginal secretions, tears, urine, bone, muscle, cartilage, CSF, skin, or any human tissue or bodily fluid.

15. An isolated, immunogenic *Treponema pallidum* peptide, said immunogenic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6-18, and conservative variations thereof.

16. The immunogenic peptide in accordance with Claim 15, wherein the *Treponema pallidum* is selected from the group consisting of

Treponema pallidum subspecies *pallidum*, *Treponema pallidum* subspecies *pertenue*, and *Treponema pallidum* subspecies *endemicum*.

17. An antibody capable of binding to a *Treponema pallidum* acidic repeat protein or immunogenic peptide of the acidic repeat protein.

18. The isolated antibody of Claim 17, wherein the immunogenic peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6-18, and conservative variations thereof.

19. The isolated antibody of Claim 17, wherein the immunogenic peptide is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 1, 3, and 5.

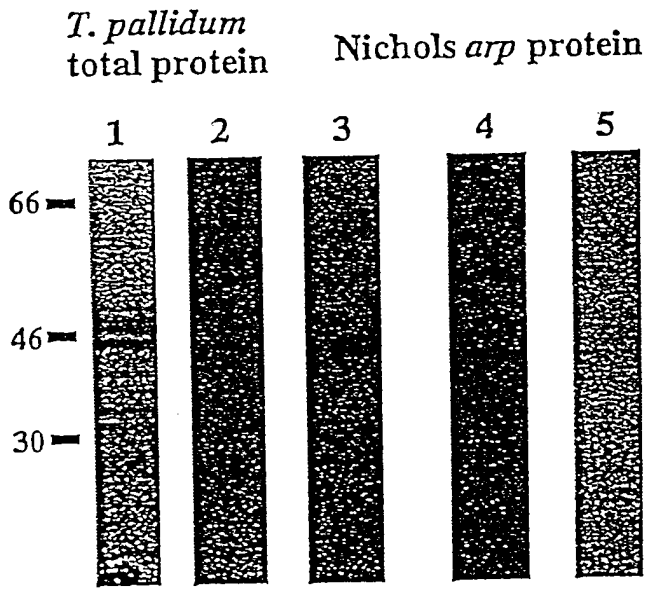
20. The isolated antibody of Claim 17, wherein the antibody is a monoclonal antibody.

21. An immunogenic composition comprising a pharmaceutically acceptable carrier and an isolated, immunogenic *Treponema pallidum* peptide in an amount sufficient to induce a protective immune response to *Treponema pallidum* in a mammal, said immunogenic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6-18, and conservative variations thereof.

22. The composition of Claim 21, wherein the *Treponema pallidum* is selected from the group consisting of *Treponema pallidum* subspecies *pallidum*, *Treponema pallidum* subspecies *pertenue*, and *Treponema pallidum* subspecies *endemicum*.

23. The composition of Claim 21, wherein the presence of *Treponema pallidum* in a mammal causes a disease comprising syphilis, yaws, or bejel.

24. The composition of Claim 21, wherein the immunogenic peptide is conjugated to a carrier protein.



1, 4 anti-*T. pallidum* serum
2, 3 anti-*arp* peptide Ab
5 pre-bled (rabbit)

FIGURE 1

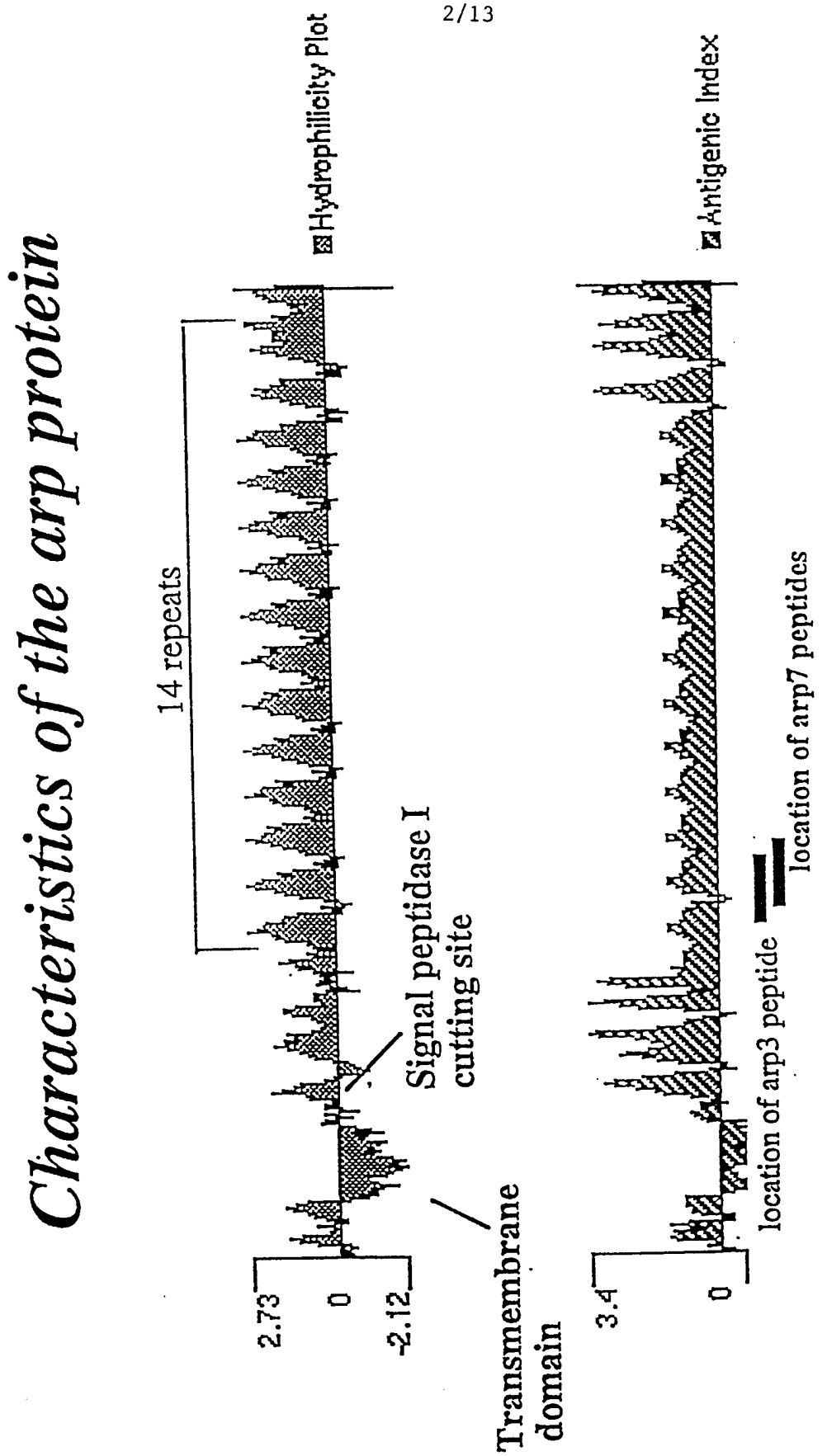

Characteristics of the arp protein
Hydrophilicity Plot
Antigenic Index
14 repeats
Signal peptidase I cutting site
Transmembrane domain
2.73
0
-2.12
3.4
0
location of arp3 peptide
location of arp7 peptides

FIGURE 2

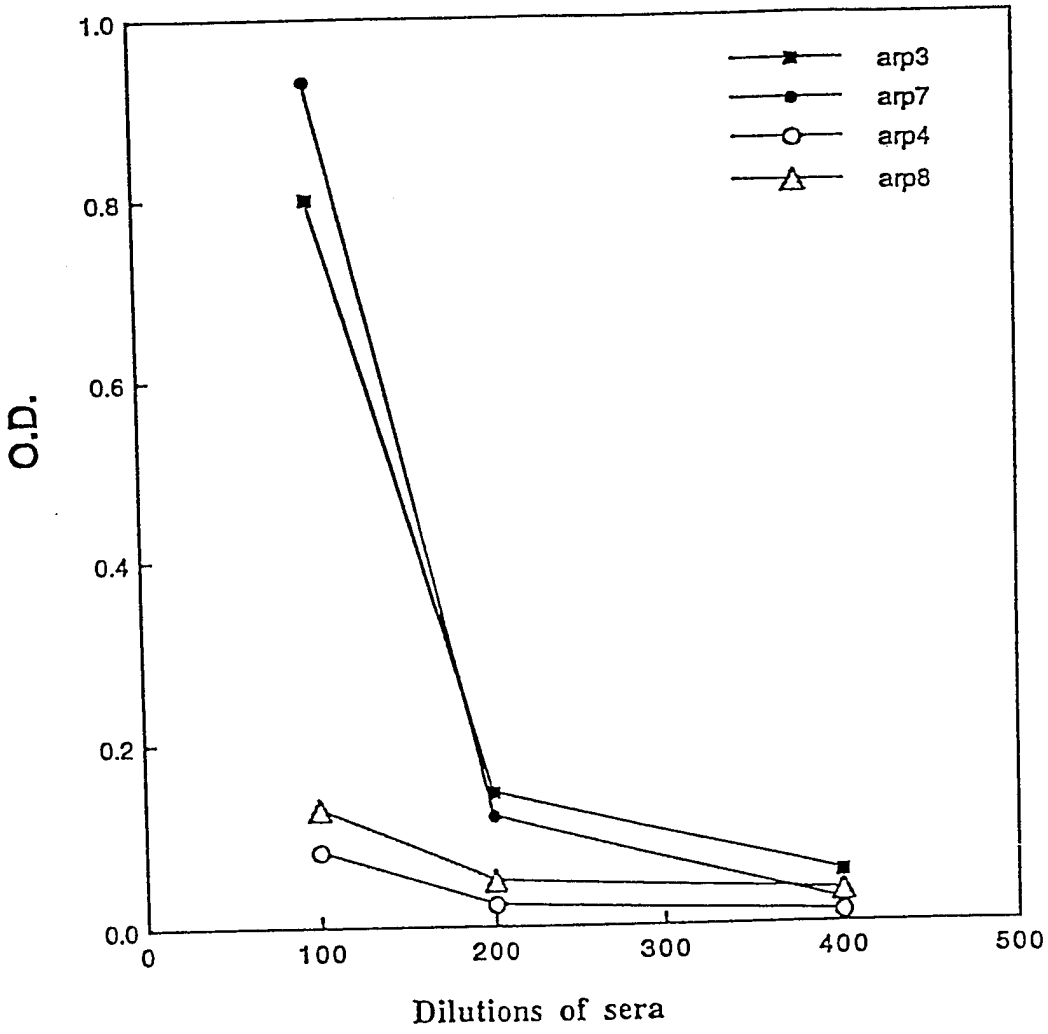

1.0
0.8
0.6
0.4
0.2
0.0
O.D.
0
100
200
300
400
500
Dilutions of sera
arp3
arp7
arp4
arp8

FIGURE 3

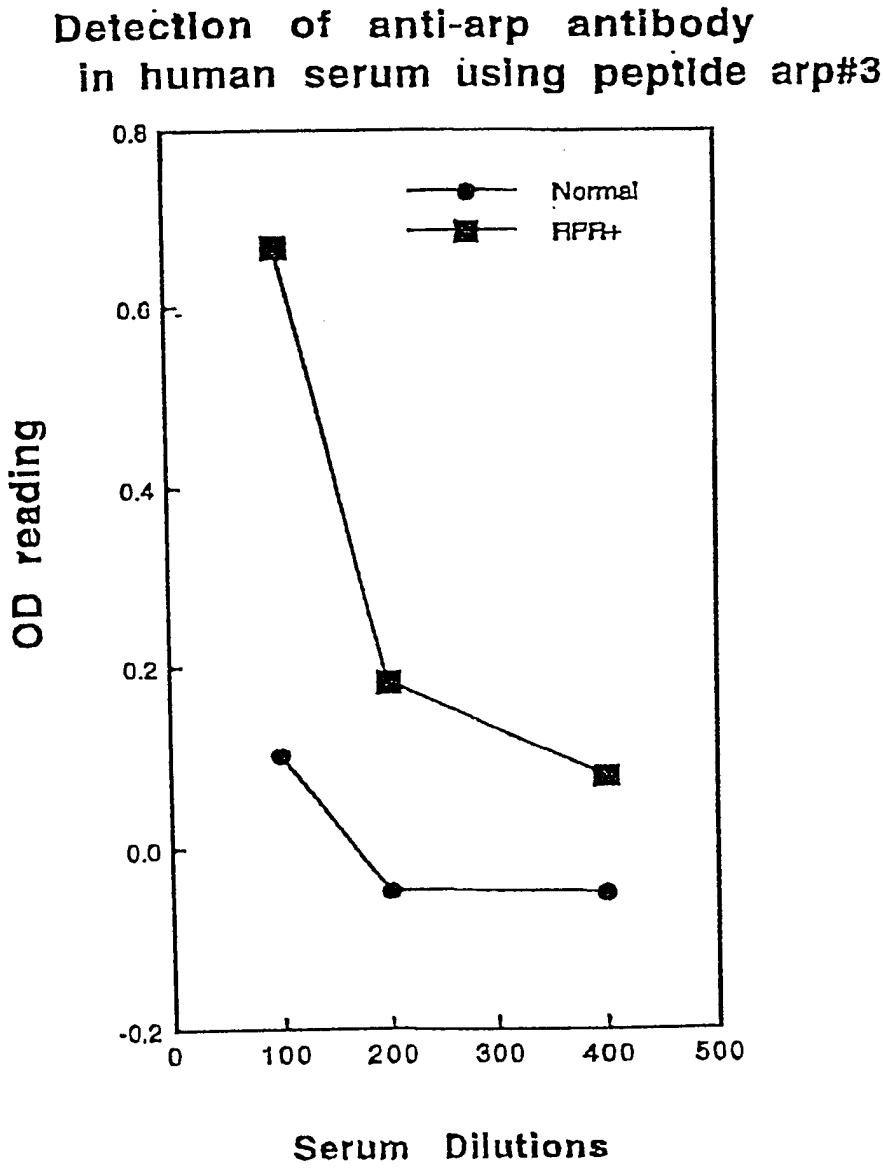

Detection of anti-arp antibody
in human serum using peptide arp#3
Normal
RPR+
OD reading
0.8
0.6
0.4
0.2
0.0
-0.2
0
100
200
300
400
500
Serum Dilutions

FIGURE 4

GTCGATGCAC AGCTGACGCT CTCAGGTCTT GCACATATTG CGCGGCTGGT GCCGACATCT
CTCCTGCCAC CTGCTACAGT GTCAGGTTCA TCGGGGAATT GAGGAAACTG TTATCCGCGC
TCCCCATCTT CCGATACTGG ATCGGTGTCG GGGGGAGTAG GAGTGGGGAA GCGTCTGTGC
TGTATCGCGC TGGTGATGCG CGCGTTCTGG TACCTCAGTG CGAAGGGAGT CAGTATCGCT
TACGTGCCCG TTCATCGCAG TGGGGGCTCT CAAGATTCGA GCATGAGCAC AGCAGTGGGC
GATACGCTCC TTAACGCCTT CTTCGACGAG GGAATGGTGG TTACGGCAGT ACCGCCGGGT
GTACACGACG GCCAGACTAT AGCAGAAATT GCTGCATGTT TTGAAGTAAT GCCCGATTAC
GCGTTGTTGG TGCAGTTTCA TTCCGCTCGT CTCCCTGGTG GGGAAAGCCC TACCTCCCGT
GCCCGCGGCG CTTGGTCTTC AGAGAGGTTC CGTGCTGTGT GGACATTAGT GGATTTGCAT
ACGCAGCGCG CGTGTGTCTA TGCGTGTGTC GCCCCATACA GGGAGAGTAT TCCCGTTTCT
GAGTGTGTTG ACGTCGTTAC CCGTTGTATT GCGGAGCAGG CAATTTCGTA CATACGGGTG
GGCACGAGCA CCGATACAGC CGGAGTTCAG TTATAGAAAA TAGGGAATAC GTAAGGTGTC
TGCAGCGTCG CTTCAGCTGG GAGGAGTCTT ATGATTAAAC GCCACATGTT CGCAAAAAGG
GGTGTCAAAG GAAGATCTTA CCTGGTTAGG GTGAACACTG CGTTCTTAGT GCTTTGTGTT
GCTTCTGTCA CGCCGCTTTG GGCTGTGTGG GAAGGGAATG CAGAAATTGG CCCCCAGGGA
AGTTTTCTGC AGGACGGC

(predicted start of arp) A TGTTTGTGCG CAGTGACATG TTCCCCAAAA ACACTGCTGT
TGAAATTAGC AACTTAGAAA AGAATGCCAA GGCTCAGGCA GTGGTTATTG GGCACGCAGG
GATCCCCGGT CTTCTAGTTA GCCTTGCACC CGCTGCTGCA GCACAGCTTG GGATTGGCGT
ATACCAAGCT GTGCGTGTAC GCGTACGTAC CTTGGGTACC GTGCGCGGTG GGTCTCAAAC
AAGTCAGGAC GGACTGTCCC TTGCATCTTT GCCGTCCCGT GTGCCTGCGC GCCCCGCGCA
GCGTGATCCT CTGTCATCCC CGCCGGCAGG TCACACTGTA CCGGAATATC GCGATACGGT
TATTTTCGAT GACCCGCGTT TGGTTTCCCC TTTGTCTCGT GAGGTGGAGG ACGCGCCGAA
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GGTAGTGGAG CCGGCCTCTG AGCGTGAGGG AGGGGAGCGT GAGGTGGAGG ACGTGCCGAA
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GGTAGTGGAG CCGGCCTCTG AGCGTGAGGG AGGGGAGCGT GAGGTGGAGG ACGTGCCGGG
GGTAGTGGAG CCGGCCTCTG GGCATGAAGG AGGGGAGCGT GAGGTGGAGG ACGTGCCGGG
GGTAGTGGAG CCGGCCTCTG GGCATGAAGG AGGGGAGCGT GAGGTCGCTT CTCAGCATAC
GAAGCAGCCA TCCCACTCGG TTTCCAACTC AGCTCCCAAT CAGTTTCGGA AACCCTGA
(end of arp)

GG GGGAACTCCC CTTTACGCTC CCTGACCTAT CCGAGTCAGA AATTGTGGTT CCGGAGGAAC
AGAAAGGACG TGCGCATCCC CAGGTGATAC CCGAGGGTGC GCCACGTGGA CTGCAACCTG
GTGAATACTA CGTACAGATT GCAGTCTTTC ATGACGCTAT CCAGGTGCAG AGCATTGTCC
ACCGTTACGG GGTAGAATAC CCCATCGCAG TGGAGCAGGA CATCCATGAA GGTAAGGTGC
GTTTCACCGT ATGCGTCGGT CCTGTCCAAA AAGACGAACG CGGCGCGGTA CTAGAGAACT
TCCAAAGGTT TGGATTCAAG GACGCCTTTC TGAAAAAGGC GCGATGATCA GGTCGGCCCT
CCTCTTCCCC TCGTGACCGT GGTGACTCGC CCCGAAGGGG GCGCACAGAG CCCGAAGGAA
CGGAAGGGAA GGGGCAGACT TAACTATTTC TTTGTTTTTT TGAGCACGTA AAACGGCGCC
ATCTCCTTTG AAGGCTTTCC TGCGCCGGGA GCGCCCATGT AGCGAACGGA GTTACTGTCT
ATCAGCTCGT ACAGCTCTTT CTCGTGCGGT GCCTTCGATT GCTCCGAGGA CACAAGCGAG
AGTTCGACAA TTCCGTCTTC ACGTACCATC CACGTACCGC GATACGTAAG AGGAGAAGGT
GCCGACTTCT TCTCAAGGGC AAGCTCTACC TTTTGCGCAG TGCCATCCGC GTTGAACGTC ACAGTC

FIGURE 5

T. pallidum ssp. Pallidum (Ni)-*arp* protein sequence

MFVRSDMFPK NTAVEISNLE KNAKAQAVVI GHAGIPGLLV SLAPAAAAQL
GIGVYQAVRV RVRTLGTVRG GSQTSQDGLS LASLPSRVPA RPAQRDPLSS
PPAGHTVPEY RDTVIFDDPR LVSPLSR

Type I:	1, 2, 4, 7, 8
Type II:	3, 5, 9, 10, 11, 12
Type III:	13, 14
Type IV:	6

EVE DAPKVVEPAS EREGGER
EVE DAPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DAPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE NVPKVVEPAS EREGGER
EVE DAPKVVEPAS EREGGER
EVE DAPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPGVVEPAS GHEGGER
EVE DVPGVVEPAS GHEGGER

EVA SQHTKQPSHS VSNSAPNQFR KP

FIGURE 6

T. pallidum ssp. Pertenue (CDC-2) nucleotide sequence

ATGTTTGTGC	GCAGTGACAT	GTTCCCCAAA	AACACTGCTG	TTGAAATTAG
CAACTTAGAA	AAGAATGCCA	AGGCTCAGGC	AGTGGTTATT	GGGCACGCAG
GGATCCCCGG	TCTTCTAGTT	AGCCTTGCAC	CCGCTGCTGC	AGCACAGCTT
GGGATTGGCG	TATACCAAGC	TGTGCGTGTA	CGCGTACGTA	CCTTGGGTAC
CGTGCGCGGT	GGGTCTCAAA	CAAGTCAGGA	CGGACTGTCC	CTTGCATCTT
TGCCGTCCCG	TGTGCCTGCG	CGCCCCGCGC	AGCGTGATCC	TCTGTCATCC
CCGCCGGCAG	GTCACACTGT	ACCGGAATAT	CGCGATACGG	TTATTTTCGA
TGACCCGCGT	TTGGTTTCCC	CTTTGTCTCG	TGAGGTGGAG	GACGTGCCGA
AGGTAGTGGA	GCCGGCCTCT	GAGCGTGAGG	GAGGGGAGCG	TGAGGTGGAG
GACGTGCCGA	AGGTAGTGGA	GCCGGCCTCT	GAGCGTGAGG	GAGGGGAGCG
TGAGGTGGAG	GACGTGCCGA	AGGTAGTGGA	GCCGGCCTCT	GAGCGTGAGG
GAGGGGAGCG	TGAGGTGGAG	GACGTGCCGA	AGGTAGTGGA	GCCGGCCTCT
GAGCGTGAGG	GAGGGGAGCG	TGAGGTCGCT	TCTCAGCATA	CGAAGCAGCC
ATCCCACTCG	GTTTCCAACT	CAGCTCCCAA	TCAGTTTCGG	AAACCCTGA

FIGURE 7

T. pallidum ssp. Pertenue (CDC-2) *arp* protein sequence

MFVRSDMFPK NTAVEISNLE KNAKAQAVVI GHAGIPGLLV SLAPAAAAQL
GIGVYQAVRV RVRTLGTVRG GSQTSQDGLS LASLPSRVPA RPAQRDPLSS
PPAGHTVPEY RDTVIFDDPR LVSPLSR

EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER

EVA SQHTKQPSHS VSNSAPNQFR KP

FIGURE 8

T. pallidum ssp. endemicum (Bosnia) nucleotide sequence

ATGTTTGTGC GCAGTGACAT GTTCCCCAAA AACACTGCTG TTGAAATTAG
CAACTTAGAA AAGAATGCCA AGGCTCAGGC AGTGGTTATT GGGCACGCAG
GGATCCCCGG TCTTCTAGTT AGCCTTGCAC CCGCTGCTGC AGCACAGCTT
GGGATTGGCG TATACCAAGC TGTGCGTGTA CGCGTACGTA CCTTGGGTAC
CGTGCGCGGT GGGTCTCAAA CAAGTCAGGA CGGACTGTCC CTTGCATCTT
TGCCGTCCCG TGTGCCTGCG CGCCCCGCGC AGCGTGATCC TCTGTCATCC
CCGCCGGCAG GTCACACTGT ACCGGAATAT CGCGATACGG TTATTTTCGA
TGACCCGCGT TTGGTTTCCC CTTTGTCTCG TGAGGTGGAG GACGTGCCGA
AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG GAGGGGAGCG TGAGGTGGAG
GACGTGCCGA AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG GAGGGGAGCG
TGAGGTGGAG GACGTGCCGA AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG
GAGGGGAGCG TGAGGTGGAG GACGTGCCGA AGGTAGTGGA GCCGGCCTCT
GCCGGCCTCT GAGCGTGAGG GAGGGGAGCG TGAGGTGGAG GACGTGCCGA
AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG GAGGGGAGCG TGAGGTGGAG
GACGTGCCGA AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG GAGGGGAGCG
TGAGGTGGAG GACGTGCCGA AGGTAGTGGA GCCGGCCTCT GAGCGTGAGG
GAGGGGAGCG TGAGGTCGCT TCTCAGCATA CGAAGCAGCC ATCCCACTCG
GTTTCCAACT CAGCTCCCAA TCAGTTTCGG AAACCCTGA

FIGURE 9

T. pallidum ssp. endemicum (Bosnia) *arp* protein sequence

MFVRSDMFPK NTAVEISNLE KNAKAQAVVI GHAGIPGLLV SLAPAAAAQL
GIGVYQAVRV RVRTLGTVRG GSQTSQDGLS LASLPSRVPA RPAQRDPLSS
PPAGHTVPEY RDTVIFDDPR LVSPLSR

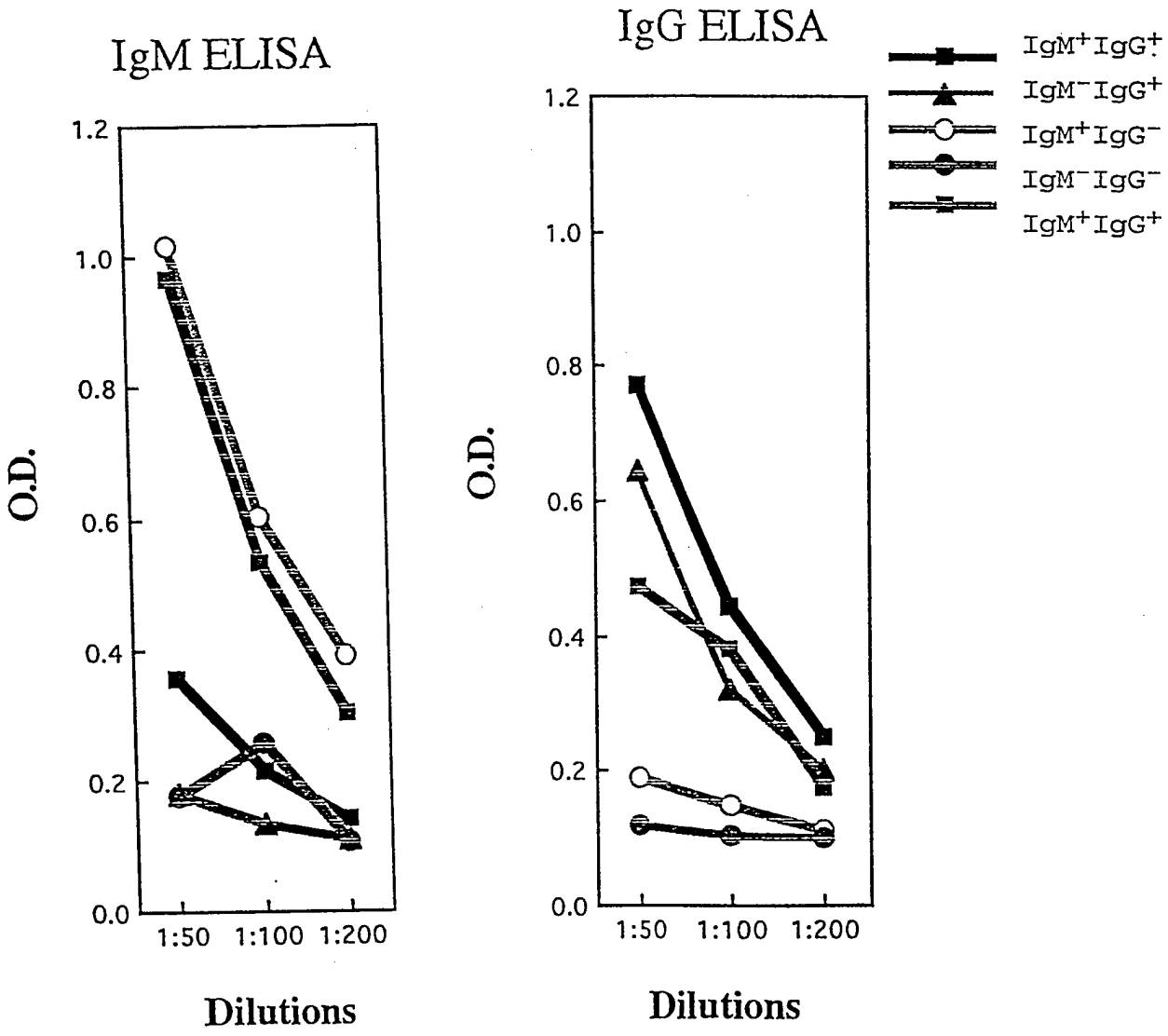
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER
EVE DVPKVVEPAS EREGGER

EVA SQHTKQPSHS VSNSAPNQFR KP

FIGURE 10

arp #1 SEQ ID NO: 7	LVSPL REVEDAPKVVEPAS-
arp #2 SEQ ID NO: 8	-SR-EVED APKVVEPASEREGG-
arp #3 SEQ ID NO: 9	-PK VVEPASEREGGEREVEDA-
TP-arp #4 SEQ ID NO: 10	PKNTAVEISNLE KNAKAQAVV
TP-arp #5 SEQ ID NO: 11	GHAGIPGLLV SLAPAAAAQLGIGVY
TP-arp #6 SEQ ID NO: 12	VPA RPAQRDPLSS PPAGHTVPEY RD
TP-arp #7 SEQ ID NO: 13	VVEPAS EREGGEREVE DVPKV
TP-arp #8 SEQ ID NO: 14	VVEPASGHEGGEREVA SQHT KQPSHS
TP-arp #9 SEQ ID NO: 15	EVEDVPKVVEPASEREGGER
TP-arp #10 SEQ ID NO: 16	EVENVPKVVEPASEREGGER
TP-arp #11 SEQ ID NO: 17	EVEDAPKVVEPASEREGGER
TP-arp #12 SEQ ID NO: 18	EVEDVPGVVEPASGHEGGER

FIGURE 11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IgG ELISA
IgM+IgG+
IgM-IgG+
IgM+IgG-
IgM-IgG-
IgM+IgG+
O.D.
1.2
1.0
0.8
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0.2
0.0
1:50 1:100 1:200
Dilutions
A
B

FIGURE 12

Flowcytometry analysis of arp 9

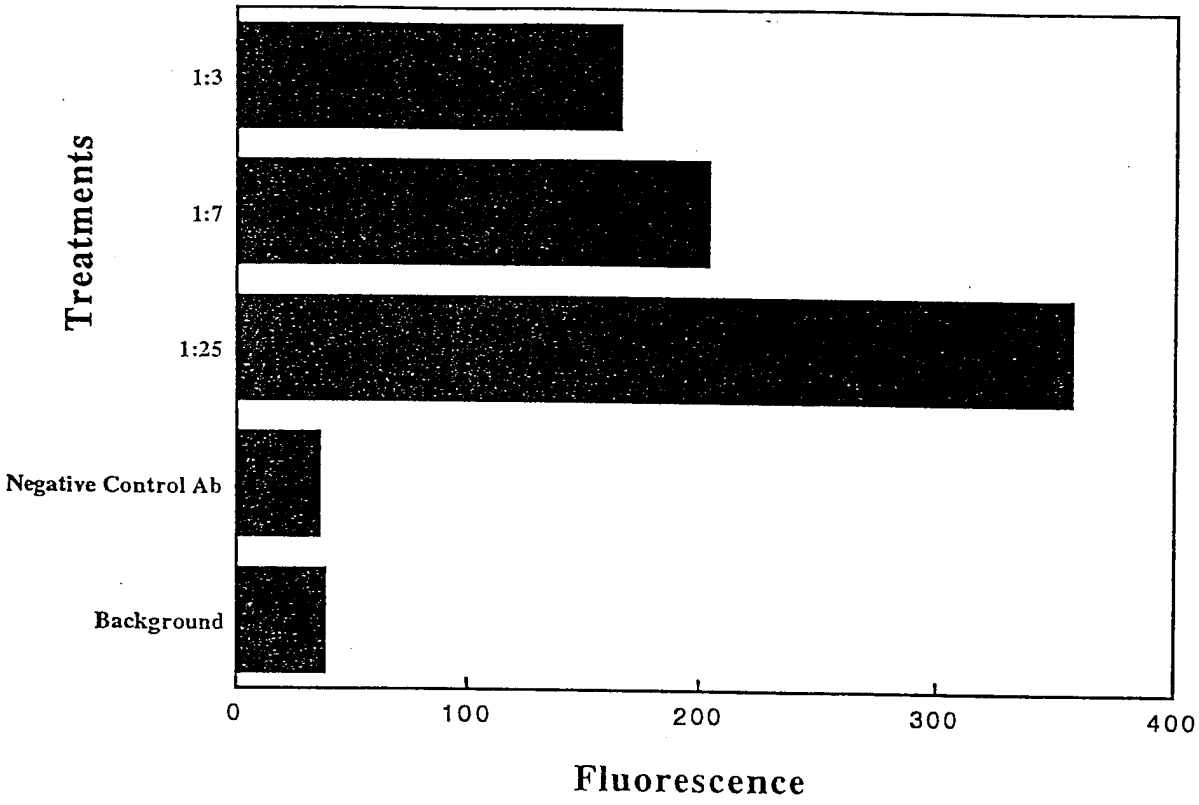

FIGURE 13

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<170> PatentIn Ver. 2.0

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ggtgtcaaag gaagatctta cctggttagg gtgaacactg cgttcttagt gctttgtgtt 840

gcttctgtca cgccgctttg ggctgtgtgg gaagggaatg cagaaattgg cccccaggga 900

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Thr Ala Val Glu Ile Ser Asn Leu Glu Lys Asn Ala Lys Ala Gln Ala
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30 35 40

ccc gct gct gca gca cag ctt ggg att ggc gta tac caa gct gtg cgt 1095
Pro Ala Ala Ala Ala Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg
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gta cgc gta cgt acc ttg ggt acc gtg cgc ggt ggg tct caa aca agt 1143
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cag gac gga ctg tcc ctt gca tct ttg ccg tcc cgt gtg cct gcg cgc 1191
Gln Asp Gly Leu Ser Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg
80 85 90

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95 100 105

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Pro Glu Tyr Arg Asp Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser
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cct ttg tct cgt gag gtg gag gac gcg ccg aag gta gtg gag ccg gcc 1335
Pro Leu Ser Arg Glu Val Glu Asp Ala Pro Lys Val Val Glu Pro Ala
125 130 135

tct gag cgt gag gga ggg gag cgt gag gtg gag gac gcg ccg aag gta 1383
Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Ala Pro Lys Val
140 145 150 155

gtg gag ccg gcc tct gag cgt gag gga ggg gag cgt gag gtg gag gac 1431
Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp
160 165 170

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Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg
175 180 185

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190 195 200

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Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala

205 210 215

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285 290 295

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335 340 345

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Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu
350 355 360

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Gly Gly Glu Arg Glu Val Glu Asp Val Pro Gly Val Val Glu Pro Ala
365 370 375

tct ggg cat gaa gga ggg gag cgt gag gtg gag gac gtg ccg ggg gta 2103
Ser Gly His Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Gly Val
380 385 390 395

gtg gag ccg gcc tct ggg cat gaa gga ggg gag cgt gag gtc gct tct 2151
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cag cat acg aag cag cca tcc cac tcg gtt tcc aac tca gct ccc aat 2199
Gln His Thr Lys Gln Pro Ser His Ser Val Ser Asn Ser Ala Pro Asn
415 420 425

cag ttt cgg aaa ccc tga gggggaactc ccctttacgc tccctgacct 2247
Gln Phe Arg Lys Pro
430

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tcatgacgct atccaggtgc agagcattgt ccaccgttac ggggtagaat accccatcgc 2427

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aaaagacgaa cgcggcgcgg tactagagaa cttccaaagg tttggattca aggacgcctt 2547

tctgaaaaag gcgcgatgat caggtcggcc ctcctcttcc cctcgtgacc gtggtgactc 2607

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gagcgcccat gtagcgaacg gagttactgt ctatcagctc gtacagctct ttctcgtgcg 2787

gtgccttcga ttgctccgag gacacaagcg agagttcgac aattccgtct tcacgtacca 2847

tccacgtacc gcgatacgta agaggagaag gtgccgactt cttctcaagg gcaagctcta 2907

ccttttgcgc agtgccatcc gcgttgaacg tcacagtc 2945

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Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg Val Arg Val Arg Thr
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Leu Gly Thr Val Arg Gly Gly Ser Gln Thr Ser Gln Asp Gly Leu Ser
65 70 75 80

Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg Pro Ala Gln Arg Asp
85 90 95

Pro Leu Ser Ser Pro Pro Ala Gly His Thr Val Pro Glu Tyr Arg Asp
100 105 110

Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser Pro Leu Ser Arg Glu
115 120 125

Val Glu Asp Ala Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
130 135 140

Gly Glu Arg Glu Val Glu Asp Ala Pro Lys Val Val Glu Pro Ala Ser
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165 170 175

Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Ala
180 185 190

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
195 200 205

Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
210 215 220

Gly Glu Arg Glu Val Glu Asn Val Pro Lys Val Val Glu Pro Ala Ser
225 230 235 240

Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Ala Pro Lys Val Val
245 250 255

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260 265 270

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275 280 285

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Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
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Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
340 345 350

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
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Gly Glu Arg Glu Val Glu Asp Val Pro Gly Val Val Glu Pro Ala Ser
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<220>

<221> CDS

<222> (1)..(699)

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Ser Asn Leu Glu Lys Asn Ala Lys Ala Gln Ala Val Val Ile Gly His
20 25 30

gca ggg atc ccc ggt ctt cta gtt agc ctt gca ccc gct gct gca gca 144
Ala Gly Ile Pro Gly Leu Leu Val Ser Leu Ala Pro Ala Ala Ala Ala
35 40 45

cag ctt ggg att ggc gta tac caa gct gtg cgt gta cgc gta cgt acc 192
Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg Val Arg Val Arg Thr
50 55 60

ttg ggt acc gtg cgc ggt ggg tct caa aca agt cag gac gga ctg tcc 240
Leu Gly Thr Val Arg Gly Gly Ser Gln Thr Ser Gln Asp Gly Leu Ser
65 70 75 80

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Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg Pro Ala Gln Arg Asp
                85                  90                  95

cct ctg tca tcc ccg ccg gca ggt cac act gta ccg gaa tat cgc gat   336
Pro Leu Ser Ser Pro Pro Ala Gly His Thr Val Pro Glu Tyr Arg Asp
            100                 105                 110

acg gtt att ttc gat gac ccg cgt ttg gtt tcc cct ttg tct cgt gag   384
Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser Pro Leu Ser Arg Glu
        115                 120                 125

gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct gag cgt gag gga   432
Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
    130                 135                 140

ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct   480
Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
145                 150                 155                 160

gag cgt gag gga ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg   528
Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
                165                 170                 175

gag ccg gcc tct gag cgt gag gga ggg gag cgt gag gtg gag gac gtg   576
Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
            180                 185                 190
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ccg aag gta gtg gag ccg gcc tct gag cgt gag gga ggg gag cgt gag 624
Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
195 200 205

gtc gct tct cag cat acg aag cag cca tcc cac tcg gtt tcc aac tca 672
Val Ala Ser Gln His Thr Lys Gln Pro Ser His Ser Val Ser Asn Ser
210 215 220

gct ccc aat cag ttt cgg aaa ccc tga 699
Ala Pro Asn Gln Phe Arg Lys Pro
225 230

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<213> Treponema pallidum

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1 5 10 15

Ser Asn Leu Glu Lys Asn Ala Lys Ala Gln Ala Val Val Ile Gly His
20 25 30

Ala Gly Ile Pro Gly Leu Leu Val Ser Leu Ala Pro Ala Ala Ala Ala
35 40 45

Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg Val Arg Val Arg Thr

50 55 60

Leu Gly Thr Val Arg Gly Gly Ser Gln Thr Ser Gln Asp Gly Leu Ser
65 70 75 80

Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg Pro Ala Gln Arg Asp
85 90 95

Pro Leu Ser Ser Pro Pro Ala Gly His Thr Val Pro Glu Tyr Arg Asp
100 105 110

Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser Pro Leu Ser Arg Glu
115 120 125

Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
130 135 140

Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
145 150 155 160

Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
165 170 175

Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
180 185 190

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
195 200 205

Val Ala Ser Gln His Thr Lys Gln Pro Ser His Ser Val Ser Asn Ser
210 215 220

Ala Pro Asn Gln Phe Arg Lys Pro
225 230

<210> 5
<211> 939
<212> DNA
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<220>
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<220>
<223> Subspecies: endemicum (Bosnia strain)

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Met Phe Val Arg Ser Asp Met Phe Pro Lys Asn Thr Ala Val Glu Ile
1 5 10 15

agc aac tta gaa aag aat gcc aag gct cag gca gtg gtt att ggg cac 96
Ser Asn Leu Glu Lys Asn Ala Lys Ala Gln Ala Val Val Ile Gly His
20 25 30

gca ggg atc ccc ggt ctt cta gtt agc ctt gca ccc gct gct gca gca 144

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Ala Gly Ile Pro Gly Leu Leu Val Ser Leu Ala Pro Ala Ala Ala Ala
         35                  40                  45

cag ctt ggg att ggc gta tac caa gct gtg cgt gta cgc gta cgt acc   192
Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg Val Arg Val Arg Thr
     50                  55                  60

ttg ggt acc gtg cgc ggt ggg tct caa aca agt cag gac gga ctg tcc   240
Leu Gly Thr Val Arg Gly Gly Ser Gln Thr Ser Gln Asp Gly Leu Ser
 65                  70                  75                  80

ctt gca tct ttg ccg tcc cgt gtg cct gcg cgc ccc gcg cag cgt gat   288
Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg Pro Ala Gln Arg Asp
                 85                  90                  95

cct ctg tca tcc ccg ccg gca ggt cac act gta ccg gaa tat cgc gat   336
Pro Leu Ser Ser Pro Pro Ala Gly His Thr Val Pro Glu Tyr Arg Asp
            100                 105                 110

acg gtt att ttc gat gac ccg cgt ttg gtt tcc cct ttg tct cgt gag   384
Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser Pro Leu Ser Arg Glu
        115                 120                 125

gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct gag cgt gag gga   432
Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
    130                 135                 140

ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct   480
Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
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145 150 155 160

gag cgt gag gga ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg 528
Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
165 170 175

gag ccg gcc tct gag cgt gag gga ggg gag cgt gag gtg gag gac gtg 576
Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
180 185 190

ccg aag gta gtg gag ccg gcc tct gag cgt gag gga ggg gag cgt gag 624
Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
195 200 205

gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct gag cgt gag gga 672
Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
210 215 220

ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg gag ccg gcc tct 720
Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
225 230 235 240

gag cgt gag gga ggg gag cgt gag gtg gag gac gtg ccg aag gta gtg 768
Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
245 250 255

gag ccg gcc tct gag cgt gag gga ggg gag cgt gag gtg gag gac gtg 816
Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
260 265 270

ccg aag gta gtg gag ccg gcc tct gag cgt gag gga ggg gag cgt gag 864
Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
275 280 285

gtc gct tct cag cat acg aag cag cca tcc cac tcg gtt tcc aac tca 912
Val Ala Ser Gln His Thr Lys Gln Pro Ser His Ser Val Ser Asn Ser
290 295 300

gct ccc aat cag ttt cgg aaa ccc tga 939
Ala Pro Asn Gln Phe Arg Lys Pro
305 310

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<211> 312
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Met Phe Val Arg Ser Asp Met Phe Pro Lys Asn Thr Ala Val Glu Ile
1 5 10 15

Ser Asn Leu Glu Lys Asn Ala Lys Ala Gln Ala Val Val Ile Gly His
20 25 30

Ala Gly Ile Pro Gly Leu Leu Val Ser Leu Ala Pro Ala Ala Ala Ala
35 40 45

Gln Leu Gly Ile Gly Val Tyr Gln Ala Val Arg Val Arg Val Arg Thr
50 55 60

Leu Gly Thr Val Arg Gly Gly Ser Gln Thr Ser Gln Asp Gly Leu Ser
65 70 75 80

Leu Ala Ser Leu Pro Ser Arg Val Pro Ala Arg Pro Ala Gln Arg Asp
85 90 95

Pro Leu Ser Ser Pro Pro Ala Gly His Thr Val Pro Glu Tyr Arg Asp
100 105 110

Thr Val Ile Phe Asp Asp Pro Arg Leu Val Ser Pro Leu Ser Arg Glu
115 120 125

Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
130 135 140

Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
145 150 155 160

Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
165 170 175

Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
180 185 190

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
195 200 205

Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly
210 215 220

Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser
225 230 235 240

Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val Pro Lys Val Val
245 250 255

Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu Asp Val
260 265 270

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
275 280 285

Val Ala Ser Gln His Thr Lys Gln Pro Ser His Ser Val Ser Asn Ser
290 295 300

Ala Pro Asn Gln Phe Arg Lys Pro
305 310

<210> 7
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<213> Treponema pallidum

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Leu Val Ser Pro Leu Arg Glu Val Glu Asp Ala Pro Lys Val Val Glu
1 5 10 15

Pro Ala Ser

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<211> 20

<212> PRT

<213> Treponema pallidum

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Ser Arg Glu Val Glu Asp Ala Pro Lys Val Val Glu Pro Ala Ser Glu
1 5 10 15

Arg Glu Gly Gly
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<210> 9

<211> 20

<212> PRT

<213> Treponema pallidum

<400> 9

Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu
1 5 10 15

Val Glu Asp Ala
20

<210> 10
<211> 21
<212> PRT
<213> Treponema pallidum

<400> 10
Pro Lys Asn Thr Ala Val Glu Ile Ser Asn Leu Glu Lys Asn Ala Lys
1 5 10 15

Ala Gln Ala Val Val
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<210> 11
<211> 25
<212> PRT
<213> Treponema pallidum

<400> 11
Gly His Ala Gly Ile Pro Gly Leu Leu Val Ser Leu Ala Pro Ala Ala
1 5 10 15

Ala Ala Gln Leu Gly Ile Gly Val Tyr
20 25

<210> 12

<211> 25

<212> PRT

<213> Treponema pallidum

<400> 12

Val Pro Ala Arg Pro Ala Gln Arg Asp Pro Leu Ser Ser Pro Pro Ala
1 5 10 15

Gly His Thr Val Pro Glu Tyr Arg Asp
20 25

<210> 13

<211> 21

<212> PRT

<213> Treponema pallidum

<400> 13

Val Val Glu Pro Ala Ser Glu Arg Glu Gly Gly Glu Arg Glu Val Glu
1 5 10 15

Asp Val Pro Lys Val
20

<210> 14

<211> 26

<212> PRT

<213> Treponema pallidum

<400> 14

Val Val Glu Pro Ala Ser Gly His Glu Gly Gly Glu Arg Glu Val Ala
1 5 10 15

Ser Gln His Thr Lys Gln Pro Ser His Ser
20 25

<210> 15

<211> 20

<212> PRT

<213> Treponema pallidum

<400> 15

Glu Val Glu Asp Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu
1 5 10 15

Gly Gly Glu Arg
20

<210> 16

<211> 20

<212> PRT

<213> Treponema pallidum

<400> 16

Glu Val Glu Asn Val Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu
1 5 10 15

Gly Gly Glu Arg
20

<210> 17

<211> 20

<212> PRT

<213> Treponema pallidum

<400> 17

Glu Val Glu Asp Ala Pro Lys Val Val Glu Pro Ala Ser Glu Arg Glu
1 5 10 15

Gly Gly Glu Arg
20

<210> 18

<211> 20

<212> PRT

<213> Treponema pallidum

<400> 18

Glu Val Glu Asp Val Pro Gly Val Val Glu Pro Ala Ser Gly His Glu
1 5 10 15

Gly Gly Glu Arg

20

专利名称(译)	用于检测梅毒螺旋体的组合物和方法		
公开(公告)号	EP1240519A2	公开(公告)日	2002-09-18
申请号	EP2000941424	申请日	2000-06-14
[标]申请(专利权)人(译)	美国卫生及公共服务部		
申请(专利权)人(译)	美利坚合众国政府作为代表局局长，卫生与公众服务部		
当前申请(专利权)人(译)	美利坚合众国政府作为代表局局长，卫生与公众服务部		
[标]发明人	LIU HSI STEINER BRET RHODES BERTA		
发明人	LIU, HSI STEINER, BRET RHODES, BERTA		
IPC分类号	A61K39/002 A61K39/02 C07K14/20 C07K16/12 C07K16/20 C12P21/08 G01N33/53 G01N33/569 G01N33/571		
CPC分类号	C07K14/20 G01N33/571 G01N2333/20		
优先权	60/138981 1999-06-14 US		
其他公开文献	EP1240519B1		
外部链接	Espacenet		

摘要(译)

本发明提供了对梅毒螺旋体感染进行特异性和高灵敏度检测的方法，包括使用特异性抗原蛋白和对梅毒螺旋体特有的肽。特别地，提供了基于酸性重复蛋白识别的检测分析。本发明的方法特别适用于在感染的早期阶段检测一期梅毒。此外，本发明的方法和组合物涉及特异性密螺旋体感染的差异检测，使得能够鉴定特定的密螺旋体疾病状态的病原体：梅毒梅毒螺旋体亚种苍白球，偏航梅毒螺旋体亚种，和梅毒螺旋体亚种地中海种子。