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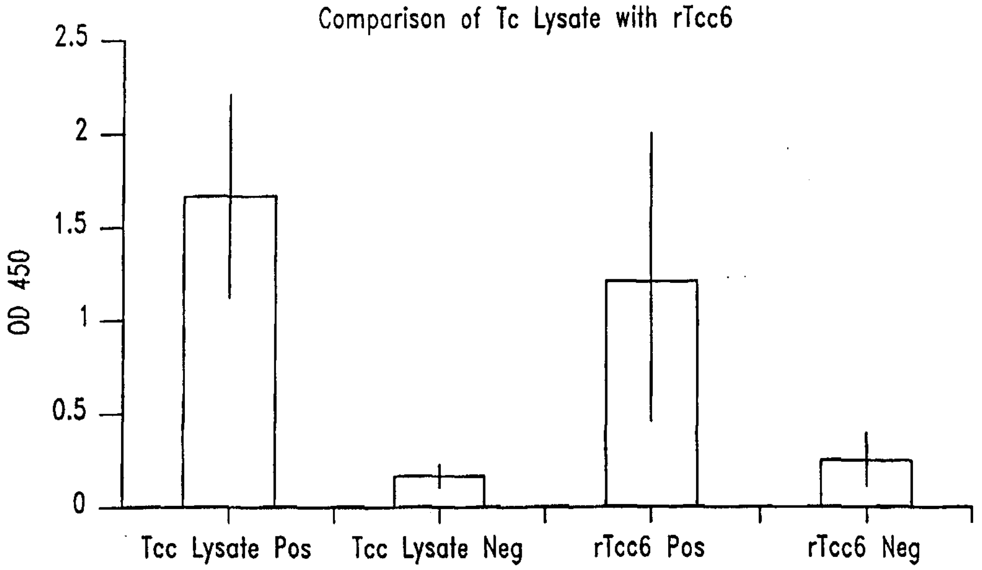

(57) Abstract

Compounds and methods are provided for diagnosing *Trypanosoma cruzi* infection. The disclosed compounds are polypeptides, or antibodies thereto, that contain one or more epitopes of *T. cruzi* antigens. The compounds are useful in a variety of immunoassays for detecting *T. cruzi* infection. The polypeptide compounds are further useful in vaccines and pharmaceutical compositions for inducing protective immunity against Chagas' disease in individuals exposed to *T. cruzi*.

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Description

COMPOUNDS AND METHODS FOR THE DETECTION AND PREVENTION OF *T. CRUZI* INFECTION

TECHNICAL FIELD

The present invention relates generally to the diagnosis of *T. cruzi* infection. The invention is more particularly related to the use of one or more *T. cruzi* antigenic peptides, or antibodies thereto, in methods and diagnostic kits to screen individuals and blood supplies for *T. cruzi* infection. The invention is also directed to vaccine compositions for immunizing an individual to prevent Chagas' disease.

BACKGROUND OF THE INVENTION

Protozoan parasites are a serious health threat in many areas of the world. *Trypanosoma cruzi* (*T. cruzi*) is one such parasite that infects millions of individuals, primarily in Central and South America. Infections with this parasite can cause Chagas' disease, which may result in chronic heart disease and a variety of immune system disorders. It is estimated that 18 million people in Latin America are infected with *T. cruzi*, but there is no reliable treatment for the clinical manifestations of infection. No vaccine for the prevention of Chagas’ disease is currently available.

The most significant route of transmission in areas where the disease is endemic is through contact with an infected triatomid bug. In other areas, however, blood transfusions are the dominant means of transmission. To inhibit the transmission of *T. cruzi* in such regions, it is necessary to develop accurate methods for diagnosing *T. cruzi* infection in individuals and for screening blood supplies. Blood bank screening is particularly important in South America, where 0.1%-62% of samples may be infected and where the parasite is frequently transmitted by blood transfusion. There is also increasing concern that the blood supply in certain U.S. cities may be contaminated with *T. cruzi* parasites.

The diagnosis of *T. cruzi* infection has been problematic, since accurate methods for detecting the parasite that are suitable for routine use have been unavailable. During the acute phase of infection, which may last for decades, the infection may remain quiescent and the host may be asymptomatic. As a result, serological tests for *T. cruzi* infection are the most reliable and the most commonly used.

Such diagnoses are complicated, however, by the complex life cycle of the parasite and the diverse immune responses of the host. The parasite passes through an epimastigote stage in the insect vector and two main stages in the mammalian host. One host stage is present in blood (the trypomastigote stage) and a second stage is intracellular (the amastigote stage). The multiple stages result in a diversity of antigens presented by the parasite during infection. In addition, immune responses to protozoan infection are complex, involving both humoral and cell-mediated responses to the array of parasite antigens.

While detecting antibodies against parasite antigens is the most common and reliable method of diagnosing clinical and subclinical infections, current tests are expensive and difficult. Most serological tests use whole or lysed *T. cruzi* and require positive results on two of three tests, including complement fixation, indirect immunofluorescence, passive agglutination or ELISA, to accurately detect *T. cruzi* infection. The cost and difficulty of such tests has prevented the screening of blood or sera in many endemic areas.

Accordingly, there is a need in the art for more specific and sensitive methods of detecting *T. cruzi* infections in blood supplies and individuals. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, this invention provides compounds and methods for detecting and protecting against *T. cruzi* infection in individuals and in blood supplies, and for screening for *T. cruzi* infection in biological samples. In one aspect, the present invention provides methods for detecting *T. cruzi* infection in a biological sample, comprising (a) contacting the biological sample with a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

In another aspect of this invention, polypeptides are provided comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:21, or a variant of such an antigen that differs only in conservative substitutions and/or modifications.

Within related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides diagnostic kits for detecting *T. cruzi* infection in a biological sample, comprising (a) a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) a detection reagent.

In yet another aspect of the invention, methods for detecting the presence of *T. cruzi* infection in a biological sample are provided, comprising (a) contacting a biological sample with a monoclonal antibody that binds to an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of such an antigen that differs only in conservative substitutions and/or modifications; and (b) detecting in the biological sample the presence of *T. cruzi* parasites that bind to the monoclonal antibody.

Within related aspects, pharmaceutical compositions comprising the above polypeptides and a physiologically acceptable carrier, and vaccines comprising the above polypeptides in combination with an adjuvant, are also provided.

The present invention also provides, within other aspects, methods for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

Within other aspects, the present invention provides methods for detecting *T. cruzi* infection in a biological sample, comprising (a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; (b) contacting the biological sample with one or more additional polypeptides comprising one or more epitopes of other *T. cruzi* antigens, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample. In one embodiment, the additional polypeptide comprises an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications. In another embodiment, the additional polypeptides comprise an epitope of TcD (or a variant thereof that differs only in conservative substitutions and/or modifications) and an epitope of TcE (or a variant thereof that differs only in conservative substitutions and/or modifications). In yet another embodiment, the additional polypeptides comprise an epitope of TcD (or a variant thereof that differs only in conservative substitutions and/or modifications) and PEP-2 (or a variant thereof that differs only in conservative substitutions and/or modifications).

In yet further aspects, the present invention provides combination polypeptides comprising two or more polypeptides, each polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant thereof that differs only in conservative substitutions and/or modifications. Combination polypeptides comprising at least one epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant thereof that differs only in conservative substitutions and/or modifications, and at least one epitope selected from the group consisting of TcD epitopes, TcE epitopes, PEP-2 epitopes and variants thereof that differ only in conservative substitutions and/or modifications are also provided. In specific embodiments, combination polypeptides comprising an amino acid sequence of SEQ ID NO: 82 or 95 are provided. Such combination polypeptides may be prepared either by synthetic means or using recombinant DNA technology.

In related aspects, methods are provided for detecting *T. cruzi* infection in a biological sample, comprising (a) contacting the biological sample with at least one of the above combination polypeptides and (b) detecting in the biological sample the presence of antibodies that bind to the combination polypeptide.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph comparing the reactivity of *T. cruzi* lysate and a representative polypeptide of the present invention (rTcc6) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. The bars represent ± 1 standard deviation.

Figure 2 is a graph presenting a comparison of the reactivity of representative polypeptides of the subject invention in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. Experiment 1 shows a comparison of rTcc22 and the peptides Tcc22-1 and Tcc22-1+; Experiment 2 shows a comparison of rTcc22, rTcHi12 and the peptides Tcc22-1, Tcc22-1+ and Tcc22-2.1. The bars represent ± 1 standard deviation.

Figure 3 is a graph depicting a comparison of the reactivity of *T. cruzi* lysate and a representative polypeptide (Tcc38) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals, as well as using sera from individuals with visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), tuberculosis (TB) and malaria. The bars represent ± 1 standard deviation.

Figure 4 is a graph presenting a comparison of the reactivity of *T. cruzi* lysate and several polypeptides of the present invention, representing different reading frames of the TcLo1 and TcHi10 antigens, in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals. The bars represent ± 1 standard deviation.

Figure 5 is a graph comparing the reactivity of *T. cruzi* lysate and a representative polypeptide (TccLo1.2) in an ELISA assay performed using sera from *T. cruzi*-infected (Pos) and uninfected (Neg) individuals, as well as sera from individuals with visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), malaria and tuberculosis (TB).

Figure 6 is a graph depicting the ELISA reactivity of a series of polypeptide combinations with *T. cruzi* positive and negative sera.

Figure 7 is a graph presenting the ELISA reactivity of a series of TcE polypeptide variants with *T. cruzi* positive and negative sera.

Figure 8 is a graph comparing the ELISA reactivity of two dipeptides, a tripeptide and a tetrapeptide of the present invention with *T. cruzi* positive and negative sera.

Figure 9 is a graph presenting the ELISA reactivity of a representative polypeptide of the present invention (TcHi29) and of TcE with sera from normal individuals, *T. cruzi* patients, and patients with other diseases.

Figure 10 is a graph comparing the ELISA reactivity of two representative dipeptide mixtures with *T. cruzi* positive and negative sera, one mixture including a TcE epitope and the other including a TcHi29 epitope of the present invention.

Figure 11 is a graph comparing the ELISA reactivity of the recombinant fusion polypeptide TcF with sera from *T. cruzi* patients and from normal donors with the reactivity of the synthetic branched tetrapeptide 2/D/E/Lo1.2.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compounds and methods for detecting and protecting against *T. cruzi* infection in individuals and in blood supplies. The compounds of this invention generally comprise one or more epitopes of *T. cruzi* antigens. In particular, polypeptides comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22 are preferred. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length (*i.e.,* native) antigens. Thus, a polypeptide comprising an epitope may consist entirely of the epitope or may contain additional sequences. The additional sequences may be derived from the native antigen or may be heterologous, and such sequences may (but need not) be antigenic. A protein "having" a particular amino acid sequence is a protein that contains, within its full length sequence, the recited sequence. Such a protein may, or may not, contain additional amino acid sequence. The use of one or more epitopes from additional *T. cruzi* proteins, prior to or in combination with one or more epitopes of sequences recited herein, to enhance the sensitivity and specificity of the diagnosis, is also contemplated.

An "epitope," as used herein, is a portion of a *T. cruzi* antigen that reacts with sera from *T. cruzi*-infected individuals (*i.e.,* an epitope is specifically bound by one or more antibodies within such sera). Epitopes of the antigens described in the present application may generally be identified using methods known to those of ordinary skill in the art, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. For example, a polypeptide derived from a native *T. cruzi* antigen may be screened for the ability to react with pooled sera obtained from *T. cruzi*-infected patients. Suitable assays for evaluating reactivity with *T. cruzi*-infected sera, such as an enzyme linked immunosorbent assay (ELISA), are described in more detail below, and in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. An epitope of a polypeptide is a portion that reacts with such antisera at a level that is substantially similar to the reactivity of the full length polypeptide. In other words, an epitope may generate at least about 80%, and preferably at least about 100%, of the response generated by the full length polypeptide in an antibody binding assay (*e.g.,* an ELISA).

The compounds and methods of this invention also encompass variants of the above polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A nucleotide “variant” is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA, 2*:183, 1983). Nucleotide variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The polypeptides provided by the present invention include variants that are encoded by DNA sequences which are substantially homologous to one or more of the DNA sequences specifically recited herein. "Substantial homology," as used herein, refers to DNA sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing DNA sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode an immunogenic polypeptide that is encoded by a hybridizing DNA sequence.

Two nucleotide or polypeptide sequences are said to be “identical” if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks *Proc. Natl. Acad., Sci. USA 80*:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

In a related aspect, combination polypeptides comprising epitopes of multiple *T. cruzi* antigens are disclosed. A "combination polypeptide" is a polypeptide in which epitopes of different *T. cruzi* antigens, or variants thereof, are joined, for example through a peptide linkage, into a single amino acid chain. The amino acid chain thus formed may be either linear or branched. The epitopes may be joined directly (*i.e.,* with no intervening amino acids) or may be joined by way of a linker sequence (*e.g.,* Gly-Cys-Gly) that does not significantly alter the antigenic properties of the epitopes. The peptide epitopes may also be linked through non-peptide linkages, such as hetero- or homo-bifunctional agents that chemically or photochemically couple between specific functional groups on the peptide epitopes such as through amino, carboxyl, or sulfhydryl groups. Bifunctional agents which may be usefully employed in the combination polypeptides of the present invention are well known to those of skill in the art. Epitopes may also be linked by means of a complementary ligand/anti-ligand pair, such as avidin/biotin, with one or more epitopes being linked to a first member of the ligand/anti-ligand pair and then being bound to the complementary member of the ligand/anti-ligand pair either in solution or in solid phase. A combination polypeptide may contain multiple epitopes of polypeptides as described herein and/or may contain epitopes of one or more other *T. cruzi* antigens, such as TcD, TcE or PEP-2, linked to an epitope described herein.

In general, *T. cruzi* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, a *T. cruzi* cDNA or genomic DNA expression library may be screened with pools of sera from *T. cruzi*-infected individuals. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989. Briefly, the bacteriophage library may be plated and transferred to filters. The filters may then be incubated with serum and a detection reagent. In the context of this invention, a "detection reagent" is any compound capable of binding to the antibody-antigen complex, which may then be detected by any of a variety of means known to those of ordinary skill in the art. Typical detection reagents for screening purposes contain a "binding agent," such as Protein A, Protein G, IgG or a lectin, coupled to a reporter group. Preferred reporter groups include, but are not limited to, enzymes, substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. More preferably, the reporter group is horseradish peroxidase, which may be detected by incubation with a substrate such as tetramethylbenzidine or 2,2'-azino-di-3-ethylbenzthiazoline sulfonic acid. Plaques containing cDNAs that express a protein that binds to an antibody in the serum may be isolated and purified by techniques known to those of ordinary skill in the art. Appropriate methods may be found, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1989.

DNA molecules having the nucleotide sequences recited in SEQ ID NO:1 - SEQ ID NO:18 may be isolated by screening a *T. cruzi* genomic expression library with pools of sera from *T. cruzi*-infected individuals, as described above. More specifically, DNA molecules having the nucleotide sequences recited in SEQ ID NO:1 - SEQ ID NO:16 may be isolated by screening the library with a pool of sera that displays serological reactivity (in an ELISA or Western assay) with parasite lysate and/or one or both of the *T. cruzi* antigens TcD and TcE, described in U.S. Patent No. 5,304,371 and U.S. Serial No. 08/403,379, filed March 14, 1995. A subsequent screen is then performed with patient sera lacking detectable anti-TcD antibody. A DNA molecule having the nucleotide sequences recited in SEQ ID NO:17 (5' end) and SEQ ID NO:18 (3' end) may be isolated by screening the genomic expression library with a pool of sera that displays lower serological reactivity (*i.e.,* detects a signal less than 3 standard deviations over background reactivity in an ELISA or Western assay) with lysate, TcD and TcE, followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody.

DNA molecules having the sequences recited in SEQ ID NO:19 - SEQ ID NO:22 may be obtained by screening an unamplified *T. cruzi* cDNA expression library with sera (both higher and lower serological reactivity) from *T. cruzi*-infected individuals, as described above.

Alternatively, DNA molecules having the sequences recited in SEQ ID NO:1 - SEQ ID NO:22 may be amplified from *T. cruzi* genomic DNA or cDNA via polymerase chain reaction. For this approach, sequence-specific primers may be designed based on the sequences provided in SEQ ID NO:1 - SEQ ID NO:22, and may be purchased or synthesized. An amplified portion of the DNA sequences may then be used to isolate the full length genomic or cDNA clones using well known techniques, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1989).

Epitopes of antigens having amino acid sequences encoded by the above DNA sequences may generally be identified by generating polypeptides containing portions of the native antigen and evaluating the reactivity of the polypeptides with sera from *T. cruzi*-infected individuals, as described above. In many instances, peptides comprising one or more repeat sequences found in the native antigen contain an epitope. Such repeat sequences may be identified based on inspection of the above nucleotide sequences. Representative repeat sequences for antigens encoded by the above DNA sequences are provided in SEQ ID NO:23 - SEQ ID NO:36 and SEQ ID NO:47 - SEQ ID NO:49. More specifically, repeat sequences for the sequence recited in SEQ ID NO:3 are provided in SEQ ID NO:23 (Frame 1), SEQ ID NO:24 (Frame 2) and SEQ ID NO:25 (Frame 3). Repeat sequences for the sequence recited in SEQ ID NO:4 are provided in SEQ ID NO:26 (Frame 1) and SEQ ID NO:27 (Frame 3) and repeat sequences for SEQ ID NO:9 are provided in SEQ ID NO:47 (Frame 1), SEQ ID NO:48 (Frame 2) and SEQ ID NO:49 (Frame 3). For SEQ ID NO:12, repeat sequences are provided in SEQ ID NO:28 (Frame 1), SEQ ID NO:29 (Frame 2) and SEQ ID NO:30 (Frame 3). SEQ ID NO:31 recites a repeat sequence for SEQ ID NO:15. For SEQ ID NO:16, repeat sequences are provided in SEQ ID NO:32 (Frame 2) and SEQ ID NO:33 (Frame 3). Finally, repeat sequences for SEQ ID NO:18 are provided in SEQ ID NO:34 (Frame 1), SEQ ID NO:35 (Frame 2) and SEQ ID NO:36 (Frame 3).

The polypeptides described herein may be generated using techniques well known to those of ordinary skill in the art. Polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, can be synthesized using, for example, the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems Division, Foster City, CA. Thus, for example, polypeptides comprising the above repeat sequences or portions thereof, may be synthesized by this method. Similarly, epitopes of other native antigens, or variants thereof, may be prepared using an automated synthesizer.

Alternatively, the polypeptides of this invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are *E. coli*, yeast, an insect cell line (such as *Spodoptera* or *Trichoplusia*) or a mammalian cell line, including (but not limited to) CHO, COS and NS-1. The DNA sequences expressed in this manner may encode naturally occurring proteins, such as full length antigens having the amino acid sequences encoded by the DNA sequences of SEQ ID NO:1 - SEQ ID NO:22, portions of naturally occurring proteins, or variants of such proteins. Representative polypeptides encoded by such DNA sequences are provided in SEQ ID NO:37 - SEQ ID NO:46, SEQ ID NO:52, and SEQ ID NO:65.

Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably, to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography.

In another aspect of this invention, methods for detecting *T. cruzi* infection in individuals and blood supplies are disclosed. In one embodiment, *T. cruzi* infection may be detected in any biological sample that contains antibodies. Preferably, the sample is blood, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood or serum sample obtained from a patient or a blood supply. Briefly, *T. cruzi* infection may be detected using any one or more of the polypeptides described above, or variants thereof, to determine the presence or absence of antibodies to the polypeptide or polypeptides in the sample, relative to a predetermined cut-off value.

There are a variety of assay formats known to those of ordinary skill in the art for using purified antigen to detect antibodies in a sample. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that binds to the antibody/peptide complex and contains a detectable reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (*e.g.*, in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptide may be bound to the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen. Nitrocellulose will bind approximately 100 µg of protein per cm^3.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

Once the polypeptide is immobilized on the support, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20 ™ (Sigma Chemical Co., St. Louis, MO). The immobilized polypeptide is then incubated with the sample, and antibody (if present in the sample) is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to permit detect the presence of *T. cruzi* antibody within a *T. cruzi*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many sources (*e.g.*, Zymed Laboratories, San Francisco, CA and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of *T. cruzi* antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. This cut-off value is preferably the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the mean is considered positive for *T. cruzi* antibodies and *T. cruzi* infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, p. 106-7 (Little Brown and Co., 1985). Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.,* sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.,* the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for *T. cruzi* infection.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antigen is immobilized on a membrane such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of *T. cruzi* antibodies in the sample. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

The assays discussed above may be performed using one or more of the polypeptides described herein. Alternatively, the sensitivity may be improved by using epitopes of one or more additional *T. cruzi* antigens in combination with the above polypeptide(s). In particular, epitopes of TcD (disclosed, for example, in U.S. Patent No. 5,304,371), PEP-2 and/or TcE (both of which are disclosed, for example, in U.S. Serial No. 08/403,379, filed March 14, 1995) may be used in conjunction with the above polypeptide(s). The PEP-2 antigenic epitope is also discussed in Peralta et al., *J. Clin. Microbiol. 32*:971-74, 1994. The sequence of TcD is provided in SEQ ID NO:50, the sequence of TcE is provided in SEQ ID NO:51. The TcD antigenic epitope preferably has the amino acid sequence Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser (SEQ ID NO:53)or the amino acid sequence Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro (SEQ ID NO:54). The TcE epitope preferably has the amino acid sequence Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala (SEQ ID NO: 55) or the amino acid sequence Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala (SEQ ID NO:56), and the PEP2 epitope preferably has the amino acid sequence Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala (SEQ ID NO:57).

Additional epitopes may be present within the same polypeptide (*i.e.,* in a combination polypeptide) or may be included in separate polypeptides. Combination polypeptides may be prepared either synthetically, as described below in Example 2, or using recombinant DNA technology as detailed below in Example 7. Preferably, the polypeptides are immobilized by adsorption on a solid support such as a well of a microtiter plate or a membrane, as described above, such that a roughly similar amount of each polypeptide contacts the support, and such that the total amount of polypeptide in contact with the support ranges from about 1 ng to about 10 μg. The remainder of the steps may generally be performed as described above.

The polypeptides described above may also be used following diagnosis using one or more of the epitopes from TcD, TcE and/or PEP2. In this embodiment, the polypeptides of the present invention are used to confirm a diagnosis of *T. cruzi* infection based on a screen with TcD, TcE and/or PEP2. Diagnosis of *T. cruzi* infection using epitopes from TcD, TcE and/or PEP2 is described in U.S. Serial No. 08/403,379, filed March 14, 1995.

In yet another aspect of this invention, methods are provided for detecting *T. cruzi* in a biological sample using monospecific antibodies (which may be polyclonal or monoclonal) to one or more epitopes, as described above. Antibodies to purified or synthesized polypeptides may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Monospecific antibodies to epitopes of one or more of the polypeptides described herein may be used to detect *T. cruzi* infection in a biological sample using any of a variety of immunoassays, which may be direct or competitive. Suitable biological samples for use in this aspect of the present invention are as described above. Briefly, in one direct assay format, a monospecific antibody may be immobilized on a solid support (as described above) and contacted with the sample to be tested. After removal of the unbound sample, a second monospecific antibody, which has been labeled with a reporter group, may be added and used to detect bound antigen. In an exemplary competitive assay, the sample may be combined with the monoclonal or polyclonal antibody, which has been labeled with a suitable reporter group. The mixture of sample and antibody may then be combined with polypeptide antigen immobilized on a suitable solid support. Antibody that has not bound to an antigen in the sample is allowed to bind to the immobilized antigen, and the remainder of the sample and antibody is removed. The level of antibody bound to the solid support is inversely related to the level of antigen in the sample. Thus, a lower level of antibody bound to the solid support indicates the presence of *T. cruzi* in the sample. To determine the presence or absence of *T. cruzi* infection, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. Such cut-off values may generally be determined as described above. Any of the reporter groups discussed above in the context of ELISAs may be used to label the monospecific antibodies, and binding may be detected by any of a variety of techniques appropriate for the reporter group employed. Other formats for using monospecific antibodies to detect *T. cruzi* in a sample will be apparent to those of ordinary skill in the art, and the above formats is provided solely for exemplary purposes.

In another aspect of this invention, vaccines and pharmaceutical compositions are provided for the prevention of *T. cruzi* infection, and complications thereof, in a mammal. The pharmaceutical compositions generally comprise one or more polypeptides, containing one or more epitopes of *T. cruzi* proteins, and a physiologically acceptable carrier. The vaccines comprise one or more of the above polypeptides and an adjuvant, for enhancement of the immune response.

Routes and frequency of administration and polypeptide doses will vary from individual to individual and may parallel those currently being used in immunization against other protozoan infections. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 4 doses may be administered for a 2-6 week period. Preferably, two doses are administered, with the second dose 2-4 weeks later than the first. A suitable dose is an amount of polypeptide that is effective to raise antibodies in a treated mammal that are sufficient to protect the mammal from *T. cruzi* infection for a period of time. In general, the amount of polypeptide present in a dose ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the animal, but will typically range from about 0.01 mL to about 5 mL for 10-60 kg animal.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.,* polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention.

Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Such adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Preparation of DNA Encoding *T. cruzi* Antigens

This Example illustrates the preparation of genomic and cDNA molecules encoding *T. cruzi* Antigens.

A. Preparation of Genomic Clones

A genomic expression library was constructed from randomly sheared *T. cruzi* genomic DNA (Tulahuen C2 strain) using the Lambda ZAP expression system (Stratagene, La Jolla, CA) according to the manufacturer's instructions. In one screen, the library was screened with a pool of sera from five patients that displayed high reactivity with parasite lysate and/or one or both of the *T. cruzi* antigens TcD and TcE, described in U.S. Patent No. 5,304,371 and U.S. Serial No. 08/403,379, filed March 14, 1995. Each of the five patients' sera was determined to be reactive based on Western and ELISA assays with whole lysate and/or TcD or TcE. Anti-*E. coli* reactivity was removed from the serum prior to screening by adsorption. 50,000 pfu of the unamplified library was screened with the serum pool and plaques expressing proteins that reacted with the serum were detected using protein A-horseradish peroxidase (with the ABTS substrate). A subsequent screen was then performed with a pool of sera from three patients lacking detectable anti-TcD antibody in Western and ELISA assays using recombinant TcD.

A similar screen was performed using a pool of sera that displayed low reactivity with lysate, TcD and TcE (*i.e.,* detected a signal less than 3 standard deviations over background reactivity in an ELISA or Western assay), followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody, as described above.

Twenty-eight clones that expressed proteins which reacted with both pools of sera in at least one of the above screens were then isolated. Excision of the pBSK(-) phagemid (Stratagene, Inc., La Jolla, CA) was carried out according to the manufacturer's protocol. Overlapping clones were generated by exonuclease III digestion and single-stranded templates were isolated after infection with VCSM 13 helper phage. The DNA was sequenced by the dideoxy chain termination method or by the Taq di-terminator system, using an Applied Biosystem automated sequencer, Model 373A.

Of the 28 clones, five had been reported previously, two were identical, and eight contained identical peptide sequences represented by a degenerate 42 base pair repeat. SEQ ID NO:16 shows the prototype clone containing the 42 base pair repeat sequence. Accordingly, 14 novel DNA sequences encoding *T. cruzi* antigens were prepared using the above screen with the reactive pool of sera (shown in SEQ ID NO:1 - SEQ ID NO:16, where SEQ ID NO:4 and SEQ ID NO:5 represent the 5' and 3' ends, respectively, of a single clone, SEQ ID NO:9 and SEQ ID NO:10 represent the 5' and 3' ends, respectively, of a single clone. One novel sequence was obtained with the screen employing the sera with low reactivity (shown in SEQ ID NO:17 (5' end) and SEQ ID NO:18 (3' end)).

B. Preparation of cDNA Clones

Poly A+ RNA was purified from the intracellular amastigote stage of *T. cruzi* (Tulahuen C2 strain). The RNA was reverse transcribed and used in the construction of a unidirectional cDNA expression library in the Lambda UniZap expression vector (Stratagene, La Jolla, CA) according to the manufacturer's instructions. 50,000 pfu of the unamplified library was screened with a serum pool containing patient sera that displayed both high and low serological reactivity, followed by a subsequent screen with patient sera lacking detectable anti-TcD antibody, as described above. A total of 32 clones were isolated from this screen. Twenty-five of these clones were proteins of the translational apparatus that have been previously identified as highly immunogenic, and all were different from the clones identified by screening the genomic expression library. The remaining seven are represented by the sequences provided in SEQ ID NO:19 - SEQ ID NO:22. The sequence recited in SEQ ID NO:22 is that of *T. cruzi* ubiquitin.

Example 2

Synthesis of Synthetic Polyeptides

This Example illustrates the synthesis of polypeptides having sequences derived from *T. cruzi* antigens described herein.

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HBTU (O-benzotriazole-N,N,N',N'-tetramethyuronium hexafluorophosphate) activation. A gly-cys-gly sequence may be attached to the amino or carboxyl terminus of the peptide to provide a method of conjugation or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanediol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1%TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides are characterized using electrospray mass spectrometry and by amino acid analysis.

This procedure was used to synthesize peptides such as Tcc22-1,Tcc22-1+, Tcc22-2.1 (contained within SEQ ID NO:41), TcLo1.1,1.2 and 1.3 (contained within SEQ ID NO: 34, 35 and 36) and TcHi10.1 and 10.3 (SEQ ID NO: 26 and 27) which have the following sequences:

Tcc22-1	VRASNCRKKACGHCSNLRMKKK
Tcc22-1+	EALAKKYNWEKKVCRRCYARLPVRASNCRKKACGHCSNLRMKKK
Tcc22-2.1	VLRLRGGVMEPTLEALAKKYNWEKKVCRRCYARL
TcLo1.1	GYVRGRKQRWQLHACGYVRGRKQRRQLHACGYVRGRKQRWQLHAF
TcLo1.2	GTSEEGSRGGSSMPSGTSEEGSRGGSSMPA
TcLo1.3	VRPRKEAEVAAPCLRVRPRKEAEEAAPCLR
TcHi10.1	SVPGKRLRNSHGKSLRNVHGKRPKNEHGKRLRSVPNERLR
TcHi10.3	EAEELARQESEERARQEAEERAWQEAEERAQREAEERAQR

Example 3

Serological Reactivity of *T. cruzi* Recombinant Antigens

This example illustrates the diagnostic properties of several recombinant antigens found to be serologically active. This includes studies of reactivity with *T. cruzi* positive and negative sera as well as cross reactivity studies with sera from patients with other diseases.

Assays were performed in 96 well plates (Corning Easiwash, Corning, New York). Wells were coated in 50μl of carbonate coating buffer pH 9.6. For *T.cruzi* lysate, 100ng/well was used, and for each of the recombinant antigens 200ng/well was used. The wells were coated overnight at 4°C (or 2 hours at 37°C). The plate contents were then removed and wells were blocked for 2 hours with 200µl of PBS/1%BSA. After the blocking step, the wells were washed five times with PBS/0.1% Tween 20™. 50µl of sera (either positive or negative for *T. cruzi* infection), diluted 1:50 in PBS/0.1% Tween 20/0.1%BSA was then added to each well and incubated for 30 minutes at room temperature. The plates were then washed again five times with PBS/0.1% Tween 20™.

The enzyme conjugate (horse radish peroxidase-Protein A, Zymed, San Francisco, CA) was then diluted 1:20,000 in PBS/0.1% Tween 20™/0.1%BSA, and 50µl of the diluted conjugate was added to each well and incubated for 30 minutes at room temperature. Following incubation the wells were again washed five times with PBS/0.1% Tween 20™. 100µl of the peroxidase substrate, tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added, undiluted, to each of the wells and incubated for 15 minutes. The reaction was stopped by the addition of 100µl of 1N H_2SO_4 to each well, and the plates were read at 450nm.

Figure 1 shows the reactivity of the recombinant rTcc6 (SEQ ID NO:39) as compared to that of *T. cruzi* lysate. Based on a cutoff of the mean of the negatives plus 3 standard deviations, 49 out of 50 serum samples were positive with lysate, and 34 out of 50 were positive with rTcc6. In a similar study (shown in Figure 2), the recombinant rTcc22 (SEQ ID NO:41) was found to have a sensitivity of 79.2% (38 out of 48 serum samples were positive). Comparative studies of the recombinant rTcc38 (SEQ ID NO:38) with *T. cruzi* lysate using similar criteria showed that 24/39 were positive compared with 39/39 for lysate (Figure 3). Tcc38 when tested with potentially cross reacting sera showed improved specificity over *T. cruzi* lysate.

The recombinant TcHi12 (SEQ ID NO:37) was also found to be immunoreactive (Figure 2) having a sensitivity of 62.5% (15/24).

Example 4

Serological Reactivity of *T. cruzi* Synthetic Peptide Antigens

This example illustrates the diagnostic properties of several of the peptides described in Example 2. These peptides were tested for reactivity with *T.cruzi* positive and negative sera and, in some cases, for cross reactivity with sera from patients with other, potentially cross reactive, diseases.

The first group of peptides included different reading frames to determine the most reactive repeat sequence. The peptides tested were TcLo1.1 (contained within SEQ ID NO:34), TcLo1.2 (contained within SEQ ID NO:35) and TcLo1.3 (contained within SEQ ID NO:36), representing reading frames 1, 2 and 3 of the DNA sequence provided in SEQ ID NO:18, and TcHi10.1 (SEQ ID NO:26) and

TcHi10.3 (SEQ ID NO:27) which represent two of the reading frames for the TcHi10 sequence (shown in SEQ ID NO:5). The data is shown in Figure 4. In the case of the TcLo frames, both the TcLo1.1 and 1.2 peptides were strongly reactive but the TcLo1.2 was superior in signal to noise when tested on sera from *T. cruzi* positive and negative individuals. TcLo1.3 had lower signal but also low background. In this study lysate detected 24/24 positives, TcLo1.1 detected 21/24, TcLo1.2 detected 23/24 and TcLo1.3 detected 15/24. In the same study, the two frames TcHi10.1 and 10.3 detected 19/24 and 14/24 positives respectively, but with lower signal than for TcLo1. Cross reactivity studies with these different reading frames demonstrate that TcLo1.2 has minimal cross reactivity with the sera tested (Figure 5) as compared to *T.cruzi* lysate.

As discussed in Example 2, overlapping peptides were also synthesized for rTcc22 to determine the active epitope. The peptides Tcc22-1, 1+ and 2 were tested with *T. cruzi* positive and negative sera. The results are shown in Figure 2. The Tcc22-1+ and Tcc22-2.1 peptides were more reactive than the Tcc22-1 peptide. In the first experiment, Tcc22-1 and Tcc22-1+ detected 29/48 and 36/48 positives as compared to the recombinant Tcc22 which detected 38/48 positives. In a subsequent experiment, Tcc22-2.1 was also shown to be reactive but with less signal than Tcc22-1+ at the same plate coating level.

A polypeptide having the TcHi15 frame 3 repeat sequence (SEQ ID NO:49) was also synthesized and tested in an ELISA assay using a coating level of 200 ng/well. A total of 48 *T. cruzi* positive sera and 26 negative sera were tested in order to determine the reactivity of this peptide sequence. In this study, the peptide had a sensitivity of 68.75% (detecting 33 out of 48 positives) and a specificity of 92.3% (24 out of 36 negatives), indicating that this polypeptide has potential significance in detecting *T. cruzi* infections. The results of this assay are presented in Table 1, below.

Table 1

Reactivity of TcHi15 Frame 3 Polypeptide with *T. cruzi*-Positive and Negative Sera

Sample ID	*T. cruzi* Status	OD 450	Sample ID	*T. cruzi* Status	OD 450
Tc011095-1	Positive	0.696	DL4-0106	Negative	0.167
Tc011095-2	Positive	0.699	DL4-0112	Negative	0.05
Tc011095-3	Positive	1.991	DL4-0127	Negative	0.240
Tc011095-4	Positive	3	DL4-0140	Negative	0.008
Tc011095-5	Positive	0.098	DL4-0145	Negative	0.107
Tc011095-6	Positive	0.238	DL4-0161	Negative	0.119
Tc011095-7	Positive	0.115	DL4-0162	Negative	1.187
Tc011095-8	Positive	0.156	DL4-0166	Negative	0.210
Tc011095-9	Positive	0.757	DL4-0167	Negative	0.131
Tc011095-10	Positive	1.147	DL4-0172	Negative	0.073

Sample ID	*T. cruzi* Status	OD 450	Sample ID	*T. cruzi* Status	OD 450
Tc011095-11	Positive	0.264	DL4-0175	Negative	0.117
Tc011095-12	Positive	1.7	DL4-0176	Negative	0.815
Tc011095-13	Positive	1.293	AT4-0013	Negative	0.100
Tc011095-14	Positive	0.242	AT4-0041	Negative	0.107
Tc011095-15	Positive	0.636	AT4-0062	Negative	0.28
Tc011095-16	Positive	0.44	AT4-0063	Negative	0.155
Tc011095-17	Positive	3	E4-0051	Negative	0.162
Tc011095-18	Positive	1.651	E4-0059	Negative	0.176
Tc011095-19	Positive	0.19	E4-0068	Negative	0.241
Tc011095-20	Positive	0.916	E4-0071	Negative	0.127
Tc011095-21	Positive	0.715	C4-0072	Negative	0.101
Tc011095-22	Positive	1.336	C4-0088	Negative	0.141
Tc011095-23	Positive	1.037	C4-0090	Negative	0.078
Tc011095-24	Positive	0.332	C4-0096	Negative	0.162
Tc011095-25	Positive	0.413	C4-0101	Negative	0.181
Tc011095-26	Positive	0.266	C4-0105	Negative	0.702
Tc011095-27	Positive	1.808			
Tc011095-28	Positive	0.238			
Tc011095-29	Positive	0.266			
Tc011095-30	Positive	1.563			
Tc011095-31	Positive	0.352	Sensitivity	33/48	68.75%
Tc011095-32	Positive	0.208	Specificity	24/26	92.30%
Tc011095-33	Positive	0.656	Mean Pos.	0.9188	
Tc011095-34	Positive	1.281	Std Dev Pos.	0.79	
Tc011095-35	Positive	0.907	Mean Neg.	0.1508	
Tc011095-36	Positive	0.429	Std Dev Neg.	0.06695	
Tc011095-37	Positive	0.454			
Tc011095-38	Positive	0.725			
Tc011095-39	Positive	0.703			
Tc0394-7	Positive	0.186			
Tc0394-8	Positive	1.06			
Tc0394-9	Positive	1.813			
Tc0394-10	Positive	0.131			
Tc0394-11	Positive	1.631			
Tc0394-12	Positive	0.613			
Tc0394-13	Positive	3			
Tc0394-14	Positive	0.268			
Tc0394-15	Positive	2.211			

Example 5

Serological Reactivity of Peptide Combinations

This example illustrates the diagnostic properties of several peptide combinations.

The TcLo1.2 peptide (contained within SEQ ID NO:35) was tested in combination with the synthetic peptide TcD and also the dual epitope peptides D/2 (which contains the TcD and the PEP-2 sequences) and D/E (which contains TcD and

TcE sequences). These combinations were compared with the individual peptides as well as the tripeptide 2/D/E, which contains TcD, TcE and PEP-2. The TcD sequence used was Ala Glu Pro Lys Ser Ala Glu Pro Lys Pro Ala Glu Pro Lys Ser (SEQ ID NO:53), the TcE sequence was Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Thr Ala Pro Ala (SEQ ID NO: 55), and the PEP2 sequence was Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala Ala Ala Gly Asp Lys Pro Ser Pro Phe Gly Gln Ala (SEQ ID NO: 57).

The data are shown in Figure 6. The results show that TcLo1.2 can augment the reactivity of TcD, D/2 and D/E, as summarized in Table 2.

Table 2

Sensitivity of Peptide Combinations in the Detection of *T. cruzi* Infection

Peptides	Number of Positives
TcD	62/67
TcE	50/67
PEP-2	66/67
TcLo1.2	61/67
TcD+TcLo1.2	66/67
D/2+TcLo1.2	67/67
D/E+TcLo1.2	67/67
2/D/E	67/67

These results demonstrate the use of *T. cruzi* antigens as described herein to enhance the serodiagnostic properties of other antigens.

Example 6

Serological Reactivity of TcE Repeat Sequences

This example illustrates the diagnostic properties of several TcE repeat sequences.

The repeat sequence region of the recombinant TcE contains several degeneracies, resulting in residues where an A (alanine), T (threonine) or I (isoleucine) can be present in the repeat sequence. In order to represent all degeneracies, the original sequence for the synthetic TcE peptide was made with an A, T and I in a single peptide containing three repeats (*see* Example 5). In order to further epitope map the repeat region and to determine the number of repeats required for serological activity, the following peptides were prepared as described in Example 2:

original TcE	KAAIAPAKAAAAPAKAATAPA (SEQ ID NO:55)
TcE(3A)	KAAAAPAKAAAAPAKAAAAPA (SEQ ID NO:58)
TcE(3T)	KAATAPAKAATAPAKAATAPA (SEQ ID NO:59)
TcE(3I)	KAAIAPAKAAIAPAKAAIAPA (SEQ ID NO:60)
TcE(2A)	KAAAAPAKAAAAPA (SEQ ID NO:61)
TcE(AT)	KAAAAPAKAATAPA (SEQ ID NO:62)

The serological reactivity of these peptides was then compared. A total of 24 positive and 21 negative sera were tested with each of the TcE variants as the solid phase in an ELISA assay performed as described in Example 3, using 25 ng/well of peptide. The reactivity of the different peptides is shown in Figure 7. The highest reactivity was seen with the 3-repeat peptide in which each repeat contained an A at the degenerate residue (TcE(3A)). This peptide displayed even higher reactivity than the original TcE sequence containing an A, T and I residue in the three repeats. The 3I and 3T variants by contrast were essentially negative with the *T. cruzi* positive samples tested. The sequence containing two repeats with A (TcE(2A)) was clearly less reactive than the 3A sequence and the two repeat sequence with an A and a T (TcE(AT)) was negative. Based on a cutoff of the mean of the negatives plus three standard deviations, the original TcE (A,T,I) detected 17 out of 24 positives and the 3A variant detected 19 out of 24 positives. It also appears that to obtain maximal serological activity at least three repeats are required.

Example 7

Serological Reactivity of Multi-epitope Peptide Combinations

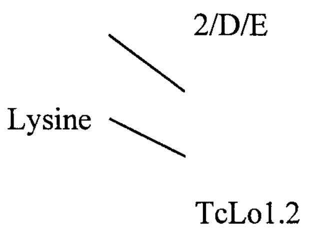
This example illustrates the diagnostic properties of several multi-epitope peptide combinations.

Two dipeptides PEP-2/TcLo1.2, which contains the PEP-2 (SEQ ID NO:57) and TcLo1.2 (SEQ ID NO:35) sequences, and TcD/TcE, which contains the TcD (SEQ ID NO:53) and TcE (SEQ ID NO:55) sequences, were synthesized as described above in Example 2. The reactivity of these two dipeptides with *T. cruzi* antibody-positive sera was compared to that of the tripeptide 2/D/E. ELISA's were performed as described in Example 3 using PEP-2/TcLo1.2 at 250ng/well and TcD/TcE at 50ng/well. The results of this study are shown in Figure 8. One *T. cruzi* positive serum found not to react with the tripeptide 2/D/E was used in screening for the

TcLo1.2 epitope. This serum was detected by the TcLo1.2 epitope and also by the dipeptide mix (PEP-2/TcLo1.2 together with TcD/TcE) as expected.

A tetrapeptide containing the four immunoreactive *T. cruzi* epitopes PEP-2, TcD, TcE and TcLo1.2 in a linear sequence, herein after referred to as 2/Lo/2E/D (SEQ ID NO:63) was synthesized as described in Example 2. This tetrapeptide was coated at 100ng/well and its reactivity with T. *cruzi* positive and negative sera was assayed as described in Example 3. The reactivity of the tetrapeptide 2/Lo/2E/D is shown in Figure 8. The one *T. cruzi* positive serum found not to react with the tripeptide 2/D/E was detected by the tetrapeptide as expected.

The four immunoreactive *T. cruzi* epitopes PEP-2, TcD, TcE and TcLo1.2 may also be linked into one reagent by the use of a 'branched' peptide originating from a lysine core residue. Orthogonal protection of the lysine, for example employing 9-Fluorenylmethoxycarbonyl (Fmoc) on the α-amino group and 1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) on the ε-amino group, is used to permit selective deprotection of one amino group in the presence of the other, thereby allowing the synthesis of the first peptide chain from either the α- or ε- group on the lysine. This first peptide chain is terminated with a protecting group that is not removed during the course of the synthesis of the second peptide chain. For example, a tert-Butoxy carbonyl (Boc) amino acid could be used with the Dde and Fmoc combination. The remaining lysine amino protecting group is then removed before a second amino acid chain is synthesized from the second amino moiety. For example, ε-Dde is removed with 20% hydrazine. Cleavage of the branched peptide from a solid support and removal of the N-α-Boc moiety is carried out using trifluoroacetic acid, following standard protocols. Using this approach two independent amino acid sequences can be built from a 'core' lysine residue, as shown below, thus allowing various combinations of TcD, TcE, PEP2, TcLo1.2, and other epitopes to be coupled to the core residue. Purification of the resulting peptide is performed as described in Example 2.



Example 8

Preparation of Multi-epitope Peptide Combinations using Recombinant DNA Technology

This example illustrates the preparation of a fusion polypeptide (hereinafter referred to as TcF) of the peptides PEP-2 (SEQ ID NO: 57), TcD (SEQ ID NO: 53), TcE (SEQ ID NO: 55) and TcLo1.2 (SEQ ID NO: 35) using recombinant DNA technology.

The TcF polypeptide fusion was created by synthesizing overlapping phosphorylated oligonucleotides, annealing the matched oligonucleotide pairs to create double stranded DNA, ligating the annealed pairs with vector DNA that was cut with appropriate enzymes, and transforming into suitable bacteria host strains (XL2, Stratagene, La Jolla, CA) for sequencing. Once the correct sequence was obtained, the construct was subcloned into a modified pET28 vector and transformed into BLR pLYS S (Novagen, Madison, WI) for expression and purification.

For construction of the PEP2-TcD fusion, the matched pairs of oligonucleotides PDM-95 (SEQ ID NO: 66) and PDM-98 (SEQ ID NO: 67); PDM-96 (SEQ ID NO: 68) and PDM-99 (SEQ ID NO: 69); and PDM-97 (SEQ ID NO: 70) and PDM-100 (SEQ ID NO: 71) were synthesized, kinased and annealed. These three pairs of annealed oligos were then ligated, digested with EcoRI (New England Biolabs, Beverly, MA) and cloned into a modified T vector construct with Eco72I and EcoRI. For construction of the PEP2-TcD-TcE fusion, the following matched pairs of oligonucleotides were synthesized, kinased and annealed: PDM-103 (SEQ ID NO: 72) and PDM-105 (SEQ ID NO: 73); and PDM-104 (SEQ ID NO: 74) and PDM-106 (SEQ ID NO: 75). These two pairs of annealed oligos were ligated into the pT7ΔL2PEP2-TcD construct cut with Eco47III (New England Biolabs) and EcoRI.

For construction of the PEP2-TcD-TcE-Lo1.2 fusion, the two matched pairs of oligonucleotides PDM-108 (SEQ ID NO: 76) and PDM-110 (SEQ ID NO: 77); and PDM-109 (SEQ ID NO: 78) and PDM-111 (SEQ ID NO: 79) were synthesized, kinased and annealed. These two pairs of annealed oligonucleotides were ligated with the pT7ΔL2PEP2-TcD-TcE construct which had been cut with EagI, treated for blunt ends with T4 DNA polymerase and then cut with EcoRI. Due to an internal EagI site in the pT7ΔL2 vector, a PCR reaction was performed with the ligation mix as template in order to clone the full length PEP2-TcD-TcE-Lo1.2 construct. PCR was accomplished with the primers PDM-101 and PDM-107 (SEQ ID NOS: 80 and 81, respectively) using the following conditions:

96 °C 2 minutes
96 °C 15 seconds 61 °C 15 seconds, 72 °C 1 minute X 40 cycles
72 °C 4 minutes.

Pful DNA polymerase (Stratagene, La Jolla, CA) was used for the PCR reaction. Folllowing PCR, the DNA was subjected to an ethanol precipitation, digested with SmaI and EcoRI, and ligated into a modified T vector construct which had been digested with Eco72I and EcoRI.

The resulting clone was digested with Hind III (Gibco BRL, Gaitherburg, MD) and Sph I (New England Biolabs), treated with T4 DNA polymerase for blunt ends and then religated in order to delete the vector's internal Eag I and Nsi I sites. This new clone was then digested with Eag I and Nsi I, treated with T4 DNA polymerase for blund ends, and religated in order to create a stop codon in frame at the end of the TcLo1.2 sequence. This clone was digested with NdeI and Eco RI, and subcloned into a modified pET28b vector cut with the same enzymes.

The resulting expression construct was transformed into BLR pLys *S E. coli* (Novagen) and grown overnight in LB broth with kanamycin (30 ug/ml, Sigma, St. Louis, MO) and chloramphenicol (34 ug/ml Sigma). The overnight culture (12 ml) was used to inoculate 500 ml of 2XYT with the same antibiotics and the culture was induced at an OD560 of 0.3-0.6 with IPTG to a final concentration of 1.0 mM. Four hours post-induction, the bacteria were harvested and sonicated in 20 mM Tris (8.0), 100 mM NaCl, 0.1% DOC, 20 ug/ml Leupeptin and 20 mM PMSF followed by centrifugation at 26,000 Xg. The fusion protein was found in the soluble supernatant after sonication. The supernantant was bound to a Pro-bond nickel resin column (Invitrogen, Carlsbad, CA). The column was washed with 50 ml of 20 mM Tris (8.0), 100 mM NaCl wash buffer and eluted with an increasing imidazole concentration. Specifically, the elutions were made with 50 mM, 100 mM and 500 mM imidazole in the 20 mM Tris (8.0), 100 mM NaCl wash buffer. The eluates containing the protein of interest were pooled and dialyzed against 10 mM Tris (8.0).

After dialysis, the protein was concentrated, sterile filtered and tested for endotoxins. Test results indicated a high level of endotoxin contamination. The sterile filtered protein was therefore purified over a High Q anion exchange column (Biorad, Hercules, CA), binding in 10 mM Tris (8.0) and eluting with a NaCl gradient up to 1 M in 10 mM Tris (8.0). The elutions containing the protein of interest were pooled and dialyzed against 10 mM Tris (8.0). After dialysis, the protein was reconcentrated, sterile filtered and a BCA assay (Pierce, Rockford, IL) was performed to determine protein concentration. The determined amino acid sequence of TcF is provided in SEQ ID NO: 82.

The reactivity of the recombinant fusion polypeptide TcF with sera from *T. cruzi* patients and from normal donors was examined by ELISA as described above. As shown in Figure 11, the reactivity of TcF was found to be very similar to that of the branched synthetic tetrapeptide 2/D/E/Lo1.2.

It is envisioned that the order of the peptides in the recombinant fusion polypeptide TcF could be altered without significantly changing the activity of the polypeptide. Also, the inclusion of a Gly-Cys-Gly linkage between the peptides may enhance solid phase binding without significantly affecting the activity of the polypeptide.

A fusion polypeptide (hereinafter referred to as TcF-2) of the peptides PEP-2 (SEQ ID NO: 57), TcD (SEQ ID NO: 53), TcE (SEQ ID NO: 55) and TcLo1.2 (SEQ ID NO: 35) with a glycine-cysteine-glycine amino acid spacer between each of the fused peptides was prepared by synthesizing overlapping phosphorylated oligos, annealing the matched oligo pairs to create double-stranded DNA, ligating the annealed pairs with vector DNA that was cut with appropriate enzymes and transformed into suitable bacterial host strains for sequencing (XL2, Stratagene). Once the correct sequence was obtained, the construct was subcloned into a modified pET28 vector and transformed into BLR pLYS S (Novagen, Madison, WI) for expression and purification.

More specifically, the PEP2-GCG-TcD fusion was constructed by synthesizing, kinasing and annealing the following matched pairs of oligos: PDM-95 (SEQ ID NO: 66) and PDM-98 (SEQ ID NO: 67); PDM-112 (SEQ ID NO: 83) and PDM-113 (SEQ ID NO: 84); and PDM-114 (SEQ ID NO: 85) and PDM-115 (SEQ ID NO: 86). The three pairs of annealed oligos were ligated, digested with EcoRI (New England Biolabs, Beverly, MA) and cloned into a modified pT7 blue construct with a His tag in frame when cut with Eco 72I and EcoRI. For construction of the PEP2-GCG-TcD-GCG-TcE fusion, the following matched pairs of oligos were synthesized, kinased and annealed: PDM-116 (SEQ ID NO: 87) and PDM-117 (SEQ ID NO: 88); and PDM-118 (SEQ ID NO: 89) and PDM-119 (SEQ ID NO: 90). These two pairs of matched oligos were then ligated into the above PEP2-GCG-TcD construct cut with Eco 47III (New England Labs.) and Eco RI.

The matched pairs of oligos PDM-120 (SEQ ID NO: 92) and PDM-121 (SEQ ID NO: 92), and PDM-122 (SEQ ID NO: 93) and PDM-123 (SEQ ID NO: 94) were synthesized, kinased and annealed, and then ligated with the above PEP2-GCG-TcD-GCG-TcE construct which had been cut with Eag I, treated for blunt ends with T4 DNA polymerase and then cut with Eco RI. This clone was then digested with Nde I

(Gibco BRL) and Eco RI, and subcloned into a modified pET28 vector cut with the same enzymes.

The expression construct was then transformed to BLR pLys S E. coli (Novagen) and grown overnight in LB broth with kanamycin (30 ug/ml, Sigma, St. Louis, MO) and chloramphenicol (34 ug/ml, Sigma). 12ml of the overnight culture was used to inoculate 500 ml of 2XYT with the same antibiotics and the culture was induced at an OD560 of 0.37 with IPTG to a final concentration of 1.0 mM. Four hours post-induction, the bacteria were harvested and sonicated in 20 mM Tris (8.0), 100 mM NaCl, 0.1% DOC, 20 ug.ml leupeptin, 20 mM PMSF followed by centrifugation at 26,000 Xg. The fusion protein was found in the soluble supernatant after sonication. The supernatant was then bound to Pro-bond nickel resin (Invitrogen, Carlsbad, CA). The column was washed with 50 ml of 20 mM Tris (8.0), 100 mM NaCl wash buffer and then eluted with an increasing imidazole concentration. The elutions were made with 50 mM, 100 mM and 500 mM imidazole in the 20 mM Tris (8.0), 100 mM NaCl wash buffer. The eluates containing the protein of interest were pooled and then dialyzed against 10 mM Tris (8.0).

After dialysis, the protein was taken over a High Q (Biorad, Hercules, CA) anion exchange column, binding in 10 mM Tris (8.0) and eluting with a NaCl gradient up to 1 M in 10 mM Tris (8.0). The elutions containing the protein of interest were pooled and dialyzed against 10 mM Tris (8.0). After dialysis, the protein was reconcentrated and sterile filtered, and a BCA assay (Pierce) was performed to determine protein concentration. The determined amino acid sequence for TcF-2 is provided in SEQ ID NO: 95).

Example 9

Comparison of the Serological Reactivity of TcHi29 and TcE

The antigen TcHi29 (SEQ ID NO:52) was shown to be a polymorph of the TcE repeat sequence. A TcHi29 peptide was synthesized that had the following sequence as compared to TcE.

TcE	KAAIAPAKAAAAPAKAATAPA (SEQ ID NO: 55)
TcHi29	KTAAPPAKTAAPPAKTAAPPA (SEQ ID NO: 64)

Figure 9 shows a comparison of the reactivity of these two related seqences with sera from *T. cruzi* positive patients as well as from other disease categories, as determined by ELISA using the procedure described above. The data indicate little or no cross reactivity with the other disease groups tested but the distribution of reactivity amongst the *T. cruzi* positive sera partially overlapped for the two peptides. Of the 53 consensus positive samples tested, TcE detected 31/53 and TcHi 29 36/53. Within this group TcE and TcHi29 both detected 24 of the same sera. TcE detected 7 positive sera not detected by TcHi29, which in turn detected 12 positive sera missed by TcE. A dipeptide, TcD/TcHi29, was also synthesized and used in combination with the PEP-2/TcLo1.2 dipeptide in ELISA (100ng/well TcD/TcHi29, 250ng/well PEP-2/TcLo1.2) and compared with the TcD/TcE plus PEP-2/TcLo1.2 dipeptide combination. As shown in Figure 10, the data indicates that the overall activity of the two mixes are similar for both the *T. cruzi* positive and negative populations studied and suggests that, in such peptide combinations, TcHi29 can be considered to be an alternative to TcE.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

1. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

2. The method of claim 1 wherein the biological sample is selected from the group consisting of blood, serum, plasma, saliva, cerebrospinal fluid and urine.

3. The method of claim 1 wherein the polypeptide is bound to a solid support.

4. The method of claim 3 wherein the solid support comprises nitrocellulose, latex or a plastic material.

5. The method of claim 3 wherein the step of detecting comprises:

(a) removing unbound sample from the solid support;

(b) adding a detection reagent to the solid support; and

(c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, and therefrom detecting *T. cruzi* infection in the biological sample.

6. The method of claim 5 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

7. The method of claim 6 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G, Protein A and lectins.

8. The method of claim 6 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

9. A polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:21, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

10. An isolated DNA sequence encoding a polypeptide according to claim 9.

11. A recombinant expression vector comprising a DNA sequence according to claim 10.

12. A host cell transformed or transfected with an expression vector according to claim 11.

13. The host cell of claim 12 wherein the host cell is selected from the group consisting of *E. coli*, yeast, insect cell lines and mammalian cell lines.

14. A diagnostic kit for detecting *T. cruzi* infection in a biological sample, comprising:

(a) a polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) a detection reagent.

15. The kit of claim 14 wherein the polypeptide is bound to a solid support.

16. The kit of claim 15 wherein the solid support comprises nitrocellulose, latex or a plastic material.

17. The kit of claim 14 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

18. The kit of claim 17 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G , Protein A and lectins.

19. The kit of claim 17 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

20. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting a biological sample with a monoclonal antibody that binds to an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) detecting in the biological sample the presence of *T. cruzi* parasites that bind to the monoclonal antibody, therefrom detecting *T. cruzi* infection in the biological sample.

21. The method of claim 20, wherein the monoclonal antibody is bound to a solid support.

22. The method of claim 21 wherein the step of detecting comprises:

(a) removing unbound sample from the solid support;

(b) adding a detection reagent to the solid support; and

(c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, therefrom detecting *T. cruzi* infection in the biological sample.

23. The method of claim 22 wherein the detection reagent comprises a reporter group coupled to an antibody.

24. A pharmaceutical composition comprising:

(a) a polypeptide, wherein the polypeptide comprises an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) a physiologically acceptable carrier.

25. A vaccine for stimulating the production of antibodies that bind to *T. cruzi*, comprising:

(a) a polypeptide, wherein the polypeptide comprises an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(b) an adjuvant.

26. A method for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a pharmaceutical composition according to claim 24.

27. A method for inducing protective immunity against Chagas' disease in a patient, comprising administering to a patient a vaccine according to claim 25.

28. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

29. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications;

(c) contacting the biological sample with a third polypeptide comprising an epitope of TcE, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(d) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

30. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcD, or a variant thereof that differs only in conservative substitutions and/or modifications;

(c) contacting the biological sample with a third polypeptide comprising an epitope of PEP-2, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(d) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

31. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a first polypeptide comprising an epitope of a *T. cruzi* antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second polypeptide comprising an epitope of TcE, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the biological sample the presence of antibodies that bind to one or more of said polypeptides, therefrom detecting *T. cruzi* infection in the biological sample.

32. The method of claim 31 wherein the second polypeptide comprises the amino acid sequence Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala (SEQ ID NO:56).

33. A combination polypeptide comprising two or more polypeptides according to claim 9.

34. A combination polypeptide comprising at least one epitope of a T. cruzi antigen having an amino acid sequence encoded by a nucleotide sequence recited in SEQ ID NO:1 - SEQ ID NO:22, or a variant of said antigen that differs only in conservative substitutions and/or modifications, and at least one epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications.

35. A combination polypeptide according to claim 34, wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:55-56.

36. A combination polypeptide according to claim 34, wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:53-54.

37. A combination polypeptide according to claim 34, wherein the epitope selected from the group consisting of epitopes of TcD, epitopes of TcE, epitopes of PEP-2 and variants thereof that differ only in conservative substitutions and/or modifications has an amino acid sequence recited in SEQ ID NO:57.

38. A combination polypeptide according to claim 34, wherein the polypeptide is prepared using recombinant DNA technology.

39. A combination polypeptide according to claim 34, wherein the polypeptide is prepared synthetically.

40. A combination polypeptide comprising an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 82 and 95.

41. A method for detecting *T. cruzi* infection in a biological sample, comprising:

(a) contacting the biological sample with a combination polypeptide according to any one of claims 33-40; and

(b) detecting in the biological sample the presence of antibodies that bind to the combination polypeptide, therefrom detecting *T. cruzi* infection in the biological sample.

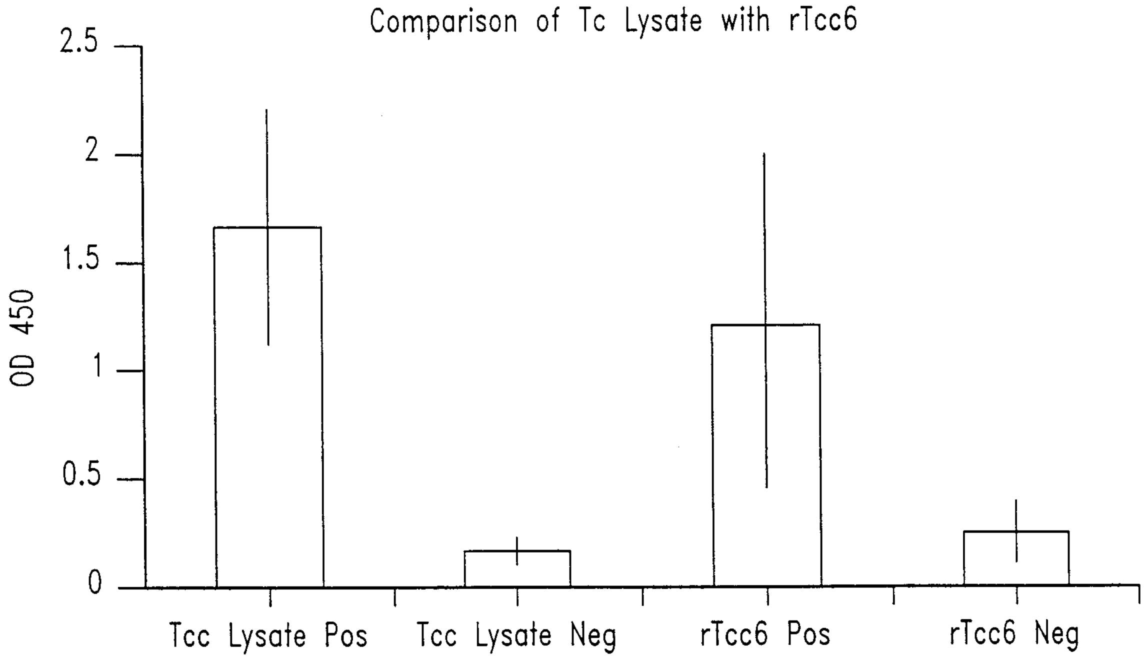

Comparison of Tc Lysate with rTcc6
OD 450
2.5
2
1.5
1
0.5
0
Tcc Lysate Pos
Tcc Lysate Neg
rTcc6 Pos
rTcc6 Neg

Fig. 1

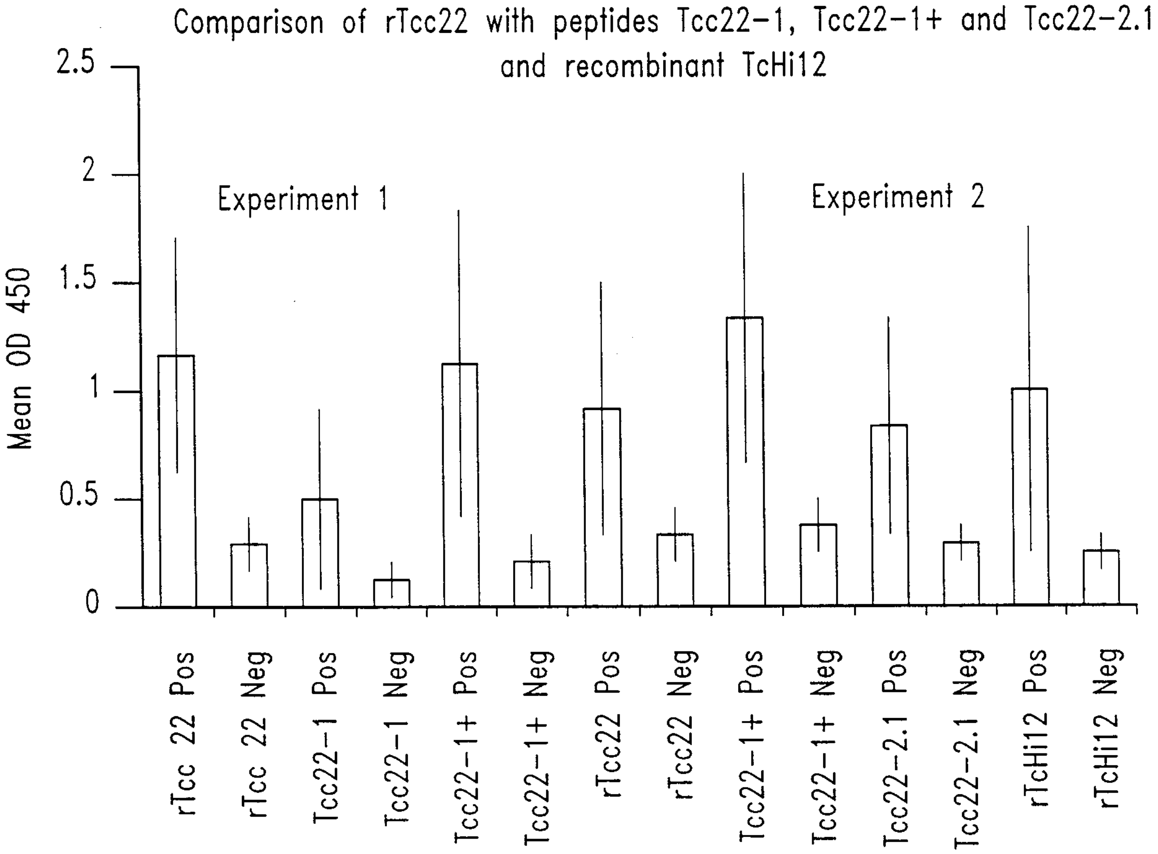

Comparison of rTcc22 with peptides Tcc22-1, Tcc22-1+ and Tcc22-2.1 and recombinant TcHi12
Mean OD 450
2.5
2
1.5
1
0.5
0
Experiment 1
Experiment 2
rTcc 22 Pos
rTcc 22 Neg
Tcc22-1 Pos
Tcc22-1 Neg
Tcc22-1+ Pos
Tcc22-1+ Neg
rTcc22 Pos
rTcc22 Neg
Tcc22-1+ Pos
Tcc22-1+ Neg
Tcc22-2.1 Pos
Tcc22-2.1 Neg
rTcHi12 Pos
rTcHi12 Neg

Fig. 2

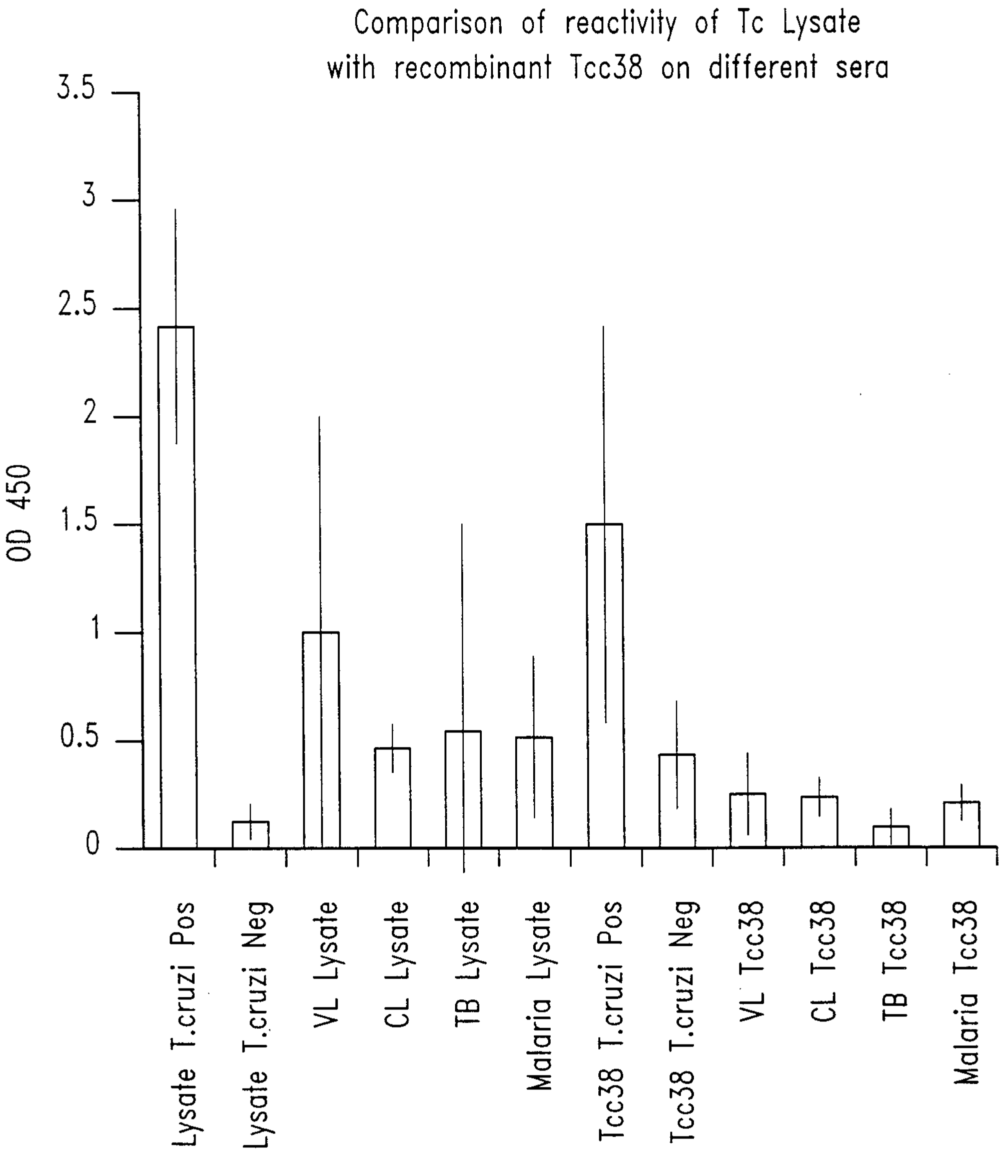

Comparison of reactivity of Tc Lysate
with recombinant Tcc38 on different sera
OD 450
3.5
3
2.5
2
1.5
1
0.5
0
Lysate T.cruzi Pos
Lysate T.cruzi Neg
VL Lysate
CL Lysate
TB Lysate
Malaria Lysate
Tcc38 T.cruzi Pos
Tcc38 T.cruzi Neg
VL Tcc38
CL Tcc38
TB Tcc38
Malaria Tcc38

Fig. 3

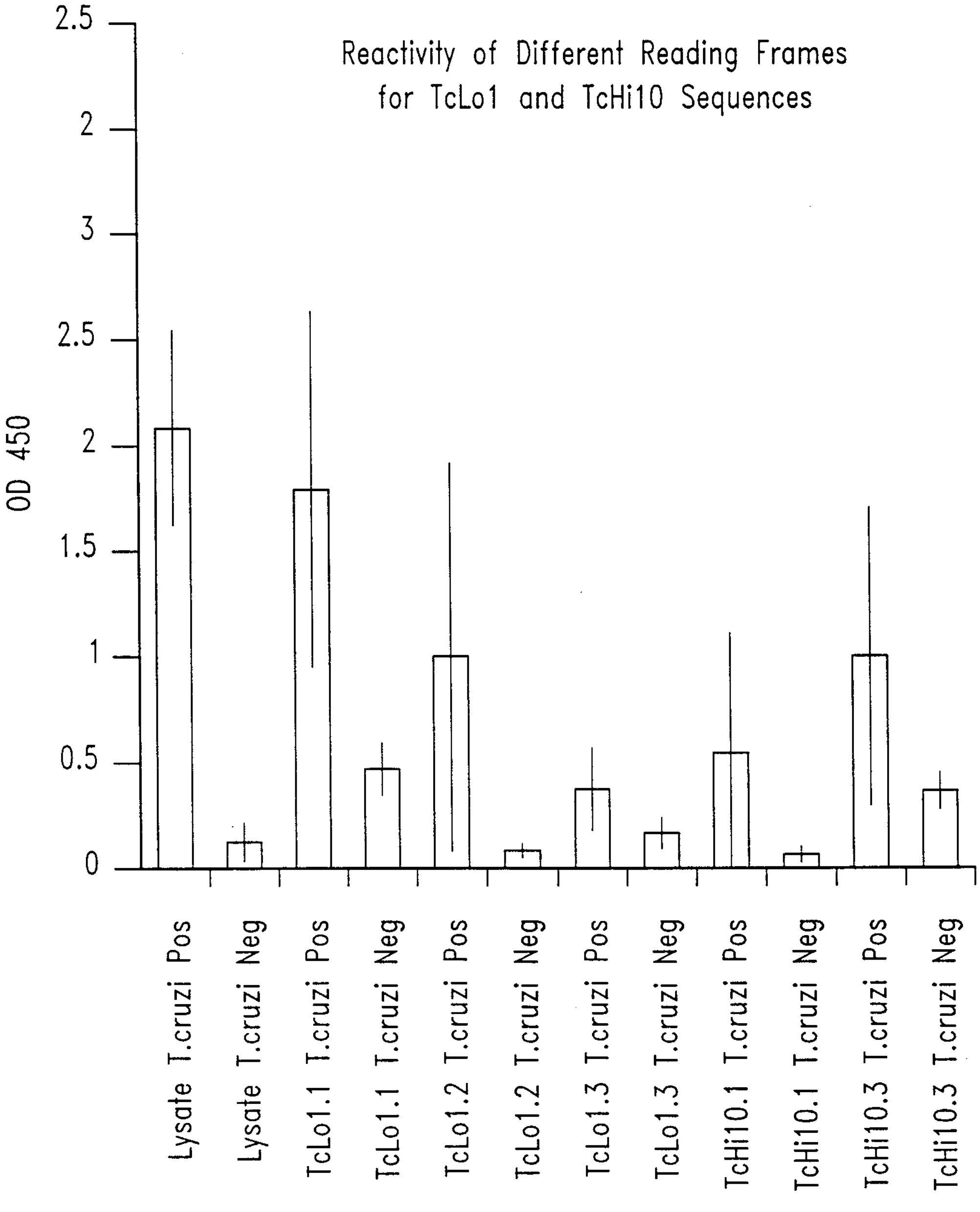

Reactivity of Different Reading Frames
for TcLo1 and TcHi10 Sequences
OD 450
2.5
2
3
2.5
2
1.5
1
0.5
0
Lysate T.cruzi Pos
Lysate T.cruzi Neg
TcLo1.1 T.cruzi Pos
TcLo1.1 T.cruzi Neg
TcLo1.2 T.cruzi Pos
TcLo1.2 T.cruzi Neg
TcLo1.3 T.cruzi Pos
TcLo1.3 T.cruzi Neg
TcHi10.1 T.cruzi Pos
TcHi10.1 T.cruzi Neg
TcHi10.3 T.cruzi Pos
TcHi10.3 T.cruzi Neg

Fig. 4

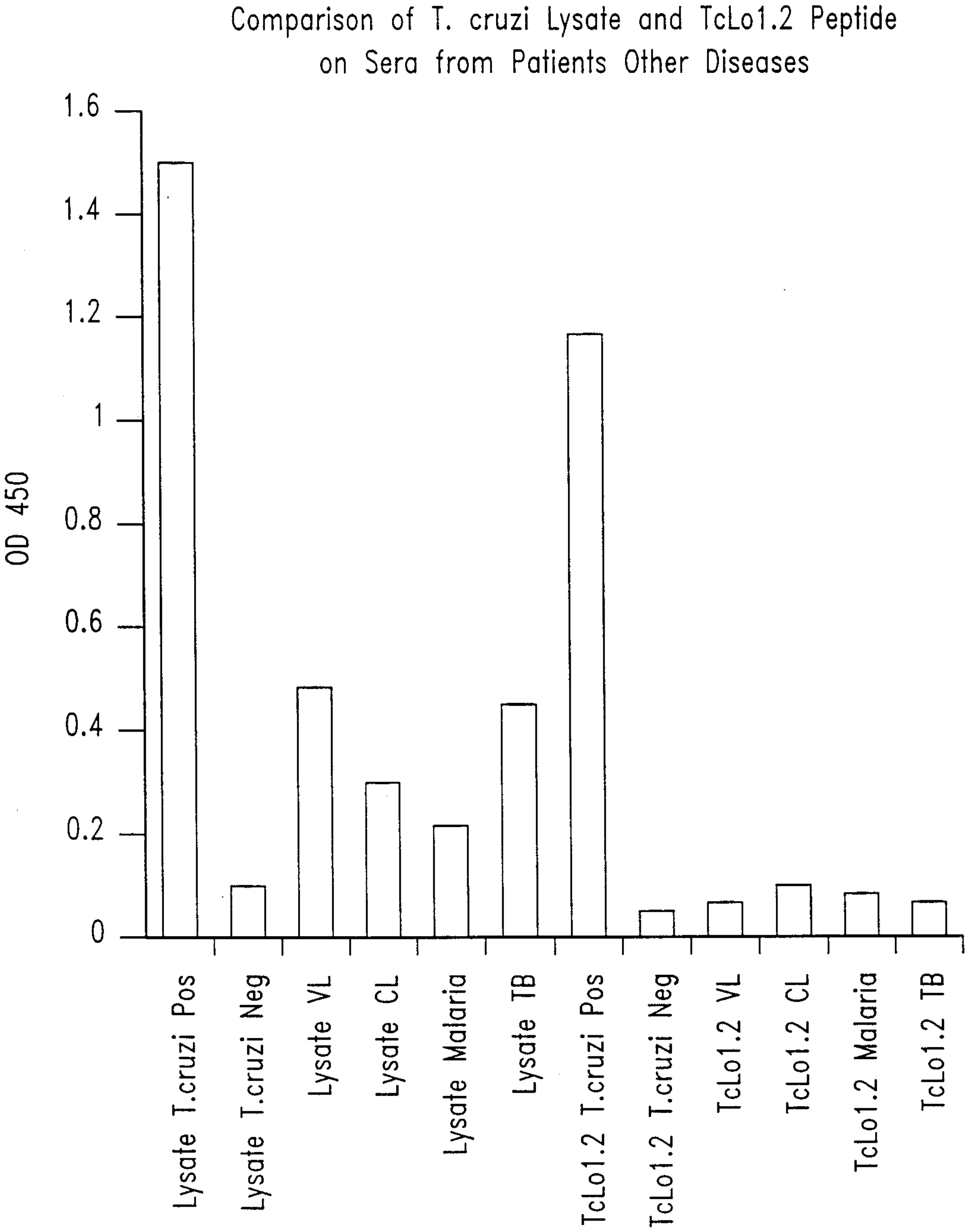

Comparison of T. cruzi Lysate and TcLo1.2 Peptide
on Sera from Patients Other Diseases
OD 450
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Lysate T.cruzi Pos
Lysate T.cruzi Neg
Lysate VL
Lysate CL
Lysate Malaria
Lysate TB
TcLo1.2 T.cruzi Pos
TcLo1.2 T.cruzi Neg
TcLo1.2 VL
TcLo1.2 CL
TcLo1.2 Malaria
TcLo1.2 TB

Fig. 5

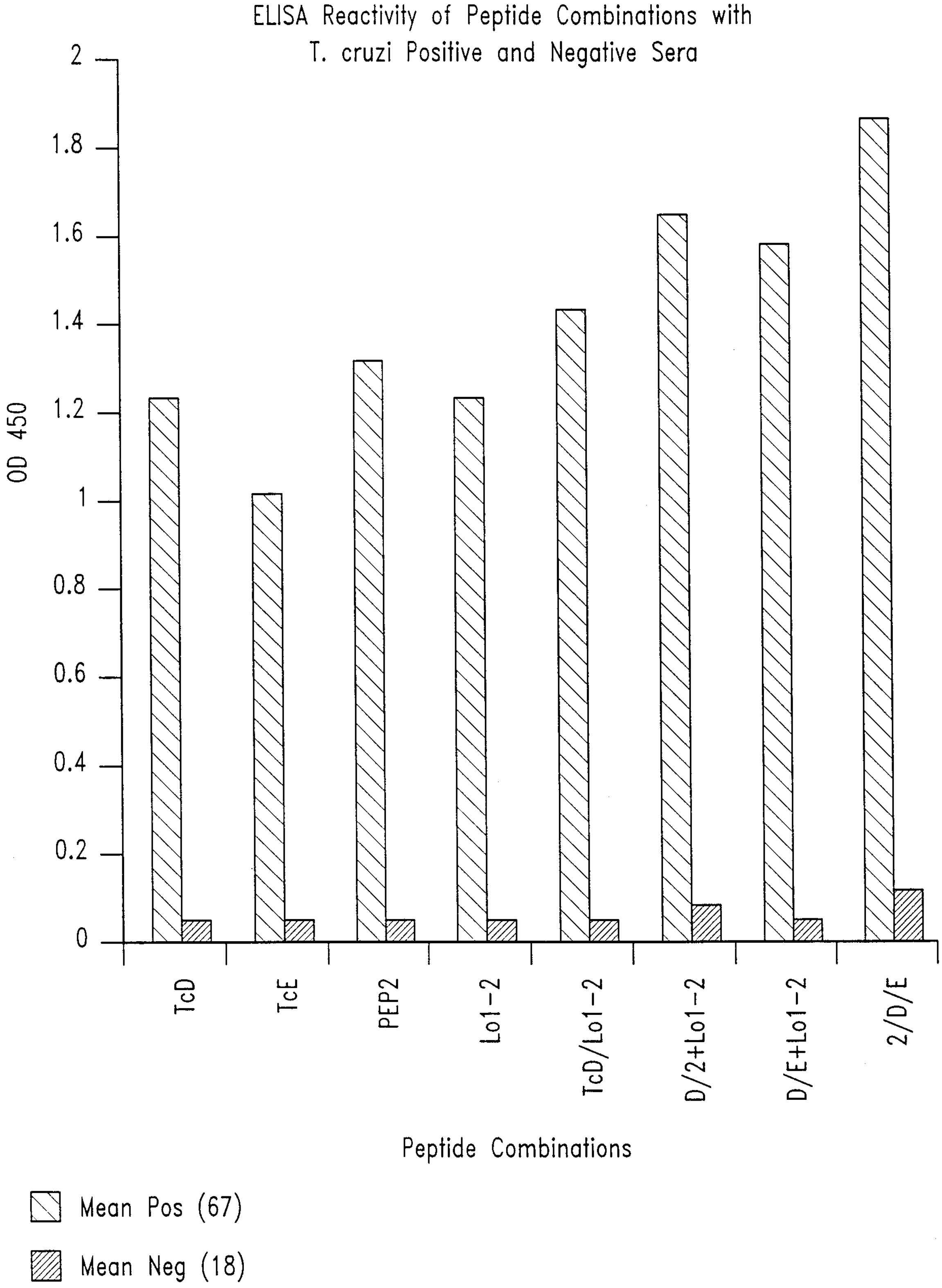

ELISA Reactivity of Peptide Combinations with T. cruzi Positive and Negative Sera
OD 450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
TcD
TcE
PEP2
Lo1-2
TcD/Lo1-2
D/2+Lo1-2
D/E+Lo1-2
2/D/E
Peptide Combinations
Mean Pos (67)
Mean Neg (18)

Fig. 6

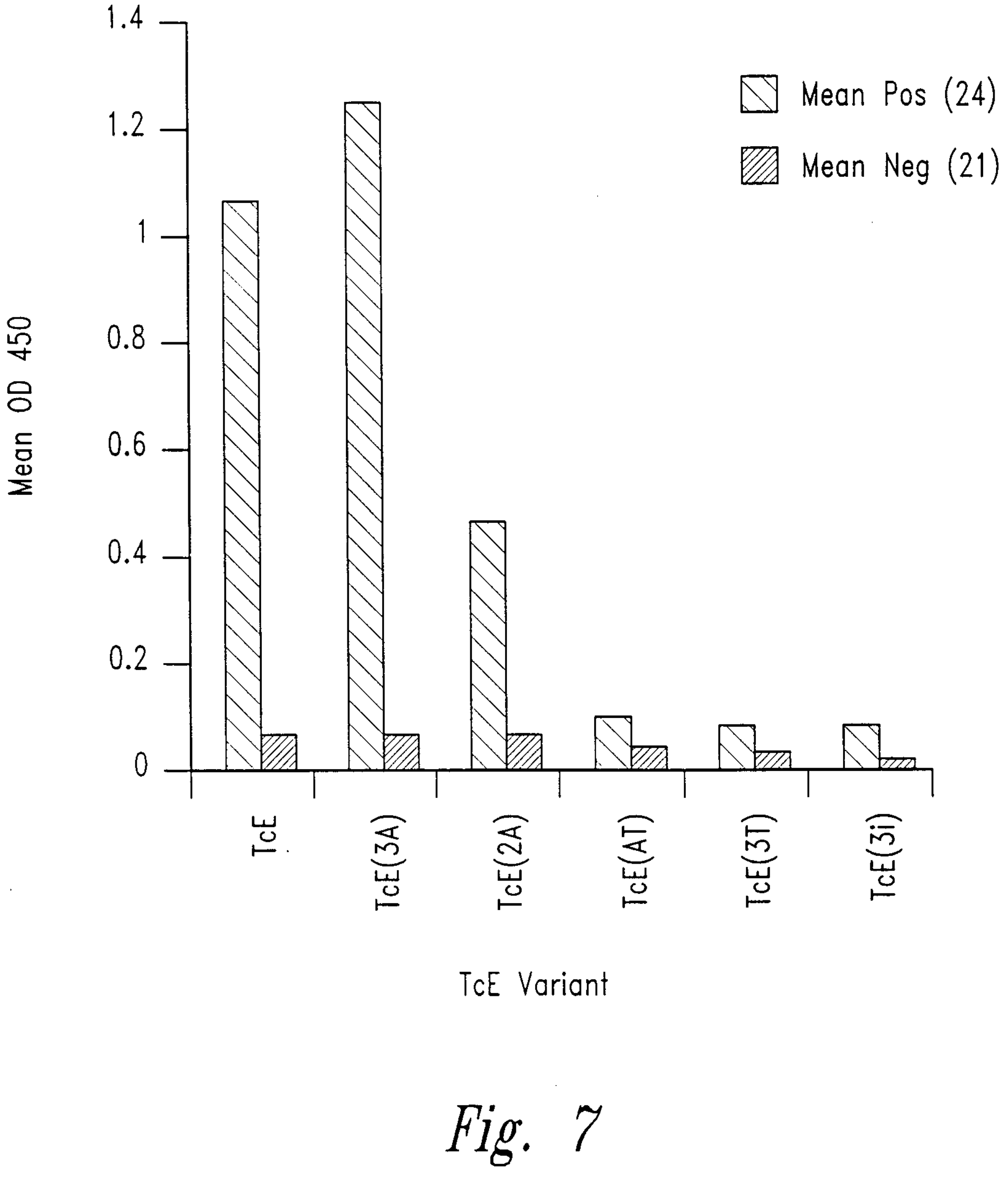

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mean OD 450
Mean Pos (24)
Mean Neg (21)
TcE
TcE(3A)
TcE(2A)
TcE(AT)
TcE(3T)
TcE(3i)
TcE Variant

Fig. 7

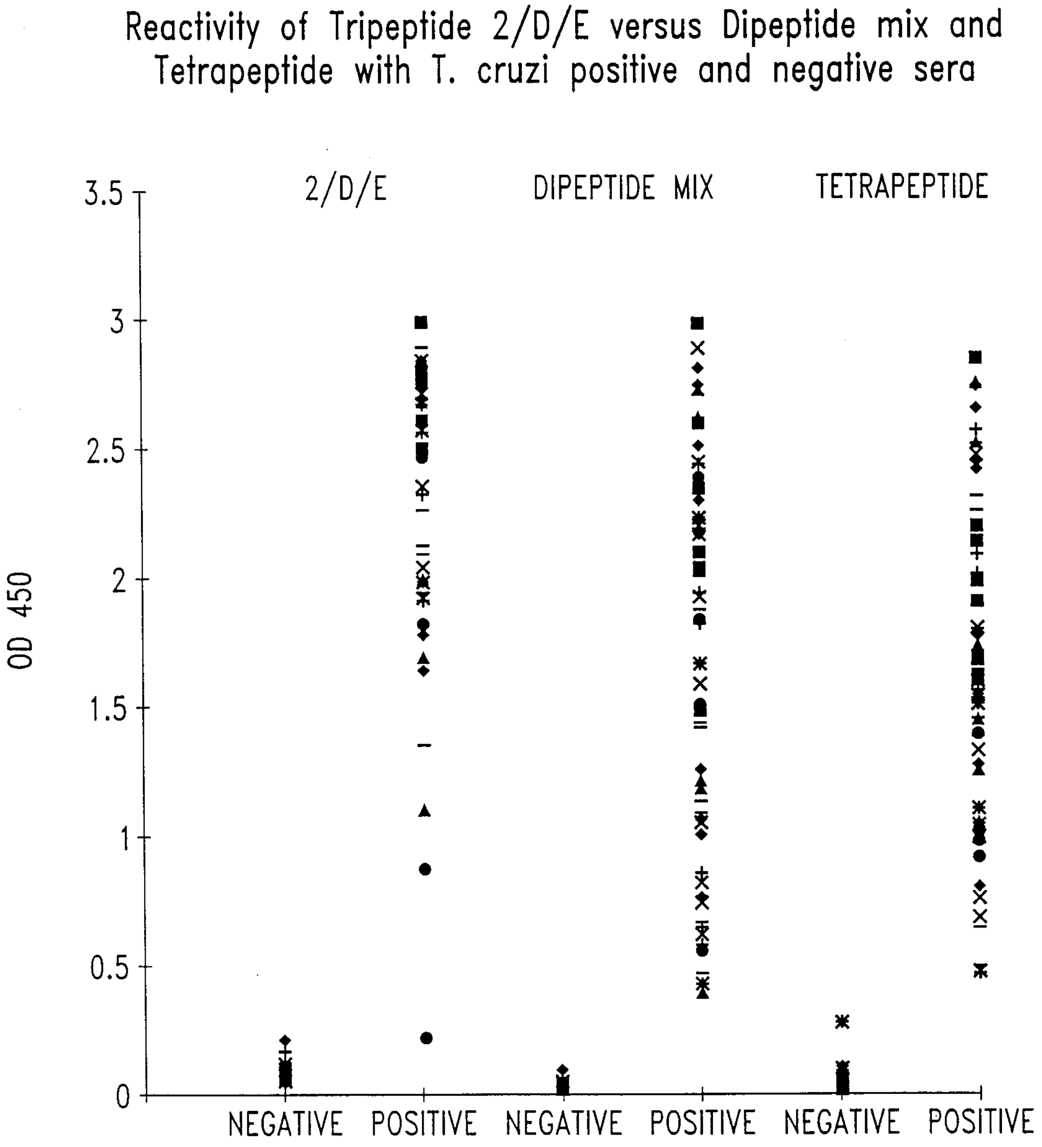

Reactivity of Tripeptide 2/D/E versus Dipeptide mix and Tetrapeptide with T. cruzi positive and negative sera
2/D/E
DIPEPTIDE MIX
TETRAPEPTIDE
OD 450
3.5
3
2.5
2
1.5
1
0.5
0
NEGATIVE
POSITIVE
NEGATIVE
POSITIVE
NEGATIVE
POSITIVE

Fig. 8

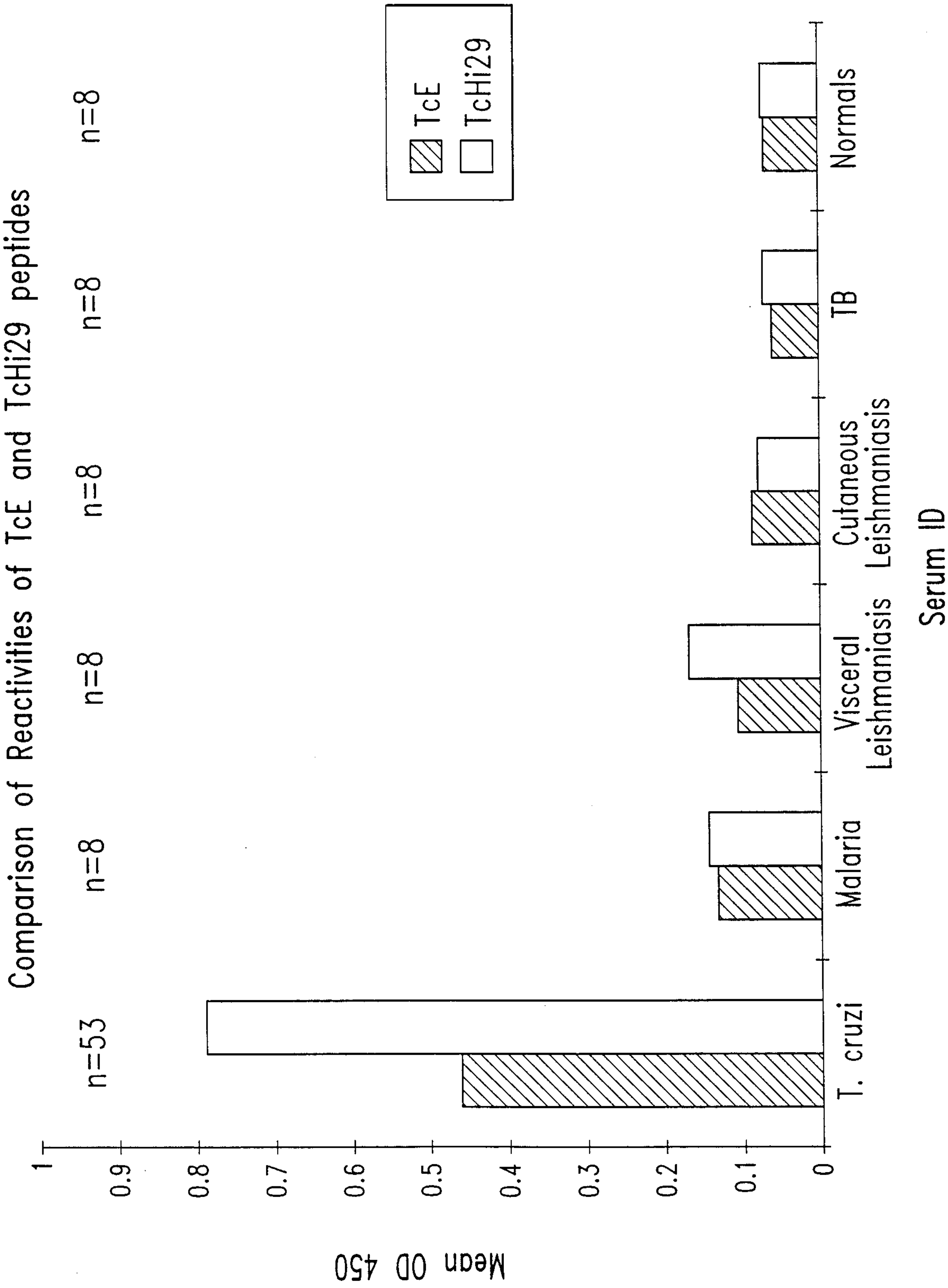

Comparison of Reactivities of TcE and TcHi29 peptides
n=53
n=8
n=8
n=8
n=8
n=8
▨ TcE
☐ TcHi29
Mean OD 450
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
T. cruzi
Malaria
Visceral Leishmaniasis
Cutaneous Leishmaniasis
TB
Normals
Serum ID

Fig. 9

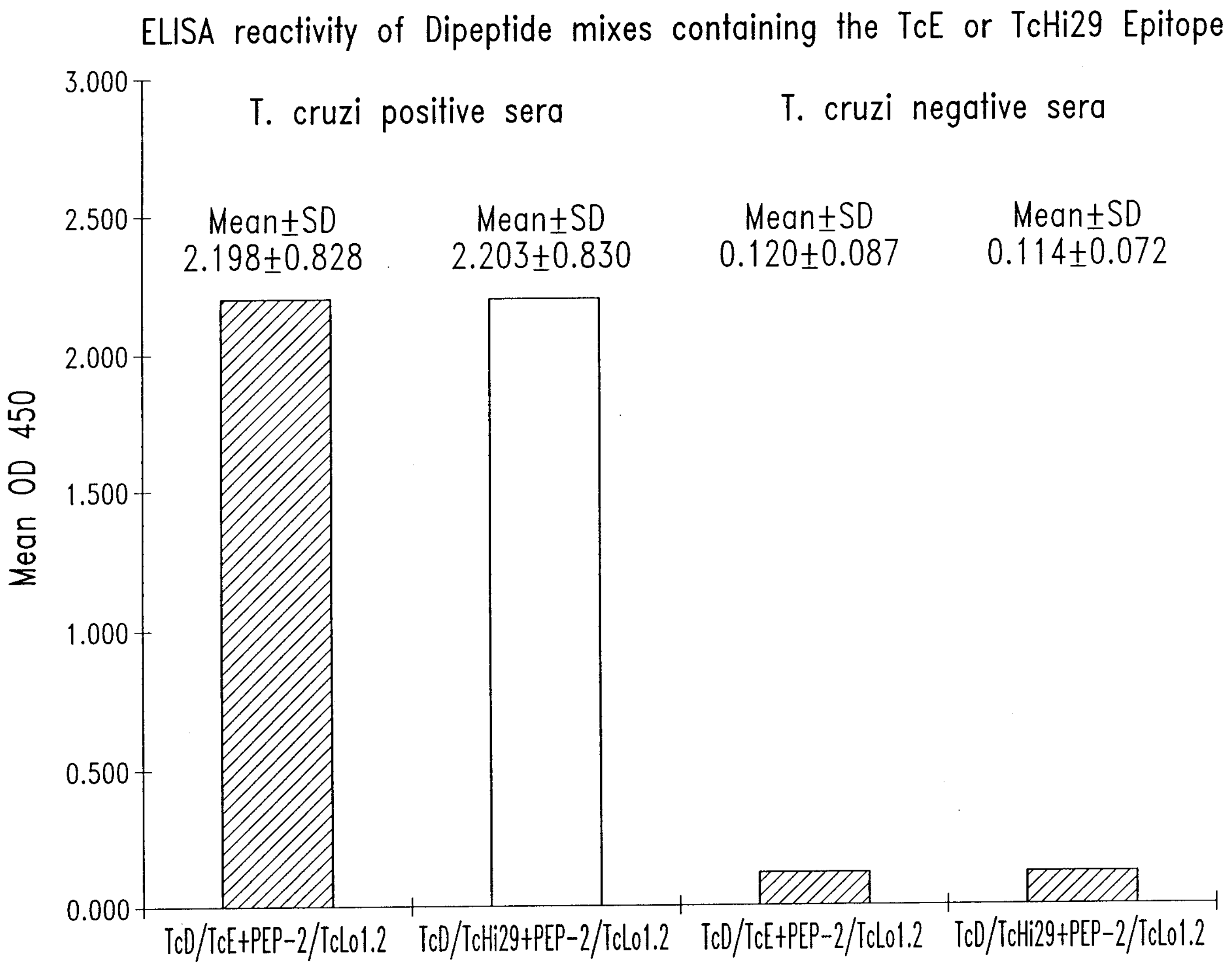

ELISA reactivity of Dipeptide mixes containing the TcE or TcHi29 Epitope
Mean OD 450
3.000
2.500
2.000
1.500
1.000
0.500
0.000
T. cruzi positive sera
T. cruzi negative sera
Mean±SD
2.198±0.828
Mean±SD
2.203±0.830
Mean±SD
0.120±0.087
Mean±SD
0.114±0.072
TcD/TcE+PEP-2/TcLo1.2
TcD/TcHi29+PEP-2/TcLo1.2
TcD/TcE+PEP-2/TcLo1.2
TcD/TcHi29+PEP-2/TcLo1.2

Fig. 10

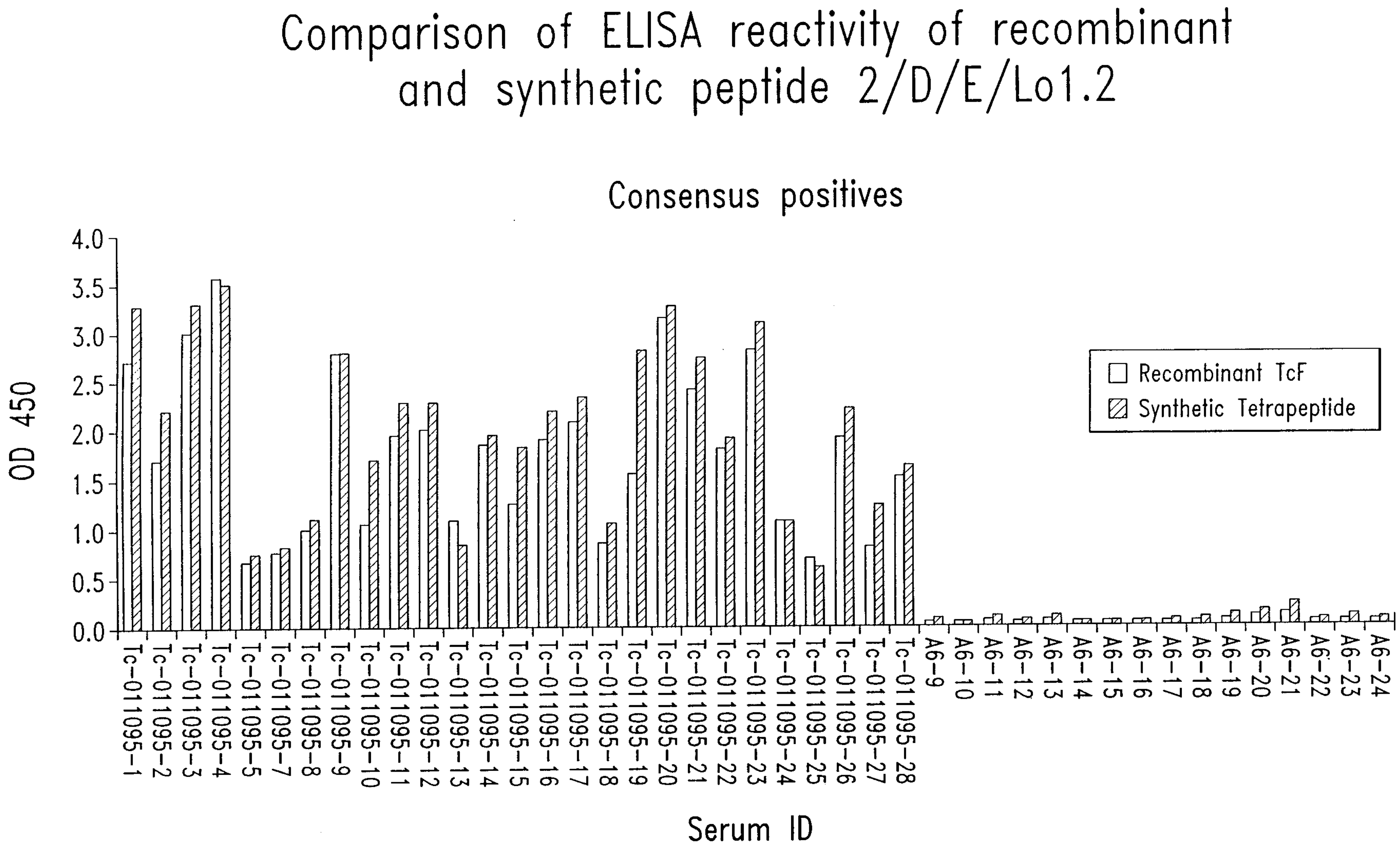

Comparison of ELISA reactivity of recombinant and synthetic peptide 2/D/E/Lo1.2
Consensus positives
OD 450
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Recombinant TcF
Synthetic Tetrapeptide
Tc-011095-1
Tc-011095-2
Tc-011095-3
Tc-011095-4
Tc-011095-5
Tc-011095-7
Tc-011095-8
Tc-011095-9
Tc-011095-10
Tc-011095-11
Tc-011095-12
Tc-011095-13
Tc-011095-14
Tc-011095-15
Tc-011095-16
Tc-011095-17
Tc-011095-18
Tc-011095-19
Tc-011095-20
Tc-011095-21
Tc-011095-22
Tc-011095-23
Tc-011095-24
Tc-011095-25
Tc-011095-26
Tc-011095-27
Tc-011095-28
A6-9
A6-10
A6-11
A6-12
A6-13
A6-14
A6-15
A6-16
A6-17
A6-18
A6-19
A6-20
A6-21
A6-22
A6-23
A6-24
Serum ID

Fig. 11

INTERNATIONAL SEARCH REPORT

Internationa plication No
PCT/US 00/04815

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/569 C07K14/44

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K

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

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

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 18475 A (CORIXA CORP) 22 May 1997 (1997-05-22) the whole document example 7 ---	1-39,41 40
A	BASTOS, C. ET AL.: "Use of recombinant peptide antigens of Trypanosoma cruzi in a diagnostic test for Charga's disease" MEMORIAS DO INSTITUTO OSWALDO CRUZ, vol. 93, no. 2, November 1998 (1998-11), pages 286-287, XP000925549 abstract --- -/--	1-41

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search 25 July 2000	Date of mailing of the international search report 04/08/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Gundlach, B

1

International ›lication No
PCT/US 00/04815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BASTOS, C. ET AL.: "Use of recombinant peptide antigens of Trypanosoma cruzi in a diagnostic test for Charga's disease" MEMÓRIAS DO INSTITUTO OSWALDO CRUZ, vol. 93, no. 2, November 1998 (1998-11), pages 234-235, XP000925550 abstract ---	1-41
X	WO 93 16199 A (REED STEVEN G) 19 August 1993 (1993-08-19) the whole document ---	1
X	WO 96 29605 A (CORIXA CORP) 26 September 1996 (1996-09-26) the whole document ---	1
P,X	WO 99 31246 A (CORIXA CORP) 24 June 1999 (1999-06-24) the whole document ---	1
P,A	HOUGHTON, R.L. ET AL.: "Multiepitope Synthetic Peptide and Recombinant Protein for the Detection of Antibodies to Trypanosoma cruzi in Patients with Treated or Untreated Chaga's Disease" THE JOURNAL OF INFECTIOUS DISEASES, vol. 181, no. 1, January 2000 (2000-01), pages 325-330, XP000925537 page 325, column 2, paragraph 2 -page 329, column 2 -----	1-41

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internationa plication No
PCT/US 00/04815

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9718475 A	22-05-1997	US 5916572 A	29-06-1999
		AU 1056897 A	05-06-1997
		EP 0874992 A	04-11-1998
		JP 2000503111 T	14-03-2000
WO 9316199 A	19-08-1993	US 5304371 A	19-04-1994
		BR 9305995 A	11-03-1997
		CA 2129747 A	15-08-1993
		DE 69319909 D	27-08-1998
		EP 0649475 A	26-04-1995
		ES 2118939 T	01-10-1998
		MX 9300787 A	01-11-1993
		US 5413912 A	09-05-1995
WO 9629605 A	26-09-1996	US 5756662 A	26-05-1998
		AU 5362696 A	08-10-1996
		BR 9607531 A	06-01-1998
		CA 2215104 A	26-09-1996
		EP 0815450 A	07-01-1998
		JP 11502923 T	09-03-1999
		US 5942403 A	24-08-1999
WO 9931246 A	24-06-1999	AU 1805799 A	05-07-1999