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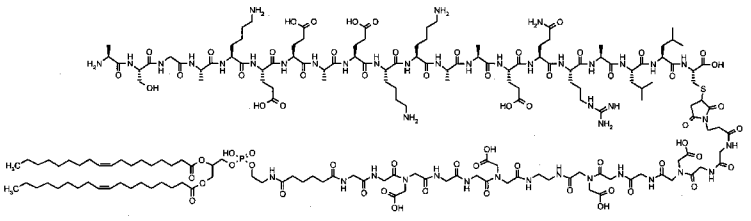


FIGURE 1

(57) Abstract: Methods for the diagnosis of infection with *Treponema palladium* using a peptide-spacer-lipid construct are disclosed, where the spacer covalently links the peptide to the lipid. The peptide is a peptide comprising the sequence AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu. The lipid is selected from a diacyl- or dialkyl- glycerolipid including glycerophospholipids. The method comprises contacting a sample of plasma or serum of a subject with a suspension of cells modified to incorporate a peptide-lipid construct to form a mixture, incubation of the mixture to allow agglutination and determining the degree of agglutination of the cells in the mixture. A particular construct is shown in figure 1.

WO 2010/143983 A1

K01.102WO I

ASSAYS FOR SEROLOGICAL DETECTION OF SYPHILIS

FIELD OF INVENTION

The invention relates to methods for the diagnosis of infection with *Treponema palladium*. In particular, the invention relates to methods for the diagnosis of infection with *Treponema palladium* in donors of blood intended for transfusion.

BACKGROUND ART

The risk of transfusion transmitted infections (TTIs) requires the testing of donor units of blood for the presence of pathogens. The testing methods employed need to be sensitive, specific and cost effective. The requirement for cost effectiveness is of particular importance given that many TTIs are prevalent in economically disadvantaged regions of the world.

A pathogen of particular concern in transfusion medicine is *Treponema palladium*. This organism is the causal agent of syphilis. Research has been performed to identify those antigens of *Treponema palladium* that may be responsible for eliciting an immunogenic response. The presence of antibody in the sera of donors is indicative of infection.

Known assays for the serological diagnosis of syphilis include the rapid plasma reagin (RPR) card test (Becton Dickinson Microbiology Systems, Cockeysville, MD) and the solid phase erythrocyte adherence (SPEA) assay CAPTURE-S™ (Immucor, Inc., Norcross, GA) (Stone *et al* (1997)).

These two assays compare favourably in terms of sensitivity and specificity. The solid phase erythrocyte adherence assay provides additional benefits of ease of use, accommodation of high-volume testing, and the potential for automation, e.g. the GALILEO ECHO™ platform (Immucor, Inc., Norcross, GA).

The specification accompanying international application no. PCT/NZ2008/000239 (publ. no. WO 2009/035347) describes a

diagnostic method based on the use of peptide-lipid constructs that is adaptable for use on existing tube serology platforms. Additional peptide-lipid constructs suitable for use in the diagnostic method disclosed are described in the specification accompanying international application no. PCT/NZ2008/000266 (publ. no. WO 2009/048343).

It is an object of the invention to provide an improved assay for serological diagnosis of infection with *Treponema palladium*.

It is an object of the invention to provide an improved assay for the detection of antibodies in donated blood that are indicative of the donor being infected with *Treponema palladium*.

These objects are to be read disjunctively with the object to at least provide a useful choice.

STATEMENTS OF INVENTION

In a **first** aspect the invention provides a method for determining the likelihood of infection of a subject with *Treponema palladium* comprising the steps of:

- Contacting a sample of the plasma or serum of the subject with a suspension of cells modified to incorporate a peptide-lipid construct of the structure F-S-L or L-S-F to provide a mixture;
- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and
- Determining the degree of agglutination of the cells in the mixture;

where:

F is a peptide comprising the sequence:

AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO: 3);

S is a spacer covalently linking F to L;

K01.102WO I

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

the degree of agglutination indicates the likelihood of infection of the subject.

Preferably, the method includes prior to determining the degree of agglutination of the cells of the mixture the intermediate step of:

- Adding an anti-subject globulin antibody to the mixture.

Preferably, the subject is a human.

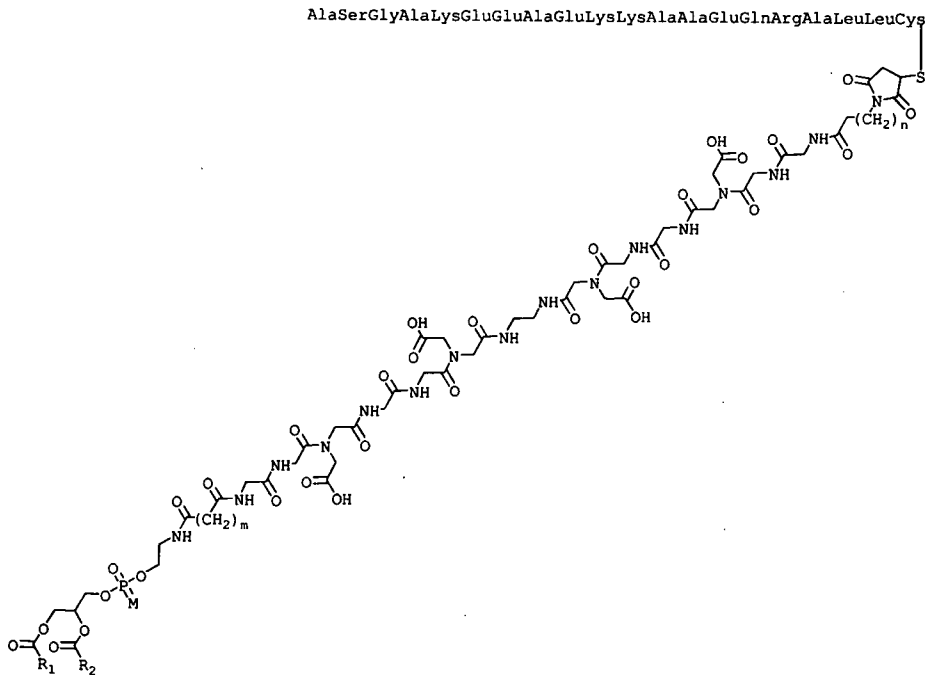
Preferably, the cells are RBCs.

Preferably, the plasma or serum of a subject is obtained from a donated sample of blood.

Preferably, the anti-subject globulin antibody is anti-human globulin (AHG) antibody.

S is selected to provide a construct that is water soluble.

Preferably, the peptide-lipid construct is of the structure:



K01.102WO I

where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids *trans*-3-hexadecenoic acid, *cis*-5-hexadecenoic acid, *cis*-7-hexadecenoic acid, *cis*-9-hexadecenoic acid, *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid, *trans*-9-octadecenoic acid, *trans*-11-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-11-eicosenoic acid or *cis*-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH_4^+.

Preferably, m is the integer 5 and n is the integer 2.

In an embodiment of the first aspect the invention provides a method for determining the likelihood of infection of a human subject with *Treponema palladium* comprising the steps of:

- Contacting a sample of the plasma or serum of the subject with a suspension of RBCs modified to incorporate the peptide-lipid construct designated FSL-SYPH3;
- Incubating the contacted sample of the plasma or serum and the suspension of RBCs for a time and at a temperature sufficient to allow binding of antibodies present in the sample to the RBCs;
- Preparing a suspension of washed RBCs;
- Adding an amount of anti-human globulin (AHG) antibody to the suspension to provide a mixture;
- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and
- Determining the degree of agglutination of the cells in the mixture to indicate the likelihood of infection.

K01.102WO I

In a **second** aspect the invention provides a method of testing donated blood for the presence of antibodies indicative of the donor being infected with *Treponema palladium* comprising the steps of:

- Contacting a sample of plasma or serum of the donated blood with an immobilized layer of modified RBCs;
- Incubating the contacted sample of plasma or serum and the immobilized layer of modified RBCs for a time and at a temperature sufficient to allow binding of the antibodies;
- Washing the immobilized layer of modified RBCs to remove the sample of plasma or serum;
- Contacting the immobilized layer of modified RBCs with a suspension of anti-immunoglobulin coated indicator cells;
- Incubating the immobilized layer of modified RBCs and the suspension of anti-immunoglobulin coated indicator cells for a time and at a temperature sufficient to allow binding of the anti-immunoglobulin coated indicator cells to the immobilized layer of modified RBCs; and
- Determining the degree of adherence of the anti-immunoglobulin coated indicator cells to the immobilized layer of modified RBCs,

where the modified RBCs have been modified to incorporate a peptide-lipid construct of the structure F-S-L where:

F is a peptide comprising the sequence:

AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO: 3);

S is a spacer covalently linking F to L;

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

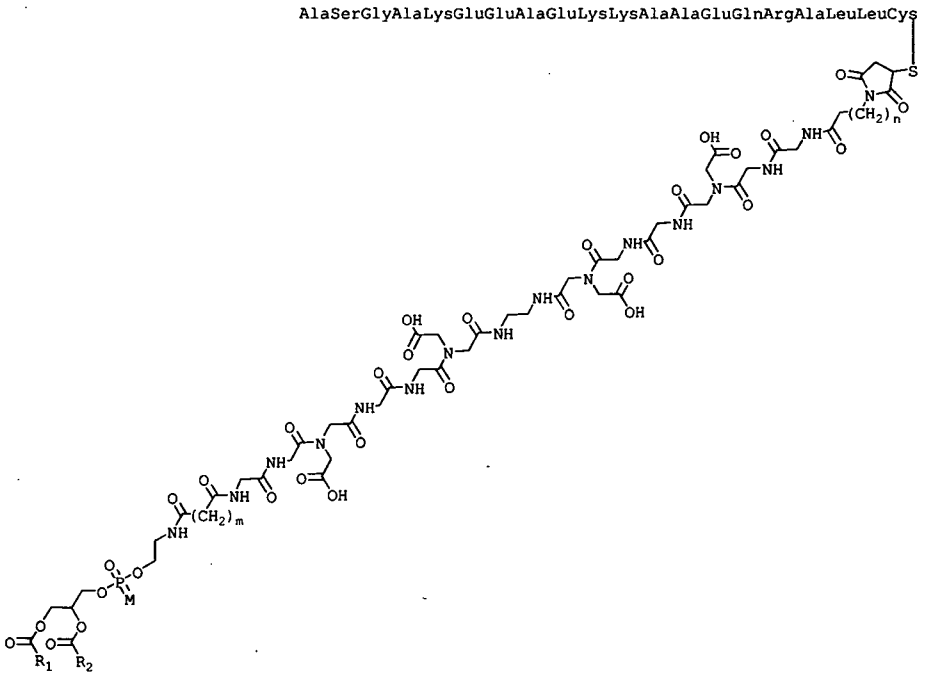
the degree of adherence is indicative of the likelihood of the antibodies being present.

K01.102WO I

Preferably, the determining the degree of adherence of the anti-globulin coated indicator cells to the immobilized layer of modified RBCs is by centrifugation to pellet unbound anti-globulin coated indicator cells.

S is selected to provide a construct that is water soluble.

Preferably, the peptide-lipid construct is of the structure:



where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids *trans*-3-hexadecenoic acid, *cis*-5-hexadecenoic acid, *cis*-7-hexadecenoic acid, *cis*-9-hexadecenoic acid, *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid, *trans*-9-octadecenoic acid, *trans*-11-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-11-eicosenoic acid or *cis*-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH_4^+.

K01.102WO I

Preferably, m is the integer 5 and n is the integer 2.

In a **third** aspect the invention provides a water soluble peptide-lipid construct of the structure F-S-L where:

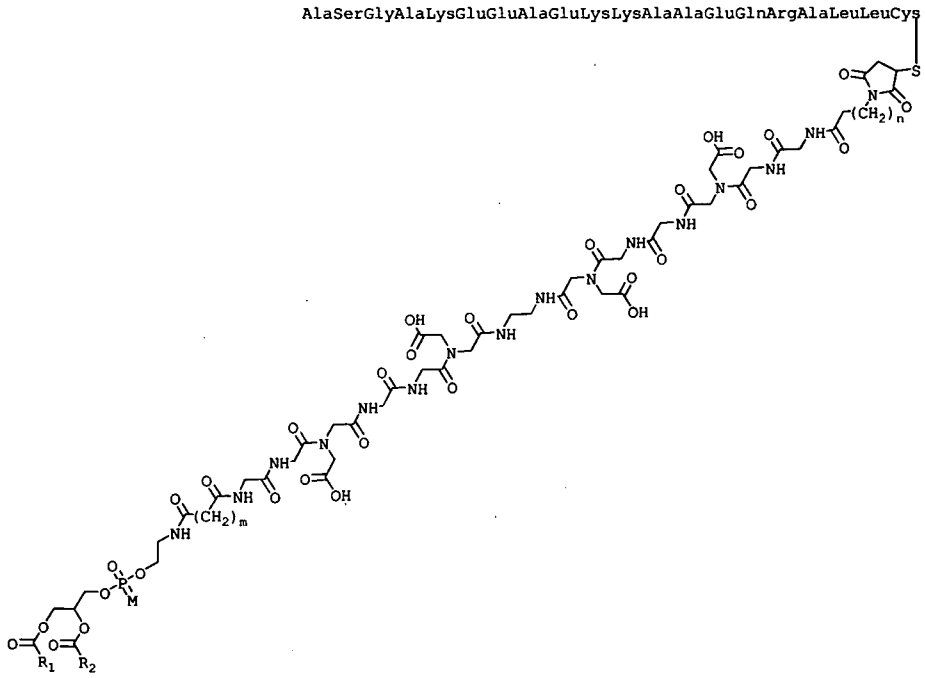
F is a peptide comprising the sequence:

AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO:3);

S is a spacer covalently linking F to L;

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

Preferably, the water soluble peptide-lipid construct is of the structure:



where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids *trans*-3-hexadecenoic acid, *cis*-5-hexadecenoic acid, *cis*-7-hexadecenoic acid, *cis*-9-hexadecenoic acid, *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid, *trans*-9-

octadecenoic acid, *trans*-11-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-11-eicosenoic acid or *cis*-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH_4^+.

Preferably, m is the integer 5 and n is the integer 2.

"Antibody reactive to an antigen" means an immunoglobulin, the presence of which in the serum of a subject is indicative of a phenotype or pathological condition of the subject.

"PCV" or "pcv" denotes packed cell volume.

"Plasma" means the colourless fluid part of blood or lymph, in which corpuscles or fat globules are suspended.

"RBC" or "rbc" means red blood cell.

"Saline" means a solution of one or more salts.

"Serum" means the amber-coloured, protein-rich liquid which separates out when blood coagulates.

"Solid phase immunoassay" means an assay in which one component of the immunological reaction, either antigen or antibody, is immobilized onto the surface of a solid phase support and in this context the term "antigen" includes mammalian cells such as erythrocytes (RBCs), leukocytes, lymphocytes, platelets and components of such cells including components prepared by lysing the cells.

"Solid phase support" means an article of manufacture composed of an organic polymer such as polystyrene, polypropylene, polyvinylchloride or nylon, or other materials that are capable of binding a monolayer of mammalian cells, or capable of being adapted to bind a monolayer of mammalian cells, such as glass.

"Synthetic" means made by chemical synthesis.

"Water soluble" means a stable, single phase system is formed when the construct is contacted with water or saline (such as PBS) at a concentration of at least 100 µg/ml and in the absence of organic solvents or detergents. The terms "soluble" and "dispersible" are used synonymously.

The amino acid residues of peptides are identified according to Table 3 of Appendix 2 of Annex C of the Administrative Instructions under the Patent Cooperation Treaty dated 7 February 2007 and in accordance with the convention:

H_2N-XaaXaaXaa.....XaaXaaXaa-COOH

Exemplifying embodiments of the invention will now be described in detail with reference to the Figures of the accompanying drawings pages.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. The structure of a peptide-lipid construct (F-S-L) of the invention comprising the peptide sequence:

AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu(SEQID NO: 3)

with conjugation via the sulfhydryl residue of a Cys residue located at the carboxy terminus of the sequence designated FSL-SYPH3.

Figure 2. Degrees of adherence of anti-immunoglobulin coated indicator cells to an immobilized layer of modified RBCs in Microtitre plate wells following centrifugation.

DETAILED DESCRIPTION

Many peptide sequences are known to be immunogenic, i.e. capable of eliciting the production of antibody. However, immunogenic peptide sequences are not equally capable of interacting with elicited antibody and therefore providing the basis for a diagnostic assay.

The following peptide has been shown to be particularly capable of interacting with antibodies, the presence of which in the serum of a subject is considered to be indicative of infection by *Treponema palladium*:

AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO:3)

This selected peptide sequence (designated SYPH3) has been demonstrated to be superior to the following peptide sequences when used in the diagnostic method described in the specification accompanying international application no. PCT/NZ2008/000239 (publ. no. WO 2009/035347):

ArgValMetTyrAlaSerSerGly (SEQID NO:1)

designated SYPH1 and:

ProGluLysAlaPheArgGluLeu (SEQID NO:2)

designated SYPH2.

The peptides were prepared in the form of peptide-lipid constructs (F-S-L) as described in the specification accompanying international application no. PCT/NZ2008/000266 (publ. no. WO 2009/048343). Each peptide was conjugated via the sulfhydryl residue of a Cys residue located at the carboxy terminus of the sequence (*cf.* Figure 1).

EXAMPLE 1

In a first study, a comparison of RBCs modified to incorporate peptide-lipid constructs (F-S-L) comprising one of the peptide (F) sequences designated SYPH1, SYPH2 and SYPH3 was performed.

RBCs were modified by contacting a suspension of the cells (1 part pcv) with a dispersion of construct at a concentration of 100 µg/mL or 50 µg/mL (2 parts pcv) at 37 °C for 1 hour. Modified cells (kodecytes) were washed three times in phosphate buffered saline (PBS) and resuspended.

The reactivity of clinical samples (n=95) indicated to be positive for Syphilis by EIA (BioKit or ICE) was assessed employing the diagnostic method described in the specification accompanying international application no. PCT/NZ2008/000239 (publ. no. WO 2009/035347).

K01.102WO I

Each of the 95 samples was assessed against RBCs modified to incorporate the FSL-SYPH3 construct at concentrations of either 100 µg/mL (SYPH3-100 kodecytes) or 50 µg/mL (SYPH3-50 kodecytes).

In addition 10 of the samples were assessed against RBCs modified to incorporate the FSL-SYPH1 construct at a concentration of 100 µg/mL (SYPH1-100 kodecytes) and 41 of the samples were assessed against RBCs modified to incorporate the FSL-SYPH2 construct at a concentration of 100 µg/mL (SYPH2-100 kodecytes).

Briefly, a suspension of 3% (pcv/v) kodecytes is prepared in PBS and 30 µl of the suspension mixed with 30 µl of sample plasma or serum. The mixtures are then incubated for 45 min at 37 °C. Following incubation the kodecytes are centrifuged for 10 s in an Immufuge™ (setting: "high") and observed for agglutination before being washed 3 times with PBS.

After washing one drop of Epiclone™ anti-human globulin (AHG) is added and the tubes then centrifuged for 10 s in an Immufuge™ (setting: "high"). Tubes are then read and serology scores recorded.

The reactivity of the samples is recorded in Table 1.

Method	n	Reactive	% Reactive
EIA (BioKit or ICE)	95	95	100
TPHA	86	79	92
TPPA	22	19	86
RPR	94	53	56
SYPH1-100 kodecytes	10	0	0
SYPH2-100 kodecytes	41	0	0
SYPH3-100 kodecytes	42	37	88
SYPH3-50 kodecytes	95	56	59

Table 1. Reactivity of EIA Syphilis positive clinical samples confirmed positive by reactivity with either TPHA or TPP.

Although none of the tested samples were reactive when assessed using the SYPH1-100 kodecytes and SYPH2-100 kodecytes, rates of reactivity when assessed using the SYPH3-100 kodecytes were comparable with those obtained for the confirmatory assays RPR, TPHA and TPPA.

Notably when the reactivity of samples determined to be false positives (negative confirmatory assay) was assessed against SYPH3-100 kodecytes all samples were determined to be negative.

EXAMPLE 2

In a second study, the reactivity of syphilis Qualification Panel samples (n=5) (SeraCare Life Sciences) confirmed to be positive using the Olympus PK TP System, and syphilis validation samples (n=20) (Plasma Services Group, Inc) confirmed to be positive using the Arlington Scientifice RPR and EuroimmunTP IgG and IgM ELISA assays, was compared in two solid phase immunoassays ("Test Method 1" and "Test Method 2").

Both Test Method 1 and Test Method 2 were performed on a GALILEO ECHO™ automated blood bank instrument (Immucor Gamma). In Test Method 1 the reactivity of the samples (n=25) was evaluated using CAPTURE-S™ plates (Immucor Gamma). In Test Method 2 the reactivity of the samples (n=25) was evaluated using SYPH3-10 kodecytes immobilized onto the surface of plates (CAPTURE-FSL-SYPH3™ plates).

SYPH3-10 kodecytes were created by suspending a packed cell volume (pcv) of group O red blood red cells in an equal volume of a solution containing the FSL-SYPH3 construct at a concentration of 10 µg/ml and incubating for 120 mins at 37°C, followed by washing.

The CAPTURE-FSL-SYPH3™ plates were then prepared *mutatis mutandis* according to the methods described in Sinor *et al* (1989), Sinor *et al* (1990) and Sinor and Eatz (1991).

Briefly, a MICROTITRE™ plate having a plurality of recessed wells is first treated with a sufficient quantity of Alcian

yellow at a concentration of 0.1 mg/ml so that all of the wells are covered by the Alcian yellow solution. The Alcian yellow solution is allowed to remain in contact with the MICROTITRE™ plate for about 30 minutes and excess dye solution is removed.

A monolayer of the SYPH3-10 kodecytes is formed on the stained plate by addition of 100 µL of a 0.2% (v/v) suspension of the SYPH3-10 kodecytes in saline or reagent RBC diluent per well. The SYPH3-10 kodecytes are allowed to settle by gravity for one hour at room temperature. The plate is then washed at least two times with isotonic saline to remove unbound SYPH3-10 kodecytes by an automatic washing apparatus or by manual methods.

One hundred microlitres (100 µL) of a drying solution containing 1.0 M dextrose and 154 mM sodium chloride is added to each well. Immediately following addition of the drying solution, each well is aspirated to remove any excess drying solution.

The MICROTITRE™ plate is then placed in an inverted position into a foil packet or pouch having at least two, 2 g capacity molecular sieve dessicant packets therein. The pouch is sealed with heat and dried for seven days at 2°to 8°C.

The CAPTURE-S plates and the CAPTURE-FSL-SYPH3 plates are each used according to the standard test method. Briefly, the foil package containing each plate is opened and one drop of a biological fluid such as serum or plasma or a control is added to each. Two drops of a 19 g/L solution of glycine with a preservative and a dye for colour is also added to each well.

The solutions are allowed to incubate in the wells at 37°C for 15 minutes. After the incubation period, the wells of the MICROTITRE™ plate are washed three times with saline, preferably by an automatic washing apparatus such as the Bio-Tek model EL-402.

One drop of anti-IgG coated indicator RBCs is then added to each well. The MICROTITRE™ plate is centrifuged at 450 x G for one minute. The plate is then examined for adherence or lack of

adherence of the indicator RBCs to the erythrocyte cell monolayer (Figure 2).

A positive reaction is seen by adherence of the indicator red cells over the reaction surface. A negative reaction forms a discreet button of indicator red cells at the bottom of the wells showing no adherence (Figure 2).

Thus, if the biological fluid being tested has antibodies directed towards the erythrocyte cell monolayer, it binds to the erythrocyte monolayers. The anti-IgG coated indicator RBCs correspondingly bind to the antibody thus bound when they are added to the wells. This gives the positive reaction.

If no antibodies directed toward the erythrocytes are present in the biological fluid being tested, the anti-IgG coated indicator RBCs will have no complimentary immunologically component to bind to and will collect at the bottom of the well as a discreet button.

The results obtained from Test Method 1 (CAPTURE-S) and Test Method 2 (CAPTURE-FSL-SYPH3) are presented in Table 2.

		Sample	
		+ve (n=25)	-ve (n=32)
Test method 1 (CAPTURE-S)	+ve assay	20	0
	-ve assay	5	32
Test method 2 (CAPTURE-FSL-SYPH3)	+ve assay	24	0
	-ve assay	1	32

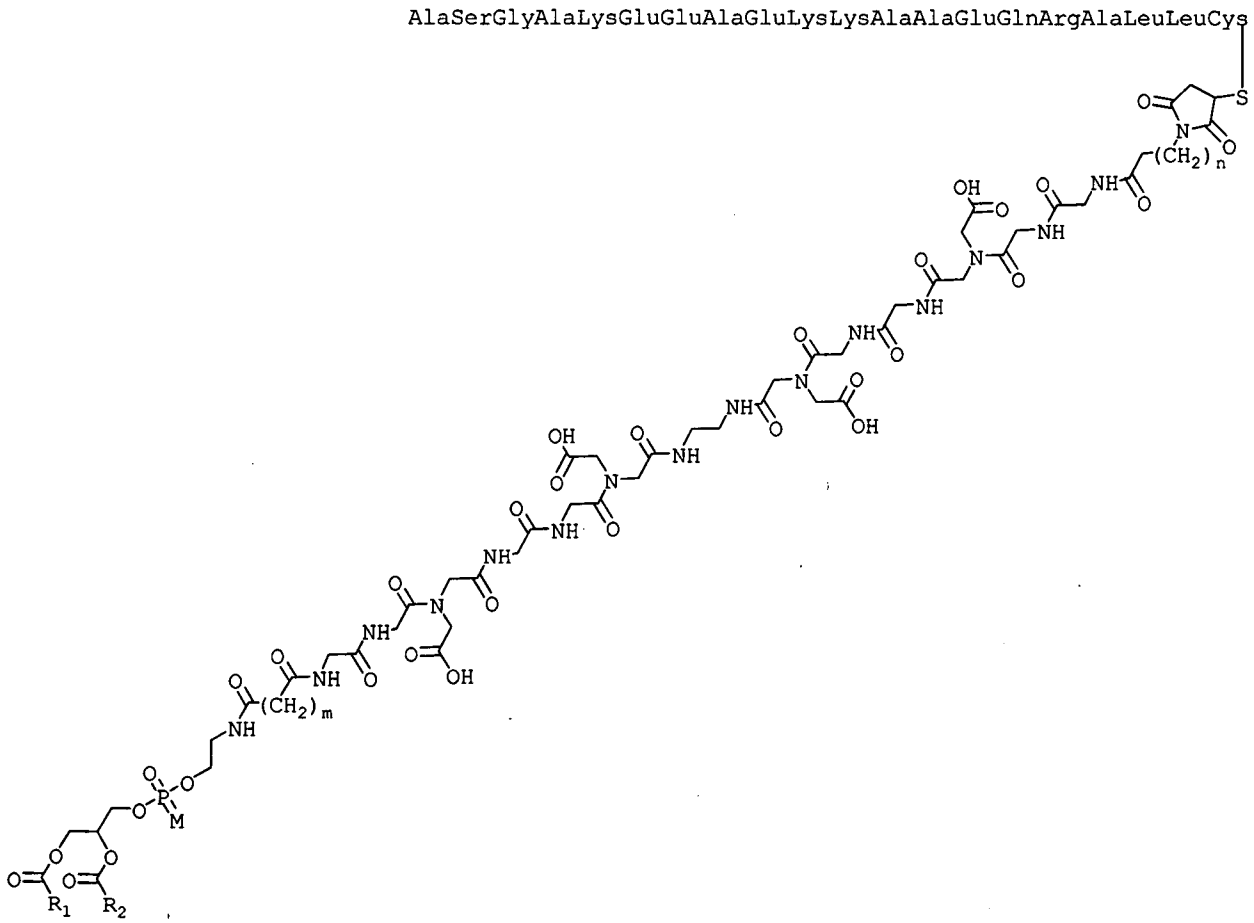
Table 2. Comparison of sensitivity and specificity of two solid phase immunoassays ("Test Method 1" and "Test Method 2").

Test Method 2 (CAPTURE-FSL-SYPH3) demonstrated improved sensitivity over Test Method 1 (CAPTURE-S). Test Method 2 (CAPTURE-FSL-SYPH3) provides the additional advantage that the introduced antigen (F) of the FSL construct is expressed against the background antigens of the naturally occurring RBCs that are modified to provide the kodecytes.

Although the invention has been described by way of examples it should be appreciated that variations and modifications may be made to the claimed methods without departing from the scope of the invention. It will be understood that for a non-specific interaction, such as the interaction between the diacyl- or dialkyl- glycerolipid portion of the functional-lipid constructs and a membrane, structural and stereo-isomers of naturally occurring lipids can be functionally equivalent.

Where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification. For example, it is contemplated that diacylglycerol 2-phosphate could be substituted for phosphatidate (diacylglycerol 3-phosphate) and that the absolute configuration of phosphatidate could be either R or S.

Enlarged representation of the structural formula presented in the foregoing *Statements of Invention* and following *Claims*:



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K01.102WO I

WHAT WE CLAIM IS:

1) A **method** for determining the likelihood of infection of a subject with *Treponema palladium* comprising the steps of:

- Contacting a sample of the plasma or serum of the subject with a suspension of cells modified to incorporate a peptide-lipid construct of the structure F-S-L or L-S-F to provide a mixture;
- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and
- Determining the degree of agglutination of the cells in the mixture;

where:

F is a peptide comprising the sequence: AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO: 3);

S is a spacer covalently linking F to L;

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

the degree of agglutination indicates the likelihood of infection of the subject.

2) The method of claim 1 where the method includes prior to determining the degree of agglutination of the cells of the mixture the intermediate step of:

- Adding an anti-subject globulin antibody to the mixture.

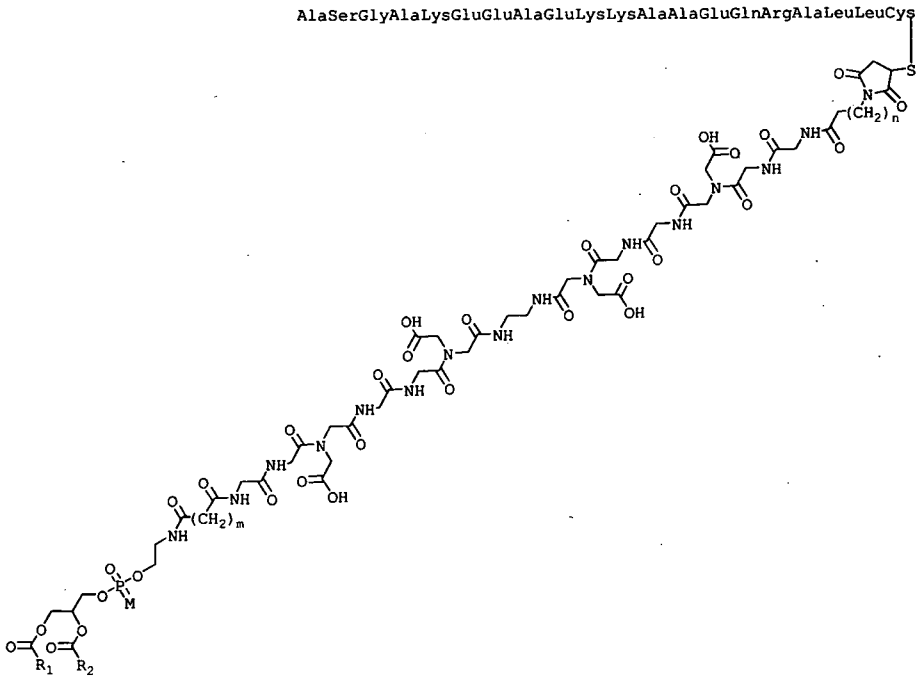
3) The method of claim 1 where the subject is a human.

4) The method of claim 1 where the cells are RBCs.

5) The method of claim 1 where the plasma or serum of a subject is obtained from a donated sample of blood.

6) The method of claim 2 where the anti-subject globulin antibody is anti-human globulin (AHG) antibody.

7) The method of claim 1 where the peptide-lipid construct is of the structure:



where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids trans-3-hexadecenoic acid, cis-5-hexadecenoic acid, cis-7-hexadecenoic acid, cis-9-hexadecenoic acid, cis-6-octadecenoic acid, cis-9-octadecenoic acid, trans-9-octadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, cis-11-eicosenoic acid or cis-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH^{4+}.

8) The method of claim 7 where m is the integer 5 and n is the integer 2.

K01.102WO I

9) A method for determining the likelihood of infection of a human subject with *Treponema palladium* comprising the steps of:

- Contacting a sample of the plasma or serum of the subject with a suspension of RBCs modified to incorporate the peptide-lipid construct designated FSL-SYPH3;
- Incubating the contacted sample of the plasma or serum and the suspension of RBCs for a time and at a temperature sufficient to allow binding of antibodies present in the sample to the RBCs;
- Preparing a suspension of washed RBCs;
- Adding an amount of anti-human globulin (AHG) antibody to the suspension to provide a mixture;
- Incubating the mixture for a time and at a temperature sufficient to allow agglutination; and
- Determining the degree of agglutination of the cells in the mixture to indicate the likelihood of infection.

10) A **method** of testing donated blood for the presence of antibodies indicative of the donor being infected with Treponema palladium comprising the steps of:

- Contacting a sample of plasma or serum of the donated blood with an immobilized layer of modified RBCs;
- Incubating the contacted sample of plasma or serum and the immobilized layer of modified RBCs for a time and at a temperature sufficient to allow binding of the antibodies;
- Washing the immobilized layer of modified RBCs to remove the sample of plasma or serum;
- Contacting the immobilized layer of modified RBCs with a suspension of anti-immunoglobulin coated indicator cells;

- Incubating the immobilized layer of modified RBCs and the suspension of anti-immunoglobulin coated indicator cells for a time and at a temperature sufficient to allow binding of the anti-immunoglobulin coated indicator cells to the immobilized layer of modified RBCs; and
- Determining the degree of adherence of the anti-immunoglobulin coated indicator cells to the immobilized layer of modified RBCs,

where the modified RBCs have been modified to incorporate a peptide-lipid construct of the structure F-S-L where:

F is a peptide comprising the sequence: AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO: 3);

S is a spacer covalently linking F to L;

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids; and

the degree of adherence is indicative of the likelihood of the antibodies being present.

11) The method of claim 10 where the determining the degree of adherence of the anti-globulin coated indicator cells to the immobilized layer of modified RBCs is by centrifugation to pellet unbound anti-globulin coated indicator cells.

12) The method of claim 10 where the peptide-lipid construct is of the structure:

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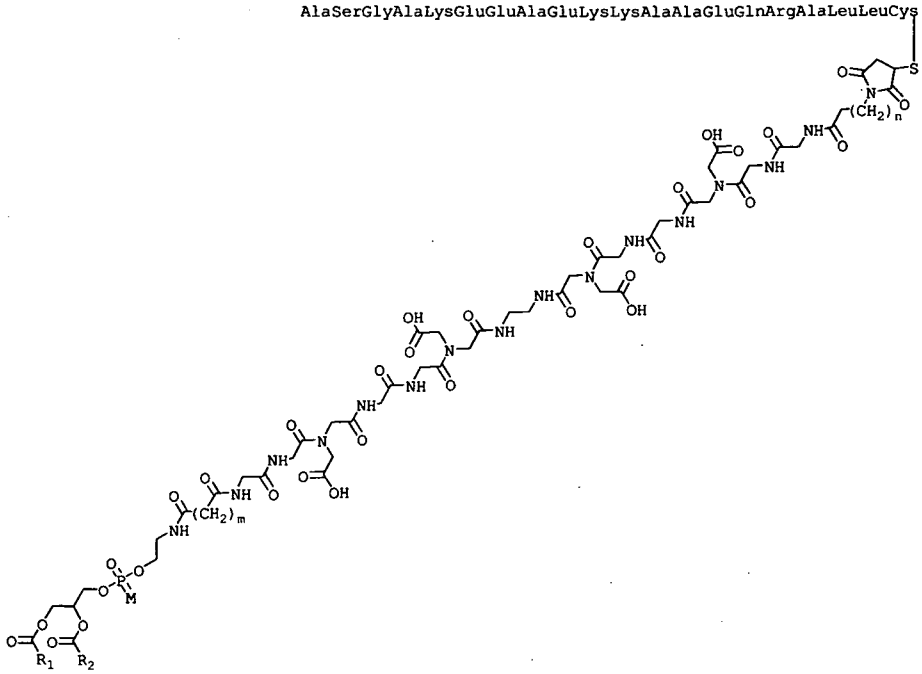


where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids trans-3-hexadecenoic acid, cis-5-hexadecenoic acid, cis-7-hexadecenoic acid, cis-9-hexadecenoic acid, cis-6-octadecenoic acid, cis-9-octadecenoic acid, trans-9-octadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, cis-11-eicosenoic acid or cis-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH^{4+}.

13) The method of claim 12 where m is the integer 5 and n is the integer 2.

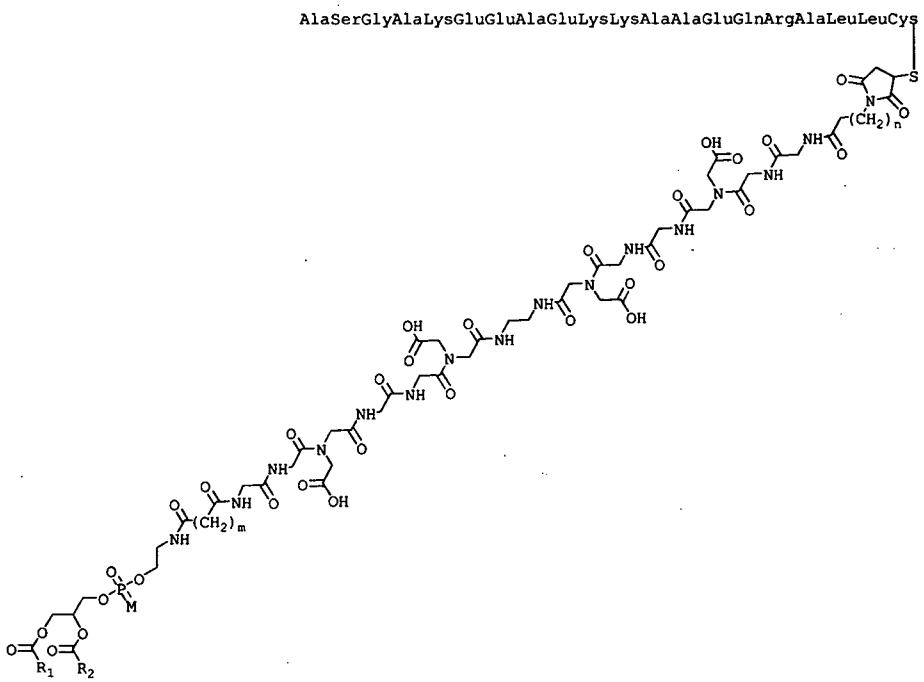
14) A water soluble peptide-lipid **construct** of the structure F-S-L where:

F is a peptide comprising the sequence: AlaSerGlyAlaLysGluGluAlaGluLysLysAlaAlaGluGlnArgAlaLeuLeu (SEQID NO:3);

S is a spacer covalently linking F to L;

L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

15) The method of claim 14 where the water soluble peptide-lipid construct is of the structure:



where:

R_1 and R_2 are independently selected from the group consisting of: alkyl or alkenyl substituents of the fatty acids trans-3-hexadecenoic acid, cis-5-hexadecenoic acid, cis-7-hexadecenoic acid, cis-9-hexadecenoic acid, cis-6-octadecenoic acid, cis-9-octadecenoic acid, trans-9-octadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, cis-11-eicosenoic acid or cis-13-docsenoic acid;

m is the integer 3, 4 or 5;

n is the integer 1, 2 or 3; and

M is a monovalent cation such as H^+, Na^+, K^+ or NH^{4+}.

16) The method of claim 15 where m is the integer 5 and n is the integer 2.

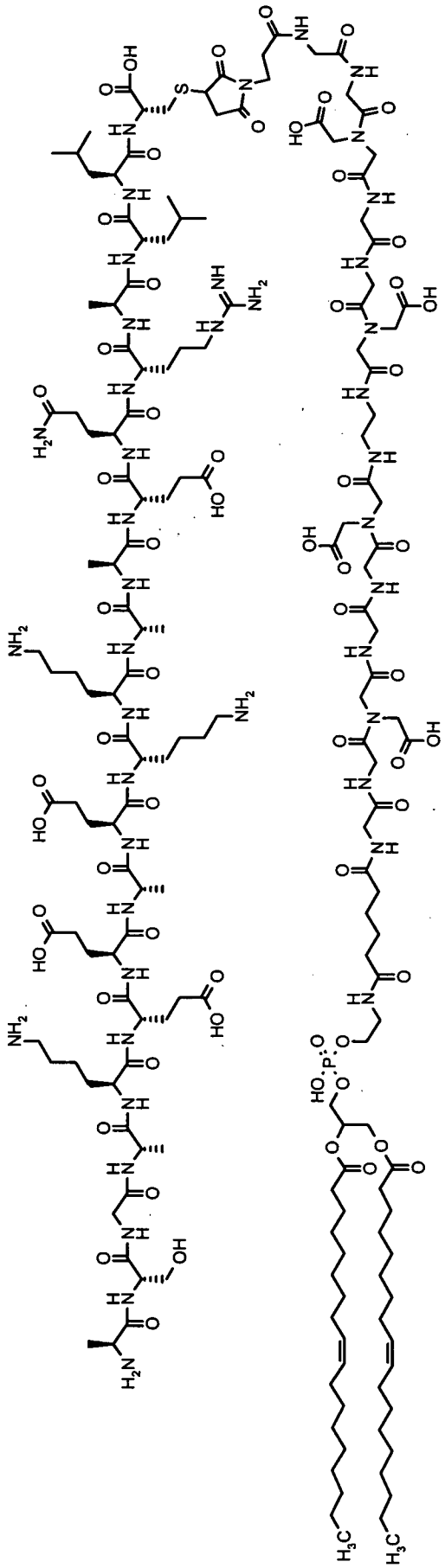


FIGURE 1

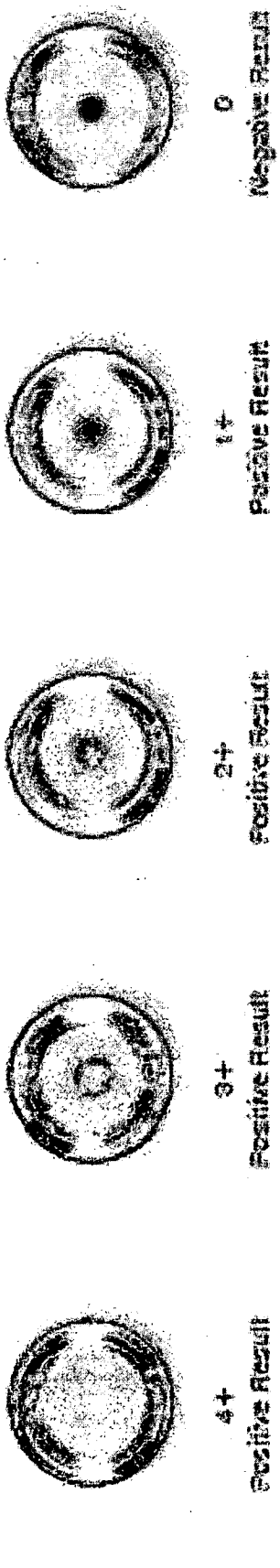

0
Negative Result
1+
Positive Result
2+
Positive Result
3+
Positive Result
4+
Positive Result

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2010/000111

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 33/92 (2006.01) *C07K 7/08* (2006.01) *C07K 17/06* (2006.01)

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GenomeQuest: Sequence search for SEQ ID No. 3
STN: partial substructure search for peptide-lipid construct of claim 7, keywords based on: red blood cell, lipid, trepenoma palladium, peptide, protein, linker, space

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/048343 A1 (BOVIN *et al*) 16 April 2009 See whole document, especially pages 8, 9 and 19	
A	WO 2009/035347 A1 (WEINBERG *et al*) 19 March 2009 See whole document, especially page 5, claim 1	
A	JP 2001-264334 A (SEKISUI CHEMICAL CO LTD) 26 September 2001 SEQ ID No. 30, paragraph [0001], claim 3	
A	WO 2006/138324 A2 (BAYLOR COLLEGE FOR MEDICINE) 28 December 2006 SEQ ID No. 31, paragraphs [0163] and [0164]	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search
29 September 2010

Date of mailing of the international search report 05 OCT 2010

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2010/000111

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   [X] on paper

   [ ] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2010/000111

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/0229017 A1 (WU *et al*) 11 December 2003 See whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2010/000111

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2009048343	AU	2008311480	CA	2702470	EP	2201025
WO	2009035347	AU	2008297660	CA	2699366	EP	2198301
JP	2001264334	NONE					
WO	2006138324	NONE					
US	2003229017	CA	2413629	CN	1453293	EP	1319667
		US	7153933	US	2003229013	US	2007106064

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX