(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 October 2008 (09.10.2008)

PCT

(10) International Publication Number
WO 2008/120006 A2

(51) International Patent Classification:
G01N 33/72 (2006.01) *G01N 33/50* (2006.01)
G01N 33/53 (2006.01) *C07K 7/00* (2006.01)

(21) International Application Number:
PCT/GB2008/001200

(22) International Filing Date: 3 April 2008 (03.04.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0706558.4 3 April 2007 (03.04.2007) GB

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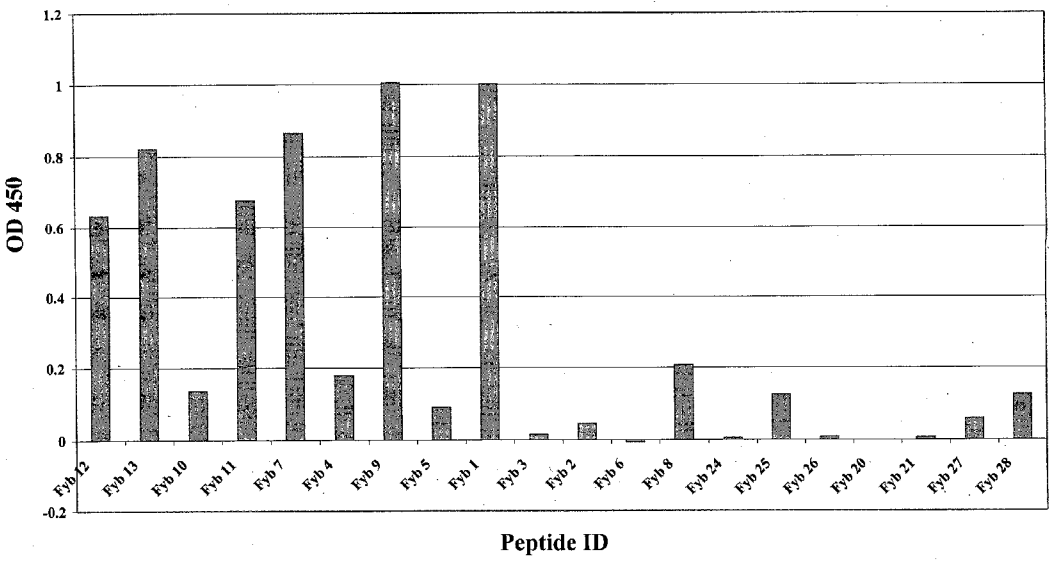
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: DIAGNOSTIC ASSAY

Figure 1: Fy^b Phage ELISA



(57) Abstract: The present invention relates to mimotopes of blood group antigens, methods for identifying mimotopes of blood group antigens and methods for identifying antibodies to blood group antigens using said mimotopes.

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Diagnostic Assay

[001] The present invention relates to mimotopes of blood group antigens, and uses thereof.

[002] Blood group antigens exist on membrane proteins on the surface of erythrocytes. The genes encoding all but one of the 29 blood group systems (Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt, Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside and GIL) have been cloned, sequenced, the amino acid sequences deduced, and the membrane structure predicted.

[003] Of the Rhesus, Kell, Duffy and Kidd blood systems etc, the Rhesus antigens are the most complex being the products of two highly homologous genes, RHD and RHCE. The antigens are predicted to have six extracellular loops that are available for antibody binding.

[004] The RhD polypeptide, the most commonly expressed of the Rhesus antigens, differs by 36 amino acids from its nearest equivalent, the RhCcEe polypeptide. Both are co-expressed with the Rhesus-associated glycoprotein (RAG) polypeptide.

[005] The RHCE gene product exists in four allelic forms and each allele determines the expression of two antigens in combination i.e. Ce, ce, cE or CE. The RhC antigen differs from Rhc by one amino acid in loop 2 (Ser103Pro), and for E/e, a single exofacial amino acid difference is predicted in loop 4 (Pro226Ala).

[006] The Kell protein is a single pass membrane structure, and the K/k alleles are the result of a single nucleotide polymorphism (SNP) resulting in a single amino acid change (Met193Thr) in the extracellular region.

[007] The Duffy protein is predicted to have 3 extracellular loops with the N-terminus being extracellular, and the Fy^a/Fy^b alleles are the result of a SNP giving rise to an amino acid substitution (Asp42Gly) in the N-terminal region.

[008] The Kidd glycoprotein is predicted to have 10 membrane-spanning domains with intracellular N- and C-termini. The Jk^a/Jk^b polymorphism results from a SNP giving rise to a single amino acid substitution (Asp280Asn) on the fourth extracellular loop.

[009] The immunological determinants on an antigen that

bind antibodies are defined as B-cell epitopes since they are recognised by the surface-membrane immunoglobulin receptors of B-lymphocytes. These epitopes may be linear i.e. a continuous stretch of amino acids from the protein sequence, or conformational and depend on the spatial juxtaposition of amino acids which are not contiguous. Study of the B-cell epitope structure of blood group antigens has largely been restricted to the RhD antigen, and to date over 30 epitopes have been identified serologically.

[0010] A single amino acid substitution can affect more than one epitope, even though the predicted loops involved are some distance apart in the linear sequence, and it is concluded that the RhD B-cell epitopes are conformational.

[0011] The B-cell epitopes on C/c and E/e are predicted to be fewer, but are likely to be conformational since full antigenicity requires co-expression of the RAG polypeptide.

[0012] The K/k polymorphism is predicted to disrupt a glycosylation site, which may account for the antigenicity of the K allele, and it is anticipated that the epitopic determinants will also be conformational.

[0013] Blood group antigens may induce the production of alloantibodies that can cause destruction of transfused red

blood cells. Reactions to transfused blood may, therefore, occur when a recipient of transfused erythrocytes has antibodies to those erythrocytes, resulting in their destruction. An immediate reaction including fever, hypotension, nausea and vomiting, and pain in the back and chest can occur, with the severity of the reaction depending on the class and amount of antibodies involved.

[0014] Alloantibodies to blood group antigens may also cross the placenta of a pregnant woman and give rise to haemolytic disease of the newborn (HDNB). Haemolytic disease of the newborn (HDNB) can occur when the mother has been sensitised to antigens on the infant's erythrocytes and makes IgG antibodies to these antigens. These antibodies can cross the placenta and react with the foetal erythrocytes causing their destruction. RhD is the most commonly involved antigen.

[0015] Blood group antigens may also induce the production of autoantibodies involved in autoimmune diseases such as autoimmune haemolytic anaemia. The trigger for this autoimmune disease is unknown and therefore it may occur at anytime and results in the body producing autoantibodies of broad Rh group specificity which attack the body's own red blood cells.

[0016] The most important blood groups associated with

alloantibody generation are Rhesus, Kell, Duffy, and Kidd.

[0017] Current methods for detecting alloantibodies that may destroy transfused erythrocytes or cross the placenta to cause HDNB, or for detecting autoantibodies, rely on blood donor red cell reagents. These have a short shelf-life and vary considerably in the antigen expression between donors. Use of human blood products also introduces safety implications, for example, involving the potential transfer of viral infections. The rigorous testing and screening required when using human reagents, together with inconsistencies between samples slows the availability of reagents for detecting alloantibodies, as well as increasing the cost of obtaining suitable reagents.

[0018] The present invention, accordingly, seeks to overcome the problems associated with the prior art, in particular, the problems associated with existing methods for detecting alloantibodies and autoantibodies.

[0019] According to an aspect of the present invention there is provided a mimotope of a blood group antigen.

[0020] The term "mimotope" refers to a peptide which is capable of mimicking an epitope of a native peptide. Usually such peptides are synthetic peptides. In this respect,

"synthetic peptide" includes any peptides which are synthesised rather than expressed natively in a biological system. Synthesis may be by recombinant technology such as phage display, F-moc chemistry or any other method for expressing or synthesising foreign peptides in biological or non-biological systems or environments.

[0021] The present invention thus provides novel peptides which are capable of binding to antibodies which have been raised to blood group antigens. These peptides are not necessarily from natural sources such as human blood and, therefore, do not carry the health risks, storage problems and variability associated therewith.

[0022] Conveniently, the blood group antigen is selected from Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt, Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside or GIL.

[0023] The deduced amino acid structure of a mimotope may not necessarily correspond to the amino acid structure of the antigen which it mimics. Indeed, in the present study it was discovered that the majority of the blood group mimotopes identified have amino acid sequences which differ widely from the amino acid sequence of the native antigen that they

mimic.

[0024] As the epitopes of many blood group antigens are thought to be complex in conformation, it is surprising that the synthetic peptides, identified in the present invention by means of immunopanning, are capable of binding antibodies raised to native antigens in a specific manner. This finding suggests that the synthetic blood group mimotopes assume and maintain a three dimensional conformation which allows them to specifically bind the antibodies raised to their corresponding antigen.

[0025] Conveniently, the mimotope is synthesised. This removes the problems associated with using human blood reagents, for example, the potential risk of contamination and infection, difficulties regarding storage, donor identification, variability and screening. These problems have associated costs which will be reduced through use of synthetic mimotopes. Use of synthetic mimotopes also provides a greater degree of control over the reagents that are available for use, ensuring that shortages never occur and unnecessary waiting for reagents can be avoided.

[0026] Through the present study, numerous mimotopes of blood group antigens were identified. Some of the peptides conveniently identified as blood group mimotopes are

identified in Tables 2-5 and include peptide fragments, immunoreactive analogues or derivatives thereof.

[0027] According to a further aspect of the present invention there is provided a method for identifying a mimotope of a blood group antigen, the method comprising contacting a random phage display library with an antibody to a blood group antigen and detecting binding of the antibody to peptides in the phage display library, wherein binding of an antibody to a peptide is indicative of the peptide being a mimotope of a blood group antigen.

[0028] Preferably, the blood group antigen is Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt, Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside or GIL. These are the blood group antigens associated with the generation of alloantibodies and autoantibodies.

[0029] The antibody to which the mimotope binds may be a monoclonal antibody or a preparation of polyclonal antibodies. Methods for generating monoclonal antibodies and polyclonal antibodies are well known in the art.

[0030] Random phage display libraries comprising 7-mer, 12-

mer and 15-mer peptides were used to identify peptides which could bind antibodies raised to native blood group antigens. Identification and sequencing of peptides which bound to the antibodies enabled identification of potential blood group antigen mimotopes. Subsequent analysis of these peptides by ELISA followed by synthesis of these peptides by SPOTs and analysis of the membrane-bound SPOTs confirmed the strength and specificity of the peptide-antibody binding. This confirmed that synthetic peptides structurally different from the native antigens which they mimicked were successfully able to specifically bind antibodies raised to native blood group antigens, thus representing effective mimotopes of the blood group antigens in question. Immobilisation of the mimotopes onto, for example, microsphere beads or superparamagnetic beads did not affect the ability of the mimotopes to specifically bind antibodies raised to native blood group antigens. The method described therefore represents an effective and efficient method for identifying synthetic mimotopes of blood group antigens which can be used in a clinical setting, for example, in the diagnosis of diseases associated with the production of alloantibodies or autoantibodies.

[0031] Binding of antibodies to the phage-peptides may be detected by any means suitable, such means being known to a person skilled in the art. Examples of the type of binding

which can be used is described in the Examples.

[0032] According to a further aspect of the present invention there is provided a method for detecting an antibody to a blood group antigen in a sample. The method comprising reacting a mimotope of said blood group antigen with the sample to be tested, detecting any reaction between the sample and the mimotope, wherein a reaction between the sample and the mimotope is indicative of the presence of an antibody to the blood group antigen.

[0033] Conveniently before carrying out the above identified method the mimotope is immobilised on a solid support. Immobilisation of the mimotopes onto a solid support such as microsphere beads or superparamagnetic beads did not affect the ability of the mimotopes to specifically bind antibodies raised to native blood group antigens. In this connection, the method comprises immobilising a mimotope of a blood group antigen on a solid support, contacting the immobilised mimotope with the sample to be tested and detecting binding of the sample to the mimotope, wherein binding of the sample to the mimotope is indicative of the presence of an antibody to the blood group antigen mimicked by the mimotope.

[0034] Conveniently, the blood group antigen is Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt,

Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside or GIL.

[0035] In this respect, given that many epitopes of native blood group antigens are thought to be conformational it is surprising that the synthetic mimotopes identified herein are capable of presenting a three dimensional conformation which specifically bind antibodies raised to native blood group antigens, even when bound to a solid support. The solid support may comprise any support suitable for the purpose but conveniently comprises a membrane, glass slide or bead (glass, polystyrene etc.). Attachment to the support can be by any suitable means, for example using Ni-NTA groups attached to the surface of polystyrene microsphere beads or using superparamagnetic polystyrene beads. Polyhistidine tags may be incorporated into the C-terminal or N-terminal of mimotopes to aid attachment to the support. Membranes having a lower background and which can be successfully stripped and re-probed, for example INTAVIS AG membranes, are preferable.

[0036] The present invention, therefore, provides a method which effectively identifies antibodies in biological samples that have been raised to blood group antigens. Use of synthetic mimotopes removes the problems identified with

regard to use of human reagents. This will increase consistency in antibody detection and enable detection of rarer antibodies for little or no extra cost, compared with existing techniques for screening antibodies raised to blood group antigens. Avoiding use of human blood products also increases safety, avoids the rigorous testing required when using human blood products and increases the consistency of the reagents available for use. This reduces the cost of sourcing and clearing reagents for use. The method described is therefore more reliable, safe and convenient than existing detection methods, making detection of antibodies to blood group antigens in biological samples quicker, cheaper and easier to conduct.

[0037] The antibodies detected may be alloantibodies or autoantibodies to blood group antigens. The method described, therefore, provides improved methods of detecting and diagnosing diseases or a reaction to blood products involving the generation of alloantibodies or autoantibodies. Such diseases include haemolytic reactions to red cell transfusions (HTR), haemolytic disease of the newborn and autoimmune haemolytic anaemia.

[0038] Conveniently, the antibody is an alloantibody, allowing one to reliably and safely diagnose the risk of HTR or haemolytic disease of the newborn.

[0039] According to another aspect of the present invention there is provided a device for detecting the presence of an antibody in a sample to a blood group antigen. The device comprising a solid support (membrane, glass slide, microarray chip or bead(s) etc.) having an array of mimotopes of a blood group antigen immobilised thereon or having one mimotope on each solid support and having provided a plurality of solid supports.

[0040] An array may be constructed comprising only Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt, Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside or GIL antigen mimotopes. Alternatively, an array may be developed comprising any combination of blood group mimotopes, with mimotopes from multiple blood groups being arranged on the same diagnostic array. The composition and design of the array may depend on the intended purpose of the device and bespoke arrays may be developed as required. Arrays required for specific purposes can be constructed quickly, reliably and cost-effectively, without having to consider the availability of reagents.

[0041] With regard to the construction of arrays, different types and length of spacer for attaching peptides to a solid

membrane support can be used, for example an amino-PEG spacer or a limited number of amino acid residues could be used to link the mimotope peptide sequence to the solid support.

[0042] Conveniently, the antibody is an alloantibody. The device can therefore be used for detecting alloantibodies in a sample of blood obtained from a patient suspected of generating alloantibodies to blood group antigens, without requiring the use of human blood group reagents. Removal of the need for blood group reagents will conveniently increase the availability of reagents for detection of antibodies in blood group samples.

[0043] Antigen-capture systems as described herein can also increase the sensitivity and accuracy of antibody detection, thus providing an improvement over existing methods for the detection of blood group.

[0044] By showing that phage-peptides, selected by biopanning, specifically bind the antibodies originally used to identify them, the experiments described herein confirm the potential value of the selected phage-peptides as diagnostic reagents.

[0045] The mimotopes identified in the present study, when coupled to a suitable solid phase, can be used as screening

reagents to detect antibodies to blood group antigens.

[0046] In this respect, the mimotopes may be used to diagnose diseases associated with blood group antigens, such as diseases associated with the production of alloantibodies or autoantibodies.

[0047] Further potential uses of the mimotopes identified and described herein, together with uses of the arrays formed therewith, will be evident to a person skilled in the art.

[0048] The present invention will now be described by way of illustration only with reference to the accompanying drawings:

[0049] Figure 1 shows the results of phage ELISA, confirming binding of the anti-Fy^b antibody (LM447) to 14 of 20 Fy^b phage-peptides assayed.

[0050] Figure 2 shows the results of SPOTs hybridisation experiments. 20 Fyb peptide mimotopes were synthesised in duplicate. Binding experiments confirmed the ability of Fyb peptide mimotopes to recognise and bind the anti- Fy^b antibody, when synthesised on a membrane support.

[0051] Figure 3 shows the results of phage ELISA, confirming

binding of the anti-Fy^a antibody (LM487) to 16 of 19 Fya phage-peptides assayed.

[0052] Figure 4 shows the results of a SPOTs hybridisation experiment. A series of 37 Fya peptide mimotopes were synthesised on a membrane and tested for their ability to bind the anti-Fy^a antibody (LM487).

[0053] Figure 5 shows the results of reciprocal hybridisation of Fya peptide mimotopes with the anti-Fy^a and anti-Fy^b monoclonal antibodies, LM447 and LM487. A series of Fy^a and peptide mimotopes were synthesised in duplicate and hybridisation of the membranes with the 2 monoclonals identified antibody-specific and cross-reactive peptide mimotopes.

[0054] Figure 6 shows the results of amino acid substitution experiments. A series of 16 synthetic peptides were synthesised in duplicate by SPOTs. These sequences were derived from the Fyb Consensus I sequence and generated by amino acid substitution. The synthetic peptide mimotopes were tested for their ability to bind the anti- Fy^a and anti-Fy^b monoclonal antibodies.

[0055] Figure 7 shows the results following hybridisation of a subset of the anti-RhD antibody-selected peptide mimotopes

with the monoclonal antibody T10. 40 peptides (RhD41-RhD80) were synthesised and positive signals were observed in 11 spots (Spots 1-3, 8, 17, 27, 28, 31, 37, 39 & 40; see Table for peptide sequences).

[0056] Figure 8 shows the results of a SPOTs hybridisation experiment. A series of 28 RhD peptide mimotopes were synthesised by SPOTs and hybridisation of the membrane with the polyclonal anti-RhD antisera preparation identified antibody-reactive peptides.

[0057] Figure 9 shows the results of a SPOTs hybridisation experiment. A series of 36 RhE peptide mimotopes were synthesised by SPOTs. Hybridisation of the membrane with the monoclonal antibody E0002 identified antibody-specific peptide mimotopes.

[0058] Figure 10 shows the results of a SPOTs hybridisation experiment. A series of 16 Rhe peptide mimotopes were synthesised by SPOTs. Hybridisation of the membrane with the monoclonal antibody e0002 identified antibody-specific peptide mimotopes.

[0059] Figure 11 shows specific binding of anti-Fy^b monoclonal antibody (LM447) binding to peptide mimotope Fyb16 immobilised onto polystyrene LiquiChip Ni-NTA microsphere

beads. The intensity of the signal equates to the quantity of monoclonal antibody bound to the immobilised peptide mimotope.

[0060] Figure 12 shows specific binding of anti-RhD monoclonal antibody (T27) binding to peptide mimotope RhD12 immobilised onto polystyrene LiquiChip Ni-NTA microsphere beads. The intensity of the signal equates to the quantity of monoclonal antibody bound to the immobilised peptide mimotope.

[0061] Figure 13 shows the specific binding of the anti-RhD monoclonal antibody (T27) to peptide mimotope RhD12 immobilised onto superparamagnetic polystyrene beads in a modified gel agglutination assay.Chamber 1: Beads + His-tagged peptide mimotope RhD12 only; Chamber 2: Beads + His-tagged RhD12 + anti-Fy^b monoclonal antibody (LM447); Chamber 3: Beads + His-tagged RhD12 + anti-RhD monoclonal antibody (T27).

[0062] Figure 14 shows the specific binding of the anti-Fy^b monoclonal antibody (LM447) to peptide mimotope Fyb16 immobilised onto superparamagnetic polystyrene beads in a modified gel agglutination assay. Chamber 1: Beads + His-tagged peptide mimotope Fyb16 only; Chamber 2: Beads + His-tagged Fyb16 + anti-Fy^b monoclonal antibody (LM447); Chamber

3: Beads + His-tagged Fyb16 + anti-RhD monoclonal antibody (T27).

[0063] Examples

[0064] Methodology

[0065] Identification of mimotopes of blood group antigens commenced with biopanning of four phage-peptide libraries with a series of blood group antigen-specific monoclonal antibodies (3x anti-RhD, 3x RhE, 2x Rhe, 1x Fy^a, 1x Fy^b) and an RhD polyclonal preparation. In total, 490 phage were selected and initially characterised by DNA sequencing and ELISA. DNA sequence analysis identified 295 unique phage-peptide sequences, which were synthesised on membranes by SPOTS technology. The specificity of the peptide/antibody reaction was tested by ELISA and SPOTS assays and identified 84 peptides capable of detecting specific RhD, RhE, Rhe, Fy^a & Fy^b antibodies.

[0066] Biopanning

[0067] Three different phage libraries from New England Biolabs. (Ph.D. 7 and Ph.D. 12 displaying linear 7- and 12-mer peptides, respectively and Ph.D. C7C displaying 7-mer peptides constrained by a disulphide bridge at their termini)

and a linear 15mer library (kindly provided by Prof. George P. Smith, University of Missouri) were used in biopanning experiments with 10 different monoclonal antibodies and a polyclonal preparation (Table 1).

[0068] Table 1: Antibodies Used in Biopanning.

[0069]

Specificity	Epitope	Class	ID	Source
Anti-RhD	epD3 [3.1]	IgGI/κ	T10 (LHM76/55)	Human
Anti-RhD	epD6/7[6.3]	IgGI/λ	T22 (LHM 169/80)	Human
Anti-RhD	epD1 [1.2]	IgGI/κ	T27 (LHM169/81)	Human
Anti-RhD	Polyclonal	-	Human Anti-D Ig	Human
Anti-RhE	Not known	IgG1	E0001 (HIRO17)	Human
Anti-RhE	Not known	IgM	E0002 (NaTH110-1D6)	Human
Anti-RhE	Not known	IgM	E0003 (NaTH110-IH4)	Human
Anti-Rhe	Not known	IgM	e0002 (MS62)	Human
Anti-Rhe	Not known	IgM	e0003 (MS69)	Human
Anti-Fy^a	Not known	IgG1	LM487	Murine
Anti-Fy^b	Not known	IgG1	LM447	Murine

[0070] All monoclonal antibodies listed were purified from culture supernatants. Polyclonal anti-RhD preparation was obtained from the Scottish National Blood Transfusion

Service.

[0071] For each target antibody/library combination, a separate well of a 96-well Immulon 4 flat-bottomed ELISA plate (Thermo Labsystems) was coated with 5µg of antibody in 150µl ELISA coating buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, pH9.6). Plates were sealed and incubated at 4°C overnight. Unbound antibody was discarded and 350µl of blocking buffer (0.1M $NaHCO_3$/3% skimmed milk powder) was added to each well. Plates were sealed and incubated for 1 hour at 4°C. The blocking buffer was removed and the wells were washed six times with 300µl TBS/0.1% Tween 20. Approximately $2x10^{11}$ plaque forming units (pfu) in 100µl TBS/0.1% Tween 20 were added to each well and the plates were incubated at room temperature for 1 hour. Wells were washed ten times with 300µl TBS/0.1% Tween 20. Bound phage were eluted by incubation with 100µl of 0.2M glycine-HCl/BSA, pH2.2 (1mg/ml) for 10 minutes, then neutralised with 15µl of 1M Tris-HCL (pH9.1). An aliquot of the Round 1 eluate was removed and titred. The remainder of the eluted phage were amplified by infection of *E. coli* ER2538 cells for 4.5 hours at 37°C. The cultures were centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatants were recovered, 1/6 volume PEG/NaCl (20% polyethylene glycol 8000; 2.5M NaCl) added, then incubated overnight at 4°C. The PEG-precipitated phage were centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was

removed and the phage pellet resuspended in 200µl TBS. Two further rounds of panning were undertaken and approximately 2 x 10^{11} pfu of the Round 2 or Round 3 amplified eluates were used as input phage. Round 3 eluates were titrated but not amplified. Individual plaques were amplified and subjected to PCR amplification, prior to DNA sequence analysis. The specificity of peptide-antibody binding was subsequently determined using both phage ELISA and SPOTs techniques.

[0072] Phage Culture

[0073] Individual phage clones were randomly selected from the Round 3 titration plates and amplified in 1ml volumes of 1:100 dilution of an overnight culture of *E. coli* ER2538, grown in L-Broth. Cultures were incubated at 37°C for 4.5 hours, then centrifuged at 13000rpm for 1 minute. The phage supernatants were recovered for further analysis by PCR amplification, DNA sequencing and phage ELISA.

[0074] PCR Amplification

[0075] To determine the peptide sequences displayed in each of the selected phage clones, the DNA insert encoding the peptide sequence was obtained by PCR. PCR amplification was performed in a final volume of 25µl containing 1x buffer (Bioline), 400µM dNTPs, 1.5mM $MgCl_2$, 1 unit of Taq DNA

polymerase (Bioline), 1µl of phage culture supernatant and 0.5µM each of the appropriate forward and reverse primers. The primers 12MER1For: 5-CGCAATTCCTTTAGTGGTAC-3 and 12MER2Rev: 5-CCCTCATAGTTAGCGTAACG-3 were used for amplification of the C7C, linear 7-mer and 12-mer phage/peptides. The primers Fuse5For1: 5′-ACCGATACAATTAAAGGCTC-3′ and Fuse5Rev1: 5′-TGAATTTTCTGTATGAGG-3′ were used for the amplification of the 15-mer phage/peptides. PCR was carried out in a thermal cycler (Techgene, model FTGene2D; Techne, Cambridge, UK) under the following conditions: denaturation at 95°C for 1 minute; annealing at 55°C for 1 minute; and extension at 72°C for 3 minutes. After 30 cycles, extension was continued at 72°C for an additional 9 minutes. PCR products were recovered using the QIAquick PCR Purification Kit (Qiagen) for sequence analysis.

[0076] Sequence Analysis

[0077] The nucleotide sequences of the phage peptides were obtained using the BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI Prism 377 DNA Sequencer (Applied Biosystems). Primer 12MER2Rev was used to determine the random peptide sequences of the C7C, linear 7-mer and 12-mer PCR products. Primer Fuse5Rev1 was used for the 15-mer PCR products. Amino acid sequences were deduced from the

nucleotide sequences using Lasergene software (DNASTAR Inc., Wisconsin, USA).

[0078] Phage ELISA

[0079] The ability of the recovered phage-peptides to react with the antibodies used in their selection was confirmed by capture ELISA. A 10µg/ml stock of appropriate monoclonal antibody was prepared in ELISA coating buffer and 150µl aliquots were added to wells of an Immulon 4 ELISA plate, and then incubated overnight at 4°C. Unbound antibody was discarded and the wells were blocked with 350µl of blocking buffer (0.1M $NaHCO_3$/3% skimmed milk powder) for 1 hour at 4°C. The wells were washed three times with 300µl TBS/0.5% Tween 20 and 100µl of selected phage culture supernatants were added to appropriate wells for 1 hour at room temperature. Unbound phage were removed and the wells washed six times with 300µl TBS/0.5% Tween 20. A 1:5000 dilution of horseradish peroxidase (HRP) conjugated anti-M13, secondary antibody was prepared in blocking buffer and 100µl aliquots added to each well. Following incubation for 1 hour at room temperature the wells were washed six times with 300µl TBS/0.5% Tween 20. To develop the reactions, a 200µl aliquot of *o*-phenylenediamine dihydrochloride (OPD) substrate (Sigma) was added to each well. The absorbance at 450nm was measured, following incubation at room temperature for 1 hour, by a

microplate ELISA reader. An OD450 signal greater than 0.1 was considered positive.

[0080] SPOT Synthesis

[0081] The peptide mimotopes were synthesised by the SPOT method (Frank; 1992) using F-moc chemistry. The peptides were anchored to an amino-PEG derivatised cellulose membrane (Intavis AG) and synthesised as discrete spots. Synthesis was by stepwise elongation of the peptide chain from C-terminus to the N-terminus, by manual pipetting of the respective amino acids, according to the Sigma Genosys protocol. Incubation times were modified when using Intavis AG membranes. After addition of the final amino acid, the terminal residues and side chains of the peptides were deprotected and the membrane was washed with methanol; air dried and then stored at -20°C prior to use.

[0082] Screening of SPOT membranes

[0083] A membrane was placed in a 50ml Falcon tube and blocked overnight at 4°C with gentle agitation in TBS/0.25% Tween 20/5% skimmed milk powder. A 2µg/ml dilution of the primary (1°) antibody (monoclonal or polyclonal) was prepared in TBS/0.25% Tween20/5% skimmed milk powder before incubation with the membrane for 1 hour at room temperature on a spiral

rotor. Following 4 washes, each for 5 minutes with 20ml of TBS/0.25% Tween20, the membrane was incubated for 1 hour at room temperature with a 1/5000 dilution of the secondary (2°) antibody, prepared in TBS/0.25% Tween20/5% skimmed milk powder. Peroxidase labelled rabbit anti-mouse IgG (Sigma) was used for the detection of the murine anti-Fy^a (LM487) and anti-Fy^b (LM447) 1° antibodies and peroxidase labelled goat anti-human IgG (Pharmacia) for the detection of the human 1° antibodies. Following 4 washes, each for 5 minutes with 20ml of TBS/0.25% Tween20, the membrane was blotted to remove excess fluid. Bound antibody was detected by chemiluminescence. ECL detection reagents (Amersham Biosciences) were mixed and overlaid on the membrane for 2 minutes. The chemiluminescent substrate was removed and the membrane blotted to remove excess fluid. Positive spots were visualised following exposure to Hyperfilm ECL (Amersham Biosciences) for a series of short incubation times (5 seconds to 10 minutes).

[0084] Example 1

[0085] Identification of phage-peptides which mimic the Duffy antigenic epitopes

[0086] The anti-Fy^a (LM487) and anti-Fy^b (LM447) -specific monoclonal antibodies were developed using immunogenic

peptides derived from epitopes on the Duffy glycoprotein (Colligan *et al*, 1998). These antibodies were used to pan phage libraries for peptides that mimicked the native Duffy antigens. Clones from the final third round of each panning experiment were chosen at random for amplification and DNA sequencing. The amino acid sequences encoded by the phage-peptides were deduced from the DNA sequence data.

[0087] Biopanning of each of the 4 phage-peptide libraries was undertaken with the anti-Fy^b monoclonal antibody (LM447) and resulted in the identification of multiple copies of particular sequences from each of the libraries. In total, 100 unique Fyb peptide mimotopes were identified (see Table 2/ peptides Fyb1-71 & Fyb88-116). None of the sequences identified resembled the 14-mer immunisation peptide (PDGDYDANLEAAAP; CPep75) used in the development of the LM447 antibody. However, analysis of the amino acid sequences of these "Fyb" peptide mimotopes identified 2 consensus sequences:

[0088] Consensus 1: **M**(F/Y)**QPD**(N/P)(P/L)(T/P)**T**(K/L)(N/Q)(P/V/A/S)

[0089] Consensus 2: **D**(H/M/V)**HYT**(S/N)**NTDPL**(H/N/R)(A/P/V)**P**

[0090] Certain positions within each consensus sequence had a limited number of amino acids (shown in brackets). These

occurred with equal frequency amongst the total population of Fyb peptide mimotopes selected.

[0091] Table 2: Fyb peptides

SEQ ID NO	Pep ID	Panning Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Fy b1	Fy^b	M	Y	Q	D	T	P	P	P	R	K	N	V			
2	Fy b2	Fy^b	M	Y	Q	P	G	P	Q	G	L	P	Q	S			
3	Fy b3	Fy^b	M	Y	Q	P	P	S	T	T	R	M	Y	P			
4	Fy b4	Fy^b	M	Y	Q	D	A	G	P	T	E	W	S	Y			
5	Fy b5	Fy^b	M	W	Q	P	A	P	P	S	V	R	G	A			
6	Fy b6	Fy^b	M	F	Q	S	A	G	Y	P	S	S	F	R			
7	Fy b7	Fy^b	M	F	Q	E	H	T	P	A	F	G	G	L			
8	Fy b8	Fy^b	M	F	Q	E	P	L	P	P	F	K	A	S			
9	Fy b9	Fy^b	M	F	Q	P	T	H	W	G	T	R	L	S			
10	Fy b10	Fy^b	M	Q	Q	P	A	G	W	K	Y	L	G	Q			
11	Fy b11	Fy^b	M	V	Q	E	L	P	F	V	L	R	S	K			
12	Fy b12	Fy^b	T	F	Q	P	R	S	W	P	N	I	D	L			
13	Fy b13	Fy^b	T	F	Q	Y	L	D	P	P	P	F	T	P			
14	Fy b14	Fy^b	T	Y	Q	H	N	N	P	D	A	F	R	L			
15	Fy b15	Fy^b	M	F	Q	S	N	T	Y	P	I	W	N	V			
16	Fy b16	Fy^b	F	Q	P	N	P	P	P	S	F	A	A	P			
17	Fy b17	Fy^b	M	Y	Q	G	R	N	D	P	H	L	L	P			
18	Fy b18	Fy^b	M	Y	Q	P	R	D	W	R	S	A	P	P			
19	Fy b19	Fy^b	M	F	N	A	D	P	T	I	P	A	Q	P			
20	Fy b20	Fy^b	H	G	T	N	N	T	D								
21	Fy b21	Fy^b	Q	L	D	N	L	T	T								
22	Fy b22	Fy^b	H	Y	Y	H	N	T	D								
23	Fy b23	Fy^b	H	A	L	P	L	H	A								
24	Fy b24	Fy^b	M	F	Q	E	H	P	I								
25	Fy b25	Fy^b	M	W	Q	P	S	T	F								

26	Fy b26	Fy^b	M	F	Q	P	A	V	Q							
27	Fy b27	Fy^b	M	Y	Q	P	L	A	L							
28	Fy b28	Fy^b	M	F	Q	P	D	F	L							
29	Fy b29	Fy^b	M	F	Q	E	L	V	I							
30	Fy b30	Fy^b	M	F	Q	A	N	T	V							
31	Fy b31	Fy^b	M	F	Q	P	L	H	R							
32	Fy b32	Fy^b	M	F	Q	P	T	D	H							
33	Fy b33	Fy^b	M	F	Q	P	L	P	S							
34	Fy b34	Fy^b	M	F	Q	P	Q	Y	H							
35	Fy b35	Fy^b	M	F	Q	P	P	L	K							
36	Fy b36	Fy^b	M	Y	Q	P	P	G	S							
37	Fy b37	Fy^b	M	Y	Q	P	R	T	L							
38	Fy b38	Fy^b	M	W	Q	P	D	A	R							
39	Fy b39	Fy^b	M	Y	Q	D	L	P	Y							
40	Fy b40	Fy^b	M	W	Q	P	Q	P	M	P	G	M	V	G		
41	Fy b41	Fy^b	M	Y	Q	P	R	N	W	S	D	A	S	G		
42	Fy b42	Fy^b	T	Y	Q	P	K	S	P	S	N	F	A	N		
43	Fy b43	Fy^b	M	F	Q	P	N	N	P	P	G	I	H	A		
44	Fy b44	Fy^b	M	F	Q	P	Y	H	P							
45	Fy b45	Fy^b	M	Y	N	P	P	W	G							
46	Fy b46	Fy^b	T	Y	Q	P	L	T	W							
47	Fy b47	Fy^b	V	S	H	T	N	N	T	D	L	R	P	P		
48	Fy b48	Fy^b	D	H	P	P	T	L	N	R	P	P	N	V		
49	Fy b49	Fy^b	H	G	V	S	N	T	D							
50	Fy b50	Fy^b	M	W	Q	P	R	T	M							
51	Fy b51	Fy^b	H	Y	H	S	N	T	D							
52	Fy b52	Fy^b	H	Y	A	S	N	T	D							
53	Fy b53	Fy^b	M	Y	Q	P	M	T	A							
54	Fy b54	Fy^b	M	Y	Q	P	N	S	T	T	Q	L	Y	N		
55	Fy b55	Fy^b	N	F	Q	P	N	D	P	P	A	R	P	S		
56	Fy b56	Fy^b	T	Y	Q	H	M	P	P	P	H	S	P	F		
57	Fy b57	Fy^b	M	Y	Q	E	S	A	P	P	R	Q	V	F		
58	Fy b58	Fy^b	M	W	Q	P	N	S	P	S	V	P	P	P		
59	Fy b59	Fy^b	I	F	N	D	P	W	M							

60	Fy b60	Fy^b	M	F	Q	P	L	P	A							
61	Fy b61	Fy^b	M	Y	Q	P	T	H	W							
62	Fy b62	Fy^b	M	F	Q	P	T	L	V	A	P	L	Q	A		
63	Fy b63	Fy^b	M	F	Q	S	S	P	P	P	L	G	H	A		
64	Fy b64	Fy^b	T	F	Q	P	R	L	F	S	S	W	V	H		
65	Fy b65	Fy^b	M	F	Q	S	R	G	P	N	Q	L	F	P		
66	Fy b66	Fy^b	M	F	Q	P	A	N	P	S	Q	S	T	A		
67	Fy b67	Fy^b	M	W	Q	P	Q	W	T							
68	Fy b68	Fy^b	M	F	Q	P	P	R	N							
69	Fy b69	Fy^b	M	F	Q	P	R	F	P							
70	Fy b70	Fy^b	M	F	Q	P	V	E	L							
71	Fy b71	Fy^b	M	Y	Q	P	N	L	M							
72	Fy b72	Fy^b	M	Y	Q	P	D	P	P	P	T	K	N	P		
73	Fy b73	Fy^b	M	Y	Q	P	D	P	P	P	T	K	N	V		
74	Fy b74	Fy^b	M	Y	Q	P	D	P	P	T	T	K	N	P		
75	Fy b75	Fy^b	M	Y	Q	P	D	P	P	T	T	K	N	V		
76	Fy b76	Fy^b	M	Y	Q	P	D	N	P	P	T	K	N	P		
77	Fy b77	Fy^b	M	Y	Q	P	D	N	P	P	T	K	N	V		
78	Fy b78	Fy^b	M	Y	Q	P	D	N	P	T	T	K	N	P		
79	Fy b79	Fy^b	M	Y	Q	P	D	N	P	T	T	K	N	V		
80	Fy b80	Fy^b	M	F	Q	P	D	P	P	P	T	K	N	P		
81	Fy b81	Fy^b	M	F	Q	P	D	P	P	P	T	K	N	V		
82	Fy b82	Fy^b	M	F	Q	P	D	P	P	T	T	K	N	P		
83	Fy b83	Fy^b	M	F	Q	P	D	P	P	T	T	K	N	V		
84	Fy b84	Fy^b	M	F	Q	P	D	N	P	P	T	K	N	P		
85	Fy b85	Fy^b	M	F	Q	P	D	N	P	P	T	K	N	V		
86	Fy b86	Fy^b	M	F	Q	P	D	N	P	T	T	K	N	P		
87	Fy b87	Fy^b	M	F	Q	P	D	N	P	T	T	K	N	V		
88	Fy b88	Fy^b	S	P	P	T	P	P	R	F	S	D	D	W		
89	Fy b89	Fy^b	L	P	T	L	H	T	A	H	P	P	V	Q		
90	Fy b90	Fy^b	G	T	Q	T	R	S	S	Y	V	L	T	S		
91	Fy b91	Fy^b	N	P	H	P	M	W	N	P	T	S	Y	L		
92	Fy b92	Fy^b	A	Y	R	A	V	T	L	N	Q	P	L	T		
93	Fy b93	Fy^b	Q	Y	L	P	D	H	R	S	S	Q	P	N		

94	Fy b94	Fy^b	L	P	H	R	T	D	Y	L	Y	T	P	E			
95	Fy b95	Fy^b	H	P	A	V	Q	P	P	L	I	Y	M	F			
96	Fy b96	Fy^b	V	P	T	Y	E	I	I	A	R	G	I	R			
97	Fy b97	Fy^b	S	P	S	D	M	F	I	S	T	Q	R	L			
98	Fy b98	Fy^b	L	L	T	Q	T	T	A	Y	A	P	M	S			
99	Fy b99	Fy^b	M	M	N	S	D	P	K	S	F	L	S	L			
100	Fy b100	Fy^b	M	R	N	D	V	P	W	I	P	W	P	V			
101	Fy b101	Fy^b	T	L	P	Y	L	K	M	H	R	N	L	L			
102	Fy b102	Fy^b	Q	S	Y	S	P	R	F								
103	Fy b103	Fy^b	L	K	Q	R	T	L	M								
104	Fy b104	Fy^b	Q	S	Y	P	Q	A	L								
105	Fy b105	Fy^b	Q	S	Y	S	P	K	Y								
106	Fy b106	Fy^b	A	M	Y	Q	P	T	H								
107	Fy b107	Fy^b	H	W	Y	H	N	T	D								
108	Fy b108	Fy^b	L	S	Y	S	P	R	Y								
109	Fy b109	Fy^b	E	S	Y	S	P	R	H								
110	Fy b110	Fy^b	Q	S	Y	P	S	V	Y								
111	Fy b111	Fy^b	D	S	Y	S	P	K	F								

112	Fy b112	Fy^b	Q	S	Y	P	A	K	F								
113	Fy b113	Fy^b	R	S	A	S	A	L	W								
114	Fy b114	Fy^b	E	S	Y	S	P	R	L								
115	Fy b115	Fy^b	P	I	F	T	K	L	H								
116	Fy b116	Fy^b	Q	S	Y	P	Y	R	G								

[0092] Biopanning with the anti-Fy^a monoclonal, LM487, identified a total of 37 unique peptide sequences (see Table 3/Fy^a peptides Fya1-37). A small number of the "Fya" phage-peptides, contained sequence identical to the Fy^a polymorphism, present within the original 14-mer immunisation peptide (PDGDYGANLEAAAP; CPep118), thus validating the use of phage display as an approach to identifying blood group antigen mimics. Sequence analysis of the amino acid sequences of these "Fya" peptide mimotopes also identified 2 putative consensus sequences:

[0093] Consensus I: **YNYQSYPNPFPV**

[0094] Consensus II: **GI**(A/S)(E/D)(D/G)**DYGALSW**

[0095] However, in contrast to the "Fyb" results, many of these "Fya" peptide mimotope sequences did not conform to either consensus sequence identified above.

[0096] Table 3: Fya peptides

[0097]

SEQ ID NO	Pep ID	Panning Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
117	Fy a1	Fy^a	W	Q	S	Y	P	N	P								
118	Fy a2	Fy^a	T	N	L	R	L	P	A								
119	Fy a3	Fy^a	Y	Q	S	Y	P	P	P								
120	Fy a4	Fy^a	Y	Q	S	Y	P	M	R								
121	Fy a5	Fy^a	M	Y	P	D	W	Q	Q								
122	Fy a6	Fy^a	L	E	R	D	P	P	V								
123	Fy a7	Fy^a	Y	Q	S	Y	P	N	W								
124	Fy a8	Fy^a	L	V	P	P	D	G	Y								
125	Fy a9	Fy^a	A	P	F	W	Q	W	M								
126	Fy a10	Fy^a	F	S	P	P	N	W	Y								
127	Fy a11	Fy^a	F	A	P	W	Y	Q	Q	R							
128	Fy a12	Fy^a	Q	S	Y	P	N	T	F								
129	Fy a13	Fy^a	D	W	L	F	K	M	S								
130	Fy a14	Fy^a	G	D	W	L	H	W									
131	Fy a15	Fy^a	S	D	G	D	Y	G	A								
132	Fy a16	Fy^a	G	P	S	W	Q	S	T								
133	Fy a17	Fy^a	V	P	Y	P	N	Q	M	C							
134	Fy a18	Fy^a	P	S	P	G	Q	I									
135	Fy a19	Fy^a	F	E	P	P	N	W	D	S	G	P	R	P			
136	Fy a20	Fy^a	G	I	A	E	D	D	Y	G	A	L	S	W			
137	Fy a21	Fy^a	Y	N	Q	Y	Q	S	Y	P	P	S	L	Q			
138	Fy a22	Fy^a	Y	Q	S	Y	P	S	R	P	P	V	R	L			
139	Fy a23	Fy^a	A	L	A	P	E	E	D	E	V	Y	Y	V			
140	Fy a24	Fy^a	V	L	L	P	E	S	D	E	S	H	R	A			

141	Fy a25	Fy^a	I	P	N	W	Q	S	C							
142	Fy a26	Fy^a	S	G	V	A	F	C	P	P	W	W	C	D	G	P	L
143	Fy a27	Fy^a	W	P	R	H	V	P	L	F	G	L	D	G	Y	V	T
144	Fy a28	Fy^a	A	R	E	Y	G	T	R	F	S	L	I	G	G	Y	R
145	Fy a29	Fy^a	A	S	R	R	L	L	P	S	D	V	R	L	P	S	S
146	Fy a30	Fy^a	L	W	D	P	P	P	F	G	L	S	R	I	F	F	G
147	Fy a31	Fy^a	D	A	R	P	L	A	W	Y	E	E	P	S	F	W	M
148	Fy a32	Fy^a	S	G	Y	A	R	P	F	Y	Q	S	Y	P	A	A	S
149	Fy a33	Fy^a	V	N	S	T	K	W	P	G	M	P	S	F			
150	Fy a34	Fy^a	V	S	P	P	E	W	Y	P	L	A	A	D			
151	Fy a35	Fy^a	T	G	P	K	L	H	C	P	P	A	V	C			
152	Fy a36	Fy^a	F	K	N	P	S	Q	S	Y	P	P	E	P			
153	Fy a37	Fy^a	F	T	M	E	R	D	P	P	I	A	R	V			

[0098] The data shows that phage-peptides were identified that mimicked the Duffy antigenic epitopes. The anti-Fy^a antibody bound phage-peptides (Peptides Fya15 & Fya20) that contained sequences identical to the Fy^a polymorphism and the anti-Fy^b antibody selected peptide mimotopes, confirming the use of phage display as a valid means of identifying blood group antigen mimics.

[0099] Example 2

[00100] Identification of phage-peptides which mimic the complex RhD antigenic epitopes

[00101] Phage-peptides that mimic RhD antigenic epitopes

were identified with 3 monoclonal anti-Ds (T10, T22 & T27). Biopanning of the 4 phage-peptide libraries with this limited number of monoclonal antibodies selected 80 RhD peptide mimotopes (see Table 4/ Peptides RhD 1-80). Additionally, biopanning with a polyclonal anti-RhD preparation identified a further 25 RhD peptide mimotopes (see Table 4/ peptides RhD81-105). Peptide RhD52 was selected with both T10 and the polyclonal anti-RhD preparation.

[00102] Table 4: RhD peptides

SEQ ID NO	Pep ID	Panning Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
154	RhD 1	RhD T27	G	L	F	K	I	Q	P								
155	RhD 2	RhD T27	L	G	H	H	N	P	H								
156	RhD 3	RhD T27	N	L	L	P	A	K	L								
157	RhD 4	RhD T27	H	T	A	L	S	T	S								
158	RhD 5	RhD T27	T	P	G	S	G	Q	F								
159	RhD 6	RhD T27	F	S	P	N	N	S	E	T	T	N	Y	T			
160	RhD 7	RhD T27	T	T	V	P	D	T	W	V	L	F	P	R			
161	RhD 8	RhD T27	S	N	V	P	L	H	W	I	L	S	P	R			
162	RhD 9	RhD T27	W	H	F	E	W	W	R	A	T	P	S	G			
163	RhD 10	RhD T27	N	Y	P	A	V	V	S	N	S	L	L	A			
164	RhD 11	RhD T27	D	V	Y	D	T	R	P	Y	H	G	F	M			
165	RhD 12	RhD T27	H	Q	N	P	W	L	L	W	P	R	G	F			
166	RhD 13	RhD T27	V	I	D	W	L	L	C	P	R	G	F	S	A	C	M
167	RhD 14	RhD T27	D	R	R	I	F	W	W	S	L	R	S	A	P	G	A
168	RhD 15	RhD T27	L	H	A	W	H	R	N	T	I	G	W	W	I	G	F
169	RhD 16	RhD T22	W	G	L	W	F	S	N								
170	RhD 17	RhD T22	W	G	I	W	W	N	N								

171	RhD 18	RhD T22	W	G	L	W	S	T	P								
172	RhD 19	RhD T22	W	V	H	L	Q	S	P								
173	RhD 20	RhD T22	W	G	L	W	W	H	T								
174	RhD 21	RhD T22	W	G	L	W	W	T	G								
175	RhD 22	RhD T22	L	L	G	L	T	E	T								
176	RhD 23	RhD T22	D	S	R	W	L	L	P								
177	RhD 24	RhD T22	F	H	W	W	P	R	T	Q	D	P	H	R			
178	RhD 25	RhD T22	F	H	W	S	W	Y	T	P	S	R	P	S			
179	RhD 26	RhD T22	H	S	S	W	W	L	A	L	A	K	P	T			
180	RhD 27	RhD T22	W	H	W	Y	P	R	F	S	P	P	S	H			
181	RhD 28	RhD T22	N	D	Y	S	D	S	N	Q	V	P	A	S			
182	RhD 29	RhD T22	F	H	W	P	R	S	W	V	T	W	Q	S			
183	RhD 30	RhD T22	H	L	S	S	R	H	L	F	V	P	L	S			
184	RhD 31	RhD T22	T	I	T	D	C	Y	P	I	C	V	S	P			
185	RhD 32	RhD T22	W	P	C	H	P	I	C	L	S	P	R	G			
186	RhD 33	RhD T22	A	M	D	C	F	P	I	C	L	W	N	P			
187	RhD 34	RhD T22	W	N	C	F	P	I	C	H	A	S	G	L			
188	RhD 35	RhD T22	V	Y	A	L	G	C	W	P	I	C	H	K			
189	RhD 36	RhD T22	H	H	V	F	T	P	N	C	Y	P	I	C			
190	RhD 37	RhD T22	W	H	W	S	S	L	S	W	P	A	L	P			
191	RhD 38	RhD T22	G	N	W	L	F	N	S	C	Y	P	L	C	F	P	L
192	RhD 39	RhD T22	A	F	L	G	H	S	H	W	F	P	S	V	A	S	R
193	RhD 40	RhD T22	Q	L	L	C	F	P	I	C	R	P	E	P	P	V	S
194	RhD 41	RhD T10	W	Y	T	K	A	P	Y								
195	RhD 42	RhD T10	W	Y	H	K	A	P	Y								
196	RhD 43	RhD T10	Y	Y	Q	R	A	P	Y								
197	RhD 44	RhD T10	H	W	K	H	P	W	G	A	W	D	T	L			
198	RhD 45	RhD T10	W	H	W	Q	W	T	P	W	S	I	Q	P			
199	RhD 46	RhD T10	W	H	K	N	W	W	P	P	S	T	P	N			
200	RhD 47	RhD T10	S	M	S	S	M	L	L	A	A	Q	T	V			
201	RhD 48	RhD T10	Q	S	H	Y	R	H	I	S	P	A	Q	V			
202	RhD 49	RhD T10	S	M	S	Q	P	K	S	Q	V	N	A	H			
203	RhD 50	RhD T10	W	H	W	T	F	Y	T	P	L	E	S	T			
204	RhD 51	RhD T10	E	S	L	S	T	D	T	Y	A	I	L	L			

205	RhD 52	RhD T10	H	S	S	W	Y	I	Q	H	F	P	P	L			
206	RhD 53	RhD T10	S	T	Y	L	N	G	P	T	G	V	D	L			
207	RhD 54	RhD T10	Q	H	K	T	S	I	T	G	H	L	E	P			
208	RhD 55	RhD T10	F	H	R	W	P	T	W	P	L	P	S	P			
209	RhD 56	RhD T10	V	P	P	W	V	S	V	R	T	G	P	G			
210	RhD 57	RhD T10	T	L	V	Y	Q	P	P	W	Y	R	I	A			
211	RhD 58	RhD T10	F	H	Q	R	L	W	W	P	T	H	T	P			
212	RhD 59	RhD T10	W	H	W	R	L	Y	S	A	N	T	P				
213	RhD 60	RhD T10	H	A	A	F	E	P	R	G	D	V	R	H	T	L	L
214	RhD 61	RhD T10	S	I	W	D	L	P	L	Q	Y	R	G	F	G	T	S
215	RhD 62	RhD T10	L	W	R	L	R	G	G	S	F	P	V	I	S	H	G
216	RhD 63	RhD T10	R	N	A	L	H	S	L	R	T	L	S	S	S	W	V
217	RhD 64	RhD T10	F	H	R	H	W	W	P	P	T	L	S	T			
218	RhD 65	RhD T10	R	P	H	L	L	D	W	E	L	N	P	V			
219	RhD 66	RhD T10	F	H	W	R	W	S	T	F	P	E	Y	P			
220	RhD 67	RhD T10	V	W	A	V	S	L	P	W	Y	R	Y	P			
221	RhD 68	RhD T10	L	D	T	Y	W	Y	R	E	H	F	R	R			
222	RhD 69	RhD T10	V	H	W	R	W	W	D	Q	R	V	P	M			
223	RhD 70	RhD T10	L	P	W	Y	Q	L	T								
224	RhD 71	RhD T10	V	P	W	F	R	A	P								
225	RhD 72	RhD T10	W	H	P	P	Q	P	S								
226	RhD 73	RhD T10	F	H	E	N	W	P	S								
227	RhD 74	RhD T10	F	W	W	Q	V	P	A								
228	RhD 75	RhD T10	T	Q	W	Y	Q	I	A								
229	RhD 76	RhD T10	L	P	W	F	Q	L	P								
230	RhD 77	RhD T10	T	P	L	S	K	S	T								
231	RhD 78	RhD T10	L	P	W	Y	A	T	P								
232	RhD 79	RhD T10	L	P	W	Y	R	H									
233	RhD 80	RhD T10	I	P	W	Y	K	I	T								
234	RhD 81	RhD polyclonal	F	H	S	T	W	P	W	R	E	A	E	G			
235	RhD 82	RhD polyclonal	F	H	A	N	W	P	Q	S	A	R	D	V			

236	RhD 83	RhD polyclonal	F	H	S	D	W	P	G	Q	T	F	T	W			
237	RhD 84	RhD polyclonal	F	H	E	N	W	S	T	R	P	T	T	R			
238	RhD 85	RhD polyclonal	F	H	S	V	Y	P	W	R	E	A	E	G			
239	RhD 86	RhD polyclonal	F	H	S	N	W	P	S	A	Y	T	A	R			
240	RhD 87	RhD polyclonal	F	H	S	N	W	P	S	L	I	R	A	R			
241	RhD 88	RhD polyclonal	A	G	Y	Q	I	G	M	P	N	P	L	L			
242	RhD 89	RhD polyclonal	F	H	W	R	Y	P	L	P	L	P	G	Q			
243	RhD 90	RhD polyclonal	S	P	T	S	F	R	Q	V	F	G	F	Y			
244	RhD 91	RhD polyclonal	A	L	P	E	L	S	S	L	P	E	S	A	R		
245	RhD 92	RhD polyclonal	L	H	W	W	P	T	Y	G	N	N	G	M			
246	RhD 93	RhD polyclonal	F	H	R	P	Y	Y	W	P	P	T	P	L			
247	RhD 94	RhD polyclonal	F	H	W	R	L	P	Y	P	L	P	S	S			
248	RhD 95	RhD polyclonal	I	H	W	W	V	K	S	P	P	P	G	S			
249	RhD 96	RhD polyclonal	S	H	W	W	T	S	I	L	A	T	P	S			
250	RhD 97	RhD polyclonal	F	H	W	H	L	Q	P	Q	L	W	S	Y			

251	RhD 98	RhD polyclonal	L	H	R	E	W	I	Y	P	Y	L	I	S			
252	RhD 99	RhD polyclonal	G	Q	K	T	H	N	P	F	H	L	H	P			
253	RhD 100	RhD polyclonal	A	T	W	S	H	H	L	S	S	A	G	L			
254	RhD 101	RhD polyclonal	F	H	R	H	Y	Y	P	W	A	L	I	Q			
255	RhD 102	RhD polyclonal	H	S	L	K	H	T	Q	M	S	Y	S	S			
256	RhD 103	RhD polyclonal	S	V	S	V	G	M	K	P	S	P	R	P			
257	RhD 104	RhD polyclonal	W	P	H	Q	V	H	K	H	I	Y	R	Q			
258	RhD 105	RhD polyclonal	A	P	P	Y	P	G	P	L	P	L	S	L			

[00103] Example 3

[00104] Identification of phage-peptides which mimic RhEe antigenic epitopes

[00105] Biopanning was undertaken with 3 anti-RhE monoclonal antibodies (1x IgG, 2x IgM; see Table 1) and resulted in the identification of multiple copies of many sequences from each of the 4 phage-peptide libraries. Sequence similarities were also evident between the selected

phage-peptides from the different libraries. Peptides RhE18 and RhE24 were selected with antibodies E0002 and E0003 and Peptide RhE19 was selected with antibodies E0001 and E0002 These results indicate that, regardless of class, antibodies raised against the same epitope will identify some phage with the identical peptide sequence. In total, 36 unique RhE peptide mimotopes were identified (see Table 5; Peptides RhE1-36). Biopanning with 2 anti-Rhe monoclonal antibodies (2x IgM; see Table 1) identified 17 unique Rhe peptide mimotopes (see Table 5; Peptides Rhe1-17).

[00106] Table 5: RhE/e

[00107]

SEQ ID NO	Pep ID	Panning Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
259	RhE1	RhE	A	A	P	P	Y	V	F	C	S	L	S	P	R		
260	RhE2	RhE	T	V	D	L	S	P	A	F	L	F	F	G	R	L	A
261	RhE3	RhE	S	L	A	P	Y	S	L	R	I	L	R	V	G	S	A
262	RhE4	RhE	R	N	V	L	P	I	F	N	D	V	Y	W	I	A	L
263	RhE5	RhE	R	N	V	P	P	I	F	N	D	V	Y	W	I	A	F
264	RhE6	RhE	I	N	N	T	F	T	W								
265	RhE7	RhE	Y	E	L	N	L	M	T								
266	RhE8	RhE	W	G	I	T	V	E	T	A	Y	G	T	A			
267	RhE9	RhE	N	Q	F	L	L	W	E	T	R	S	M	R			
268	RhE10	RhE	N	V	S	L	T	N	L	T	Y	K	P	R			
269	RhE11	RhE	Y	T	P	P	D	W	S	W	W	P	A	P			
270	RhE12	RhE	I	K	N	T	S	L	Q	Q	L	V	T	A			
271	RhE13	RhE	E	W	L	A	Y	D	G	I	R	A	Y	S			
272	RhE14	RhE	H	A	K	V	Q	V	S	S	P	F	P	P			

273	RhE15	RhE	L	S	S	Q	F	K	Q								
274	RhE16	RhE	G	T	F	K	W	Y	Q								
275	RhE17	RhE	T	K	M	D	Q	S	T								
276	RhE18	RhE	I	P	E	E	A	L	R	A	R	F	K	T			
277	RhE19	RhE	S	F	Q	D	A	L	L	S	R	W	Y	S			
278	RhE20	RhE	S	N	L	R	S	W	L	F	P	F	D	R	V	G	N
279	RhE21	RhE	P	G	P	M	F	G	G	S	Y	I	P	S	L	H	H
280	RhE22	RhE	Y	P	Q	G	L	W	R								
281	RhE23	RhE	Y	P	Q	G	E	W	R								
282	RhE24	RhE	G	P	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
283	RhE25	RhE	G	L	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
284	RhE26	RhE	W	P	P	I	S	R	H								
285	RhE27	RhE	A	H	S	W	L	P	G	A	G	L	L	M			
286	RhE28	RhE	S	L	T	H	S	P	R	T	P	I	L	A			
287	RhE29	RhE	F	P	N	L	R	E	R	H	E	P	L	F			
288	RhE30	RhE	G	H	S	Y	H	A	L	I	T	R	Y	L	G	A	A
289	RhE31	RhE	W	A	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
290	RhE32	RhE	D	F	A	Q	A	L	F	L	R	Y	V	V	S	G	L
291	RhE33	RhE	H	R	W	M	P	H	V	F	A	V	R	Q	G	A	L
292	RhE34	RhE	W	A	S	F	Y	A	S	S	Y	R	D	S	R	L	L
293	RhE35	RhE	R	L	P	A	T	I	R	A	L	L	G	R	D	V	R
294	RhE36	RhE	R	L	P	A	T	I	R	A	L	L	G	R	D	V	W
295	Rhe1	Rhe	V	P	R	H	N	P	I								
296	Rhe2	Rhe	P	K	A	F	Q	Y	G	G	R	A	V	G	G	L	W
297	Rhe3	Rhe	D	S	G	A	L	F	N	H	I	F	M	P	G	P	F
298	Rhe4	Rhe	L	H	S	S	H	L	P	P	D	D	R	R	W	G	L
299	Rhe5	Rhe	P	R	Q	F	P	L	R	D	L	Y	T	F	R	Y	W
300	Rhe6	Rhe	G	A	A	N	L	Y	V	S	S	F	L	I	P	L	H
301	Rhe7	Rhe	R	N	V	P	P	I	F	N	D	V	Y	W	I	A	L
302	Rhe8	Rhe	Q	P	R	G	V	T	V	H	G	D	A	W	R	V	A
303	Rhe9	Rhe	G	L	D	L	L	G	D	V	R	I	P	V	V	R	R
304	Rhe10	Rhe	A	Y	W	D	L	Y	G	V	G	F	A	F	S	A	P
305	Rhe11	Rhe	I	W	T	I	T	G	S	T	K	Q	A	F	D	R	S
306	Rhe12	Rhe	E	D	W	F	S	A	S	I	R	R	V	P	T	F	A

307	Rhe13	Rhe	A	V	K	L	R	P	S	S	C	W	L	K	S	T	C
308	Rhe14	Rhe	R	F	D	G	I	D	L	R	V	S	F	V	S	R	P
309	Rhe15	Rhe	A	S	L	H	P	V	P	K	T	W	F	S	L	L	S
310	Rhe16	Rhe	L	A	P	P	P	S	P								
311	Rhe17	Rhe	A	V	T	F	N	S	Y	F	G	F	S	T	T	S	V

[00108] Example 4

[00109] Specific binding of synthetic peptides to the original Duffy monoclonal antibodies

[00110] The relevance of the phage-peptides selected by biopanning was tested in immunological studies. Initially, 20 "Fy^b" phage (Peptides Fyb1-13, Fyb 20-21, Fyb 24-28; see Table 2 for sequence details) were selected for investigation by both phage ELISA and SPOTs analysis, to confirm that synthetic peptides could bind specifically the original monoclonal antibodies.

[00111] The phage ELISA results confirmed the binding of the anti- Fy^b monoclonal, LM447. A 10µg/ml stock of this monoclonal antibody was prepared in ELISA coating buffer and 150µl aliquots were added to wells of an Immulon 4 ELISA plate, and then incubated overnight at 4°C. Unbound antibody was discarded and the wells were blocked with 350µl of blocking buffer for 1 hour at 4°C. The wells were washed three times with 300µl TBS/0.5% Tween 20 and 100µl of

selected phage culture supernatants were added to appropriate wells for 1 hour at room temperature. Unbound phage were removed and the wells washed six times with 300µl TBS/0.5% Tween 20. A 1:5000 dilution of horseradish peroxidase (HRP) conjugated anti-M13, secondary antibody was prepared in blocking buffer and 100µl aliquots added to each well. Following incubation for 1 hour at room temperature the wells were washed six times with 300µl TBS/0.5% Tween 20. To develop the reactions, 200µl aliquots of *o*-phenylenediamine dihydrochloride (OPD) substrate (Sigma) was added to each well. The absorbance at 450nm was measured following incubation at room temperature for 1 hour. The absorbance values at 450nm, confirmed the binding of 14 (Peptides Fyb1, Fyb2, Fyb4-5, Fyb7-13, Fyb25, 27 & 28) of the 20 phage assayed (see Figure 1).

[00112] The 20 Fy^b peptide mimotopes were synthesised in duplicate, by SPOTs. Binding experiments using the anti-Fy^b antibody (LM447) showed that the antibody successfully bound 2 of the 20 peptide sequences (Peptides Fyb12 & Fyb13) tested (Figure 2). Binding was also observed in Spot 21 with the Peptide CPep75 (the positive control for the assay). Despite Peptides Fyb1, Fyb7, Fyb9 and Fyb11 producing phage ELISA signals equally as strong as Peptides Fyb12 and Fyb13, these 4 peptides failed to react with the anti-Fy^b antibody (LM447) when synthesised as SPOTs.

[00113] Phage ELISA analysis of 19 "Fy^a" phage-peptide clones (Peptides Fya1-18 & Fya25; see Table 3 for sequence details) confirmed the binding specificity of the LM487 antibody for the Fy^a phage-peptides. Figure 3 shows that only 3 of 19 (Peptides Fya2, Fya14 & Fya18) failed to bind the antibody. These 19 peptides were amongst 37 (Peptides Fya1-37) synthesised as SPOTs and a strong positive signal was only observed with Peptide Fya15 (Spot 15), following hybridisation of the membrane with the anti-Fy^a antibody (Figure 4).

[00114] These results mirrored the observations obtained for the Fy^b phage-peptides when tested in parallel by both phage ELISA and SPOTs. A positive phage ELISA signal did not automatically guarantee a positive signal when the corresponding peptide mimotope was synthesised and tested by SPOTs hybridisation. Moreover, sequence analysis identified no obvious sequence similarities amongst either positive or negative peptides.

[00115] The results from both the Fy^a and Fy^b phage ELISA and SPOTs assays suggested that the mode of presentation of the peptide is important in antibody binding. Peptides that form part of a fusion protein in the phage coat may adopt a different conformation when synthesised on a membrane by

SPOTs. Without the surrounding protein, antibody recognition may be subsequently affected. Therefore, rather than simply using ELISA to select phage-peptide clones for analysis by SPOTs, all phage-peptides (Fy^b, Fy^a, RhD and RhEe) selected by biopanning were characterised by DNA sequence analysis, prior to SPOTs analysis with the monoclonal antibody originally used in their selection, in order to ensure no potentially diagnostic peptide was overlooked.

[00116] Reciprocal Hybridisation to Identify Cross-reactive Peptides

[00117] A series of 44 peptides was synthesised in duplicate (Figure 5). Spots 1-37 were Fya peptide mimotopes (Fya 1-37), Spots 38-40 were repeats of Peptides Fya17, Fya25 and Fya35, respectively and Spots 41-44 were Control peptides (Spot 41 was the immunisation peptide [Cpep75] used to generate the anti-Fy^b antibody; Spot 42 an irrelevant 12-mer [Cpep76]; Spot 43 the immunisation peptide [CPep118] used to generate the anti-Fy^a antibody and Spot 44 an Fyb peptide mimotope [Fyb1]). Hybridisation of the membranes, in parallel, with the anti- Fy^a and anti-Fy^b monoclonal antibodies, confirmed the Fy^a-specificity of a number of the peptides (Peptide spots 15, 20, and 43) but also identified peptides capable of binding the anti-Fy^b antibody (Spots 36 and 41) and a further peptide displaying cross-reactivity

with both antibodies (Spot 3). The signals observed with Spots 43 and 41 were not unexpected as these 2 peptides were included as positive controls for the anti- Fy^a and anti-Fy^b monoclonal antibodies, respectively.

[00118] Generation of consensus sequence-derived synthetic Fyb peptide mimotopes by amino acid substitution

[00119] Sequence analysis of the 100 unique "Fyb" peptide mimotopes (Fyb1-71 & Fyb88-116) had identified 2 consensus sequences. Consensus 1: M(F/Y)QPD(N/P)(P/L)(T/P)T(K/L)(N/Q)(P/V/A/S) contains a series of positions (amino acid 2, 6, 7, 8, 10, 11 & 12) which have a limited number of residues occurring with equal frequency. A series of 16 peptide mimotopes (Peptides Fyb72-87; see Table 2) were synthesised by SPOTs, with amino acid substitutions at positions 2, 6, 8 and 12. On testing, 2 of these consensus sequence-derived peptide mimotopes (Peptide Fyb84; Spot 13 and Peptide Fyb85; Spot 14) proved capable of binding the anti-Fy^b antibody LM447 (see Figure 6). Analysis of these 2 synthetic, peptide mimotope sequences identified a single amino acid difference at position 12. The strength of signal observed for these 2 sequences was however dramatically lower than that achieved with Peptide CPep75 (Spot 17), the positive control used for this assay. This result shows that peptide mimotopes may also be artificially

generated through amino acid substitution experiments and not simply through biopanning.

[00120] Example 5

[00121] Specific binding of antibodies to synthetic Rh peptides determined by SPOTs

[00122] The RhD, RhE and Rhe peptide mimotopes (see Tables 4 & 5) were synthesised by SPOTs and hybridisation of the membranes was undertaken with the appropriate antibodies (Table 1). Figures 7-10 show examples of peptide mimotopes reacting specifically with the antibody originally used in their selection.

[00123] Figure 7 shows the results following hybridisation of a subset of the anti-RhD antibody-selected peptide mimotopes with the monoclonal antibody T10. 40 peptides (RhD41-RhD80) were synthesised and positive signals were observed in 11 spots (Spots 1-3, 8, 17, 27, 28, 31, 37, 39 & 40; see Table for peptide sequences).

[00124] A further 28 RhD peptide mimotopes were synthesised by SPOTs. Peptide mimotopes RhD81-105 (Spots 1-25) were originally selected with the polyclonal anti-RhD preparation and Peptides RhD68, RhD71 and RhD79 (Spots 26-28)

were identified through biopanning with the anti-RhD antibody T10. The peptides CPep75 (Spot 29) and CPep76 (Spot 30) were included in the assay as negative controls. Figure 8 identifies a series of 11 peptides recognised by the polyclonal anti-RhD (Spots 3, 5, 6, 12, 14, 16, 24, 26-28 & 30; see Table for peptide sequences). This series of peptides includes 3 peptides (Spots 26-28) originally selected with T10. This result strongly suggested that peptides identified with individual monoclonal antibodies (eg T10) mimic epitopes of the antigen to which the polyclonal antibody was raised.

[00125] Figure 9 shows the results following hybridisation of the 36 RhE peptide mimotopes with the anti-RhE monoclonal antibody E0002. The peptides CPep75 (Spot 37) and CPep76 (Spot 38) were included in the assay as negative controls. Specific binding of the antibody was achieved with 4 spots (Spot 18: Peptide mimotope RhE18; Spot 19: RhE19; Spot 24: RhE24 & Spot 25: RhE25).

[00126] Figure 10 shows the results following hybridisation of the 16 Rhe peptide mimotopes with the anti-Rhe monoclonal antibody e0002. The peptides CPep75 (Spot 17) and CPep76 (Spot 18) were included in the assay as negative controls. Strong positive signals were observed with 2 spots (Spots 10 & 13; see Table for peptide sequences). A further

3 weakly, positive spots were also noted (Spots 8, 11 & 16) however these signals were only as strong as that achieved with the control peptide CPep 76 (Spot 18) which was included in the assay as a negative control.

[00127] Overall, the results achieved with the Rh peptide mimotopes were comparable to those obtained with the Fya and Fyb peptides and Fy^a and Fy^b monoclonal antibodies. SPOTs analysis identified Rh peptides that reacted specifically with their cognitive antibody.

[00128] Example 6

[00129] Specific binding of antibodies to synthetic peptide mimotopes bound to microsphere beads

[00130] Further confirmation that the peptide mimotopes can specifically bind antibodies raised to native blood group antigens was achieved following the immobilisation of peptide mimotopes onto polystyrene LiquiChip Ni-NTA microsphere beads (Qiagen).

[00131] Peptide mimotopes (e.g. Fyb16; RhD12) were synthesised with a C-terminal tag consisting of 6 Histidine amino acids. Stock solutions (4µg/ml) of the 6xHis tagged peptide mimotopes were prepared in PBS/0.1%BSA. The peptides

were immobilised onto the beads using the Ni-NTA groups attached to the surface of the beads, as follows. A 50µl volume of a 4µg/ml peptide stock (Fyb16 or RhD12) was mixed with an equal volume of LiquiChip Ni-NTA beads (Bead 50 or Bead 52, respectively). Following incubation with mixing at 4°C overnight, the Bead/peptide mixture was diluted with 900µl of PBS/0.1%BSA. The Bead/peptide mixture was vortexed for 2 minutes and then a 10µl volume was aliquoted into the well of a 96-well, round-bottomed ELISA plate (Nunc). A 10µl volume of the test monoclonal antibody (LM447: anti-Fy^b or T27: anti-RhD) was added to the well of the ELISA plate and the reaction volume was made up to 60µl with PBS/1%BSA. The plate was incubated at room temperature for 90 minutes, in the dark and then a 10µl volume (200ng) of phycoerythrin conjugated sheep anti-mouse IgG (added to wells containing the LM447: anti-Fy^b monoclonal antibody) or phycoerythrin conjugated goat anti-human IgM (added to wells containing the T27: anti-RhD monoclonal antibody) was added into the well. The plate was incubated at room temperature for a further 90 minutes, in the dark. The plate was briefly vortexed, then loaded into a Luminex 100 Analyser (Luminex). Confirmation of bound monoclonal antibody was determined by the detection of a phycoerythrin fluorochrome signal. The intensity of the signal equates to the quantity of monoclonal antibody bound to the immobilised peptide mimotope.

The results are set out in Figures 11 and 12 where specific binding of anti-Fyb monoclonal antibody (LM447) to Peptide Mimotope Fyb16 and specific binding of the anti-RhD monoclonal antibody (T27) to Peptide Mimotope RhD12 is shown.

[00132] Example 7

[00133] Specific binding of antibodies to synthetic peptide mimotopes bound to superparamagnetic beads

[00134] The ability of peptide mimotopes to specifically bind antibodies was further tested using superparamagnetic polystyrene beads (TALON Dynabeads; Invitrogen) in a modified gel agglutination assay.

[00135] A 50µl volume of the magnetic beads was added to 700µl of TBS (50mM Tris-HCl pH7.5; 150mM NaCl). Following mixing for 5 minutes at room temperature, the magnetic beads were isolated using a Magnetic Particle Concentrator (Invitrogen). The supernatant was discarded and the beads were washed for 5 minutes in 700 µl of TBS. The beads were again separated from the buffer using the MPC magnet, and then resuspended in 700 µl of TBS. Peptide mimotopes (e.g. Fyb16; RhD12) were synthesised with a C-terminal tag consisting of 6 Histidine amino acids. Stock solutions (100µg/ml) of the 6xHis tagged peptide mimotopes were

prepared in PBS.

[00136] A 50µl volume of diluted TALON beads was placed in the reaction chamber of an NaCl gel card (ID Microtyping System; Diamed), together with a 50µl volume of diluted His-tagged peptide mimotope (e.g. RhD12) and 100µl of test human monoclonal antibody (e.g. T27: anti-RhD). The gel card was incubated at 37°C for 1 hour in an Incubator-ID (DiaMed). Following incubation, the card was centrifuged for 10 minutes in an ID Centrifuge 24S (DiaMed). Antibody-positive reactions are visualised by a layer of beads upon the surface of the gel matrix. In negative reactions, non-agglutinated beads pass through the gel matrix and form a pellet.

[00137] To test the binding of a murine monoclonal antibody (e.g. LM447: anti-Fy^b monoclonal antibody), a 1µl volume of sheep anti-mouse IgG is first added to the reaction chamber of an NaCl gel card and allowed to equilibrate with the gel matrix for 10 minutes at room temperature, prior to the addition of a 50µl volume of diluted TALON beads, a 50µl volume of diluted His-tagged peptide mimotope (e.g. Fyb16) and 100µl of the test murine monoclonal antibody (e.g. LM447: anti-Fy^b). The gel card was incubated at 37°C for 1 hour in an ID Incubator (DiaMed). Following incubation, the card was centrifuged for 10 minutes in an ID centrifuge (DiaMed). Again the reaction was read visually.

[00138] These examples demonstrate that the synthetic peptide mimotopes are capable of specifically binding antibodies raised to native blood group antigens even when bound to a solid support. In these examples the solid support used is polystyrene Ni-NTA microsphere beads or supermagnetic polystyrene beads but it is envisaged that the synthetic peptide mimotopes will be capable of specifically binding antibodies raised to native blood group antigens when immobilised on any suitable support.

The results are identified in Figures 13 and 14 whereby specific binding of anti-RhD monoclonal antibody (T27) to Peptide Mimotope RhD12 and specific binding of the anti-Fy^b monocolonal antibody (LM447) to Peptide Mimotope Fyb16 is shown.

CLAIMS

1. A mimotope selected from at least one of SEQ ID Nos 1 to 311 or a peptide fragment, an immunoreactive analogue or derivative or a cross-reactive sequence thereof.

2. A method for detecting an antibody to a blood group antigen in a sample, the method comprising:

a) reacting a mimotope of said blood group antigen with the sample to be tested,

b) detecting any reaction between the sample and the mimotope,

c) wherein a reaction between the sample and the mimotope is indicative of the presence of an antibody to the blood group antigen.

3. A method according to claim 2 wherein before step a) the mimotope is immobilised on a solid support.

4. A method according to claim 3 wherein the solid support is selected from a membrane, glass slide or bead.

5. A method according to any of claims 2 to 4 wherein the antibody is an alloantibody.

6. A method of diagnosing diseases or a reaction to blood products involving the generation of alloantibodies or

autoantibodies, the method comprising using a mimotope of a blood group antigen to identify antibodies associated with the disease.

7. A method according to claim 6 wherein the disease is HTR, haemolytic disease of the newborn or autoimmune haemolytic anaemia.

8. A method of diagnosing according to either claim 6 or 7 wherein the method of detecting is according to any of claims 2 to 5.

9. A method according to any preceding claim wherein the blood group antigen is selected from Rhesus, Kell, Duffy, Kidd, ABO, MNS, P, Lutheran, Lewis, Diego, Yt, Xg, Scianna, Dombrock, Colton, Landsteiner-Wiener, Chido-Rodgers, Hh, Kx, Gerbich, Cromer, Knops, Indian, Ok, Raph, JMH, I, Globoside or GIL antigen mimotopes.

10. A device for detecting the presence of an antibody to a blood group antigen in a sample, the device comprising an array of mimotopes of a blood group antigen.

11. A device according to claim 10 wherein the mimotope is immobilised on a solid support

Figure 1: Fy^b Phage ELISA

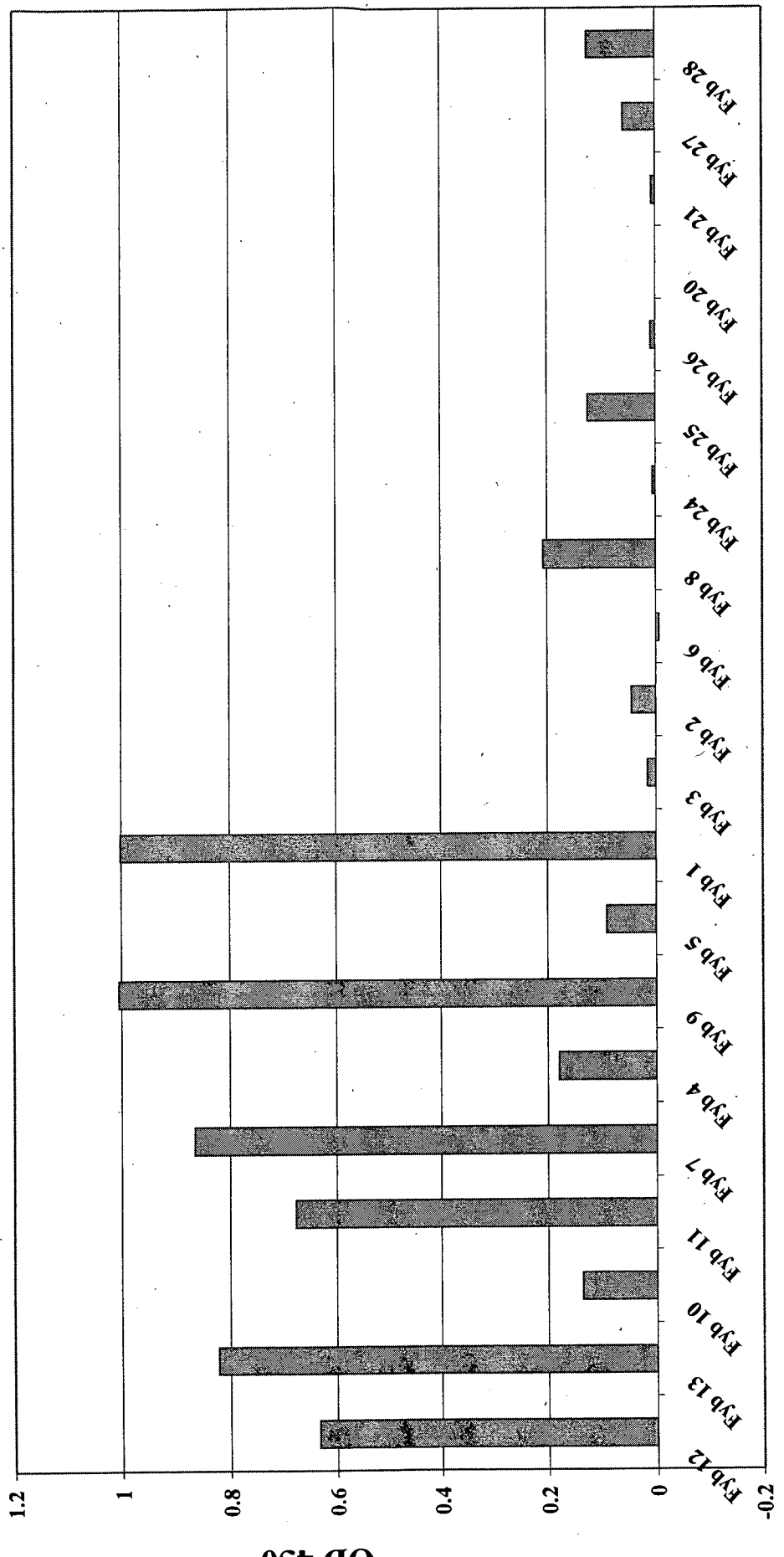
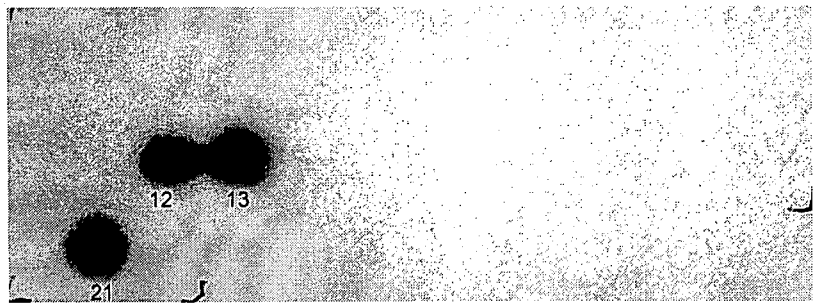


Figure 2: SPOTs Analysis of Fy^b Peptide Mimotopes

Membrane 1



Membrane 2

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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2	Fy b2	M	Y	Q	P	G	P	Q	G	L	P	Q	S			
3	Fy b3	M	Y	Q	P	P	S	T	T	R	M	Y	P			
4	Fy b4	M	Y	Q	D	A	G	P	T	E	W	S	Y			
5	Fy b5	M	W	Q	P	A	P	P	S	V	R	G	A			
6	Fy b6	M	F	Q	S	A	G	Y	P	S	S	F	R			
7	Fy b7	M	F	Q	E	H	T	P	A	F	G	G	L			
8	Fy b8	M	F	Q	E	P	L	P	P	F	K	A	S			
9	Fy b9	M	F	Q	P	T	H	W	G	T	R	L	S			
10	Fy b10	M	Q	Q	P	A	G	W	K	Y	L	G	Q			
11	Fy b11	M	V	Q	E	L	P	F	V	L	R	S	K			
12	Fy b12	T	F	Q	P	R	S	W	P	N	I	D	L			
13	Fy b13	T	F	Q	Y	L	D	P	P	P	F	T	P			
14	Fy b24	M	F	Q	E	H	P	I								
15	Fy b25	M	W	Q	P	S	T	F								
16	Fy b26	M	F	Q	P	A	V	Q								
17	Fy b20	H	G	T	N	N	T	D								
18	Fy b21	Q	L	D	N	L	T	T								
19	Fy b27	M	Y	Q	P	L	A	L								
20	Fy b28	M	F	Q	P	D	F	L								
21	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P	
22	CPep76	W	T	S	L	N	F	N	R	M	L	R	L			

Figure 3: Fy^a Phage ELISA

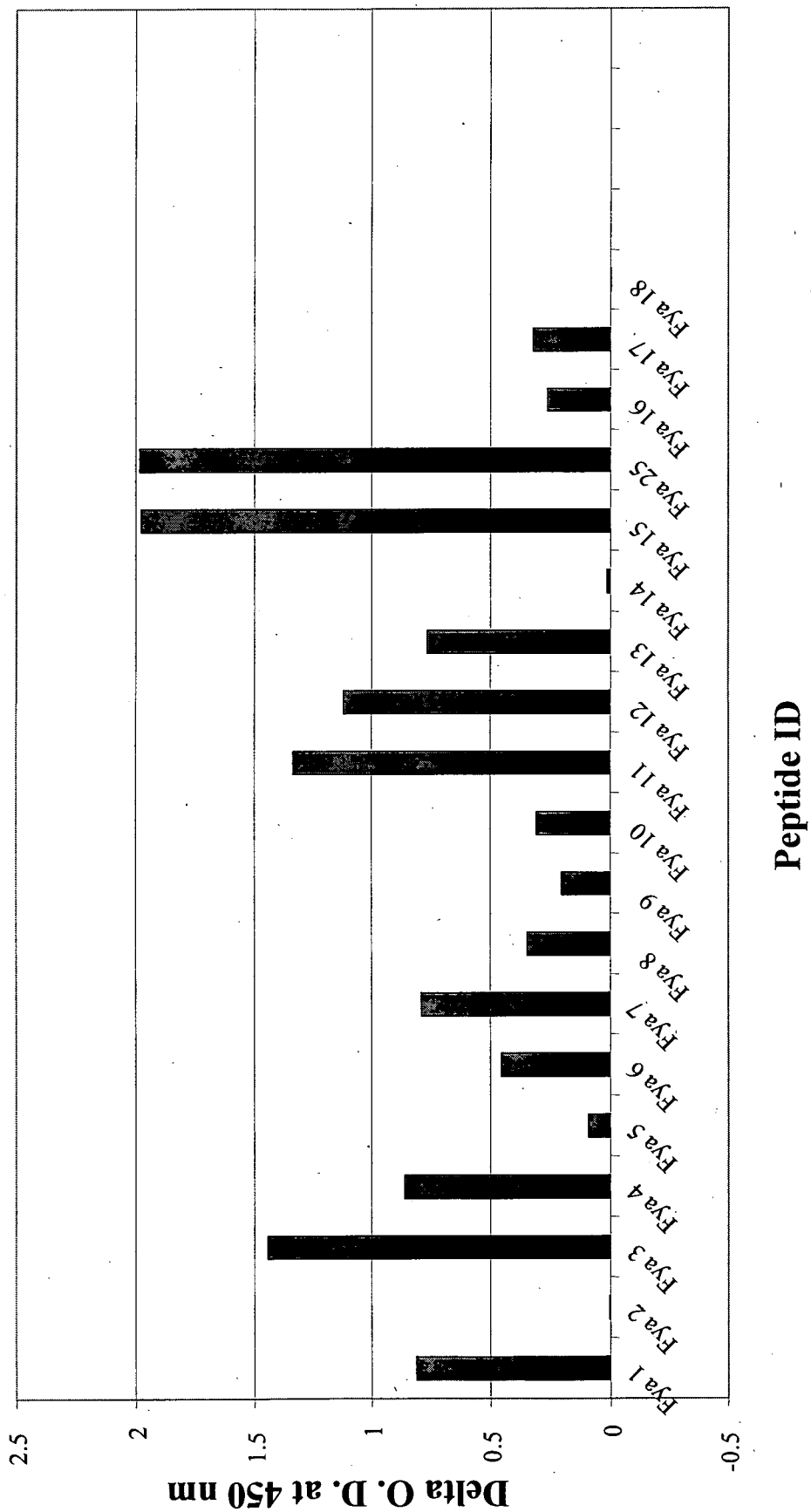


Figure 4: SPOTs Analysis of Fy^a Peptide Mimotopes

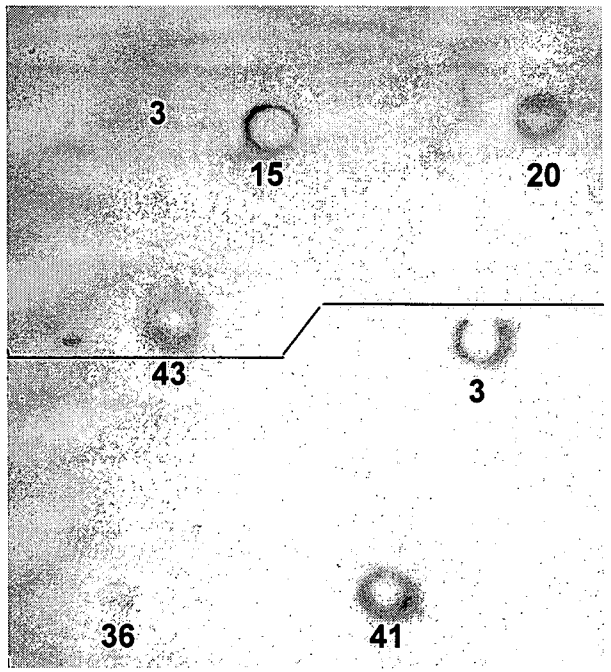
SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Fy a1	W	Q	S	Y	P	N	P								
2	Fy a2	T	N	L	R	L	P	A								
3	Fy a3	Y	Q	S	Y	P	P	P								
4	Fy a4	Y	Q	S	Y	P	M	R								
5	Fy a5	M	Y	P	D	W	Q	Q								
6	Fy a6	L	E	R	D	P	P	V								
7	Fy a7	Y	Q	S	Y	P	N	W								
8	Fy a8	L	V	P	P	D	G	Y								
9	Fy a9	A	P	F	W	Q	W	M								
10	Fy a10	F	S	P	P	N	W	Y								
11	Fy a11	F	A	P	W	Y	Q	Q	R							
12	Fy a12	Q	S	Y	P	N	T	F								
13	Fy a13	D	W	L	F	K	M	S								
14	Fy a14	G	D	W	L	H	W									
15	Fy a15	S	D	G	D	Y	G	A								
16	Fy a16	G	P	S	W	Q	S	T								
17	Fy a17	V	P	Y	P	N	Q	M	C							
18	Fy a18	P	S	P	G	Q	I									
19	Fy a19	F	E	P	P	N	W	D	S	G	P	R	P			

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20	Fy a20	G	I	A	E	D	D	Y	G	A	L	S	W			
21	Fy a21	Y	N	Q	Y	Q	S	Y	P	P	S	L	Q			
22	Fy a22	Y	Q	S	Y	P	S	R	P	P	V	R	L			
23	Fy a23	A	L	A	P	E	E	D	E	V	Y	Y	V			
24	Fy a24	V	L	L	P	E	S	D	E	S	H	R	A			
25	Fy a25	I	P	N	W	Q	S	C								
26	Fy a26	S	G	V	A	F	C	P	P	W	W	C	D	G	P	L
27	Fy a27	W	P	R	H	V	P	L	F	G	L	D	G	Y	V	T
28	Fy a28	A	R	E	Y	G	T	R	F	S	L	I	G	G	Y	R
29	Fy a29	A	S	R	R	L	L	P	S	D	V	R	L	P	S	S
30	Fy a30	L	W	D	P	P	P	F	G	L	S	R	I	F	F	G
31	Fy a31	D	A	R	P	L	A	W	Y	E	E	P	S	F	W	M
32	Fy a32	S	G	Y	A	R	P	F	Y	Q	S	Y	P	A	A	S
33	Fy a33	V	N	S	T	K	W	P	G	M	P	S	F			
34	Fy a34	V	S	P	P	E	W	Y	P	L	A	A	D			
35	Fy a35	T	G	P	K	L	H	C	P	P	A	V	C			
36	Fy a36	F	K	N	P	S	Q	S	Y	P	P	E	P			
37	Fy a37	F	T	M	E	R	D	P	P	I	A	R	V			

Figure 5: Reciprocal Hybridisation

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Fy a1	W	Q	S	Y	P	N	P								
2	Fy a2	T	N	L	R	L	P	A								
3	Fy a3	Y	Q	S	Y	P	P	P								
4	Fy a4	Y	Q	S	Y	P	M	R								
5	Fy a5	M	Y	P	D	W	Q	Q								
6	Fy a6	L	E	R	D	P	P	V								
7	Fy a7	Y	Q	S	Y	P	N	W								
8	Fy a8	L	V	P	P	D	G	Y								
9	Fy a9	A	P	F	W	Q	W	M								
10	Fy a10	F	S	P	P	N	W	Y								
11	Fy a11	F	A	P	W	Y	Q	Q	R							
12	Fy a12	Q	S	Y	P	N	T	F								
13	Fy a13	D	W	L	F	K	M	S								
14	Fy a14	G	D	W	L	H	W									
15	Fy a15	S	D	G	D	Y	G	A								
16	Fy a16	G	P	S	W	Q	S	T								
17	Fy a17	V	P	Y	P	N	Q	M	C							
18	Fy a18	P	S	P	G	Q	I									
19	Fy a19	F	E	P	P	N	W	D	S	G	P	R	P			
20	Fy a20	G	I	A	E	D	D	Y	G	A	L	S	W			
21	Fy a21	Y	N	Q	Y	Q	S	Y	P	P	S	L	Q			
22	Fy a22	Y	Q	S	Y	P	S	R	P	P	V	R	L			
23	Fy a23	A	L	A	P	E	E	D	E	V	Y	Y	V			
24	Fy a24	V	L	L	P	E	S	D	E	S	H	R	A			
25	Fy a25	I	P	N	W	Q	S	C								
26	Fy a26	S	G	V	A	F	C	P	P	W	W	C	D	G	P	L
27	Fy a27	W	P	R	H	V	P	L	F	G	L	D	G	Y	V	T
28	Fy a28	A	R	E	Y	G	T	R	F	S	L	I	G	G	Y	R
29	Fy a29	A	S	R	R	L	L	P	S	D	V	R	L	P	S	S
30	Fy a30	L	W	D	P	P	P	F	G	L	S	R	I	F	F	G
31	Fy a31	D	A	R	P	L	A	W	Y	E	E	P	S	F	W	M
32	Fy a32	S	G	Y	A	R	P	F	Y	Q	S	Y	P	A	A	S
33	Fy a33	V	N	S	T	K	W	P	G	M	P	S	F			
34	Fy a34	V	S	P	P	E	W	Y	P	L	A	A	D			
35	Fy a35	T	G	P	K	L	H	C	P	P	A	V	C			
36	Fy a36	F	K	N	P	S	Q	S	Y	P	P	E	P			
37	Fy a37	F	T	M	E	R	D	P	P	I	A	R	V			
38	Fy a17	V	P	Y	P	N	Q	M	C							
39	Fy a25	I	P	N	W	Q	S	C								
40	Fy a35	T	G	P	K	L	H	C	P	P	A	V	C			
41	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P	
42	CPep76	W	T	S	L	N	F	N	R	M	L	R	L			
43	CPep118	P	D	G	D	Y	G	A	N	L	E	A	A	A	P	
44	Fy b1	M	Y	Q	D	T	P	P	P	R	K	N	V			

Membrane 1



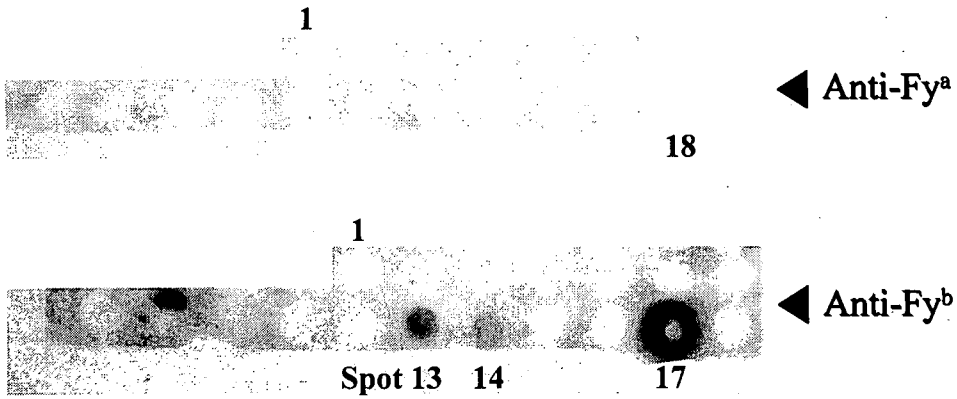
Anti-Fy^a

Anti-Fy^b

Membrane 2

Figure 6: SPOTs Analysis of Fy^b Peptide Mimotopes Generated by Amino Acid Substitution

Membrane 1



Membrane 2

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
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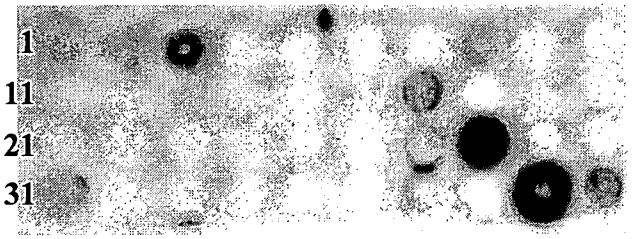
2	Fy b73	M	Y	Q	P	D	P	P	P	T	K	N	V		
3	Fy b74	M	Y	Q	P	D	P	P	T	T	K	N	P		
4	Fy b75	M	Y	Q	P	D	P	P	T	T	K	N	V		
5	Fy b76	M	Y	Q	P	D	N	P	P	T	K	N	P		
6	Fy b77	M	Y	Q	P	D	N	P	P	T	K	N	V		
7	Fy b78	M	Y	Q	P	D	N	P	T	T	K	N	P		
8	Fy b79	M	Y	Q	P	D	N	P	T	T	K	N	V		
9	Fy b80	M	F	Q	P	D	P	P	P	T	K	N	P		
10	Fy b81	M	F	Q	P	D	P	P	P	T	K	N	V		
11	Fy b82	M	F	Q	P	D	P	P	T	T	K	N	P		
12	Fy b83	M	F	Q	P	D	P	P	T	T	K	N	V		
13	Fy b84	M	F	Q	P	D	N	P	P	T	K	N	P		
14	Fy b85	M	F	Q	P	D	N	P	P	T	K	N	V		
15	Fy b86	M	F	Q	P	D	N	P	T	T	K	N	P		
16	Fy b87	M	F	Q	P	D	N	P	T	T	K	N	V		
17	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P
18	CPep76	W	T	S	L	N	F	N	R	M	L	R	L		

Figure 7: SPOTs Analysis of RhD Peptide Mimotopes



SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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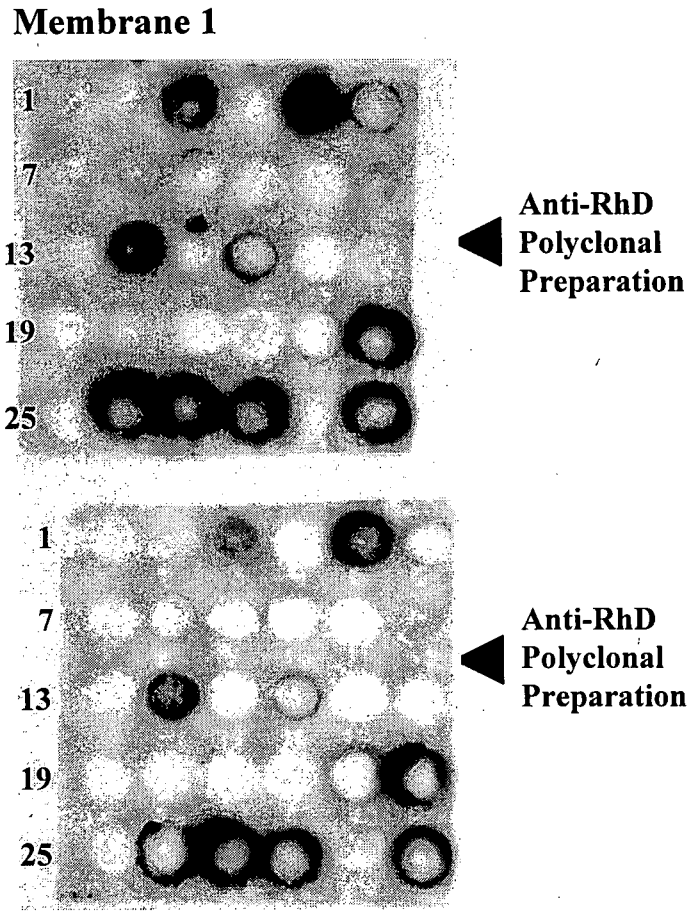
2	RhD 42	W	Y	H	K	A	P	Y								
3	RhD 43	Y	Y	Q	R	A	P	Y								
4	RhD 44	H	W	K	H	P	W	G	A	W	D	T	L			
5	RhD 45	W	H	W	Q	W	T	P	W	S	I	Q	P			
6	RhD 46	W	H	K	N	W	W	P	P	S	T	P	N			
7	RhD 47	S	M	S	S	M	L	L	A	A	Q	T	V			
8	RhD 48	Q	S	H	Y	R	H	I	S	P	A	Q	V			
9	RhD 49	S	M	S	Q	P	K	S	Q	V	N	A	H			
10	RhD 50	W	H	W	T	F	Y	T	P	L	E	S	T			
11	RhD 51	E	S	L	S	T	D	T	Y	A	I	L	L			
12	RhD 52	H	S	S	W	Y	I	Q	H	F	P	P	L			
13	RhD 53	S	T	Y	L	N	G	P	T	G	V	D	L			
14	RhD 54	Q	H	K	T	S	I	T	G	H	L	E	P			
15	RhD 55	F	H	R	W	P	T	W	P	L	P	S	P			
16	RhD 56	V	P	P	W	V	S	V	R	T	G	P	G			
17	RhD 57	T	L	V	Y	Q	P	P	W	Y	R	I	A			
18	RhD 58	F	H	Q	R	L	W	W	P	T	H	T	P			
19	RhD 59	W	H	W	R	L	Y	S	A	N	T	P				
20	RhD 60	H	A	A	F	E	P	R	G	D	V	R	H	T	L	L
21	RhD 61	S	I	W	D	L	P	L	Q	Y	R	G	F	G	T	S
22	RhD 62	L	W	R	L	R	G	G	S	F	P	V	I	S	H	G
23	RhD 63	R	N	A	L	H	S	L	R	T	L	S	S	S	W	V
24	RhD 64	F	H	R	H	W	W	P	P	T	L	S	T			
25	RhD 65	R	P	H	L	L	D	W	E	L	N	P	V			
26	RhD 66	F	H	W	R	W	S	T	F	P	E	Y	P			
27	RhD 67	V	W	A	V	S	L	P	W	Y	R	Y	P			
28	RhD 68	L	D	T	Y	W	Y	R	E	H	F	R	R			
29	RhD 69	V	H	W	R	W	W	D	Q	R	V	P	M			
30	RhD 70	L	P	W	Y	Q	L	T								
31	RhD 71	V	P	W	F	R	A	P								
32	RhD 72	W	H	P	P	Q	P	S								
33	RhD 73	F	H	E	N	W	P	S								
34	RhD 74	F	W	W	Q	V	P	A								
35	RhD 75	T	Q	W	Y	Q	I	A								
36	RhD 76	L	P	W	F	Q	L	P								
37	RhD 77	T	P	L	S	K	S	T								
38	RhD 78	L	P	W	Y	A	T	P								
39	RhD 79	L	P	W	Y	R	H									
40	RhD 80	I	P	W	Y	K	I	T								

Figure 8: SPOTs Analysis of RhD Peptide Mimotopes



SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	RhD 81	F	H	S	T	W	P	W	R	E	A	E	G			
2	RhD 82	F	H	A	N	W	P	Q	S	A	R	D	V			
3	RhD 83	F	H	S	D	W	P	G	Q	T	F	T	W			
4	RhD 84	F	H	E	N	W	S	T	R	P	T	T	R			
5	RhD 85	F	H	S	V	Y	P	W	R	E	A	E	G			
6	RhD 86	F	H	S	N	W	P	S	A	Y	T	A	R			
7	RhD 87	F	H	S	N	W	P	S	L	I	R	A	R			
8	RhD 88	A	G	Y	Q	I	G	M	P	N	P	L	L			
9	RhD 89	F	H	W	R	Y	P	L	P	L	P	G	Q			
10	RhD 90	S	P	T	S	F	R	Q	V	F	G	F	Y			
11	RhD 91	A	L	P	E	L	S	S	L	P	E	S	A	R		
12	RhD 92	L	H	W	W	P	T	Y	G	N	N	G	M			
13	RhD 93	F	H	R	P	Y	Y	W	P	P	T	P	L			
14	RhD 94	F	H	W	R	L	P	Y	P	L	P	S	S			
15	RhD 95	I	H	W	W	V	K	S	P	P	P	G	S			
16	RhD 96	S	H	W	W	T	S	I	L	A	T	P	S			
17	RhD 97	F	H	W	H	L	Q	P	Q	L	W	S	Y			
18	RhD 98	L	H	R	E	W	I	Y	P	Y	L	I	S			
19	RhD 99	G	Q	K	T	H	N	P	F	H	L	H	P			
20	RhD 100	A	T	W	S	H	H	L	S	S	A	G	L			
21	RhD 101	F	H	R	H	Y	Y	P	W	A	L	I	Q			
22	RhD 102	H	S	L	K	H	T	Q	M	S	Y	S	S			
23	RhD 103	S	V	S	V	G	M	K	P	S	P	R	P			
24	RhD 104	W	P	H	Q	V	H	K	H	I	Y	R	Q			
25	RhD 105	A	P	P	Y	P	G	P	L	P	L	S	L			
26	RhD 68	L	D	T	Y	W	Y	R	E	H	F	R	R			
27	RhD 71	V	P	W	F	R	A	P								
28	RhD 79	L	P	W	Y	R	H									
29	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P	
30	CPep76	W	T	S	L	N	F	N	R	M	L	R	L			

Figure 9: SPOTs Analysis of RhE Peptide Mimotopes

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	RhE1	A	A	P	P	Y	V	F	C	S	L	S	P	R		
2	RhE2	T	V	D	L	S	P	A	F	L	F	F	G	R	L	A
3	RhE3	S	L	A	P	Y	S	L	R	I	L	R	V	G	S	A
4	RhE4	R	N	V	L	P	I	F	N	D	V	Y	W	I	A	L
5	RhE5	R	N	V	P	P	I	F	N	D	V	Y	W	I	A	F
6	RhE6	I	N	N	T	F	T	W								
7	RhE7	Y	E	L	N	L	M	T								
8	RhE8	W	G	I	T	V	E	T	A	Y	G	T	A			
9	RhE9	N	Q	F	L	L	W	E	T	R	S	M	R			
10	RhE10	N	V	S	L	T	N	L	T	Y	K	P	R			
11	RhE11	Y	T	P	P	D	W	S	W	W	P	A	P			
12	RhE12	I	K	N	T	S	L	Q	Q	L	V	T	A			
13	RhE13	E	W	L	A	Y	D	G	I	R	A	Y	S			
14	RhE14	H	A	K	V	Q	V	S	S	P	F	P	P			
15	RhE15	L	S	S	Q	F	K	Q								
16	RhE16	G	T	F	K	W	Y	Q								
17	RhE17	T	K	M	D	Q	S	T								
18	RhE18	I	P	E	E	A	L	R	A	R	F	K	T			
19	RhE19	S	F	Q	D	A	L	L	S	R	W	Y	S			
20	RhE20	S	N	L	R	S	W	L	F	P	F	D	R	V	G	N
21	RhE21	P	G	P	M	F	G	G	S	Y	I	P	S	L	H	H
22	RhE22	Y	P	Q	G	L	W	R								
23	RhE23	Y	P	Q	G	E	W	R								
24	RhE24	G	P	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
25	RhE25	G	L	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
26	RhE26	W	P	P	I	S	R	H								
27	RhE27	A	H	S	W	L	P	G	A	G	L	L	M			
28	RhE28	S	L	T	H	S	P	R	T	P	I	L	A			
29	RhE29	F	P	N	L	R	E	R	H	E	P	L	F			
30	RhE30	G	H	S	Y	H	A	L	I	T	R	Y	L	G	A	A
31	RhE31	W	A	S	Y	Y	A	L	I	T	R	Y	L	G	A	A
32	RhE32	D	F	A	Q	A	L	F	L	R	Y	V	V	S	G	L
33	RhE33	H	R	W	M	P	H	V	F	A	V	R	Q	G	A	L
34	RhE34	W	A	S	F	Y	A	S	S	Y	R	D	S	R	L	L
35	RhE35	R	L	P	A	T	I	R	A	L	L	G	R	D	V	R
36	RhE36	R	L	P	A	T	I	R	A	L	L	G	R	D	V	W
37	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P	
38	CPep76	W	T	S	L	N	F	N	R	M	L	R	L			

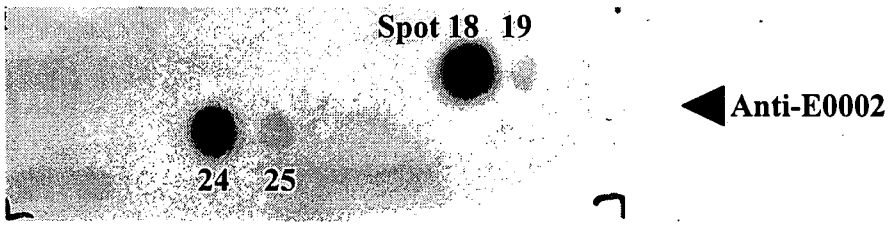


Figure 10: SPOTs Analysis of Rhe Peptide Mimotopes

SPOT No	Pep ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Rhe1	V	P	R	H	N	P	I								
2	Rhe2	P	K	A	F	Q	Y	G	G	R	A	V	G	G	L	W
3	Rhe3	D	S	G	A	L	F	N	H	I	F	M	P	G	P	F
4	Rhe4	L	H	S	S	H	L	P	P	D	D	R	R	W	G	L
5	Rhe5	P	R	Q	F	P	L	R	D	L	Y	T	F	R	Y	W
6	Rhe6	G	A	A	N	L	Y	V	S	S	F	L	I	P	L	H
7	Rhe8	Q	P	R	G	V	T	V	H	G	D	A	W	R	V	A
8	Rhe9	G	L	D	L	L	G	D	V	R	I	P	V	V	R	R
9	Rhe10	A	Y	W	D	L	Y	G	V	G	F	A	F	S	A	P
10	Rhe11	I	W	T	I	T	G	S	T	K	Q	A	F	D	R	S
11	Rhe12	E	D	W	F	S	A	S	I	R	R	V	P	T	F	A
12	Rhe13	A	V	K	L	R	P	S	S	C	W	L	K	S	T	C
13	Rhe14	R	F	D	G	I	D	L	R	V	S	F	V	S	R	P
14	Rhe15	A	S	L	H	P	V	P	K	T	W	F	S	L	L	S
15	Rhe16	L	A	P	P	P	S	P								
16	Rhe17	A	V	T	F	N	S	Y	F	G	F	S	T	T	S	V
17	CPep75	P	D	G	D	Y	D	A	N	L	E	A	A	A	P	
18	CPep76	W	T	S	L	N	F	N	R	M	L	R	L			

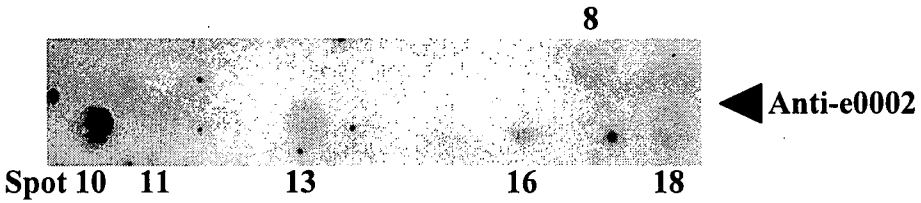


Figure 11

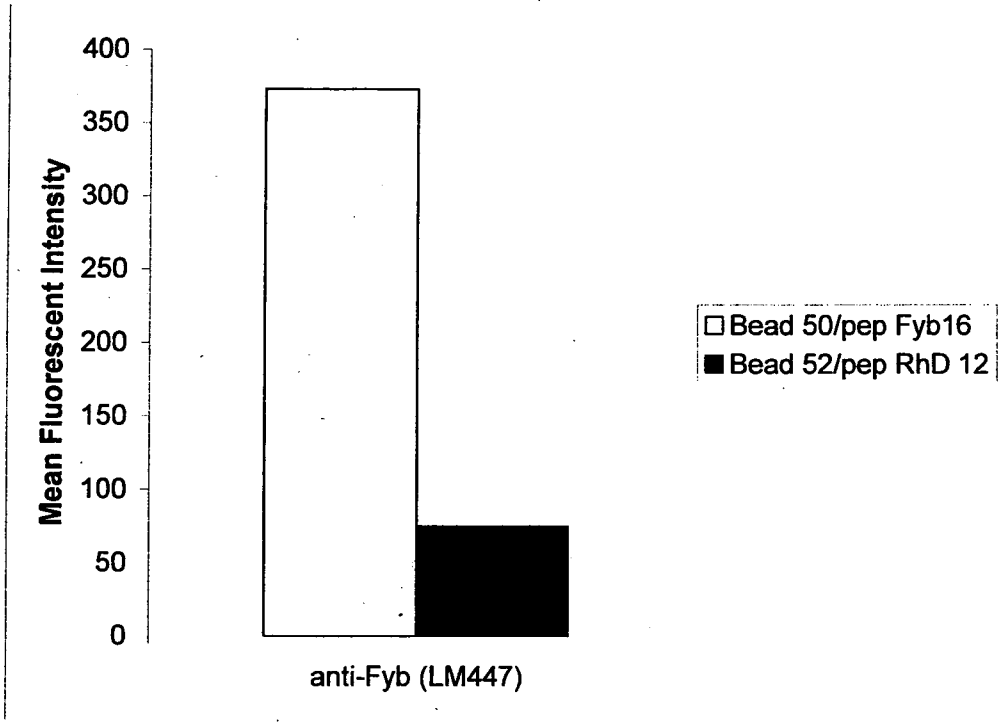

400
350
300
250
200
150
100
50
0
Mean Fluorescent Intensity
anti-Fyb (LM447)
Bead 50/pep Fyb16
Bead 52/pep RhD 12

Figure 12

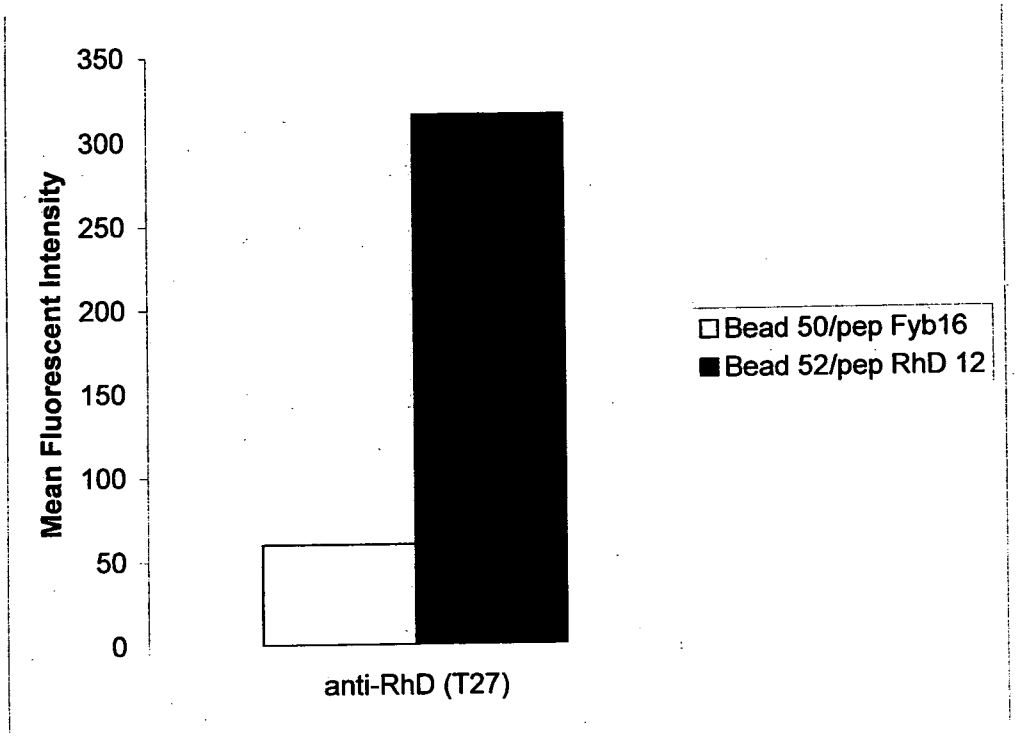

350
300
250
200
150
100
50
0
Mean Fluorescent Intensity
anti-RhD (T27)
Bead 50/pep Fyb16
Bead 52/pep RhD 12

Figure 13

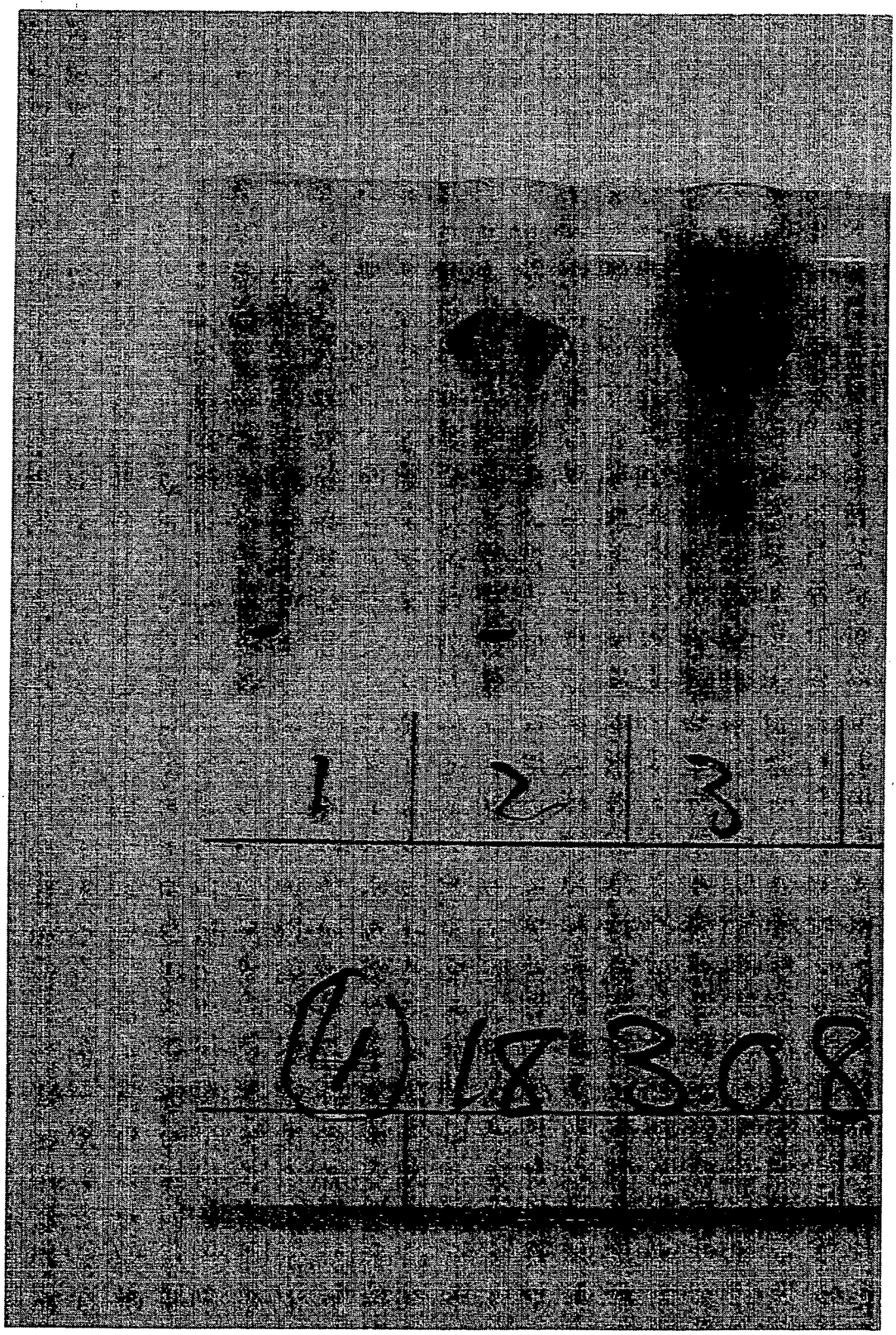
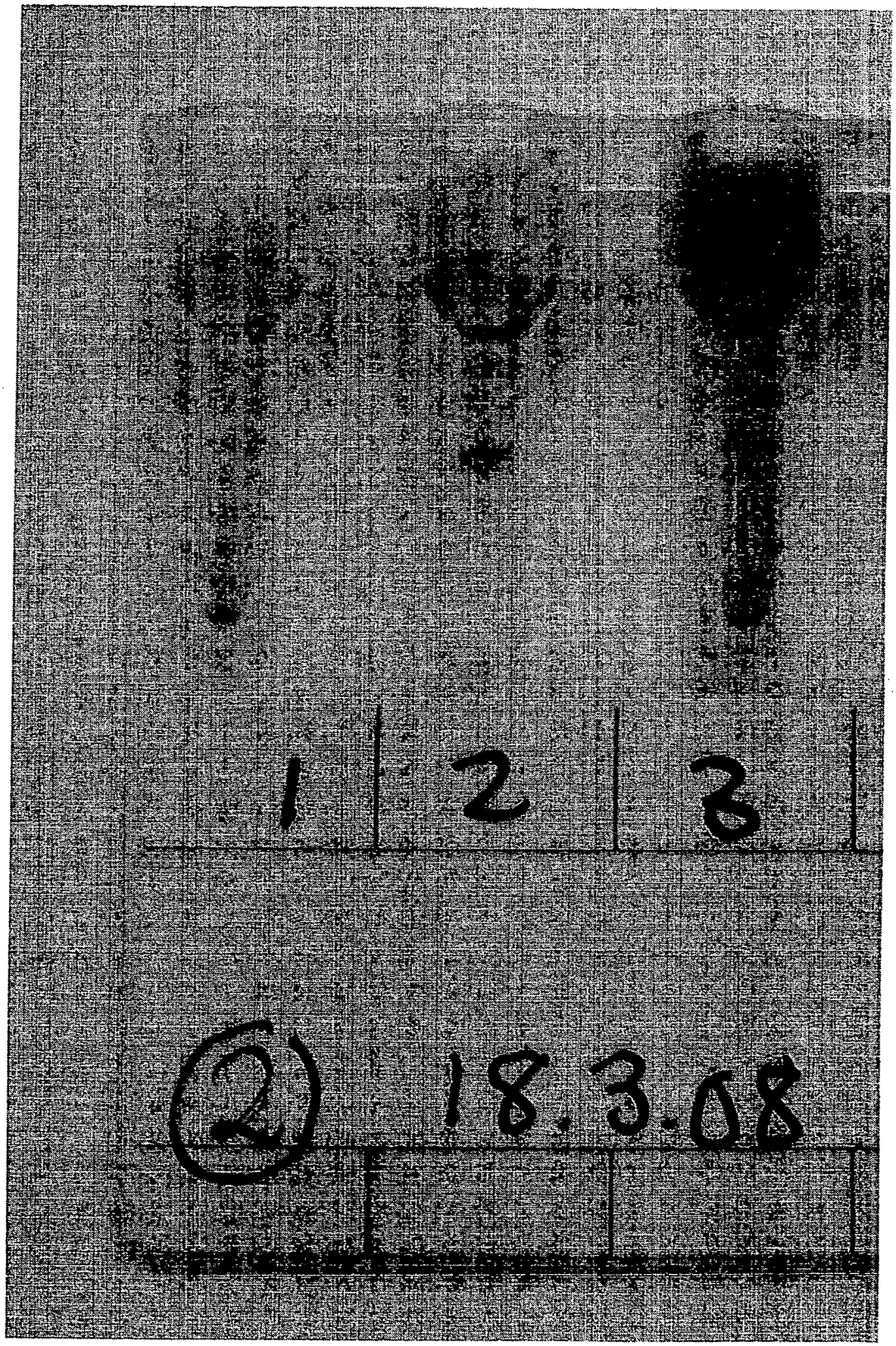


Figure 14



专利名称(译)	诊断分析		
公开(公告)号	EP2140273A2	公开(公告)日	2010-01-06
申请号	EP2008736890	申请日	2008-04-03
[标]申请(专利权)人(译)	常见的服务机构为苏格兰卫生服务		
申请(专利权)人(译)	常见的服务机构为苏格兰卫生服务		
当前申请(专利权)人(译)	常见的服务机构为苏格兰卫生服务		
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发明人	URBANIAK, STANISLAW, JOSEPH MOSS, MICHAEL, TERRANCE TAIT, EVELYN, JACQUELINE, ANNETTE		
IPC分类号	G01N33/72 G01N33/53 G01N33/50 C07K7/00		
CPC分类号	G01N33/80 C07K14/705 C07K14/715 G01N2800/245		
优先权	2007006558 2007-04-03 GB		
外部链接	Espacenet		

摘要(译)

本发明涉及血型抗原的模拟表位，鉴定血型抗原模拟表位的方法和使用所述模拟表位鉴定血型抗原抗体的方法。