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(54) **METHODS OF SCREENING FOR BINDING INTERACTION USING SETS OF MICROPARTICLES AND UNIQUE PROBES**

SCREENING-VERFAHREN FÜR BINDUNGSWECHSELWIRKUNG UNTER VERWENDUNG VON MIKROPARTIKELSETS UND EINZIGARTIGEN SONDEN

PROCEDES DE CRIBLAGE D'INTERACTIONS DE LIAISONS UTILISANT DES ENSEMBLES DE MICROPARTICULES ET DES SONDES UNIQUES

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(56) References cited:
US-A1- 2006 240 411
US-A1- 2006 292 601
US-B1- 6 514 714
US-B2- 6 551 784

- **PEI R ET AL: "Flow cytometric detection of HLA antibodies using a spectrum of microbeads." HUMAN IMMUNOLOGY DEC 1999 LNKD-PUBMED:10626745, vol. 60, no. 12, December 1999 (1999-12), pages 1293-1302, XP002586995 ISSN: 0198-8859**
- **DRAGO F ET AL: "Genotyping of the Kidd blood group with allele-specific oligodeoxynucleotides coupled to fluorescent microspheres" TRANSFUSION MEDICINE 200512 GB LNKD-DOI:10.1111/J.1365-3148.2005.00632.X, vol. 15, no. 6, December 2005 (2005-12), pages 499-501, XP002586997**
- **ITOH Y ET AL: "Four-digit allele genotyping of the HLA-A and HLA-B genes in Japanese patients with Behcet's disease by a PCR-SSOP-Luminex method" TISSUE ANTIGENS, vol. 67, no. 5, May 2006 (2006-05), pages 390-394, XP002586999 ISSN: 0001-2815**
- **XU HONGXIA ET AL: "Multiplexed SNP genotyping using the Qbead system: a quantum dot-encoded microsphere-based assay." NUCLEIC ACIDS RESEARCH 15 APR 2003 LNKD-PUBMED:12682378, vol. 31, no. 8, 15 April 2003 (2003-04-15) , page E43, XP002587000 ISSN: 1362-4962**

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(Cont. next page)

- **WATERBOER ET AL: "Suppression of non-specific binding in serological Luminex assays" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL LNKD-DOI:10.1016/J.JIM.2005.11.008, vol. 309, no. 1-2, 20 February 2006 (2006-02-20), pages 200-204, XP005282614 ISSN: 0022-1759**
- **MARTINS THOMAS B: "The application of true internal controls to multiplexed fluorescent immunoassays" JOURNAL OF CLINICAL LIGAND ASSAY, CLINICAL LIGAND ASSAY SOCIETY, WAYNE, MI, US, vol. 26, no. 2, 1 July 2003 (2003-07-01), pages 93-97, XP009132695 ISSN: 1081-1672**
- **RAHAL M ET AL: "DNA typing by microbead arrays and PCR-SSP: apparent false-negative or positive hybridization or amplification signals disclose new HLA-B and -DRB1 alleles" TISSUE ANTIGENS, vol. 71, no. 3, March 2008 (2008-03), pages 238-241, XP002587001 ISSN: 0001-2815**

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Description

Field of Invention

[0001] The present invention relates to methods for screening for binding interactions using multiple sets of microparticles, wherein said set has the same identifiable characteristic and wherein one of more sets comprise subsets of microparticles and said subset presents at least one unique probe that acts as a binding partner for a target molecule in a biological sample. In particular, the invention provides for methods of detecting tissue-typing antigens in donor tissue or recipient tissue using these multiple sets of microparticles.

Background

[0002] One such tissue-typing antigen is human leukocyte antigen (HLA).

Individuals may be sensitized to HLA antigens during pregnancy, or by blood transfusion or previous organ grafts. Testing to determine sensitivity to HLA alleles is relevant to tissue and organ transplantation where the presence in the recipient of antibodies against HLA antigens of the donor (donor specific crossmatch) is predictive of a high risk of graft rejection. It is a standard practice in the transplant field to test all potential recipients against a panel of HLA antigens selected to represent a human population and the percentage of HLA alleles against which the serum is reactive is determined. In this panel reactive antibody (PRA) testing reaction of a patient's serum against a high percentage of HLA alleles present in a normal human population is predictive of a high risk of graft rejection.

[0003] The HLA locus is highly polymorphic in nature. As disclosed in the Nomenclature for Factors of the HLA System 2000 (Hum. Immunol.; 62(4):419-68), 2001) there are 124 HLA-A alleles, 258 HLA-B alleles, 74 HLA-C alleles, 221 HLA-DRB1 alleles, 19 DRB3 alleles, 89 DRB4 alleles, 14 DRB5 alleles, 19 DQA1 alleles and 39 DQB1 alleles, with new alleles being discovered continuously. As testament to this rapid progress, a April 2007 update by the WHO nomenclature Committee for Factors of the HLA System (www.anthonynolan.com/HIG/) showed there are 545 HLA-A alleles, 895 HLA-B alleles, 307 HLA-C alleles, 8 HLA-E alleles, 12 HLA-H alleles, 9 HLA-J alleles, 6 HLA-K alleles, 4 HLA-L alleles, 4 HLA-P alleles, 3 HLA-V alleles, 3 DRA alleles, 494 DRB1 alleles, 1 DRB2 alleles , 44 DRB3 alleles, 13 DRB4 alleles, 18 DRB5 alleles, 3 DRB6 alleles, 2 DRB7 alleles, 10 DRB8 alleles, 1 DRB9 alleles, 34 DQA1 alleles, 83 DQB1 alleles, 23 DPA1, 126 DPB1 alleles, 4 DMA alleles, 7 DMB alleles, 12 DOA alleles and 9 DOB alleles.

[0004] All HLA-A, -B, and -C alleles have similar sequences. The same holds for DRB1, DRB3, DRB4 and DRB5 sequences. Because of these similarities, very often when a primer pair is used in the practice of polymerase chain reaction sequence-specific priming (PCR-SSP), two or more alleles will be amplified, or in a diagnostic sequence-specific oligonucleotide-probe detection (SSO) system, two or more alleles will hybridize. Therefore, for each allele to have a unique PCR-SSP or detection-SSO pattern, many pairs of primers or probes must be used. Further, the use of diagnostic hybridization SSO probes in HLA typing is confounded by the high levels of homology shared by the HLA alleles. Thus, many prior art typing methods such as those of Bugawan et al., Tissue Antigens 44:137-147 (1994), lack the accuracy desired for HLA typing and other applications.

[0005] PCR can be used to characterize the sequence on the target DNA template. If amplification occurs, the template DNA contains the same sequences as the primers used. If no amplification occurs, the sequences on the template DNA are different from the primer sequences. Newton et al., U.S. Patent No. 5,595,890 discloses PCR diagnostic methods for typing, including molecular typing of HLA using PCR-SSP. According to this method, an unknown allele is assigned based on the pattern of positive or negative reactions from multiple rounds of PCR. The methods disclosed by Newton are limited in their effectiveness for HLA typing, however, due to the high degree of polymorphism in HLA as described above. As a consequence two primers, each with specific sequences, frequently amplify many HLA alleles, thus increasing the number of PCR amplifications required in order to assign an unknown allele. For similar reasons, multiple diagnostic probes are required for correct typing of HLA in non-PCR contexts. PCR requires a pair of primers flanking the region on the DNA template for that region to be amplified. The ability of a primer to anneal to the desired sequence depends on the length of the primer and the annealing temperature set in the PCR thermocycling program. The longer the primer, the higher the annealing temperature it needs to achieve specific amplification of a DNA sequence. PCR-SSP uses a balance between primer length and annealing temperature to achieve the specificity of the primer-directed sequence amplification.

[0006] In the clinical use of PCR-SSP systems for HLA typing, there had existed a need to use a limited number of PCR reactions to achieve as much resolution as possible whereby the number of alleles amplified by a pair of primers would be reduced (i.e., the specificity of the primers or probes would be increased). Of interest to the present invention is the disclosure of co-owned U.S. Patent No. 6,207,379, which teaches the use of diagnostic PCR primers that are characterized by non-contiguous (gap) sequences for obtaining greater discrimination between related alleles in HLA typing. In an alternative embodiment, U.S. 6,207,379 teaches use of diagnostic primers that hybridize to non-contiguous sequences in a target nucleic acid and amplify that target by polymerase-mediated primer extension. Despite the success of the methods of U.S. 6,207,379 in carrying out more specific amplification of the target HLA sequences there still remains a desire for improved methods for detection of

HLA sequences in both PCR and non-PCR contexts.

[0007] The PCR invention described in U.S. 6,207,379 addressed the need in the art for improved methods of PCR--SSP--based molecular typing whereby the specificity of the typing can be increased so as to reduce the number of PCR reactions required for each typing. However, there still exists a need in the art for methods to probe for specific sequences in non-PCR contexts. For reasons of basic thermodynamics, probes and templates, including those with a perfect match, are in state of equilibrium between the hybridized and non-hybridized state. A probe that is at one moment attached to its target template, at another moment may not be. The polymerase in PCR plays a critical role by locking a primer in place through elongation (primer extension). In non-PCR contexts, the critical factor--the polymerase (and the subsequent elongation)--is lacking, and long-term stability of the hybridized duplex of a short probe to a target would not necessarily be expected. For these reasons it is generally considered necessary for hybridization probes to be longer than corresponding extension primers in order to assure stable duplex formation.

[0008] The U.S. Patent Publication No. US 2003-0165925 A1 provides improved methods for detecting HLA nucleic acids and T-cell receptor nucleic acid sequences whereby the specificity of diagnostic probes is increased. The specificity is increase in these methods because at least one probe is capable of recognizing two or more regions on the target and is capable of doing so without increasing the annealing temperature of the probe to the target nucleic acid sequence. The increased specificity of the probe set reduces the number of alleles detected, thus increasing the resolution of the method, and does so at lower cost.

[0009] US 6 514 714 B1 relates to a method for detecting panel reactive antibodies in serum of a subject against anti-human leukocyte antigen (HLA) class I antigens. The method comprises the steps of adding serum from a subject to an array of microbeads, each microbead presenting HLA antigens from a subpopulation presenting the same HLA antigens; incubating the serum and the microbeads for a sufficient period of time for anti-HLA antibodies in the serum to bind to the HLA antigens present on the microbeads; removing the serum components which do not specifically bind with the HLA antigens presented on the microbeads; incubating the microbeads with a labeled ligand capable of specifically binding with the anti-HLA antibodies bound to that HLA antigen; removing the labeled ligand which is not bound to said HLA antigens; and detecting the presence of labeled ligand bound to said HLA antigens by flow cytometry.

[0010] PEI R ET AL: "Flow cytometric detection of HLA antibodies using a spectrum of microbeads." HUMAN IMMUNOLOGY, vol. 60, no. 12, December 1999 (1999-12), pages 1293-1302 refers to flow cytometric detection of HLA antibodies using a spectrum of microbeads. Principally, HLA antigen coated beads containing eight levels of fluorescence were used, whereby HLA antigens isolated from eight cultured cells were coated onto these beads so that each bead was the equivalent of one cell. By using four sets of eight beads, an equivalent of 32 cells could be examined in four test tubes.

[0011] DRAGO F ET AL: "Genotyping of the Kidd blood group with allele-specific oligodeoxynucleotides coupled to fluorescent microspheres" TRANSFUSION MEDICINE, vol. 15, no. 6, December 2005, pages 499-501 relates to the genotyping of the Kidd blood group with allele-specific oligodeoxynucleotides coupled to fluorescent microspheres. The authors exploited the Luminex technology to develop a high-throughput method for single-nucleotide polymorphism genotyping of the common alleles of the gene encoding the protein carrying the antigen for the Kidd blood group. Luminex technology utilizes a mixture of oligonucleotide probes covalently attached to fluorescent microspheres, enabling the creation of an oligonucleotide hybridization assay for a polymerase chain reaction product.

[0012] ITOH Y ET AL: "Four-digit allele genotyping of the HLA-A and HLA-B genes in Japanese patients with Behcet's disease by a PCR-SSOP-Luminex method" TISSUE ANTIGENS, vol. 67, no. 5, May 2006, pages 390-394 discloses 4-digit allele genotyping of the HLA-A and HLA-B genes in Japanese patients with Behcet's disease by a PCR-sequence-specific oligonucleotide probes (SSOP)-Luminex method. In the PCR-SSOP-Luminex method, oligonucleotide probes were designed to specifically detect the nucleotide sequences of PCR products at the polymorphic cites of the HLA-A and HLA-B loci, said nucleotide sequences being covalently coupled to a different set of microbeads. After the hybridized amplicons were labeled with the fluorescent reporter molecule, reactions were analyzed on the flow cytometer to identify the fluorescent intensity on each bead, and the genotyping HLA of the sample DNA was determined.

[0013] XU HONGXIA ET AL: "Multiplexed SNP genotyping using the Qbead system: a quantum dot-encoded microsphere-based assay." NUCLEIC ACIDS RESEARCH, vol. 31, no. 8, 15 April 2003, page E43 describes a multiplexed SNP genotyping using the Qbead system of fluorescent semiconductor nanocrystals to encode microspheres with the unique spectral barcodes. For multiplexed SNP genotyping, encoded microspheres were conjugated to allele-specific oligonucleotides. After hybridization of said oligonucleotides to amplicons produced by multiplexed PCR of genomic DNA, individual microspheres are analyzed by flow cytometry and each SNP is distinguished by its unique spectral barcode.

[0014] Currently, the methods of DNA-base tissue-typing are expensive and time consuming because these methods require detection of many different labels to distinguish different SSO probes such as by flow cytometry or visual images from a camera or microscope, such as Bioarrays. While, an unlimited number of uniquely labeled microparticles presenting different oligonucleotide probes can theoretically be prepared there exist practical limitations on the number of labels that can be distin-

guished and measured during a single assay using methods such as fluorescent labeled flow cytometry. Accordingly, there exists a desire in the art to maximize the number of oligonucleotide probes that can be tested in a single assay using a limited number of uniquely labeled microparticles.

Summary of Invention

[0015] The invention provides for methods of obtaining more data from a limited set of microparticles. The sets of microparticles currently available have a limited number of identifiable characteristics (such as fluorescent labels). There is a limited number of characteristics that can be measured at one time using conventional methods. However, the number of test molecules that need to be screened continues to increase. For example, for DNA based tissue typing, the number of HLA antigens and polymorphisms is increasing daily and a complete HLA screen of a donor sample requires hundreds of probes. Using conventional methods, a complete initial HLA screen would require multiple assays The improved methods of the invention allow for screening of more target molecules using fewer distinctly labeled microparticles and thereby measuring fewer characteristics and ultimately fewer assay runs.

[0016] The improved methods of the invention utilize a set number of microparticles that each have a different identifiable characteristic. The improvement of the invention relates to presenting one or more different probes on sets of microparticles that have the same identifiable characteristic, such as a set of microparticles each embedded with the same fluorescent label. Therefore, the assay may screen for more target molecules while detecting the same or less identifiable characteristics. For example, 100 differently labeled microparticles can be used to screen for more than 100 target molecules; or alternatively, 100 target molecules can be screened using less than 100 different labels.

[0017] The probes of the method of the invention act as binding partners for target molecules within a biological sample. The probes can be any binding partner including nucleic acids, such as oligonucleotides, primers or any nucleic acid that are complementary to target molecule nucleic acids. The binding partner may also be an antigen, such as a peptide antigen or protein, that binds to a target molecule antibody, or an antibody that binds to a peptide antigen or protein target molecule. In addition, the binding partner may be any protein that binds to a target molecule protein, such as receptors and ligands or proteins that complex. The binding interaction is the event of binding between any protein binding partners or the hybridization of a nucleic acid with a SSO probe or primer or any other complementary nucleic acid.

[0018] These methods are preferably carried out using microparticles that are presenting a probe that will bind to a target molecule within a biological sample, and this binding interaction will generate a measurable signal. According to a preferred aspect of the invention, the probe may be a sequence specific oligonucleotide (SSO) probe that will hybridize (binds) to a DNA sample. Alternatively, the probe may be a peptide, antibody or protein antigen that binds to an antibody or another protein in a biological sample.

[0019] The target molecules of the invention may be any molecule of interest in a biological sample. The methods of the invention are screening for the target molecules. In one embodiment, the methods of the invention are used to screen for tissue-typing alleles. This method can be carried out using any tissue-typing allele. The HLA tissue typing allele is described throughout the specification but these methods can be carried out using any tissue-typing antigen.

[0020] The invention provides for improved methods for tissue typing using probes conjugated to microparticles, such as microspheres or beads, having unique identifiable characteristics. The methods of the invention generate more tissue-typing information with a limited number of sets of detectably labeled and distinctly identifiable microparticles by using one or more sets of microparticles having the same detectable label but wherein the set of identically labeled microparticles comprises multiple subsets of microparticles labeled with different probes.

[0021] For example, a set may comprise 10 subsets of microparticles all labeled with the same fluorescent dye wherein the subset of microparticles has a different probe immobilized to its surface. Using this approach, an increased number of different tissue-typing antigens can be screened for using a limited number of differently labeled microparticles.

[0022] In an exemplary embodiment, the improved method of the invention may screen for 1000 different tissue-typing antigens by immobilizing 1000 different probes on a multiplicity of beads (microparticles). The labeled beads are divided into 100 sets such that each set is made up of 10 subsets of beads having 10 unique probes but which are labeled with the same fluorescent dye, or unique combination of fluorescent dyes. Accordingly, many identically labeled beads (emitting the same signals) will present a different probe. Thus, in the exemplary screen, 100 different florescent signal or combinations of signals are detected, but 1000 different probes are tested.

[0023] Conversely, many fewer unique labels can be used to screen for the same number of target molecules. Therefore, this method allows for screening of many tissue-typing antigen alleles while only using a more limited number of labeled beads. In addition, the improved methods of the invention are more efficient and require less labor. Reduced labor is required for the improved methods because there is less of a need to run a low resolution test followed by a higher resolution test.

[0024] Subsequently, a biological sample contacts the multiple sets of microparticles under conditions that promote a binding interaction such as conditions that pro-

mote antibody/antigen binding or conditions that promote nucleic acid hybridization. The binding interaction between the target molecule within the biological sample can be detected, most preferably through a signal system that generates a signal indicative of binding and detection of that signal indicates that the biological sample is positive for the target molecule.

[0025] While practice of the methods of the invention increases the number of unique target molecules assayed for by a given number of uniquely labeled beads, the methods are more susceptible to false negatives reporting contrasted with prior art methods in which all of a given bead set are specific for a particular target molecule. For example, in DNA-based HLA testing, it is frequently the case that few of the SSOs will have a hybridization partner in the DNA sample. Thus, for any given set of oligonucleotide probes as few as one subset of probes will report a positive reaction with the sample, if at all. In such a case, the remainder of subsets within a given set of probes will not report a positive reaction and the overall signal, as well as the mean and median signal, provided by all the microparticles of a given set will be low. Accordingly, there exists the possibility of a false negative being reported (i.e., a failure to report the true positive reaction). While the present invention provides for the ability to screen for a greater number of target molecules, the possibility of such false negatives can lead to devastating consequences in areas such as in the tissue transplantation art.

[0026] According to a further aspect of the present invention, the risk of false negatives is reduced by choosing a selected proportion of a set generating a positive signal for further evaluation. This step is referred to as "the filtering step'. The proportion selected is some proportion of the microparticles generating the greatest intensity of signal indicative of target molecule binding. Because some proportion of the true negatives is excluded by this method the presence of true positives can more reliably be determined.

[0027] In one embodiment, the invention provides for methods of screening for a binding interaction comprising the steps of: preparing multiple sets of uniquely identifiable microparticles wherein the microparticles within a set have same unique identifiable characteristic and further, wherein one or more sets comprise two or more subsets of microparticles said subset presenting at least one unique probe that acts as a binding partner for a target molecule within a biological sample, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ration other than about 1 to 1; contacting said multiple sets of microparticles with a biological sample under conditions in which a target molecule within the biological sample binds to the probe, detecting the binding of a target molecule within the biological sample and the probe on said microparticles by generation of a signal indicative of the binding interaction between the target molecule and the probe, measuring the signal indicative of the binding interaction for said microparticles selecting a proportion of less than 100% of microparticles exhibiting the greatest signal indicative of the binding interaction for one or more sets of uniquely identifiable microparticles, determining for said set(s) of uniquely identifiable microparticles whether the signal indicative of the binding interaction for the selected proportion is greater than a selected threshold indicative of the presence of one or more target molecules in said biological sample, and determining the number of uniquely identifiable microparticle sets indicating the binding interaction.

[0028] Exemplary ratios other than 1 to 1 are about 1:2, about 1 to 3, about 1 to 4, about 1 to 5, about 2 to 3, about 3 to 4, about 4 to 5, about 7 to 8, about 8 to 9, about 1 to 10, about 1 to 25, about, 1 to 50, about 1 to 100. The rations may be integral ratios or non-integral rations.

[0029] In one embodiment of the invention, the probe is a SSO probe, the target molecule is a nucleic acid, the biological sample is a sample DNA and the binding interaction is hybridization of a nucleic acid within sample DNA and the SSO probe. In one aspect of the invention, the SSO probe is specific for a tissue typing antigen allele such as HLA, HNA, blood group, KIL or TCR. The sample DNA may be obtained from any source such as from a buccal swab or blood.

[0030] In another embodiment of the invention, the probe is a peptide or protein antigen, the target molecule is an antibody and the binding interaction is binding of the antibody and the peptide or protein antigen. In one aspect of the invention, the target molecule is an antibody specific for HLA, HNA, blood group antigens, TCR or KIL.

[0031] These methods are carried out wherein at least two subsets of microparticles within at least one set of the uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1, and wherein the ratio will determine the selected proportion of the set of microparticles. Exemplary ratios are about 1:2, about 1 to 3, about 1 to 4, about 1 to 5, about 2 to 3, about 3 to 4, about 4 to 5, about 7 to 8, about 8 to 9, about 1 to 10, about 1 to 25, about, 1 to 50, about 1 to 100. The rations may be integral ratios or non-integral ratios.

[0032] The selected proportion of a set of microparticles may be less than 50%, less than 30%, less than 20% or less than 10%. In a preferred embodiment, the selected proportion of a set of microparticles is less than or equal to the inverse of the number of subsets of microparticles presenting unique probes for said bead set.

[0033] In another embodiment, the invention provides for methods of DNA-based tissue-typing comprising the steps of: preparing multiple sets of uniquely identifiable microparticles wherein the microparticles within a set have same unique identifiable characteristic, and further wherein said sets comprise subsets of microparticles, said subset presenting at least one unique sequence specific oligonucleotides (SSOs) selected to hybridize

with tissue-typing alleles, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ration other than about 1 to 1, contacting said multiple sets of microparticles with a sample DNA under hybridizing conditions, detecting the hybridization of sample DNA and the SSOs on said microparticle by generation of a signal indicative of the hybridization of sample DNA with the SSOs, measuring the signal indicative of the hybridization of sample DNA and the SSOs for said microparticle, selecting a proportion of less than 100% of microparticles exhibiting the greatest signal indicative of sample DNA/SSO hybridization for at least one set of uniquely identifiable microparticles, determining for said set(s) of uniquely identifiable microparticles whether the signal indicative of sample DNA/SSO hybridization for the selected proportion is greater than a selected threshold indicative of the presence of one or more tissue-typing antigen alleles in said sample; and determining the number of uniquely identifiable microparticle sets indicating DNA/SSO hybridization.

[0034] These methods of DNA-based tissue typing are carried out wherein at least two subsets of microparticles within at least one set of the uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1. Exemplary ratios are about 1:2, about 1 to 3, about 1 to 4, about 1 to 5, about 2 to 3, about 3 to 4, about 4 to 5, about 7 to 8, about 8 to 9, about 1 to 10, about 1 to 25, about, 1 to 50, about 1 to 100. The rations may be integral ratios or non-integral rations.

[0035] The selected proportion of a set of microparticles may be less than 50%, less than 30%, less than 20% or less than 10%. In a preferred embodiment, the selected proportion of a set of microparticles is less than or equal to the inverse of the number of subsets of microparticles presenting unique SSO probes for said microparticle set.

[0036] In one aspect of the invention, the SSO probe is specific for a tissue typing antigen allele such as HLA, HNA, blood group, KIL or TCR. The sample DNA may be obtained from any source such as from a buccal swab or blood.

[0037] These methods of the invention may further comprise a step of reporting a signal for positive hybridization. The methods may also further comprise the step of selecting for each identifiable probe set a proportion of microparticles having the atrongest signal.

[0038] In a further embodiment, the invention provides kits for conducting a method of measuring a binding interaction comprising multiple sets of uniquely identifiable microparticles, wherein the microparticles within a set have the same unique identifiable characteristic, and wherein one or more sets comprise subsets of microparticles, said subset presenting at least one unique probe that binds to a target molecule within a biological sample, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1. The target molecule of the kit may be HLA allele, HLA allele, HNA antigen, HNA allele blood grouping antigen, TCR or KIL. In one aspect, the probe of the kit is a SSO or a primer. In another aspect, the probe of the kit is a peptide or protein antigen.

[0039] The above kit may be for conducting a method of DNA-based tissue, wherein the probe is a sequence specific oligonucleotide (SSO) selected to hybridize with a tissue-typing antigen allele. The tissue-typing antigen of the kit may be s HLA, HNA, blood grouping antigen, TCR or KIL.

Detailed Description

[0040] The invention is related to improved methods for tissue typing transplant donors and recipients using probes conjugated to microparticles or beads with unique identifiable characteristics. The method involves screening with a set of beads with identical identifiable characteristics, wherein different probes are immobilized on the bead. These methods include DNA-based and protein-based tissue-typing.

[0041] The method involves preparing multiple sets of beads, wherein each set has a unique identifiable characteristic. In a preferred embodiment, the unique identifiable characteristic is some combination of multiple embedded detectable label such as fluorescent dyes. Each of the beads within a set presents at least one probe that binds to a tissue-typing antigen, such as HLA in a sample DNA. For DNA-based tissue-typing the probe is a sequence specific oligonucleotide (SSO) which hybridizes to a sample DNA. For protein-based tissue typing, the probe is an peptide or protein antigen that binds to an antibody or another protein within a biological sample.

[0042] For DNA-based tissue typing, a sample DNA may be obtained from a biological sample or tissue from a transplant donor or transplant recipient, and the gene encoding the tissue-typing antigen of interest or a region containing the an allele or polymorphism of the tissue-typing antigen is amplified by PCR. The amplified sample DNA (or PCR product) contacts the multiple sets of beads under hybridizing conditions such as moderately stringent hybridization conditions or highly stringent hybridization conditions. Hybridization of the sample DNA and the SSO probe on the bead generates a signal indicative of hybridization and this signal is detected and measured with a method standard in the art such as flow cytometry or visual images generated by a microscope and/or a camera. In a preferred embodiment, the signal is generated because the sample DNA is labeled with a fluorescent dye, such as phycoerythrin (PE) or fluorescein isothiocyanate (FITC) and a label such as biotin, with the corresponding steptavidin conjugated with fluorescent dyes. The fluorescent dyes conjugated to hexahistidine also may be used in combination with sample DNA labeled with nickel. A positive event is one in which an SSO

probe hybridizes to the sample DNA. The signal also may be generated using immobilized SSP primers to detect the elongation events with labeled nucleotides.

[0043] A proportion of the positive subset of beads is selected. Preferably, the selected proportion exhibits the greatest signal indicative of a specific binding interaction between the target molecule and the probe, such as sample DNA/SSO hybridization or the antibody/antigen binding, for each set of uniquely identifiable beads. The signal emitted by the selected portion of positive beads is statistically analyzed to determine the mean or median (or any other statistics such as peak, trimmed mean, trimmed peak etc.) intensity of the signal emitted by the detectable label. The portion of the subset of positive beads is preferably determined by the number of different probes and number of different labeled beads used in the screening assays. The positive beads are those that emit a signal that is greater than a selected threshold, wherein the threshold is indicative of the presence of one or more tissue-typing antigen alleles in the sample DNA. Exemplary software for analyzing the signal using a Flow cytometer is available from Luminex, Inc. (Austin, TX) and BD Biosciences (San Jose, CA). Additional software examples include WinMDI (Windows Multiple Document Interface for Flow Cytometry), FCS Express (De Novo Software, Thornhill, ON Canada), FlowJO (Tree Star, Inc.). The positive subset of beads will indicate a number of tissue-typing antigens that may be present within the sample DNA or biological sample. The data may be analyzed using software that analyzes the intensity of detectable labels, which is standard in the art.

[0044] The selected threshold to determine positive beads (after the filtering step) will be the same for any single probe. The selected threshold is commonly determined by analyzing a panel of known positive samples and a panel of known negative samples, and identifying the differential between the two. A threshold is then set within that differential.

[0045] The invention provides an improved method for DNA-based tissue typing or protein-based tissue typing, which utilizes multiple probes on beads with identical identifiable characteristics. The use of a subset of the multiple probes immobilized on beads having an identical identifiable characteristics allow for detection of a stronger signal from the positive beads since only one characteristic or signal is being detected. In addition, the signal detected using the improved method of the invention is near to the true value of the intensity of the actual signal produced by the specific binging interaction, such as DNA/SSO hybridization or the antibody/antigen binding. The true value of intensity of the signal is that measured using the conventional methods that use beads each having a different identifiable characteristic, such each having a different fluorescent label, and a unique SSO probe or peptide probe. Thus, the signal detected using the improved method is similar to the that detected using the conventional method.

[0046] One advantage of this improved method of DNA-based tissue-typing or protein-based tissue-typing is that the initial tissue typing screen provides much information on which tissue-typing antigens are not present in the sample DNA or biological sample. Nevertheless, the information on which antigens are definitely present in the sample DNA or biological sample may be less precise because of the inherent ambiguity of the system. Methods by which the ambiguity of the system may be reduced are set out below. This approach is less expensive, because it involves fewer different identifiable beads and considerably narrows the overall potential positive antigens in a sample DNA or biological sample. Also, this approach reduces the cost of labor because fewer rounds of testing are needed. In addition, this approach allows for testing an increased number of tissue-typing antigens gene sequence because a greater number of probes may be used while reducing the number of beads used. In addition, the improved method allows for higher resolution typing in a shorter time which is advantageous because the tissue may be transplanted sooner.

[0047] Analyzing multiple subsets of microparticles within a set of microparticles having the identical identifiable characteristic may result in an increased number of false negatives. In particular, if a DNA sample only hybridizes to a few microparticles (such as one subset of microparticles) within a set, the intensity of the signal generated by the hybridization will be low and may be deemed as a negative event. i.e. false negative events To solve the problem of false negatives, a selected portion of a set generating a positive signal is chosen for further evaluation. This selected portion will be less than all (100%) of the positive set. Preferably, the selected portion of the positive set is less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the positive set of microparticles. The proportion selected may depend on the number of SSO probes and the number of total microparticles used in the tissue-typing screen. In addition, the proportion may be selected by detecting a drop off in signal, *i.e.* the slope of the curve for the analysis.

[0048] The inherent ambiguity of the system wherein identically labeled proteins are capable of binding with multiple different targets may be reduced by appropriate arrangement of probes in different microparticle sets and subsets. The selection of the SSO or protein-based probes on the microparticles allows for detection of particular alleles or particular antigens. For example, patterns of SSOs within a set or patterns between different sets may be designed to allow for selected elimination of certain alleles or selection of a rare alleles. Such patterns of probes may have multiple copies of the same probe within one set of microparticles or multiple copies of the same probe within different sets of microparticles. The variation in the numbers of the identical probes and the ratio of different probes allow for the generation of additional data by fewer assays.

[0049] The invention particular provides for subsets of probes within a set of microparticles, wherein the microparticles of a set have the same identifiable characteristic, and the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1. The ratio of probes between the subsets allow for complex analysis of the presence of the target molecules, such as HLA alleles, within a biological sample. Variation of the ratios of probes between subsets within a single set of microparticles allows for a larger proportion of the positive events to be selected for further statistical analysis or stated another way, a larger portion of the events will be selected in the filtering step while reducing the potential for false negative events.

[0050] In a further embodiment, the variation in probe ratios between subsets within a single set may reduce the ambiguity within the system by allowing for identification of the target molecule based on the percentage of microparticles generating certain signals.

[0051] The use of computer algorithms will allow for the analysis of the pattern of positive probes or analysis of the variations of ratios of probes to determine specific tissue typing or to determine the presence of a rare allele verses the presence of a common allele. It is more efficient to carry out complex analyses using patterns of SSOs or differing ratios of SSOs between subsets of a set of microparticles having the same identifiable characteristic, rather than using a single set of 100 microparticles each containing a different SSO and a different identifiable characteristic.

[0052] A preferred embodiment of the invention uses microparticles presenting SSO for DNA-based tissue typing. However, the invention also provides for methods of tissue-typing using microparticles presenting a tissue-typing antigen for detection of an antibody is a biological sample. The advantages of the improved invention in view of DNA-based tissue-typing are also advantageous for protein-based assays (antigen/antibody reactions). For example, a subset of beads may be labeled with the HLA*A*0201 antigen, HLA*A*0202 antigen and/or the HLA*A*0203 antigen to detect the presence of an antibody that binds these antigens in a biological sample.

[0053] The methods of the invention may be carried out with microparticles, microbeads, beads or microsphere of any material, e.g. silica, gold , latex., polymers such as polystyrene, polysulfone and polyethyl, or hydrogel. Additional exemplary microparticles are encoded with the dyes and the oligonucleotides are immobilized to the encoded microparticles, The microparticles used in the methods of the invention are commercially available from sources such from Luminex Inc., Invitrogen (Carlsbad, CA), Polysciences Inc. (Warrington, PA) and Bangs Laboratories (Fishers, IN) to name a few.

[0054] The microparticles of the invention may comprise a detectable label or another identifying characteristic. The microparticles may comprise a single fluorescent dye or multiple fluorescent dyes. In one embodiment, the microparticles are internally labeled with fluorescent dyes and contain surface carboxyl groups for covalent attachment of biomolecules. In another embodiment, the microparticles are internally labeled with fluorescent dyes and contain a surface layer of Avidin for near covalent binding of biotin and biotinylated ligands. In another embodiment, the microparticles may comprise a combination of different dyes, such as a fluorescent and a non-fluorescent dye. For example, the microparticles may be labeled with E)-5-[2-(methoxycarbonyl)ethenyl]cytidine , which is a nonfluorescent molecule, that when subjected to ultraviolet (UV) irradiation yields a single product, 3-β-D-ribofuranosyl-2,7-dioxopyrido[2,3-*d*] pyrimidine, which displays a strong fluorescence signal. In another embodiment, the microparticles may comprise bar codes as an identifiable characteristic as described in U.S. Patent Publication No. US 20070037195.

[0055] In another embodiment, the microparticles may be nanocrystals or quantum dots. These nanocrystals are substances that absorb photons of light, then re-emit photons at a different wavelength (fluorophores). In addition, additional florescent labels, or secondary antibodies may be conjugated to the nanocrystals. These nanocrystals are commercially available form sources such as Invitrogen and Evident Technologies (Troy, NY),

[0056] The identifiable characteristic of the microparticle may be any nanoparticle DNA based detection methods or any nanoparticle protein based detection method. On example, is a bio bar code, which is an ultrasensitve method of detecting proteins using nanoparticle probes that are encoded with DNA that is unique to the protein target in the biological sample (Nam et al., Science 301, 1884-1886, 2003). Examples of nanoparticle DNA-based detection include colorimetric polynucleotide detection methods based on mercaptoalkyloligonucleotide-modified gold nanoparticles (Elghanian et al., Science 277, 1078-1080, 1997), chip-based detection methods that rely upon either light scattering or silver staining (Taton et al. Science 289, 1757-1760, 2000; Taton et al., .J. Am. Chem. Soc., 123, 5164-5165, 2001) electrical detection method for DNA in which the target DNA is captured in the gap between two electrodes using a sandwich assay (Park et al., Science 295, 1503-1506, 2002) and DNA detection using chemoresponsive diffraction gratings interrogated simultaneously at multiple laser wavelengths (Cao et al., J. Am. Chem. Soc. 2003).

[0057] The invention can be carried out with any system that detect the identifiable characteristic or label, such as FLOW. Detection of fluorescent labels may also be carried out using a microscope or camera that will read the image on the microparticles, such as the Bioarray BeadChip (Bioarray Solutions, Ltd., Warren, NJ). The BeadChip format combines microparticle ("bead") chemistry with semiconductor wafer processing in which binding to the microparticle is recorded using an optical microscope and camera.

[0058] The sample DNA used in the methods of the

invention are isolated or extracted from a biological sample from a human transplant or transfusion donor or a human transplant or transfusion recipient. The sample DNA may be prepared using any conventional method in the art, such as those taught in Sambrook et al., Molecular Cloning: A Laboratory Manual, cold Springs Harbor Laboratories (New York, 1989). The tissue typing gene of interest, such as HLA, is amplified using PCR techniques standard in the art.

[0059] Biological samples includes whole blood, blood derivatives, red blood cell concentrates, plasma, serum, fresh frozen plasma, whole blood derived platelet concentrates, apheresis platelets, pooled platelets, intravenous gamma-globulin, cryoprecipitate, cebrospinal fluid, tissues and cells such as epithelial cells, such as those collected from the buccal cavity, stem cells, leukocytes, neutrophils and granulocytes. The biological samples may be obtained from a human donor of tissue or cells intended for transplantation or a human donor of blood or blood derivatives intended for transfusion. The biological sample may be obtained from a healthy bone marrow donor or a subject of a paternity test. The biological sample may also be obtained from a human subject that is an intended recipient of a transplant or transfusion, or the human subject that is donating the tissue or organ intended for transplantation or transfusion.. Alternatively, the biological sample may be obtained directly from tissues or cells that are intended for transplantation in a human recipient. In addition, the biological sample may be obtained from blood or blood derivatives that are intended for transfusion in a human recipient.

SSO Probes

[0060] To carry out the methods of the invention, SSO probes are conjugated to fluorescently labeled beads. The SSO probes may also contain a label such as biotin, streptavidin, nickel, hexahistidine, digoxigenin (DIG), DNP or a fluorescent label such as FITC , PE, 6-TAMRA, CR6G, DEAC, Texas Red, Cy3, Cy3.5, CY5, Cy5.5, Cy7, Rhodamine Green X. The term "nucleotide" as used herein can refer to nucleotides present in either DNA or RNA and thus includes nucleotides that incorporate adenine, cytosine, guanine, thymine and uracil as base, the sugar moiety being deoxyribose or ribose. It will be appreciated, however, that other modified bases capable of base pairing with one of the conventional bases, adenine, cytosine, guanine, thymine and uracil, may be used in the diagnostic probe employed in the present invention. Such modified bases include, for example, 8-azaguanine and hypoxanthine. The term "oligonucleotide" as used herein refers to a molecule that is 200 nucleotides or fewer, preferred oligonucleotides are 100 nucleotides or less, 50 nucleotides or less or 25 nucleotides or less.

[0061] The term "complementary to" is used herein in relation to nucleotides to mean a nucleotide which will base pair with another specific nucleotide. Thus, adenosine monophosphate is complementary to uridine monophosphate or thymidine monophosphate and guanosine monophosphateis complementary to cytidine monophosphate. It is appreciated that while thymidine monophosphate and guanosine monophosphate may base pair under certain circumstances, they are not regarded as complementary for the purpose of this specification. It will also be appreciated that while cytosine monophosphate and adenosine monophosphate may base pair under certain circumstances, they are not regarded as complementary for the purposes of this specification. The same applies to cytosine monophosphate and uracil monophosphate.

[0062] The SSO probe used in the methods herein are selected to be "substantially" complementary to the different strands of each specific sequence to be detected. This means that the SSO probes must be sufficiently complementary to hybridize with their respective strands of sample DNA. Therefore, the SSO probe sequence need not reflect the exact sequence of the sample DNA sequence. Thus, probe sequences do not necessarily have to be exactly complementary to the target sample DNA sequences. Thus, not all probes produce a clean negative signal similar to that of a negative control for negative alleles. Depending upon the number of mismatches and what types of mismatches (G-T mismatch occasionally produces approximately the same signal as G-C match), the fluorescent signal for 1 base-pair mismatched alleles might produce a signal substantially higher than the negative control. However, as long as the signal of the true positive alleles are significantly higher than those potentially cross-reacting alleles, usually >by 10-20%, a threshold or cut-off value can be established to distinguish between positive and negative reactions.

[0063] Generally a small number of mismatches will be tolerated in the middle of the probe sequences and will allow for hybridization. In general, the degree of mismatching tolerated depends upon the SSO probe region length, which in turn affects the denaturation temperature and the annealing temperature selected of the hybridization conditions. If the denaturation temperature of the probe is close to or higher than the annealing temperature (less stringent), then the probe will still adhere to the target sequence despite a small number of (generally one or two or at most three) mismatches. A probe region may be capable of tolerating more mismatches in the middle of the sequence but its ability to do so depends on the denaturation temperature of the probe region and the annealing temperature of the selected hybridization and detection conditions.

[0064] The term "stringent" is used herein to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at

65-68°C or 0.015 M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989).

[0065] More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6X SSC 0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

[0066] Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1 % bovine serum albumin, 0.1 % polyvinyl-pyrrolidone, 0.1 % sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO4, (SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid Hybridisation: A Practical Approach, Ch. 4, IRL Press Limited (Oxford, England). Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids.

[0067] A diagnostic sequence-specific oligonucleotide-probe detection (SSO) system is a system or device that uses a diagnostic probe to assay for the presence of a particular target nucleic acid sequence. In such a system or device, the diagnostic probes may be attached to a support using linkers such as are well known in the art including the use of poly-carbon and poly-nucleotide linkers. Alternatively, target sequences can be immobilized on a solid support, such as a nitrocellulose membrane and the diagnostic probe is in solution. The diagnostic probe in the SSO includes at least one probe region. The term "probe region" refers to a nucleotide sequence on a diagnostic probe substantially complementary to a portion of the target nucleotide sequence.

[0068] Labeling can be either on the probes immobilized on the bead or on the amplified sample DNA. Direct fluorescence compounds, biotin, FITC or Digoxigenin (Dig) can be used as the tag. For the indirect detection, fluorescence or enzyme conjugated Avidine/Strepavidine (for biotin), anti-FITC antibody (for FITC), anti-Dig antibody (for Dig) will be used for detection purposes. According to one embodiment, latex beads modified with a carboxyl group can be used to immobilize probes. The carboxyl group on the beads is first activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and then the EDC-activated carboxyl group is reacted with the amine group at the 5' end of the oligo probes. Alternative embodiments using chemistries to link amine to amine, sulfide to amine and other chemistries may also be practiced.

Tissue-Typing Antigens

[0069] The invention provides for methods of tissue-typing screening for human leukocyte antigens (HLA). The HLA antigens are present on the cell surface of nearly every cell in the body and these antigens are in high concentrations on leukocytes. HLA antigens are one of the major determinants the immune system uses for recognition and differentiation of self from non-self. As of April 2007, see the WHO nomenclature Committee for Factors of the HLA System (www.anthonynolan.com/HIG/), there are 545 HLA-A alleles, 895 HLA-B alleles, 307 HLA-C alleles, 8 HLA-E alleles, 12 HLA-H alleles, 9 HLA-J alleles, 6 HLA-K alleles, 4 HLA-L alleles, 4 HLA-P alleles, 3 HLA-V alleles, 3 DRA alleles, 494 DRB1 alleles, 1 DRB2 alleles , 44 DRB3 alleles, 13 DRB4 alleles, 18 DRB5 alleles, 3 DRB6 alleles, 2 DRB7 alleles, 10 DRB8 alleles, 1 DRB9 alleles, 34 DQA1 alleles, 83 DQB1 alleles, 23 DPA1, 126 DPB1 alleles, 4 DMA alleles, 7 DMB alleles, 12 DOA alleles and 9 DOB alleles.

[0070] The invention is not limited to methods of tissue typing comprising screening for HLA antigens. The methods of the invention can be used to screen for any antigen used for typing tissues or blood. For example, the methods can screen for killer cell immunoglobulin-like receptors (KIR), human neutrophil antigens (HNA), T Cell Receptors (TCR) and fragments thereof and blood groups, such as ABO, RH factor.

Killer cell immunoglobulin-like receptors (KIRs)

[0071] Killer cell immunoglobulin-like receptors (KIRs) are members of a group of regulatory molecules found on subsets of lymphoid cells. KIR molecules have been implicated in reduced risk of relapse in patients with acute myeloid leukemia (AML) who received hematopoietic transplants that were mismatched for KIR ligands. KIR ligand incompatibility was defined as absence in recipients of donor class I allelic groups known to be ligands for inhibitory KIRs (3DL1, 2DL2/3, 2DL1 and 3DL2). Donor-versus-recipient NK cell alloreactivity is known to be capable of eliminating leukemia relapse and graft rejection, and it also protected patients against graft-versus-host-disease.

[0072] Sequence analysis of KIR cDNA has shown that most KIR genes contain variable sites, and that some are quite polymorphic. Allelic polymorphism provides additional diversity to the extent that unrelated individuals identical for both KIR haplotypes are unlikely to be observed. The variation in KIR sequences can occur at positions encoding residues that affect interaction with HLA

class I. Variation tends to occur throughout the gene, unlike the pattern observed in HLA class I and II genes where nucleotide variation is restricted primarily to one or two exons. KIR molecules have been implicated in reduced risk of relapse in patients with acute myeloid leukemia (AML) who received hematopoietic transplants that were mismatched for KIR ligands. KIR ligand incompatibility was defined as absence in recipients of donor class I allelic groups known to be ligands for inhibitory KIRs (3DL1, 2DL2/3, 2DL1 and 3DL2). The data indicated that donor-versus-recipient NK cell alloreactivity was capable of eliminating leukemia relapse and graft rejection, and it also protected patients against graft-versus-host-disease.

[0073] KIR genotyping can be locus or allele specific. Locus only typing detects presence or absence of each gene in a given individual, thus providing a profile of the KIR repertoire (KIR profile). The PCR sequence-specific priming (PCR-SSP) method for KIR typing has been updated to account for newly discovered loci and previously undetected alleles. A PCR sequence-specific oligonucleotide probe (PCR-SSOP) method has also been developed. Inter-laboratory collaboration has helped to authenticate the molecular genotyping assay for KIR loci (100). Over 100 different KIR genotype profiles have been found so far.

[0074] Medium- to high-resolution allele-specific reactions (PCR-SSP) have been described for 2DL1, 2DL3, 3DL1 and 3DL2 (25, 55), and a single-stranded conformational polymorphism (SSCP) assay has been used to genotype 2DL4. Development of a comprehensive assay was required for 2DL5 in order to distinguish the two highly homologous loci, 2DL5A and 2DL5B.. Reverse-transcriptase PCR (RT-PCR) based on PCR-SSP is the method of choice for allotyping NK cell clones and remains largely unchanged from that described previously. Various monoclonal antibodies are available for this purpose, but specificity is limited by the high homology between KIR isotypes.

Blood Groups

[0075] Currently, there are many blood typing systems. The presence of an unfamiliar antigen on a transfused red blood cell or the presence of an antibody in donated plasma may cause a deleterious immune response which attacks the donated blood cells, causing the donated red blood cells to burst. This may also cause serious symptoms, including kidney failure and shock. Antigens also occur on other blood components, including white blood cells, platelets, and plasma proteins.

[0076] One example of current blood typing systems is the ABO system in which blood is typed based on the presence of A antigen (AA, AO), B antigen (BB or BO), both A and B antigens (AB) or the absence of both A and B (O) on the red blood cells. Additional antigen on red blood cells include MNS system (antigens M, N, S and s), the P system (antigen PI), the Lutheran system (Lu(a) and Lu(b), the Kell System (K (Kell) and k (cellano), and Kpa and Kpb), the Lewis system (Le a and Le b), the Duffy system (Fy a and Fy b) and the Kidd (JK) system (Jka and Jkb),

[0077] In addition to those listed above, blood is also typed using the RH (Rhesus) system. There are three genes making up Rhesus antigens: C, D, and E, found on chromosome 1. There are two possible alleles at each locus: c or C; d or D; and e or E. One haplotype consisting of c/C, d/D, e/E is inherited from each parent, and the resulting Rhesus type of the individual depends on their inherited genotype. The haplotypes are given a code as follows: CDe is known as R1, cDE is known as R2, CDE is known as Rz, cDe is known as Ro, Cde is known r', cdE is known as R", CdE is known as Ry and cde is know as r.

Human Neutrophil Antigen

[0078] Antibodies to human neutrophil-specific antigens (HNA) were shown to cause clinical complications after transfusions such as pulmonary transfusion reactions and in some cases transfusion related acute lung injury (TRALI) or causing neonatal alloimmune neutropenia (NAIN) (Bux, et al. Transfus. Med. 2(2): 143-9, 1992). Therefore, detection of HNA specific antigen or antibodies has important clinical applications.

[0079] Human neutrophil antigens are also known as neutrophil-specific antigens or HNA. Currently there are 5 HNA antigen systems: HNA-1, HNA-2, HNA-3, HNA-4 and HNA-5. Alleles for HNA-1, 2, 4 and 5 were identified and the corresponding glycoproteins were characterized; however, the allele for HNA-3 remains unknown (reviewed by Stroncek, ASHI Quarterly 2004). There are three HNA-1 antigens (HNA-1a, HNA-1b and HNA-1c) that are expressed solely on neutrophils and are located on low affinity Fc-y receptor IIIb. The HNA-2 system has one well established antigen (HNA-2a). HNA-2 is only expressed on neutrophils and neutrophil precursors and is located on the glycoprotein CD 177 (NB 1 gp). The HNA-3 system has one antigen, HNA-3a, that is also known as 5b. HNA-3 is expressed on neutrophils, lymphocytes, platelets, endothelial cells, kidney, spleen and placenta cells, and is known to be located on a 70 to 95 kD neutrophil glycoprotein. HNA-4 and HNA-5 are located on the β2 integrin. HNA-4 is expressed on granulocytes, monocytes and lymphocytes. (See Stroncek, ASHI Quarterly 2004)

T Cell Receptors

[0080] Antigenic peptides presented by the major histocompatibility complex (MHC) cell surface glycoproteins are recognized by T cells through the T cell receptor (TCR) complex, which is a multisubunit transmembrane surface complex made up of a T cell receptor and of the CD3 chains. The TCR directly binds the peptide/MHC complex, and activates the T cell through interactions

with the CD3 and other components of the TCR. Therefore, TCR is involved in self and foreign tissue recognition and analyzing tissues for TCR expression may be helpful for tissue-typing transplant donors and recipients.

Kits

[0081] The invention also provides for kits to carryout the methods of the invention. In particular, the disclosure provides for kit for conducting a method of DNA-based tissue typing comprising a) multiple sets of microparticles having identifiable characteristics, wherein within each set every microparticles has an identical identifying characteristic and b) SSO probes, wherein a different SSO probe is immobilized to a microparticle within a set.

[0082] Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative example.

EXAMPLE 1

Comparison of Conventional Method and the Method of the Invention Bead, Probe and Sample DNA Preparation

[0083] The LABTYPE® SSO DRB1 Locus (One Lambda Inc., Canoga Park, CA) were used for the DNA-tissue typing analysis described below. The Luminex xMAP microsphere (Austin, TX) which have two fluorescent dyes embedded within. Each microsphere (or bead) had a different concentration of each of the embedded fluorescent dyes were used.

[0084] The SSO probe was labeled with amine and was conjugated to the bead. The HLA DRB1 Exon 2 was amplified from DNA isolated from a biological or tissue sample by PCR using biotinlyated primers. The resulting PCR product (denoted herein as "sample DNA") was labeled with biotin on the 5'-end The sample DNA was contacted to the bead and hybridization of the sample DNA with the SSO probe immobilized on the bead generated a fluorescent signal indicative of hybridization of the sample DNA and the SSO probe after incubating with Streptavidin conjugated with fluorescent dye phycoerythrin (PE). This fluorescent signal was detected and measured flow cytometry. (DNA can be from any source, preferably from blood or buccal swabs)

[0085] A DNA hybridization reaction between the amplified HLA gene within the sample DNA and the SSO probes immobilized on microparticles was carried out as follows. Standard gene amplification reactions containing approximately 1 ng/microliter of genomic DNA and 10 micromolar of corresponding sequence-specific biotinylated primers were set up using a pre-optimized thermocycling program. 5 microliters of resulting mixture containing amplified DRB1*0814 was denatured, neutralized and were mixed with desired probe-bound microparticles (1000 microparticles per probe per test) in 1M NaCl and 70 mM sodium phosphate buffer. The hybridization reactions were incubated at 60°C for 15 minutes. Then the 2 volumes of 50nM NaCl solution (pre-heated at 60°C) was added to the mixtures, and the tubes were centrifuged for 5 minutes. Supernatant was removed without disturbing the pelleted microparticles. This washing step was repeated two more times.

[0086] The hybridized DNA was then labeled by addition of 3 volumes of 5 micrograms per microliter of phycoerythrin-streptavidin conjugate. The labeling mixture was incubated at 60°C for five minutes and washed as described above. Washed microparticles were resuspended to 80 microliter volume with 50 nM NaCl. The hybridization signal was detected using a Luminex 100 Flow Analyzer that excites, detects and records fluorescence signal at 580 nm for individual microparticles injected into the instrument. Approximately 500 microparticles per test were analyzed to calculate trimmed mean fluorescence intensity (MFI) for each probe. Resulting MFI for each probe used in a test are then used to calculate relative hybridization signals using the MFI from appropriate positive control probes.

[0087] Positive control probes are probes that recognize a non-polymorphic region on all alleles that can be amplified by a specific primer set. The target nucleic acid strands for this invention include allelic regions that have been amplified using the polymerase chain reaction (PCR). The positive control probes are used to provide reference signal so that variation in the amount of the amplified DNA (amplicons) can be estimated. The positive control signal is used to calculate relative signal of all diagnostic probes as diagnostic probe signals are expressed as percent of positive control signal.

Conventional Analysis

[0088] For the conventional DNA-based tissue-typing method, 100 different fluorescently labeled beads were each conjugated with a different HLA specific SSO probe as described above. The beads were contacted with the sample DNA. Hybridization of the sample DNA to any of the beads was measured using a Luminex 100 Flow Analyzer.

[0089] The flow cytometry data was statistically analyzed using the Luminex 100 Flow Analyzer software. The bead of interest (Bead 37) had a trimmed mean value of fluorescent intensity of 1707. The trimmed mean is the mean calculated when a certain percentage of high and low outliers are omitted.

Analysis Using the Method of the Invention

[0090] Using the method of DNA-based tissue typing of the invention, a number of Bead 37 (750 beads) were conjugated with one of five HLA specific SSO probes that are set out in Table 1. The beads were contacted with the sample DNA. Hybridization of the sample DNA to any of the beads is measured using Luminex 100 Flow Analyzer. As 5 probes were tested, 20% of the positive bead

were further analyzed.

Table 1

Bead ID	Probe ID	Probe Specificity
37	OLR4916	DRB1*0814
37	DR245	DRB1*0113
37	DR250	DRB1*1213
37	DR260	DRB1*0309, DRB1*0323
37	DR228	DRB1*0703

[0091] The flow cytometry data was statistically analyzed using the Luminex 100 Flow Analyzer software. This analysis included determining the size of the bead analyzed, the fluorescent intensity of each of the fluorescence dyes embedded within the bead, and a determination if the fluorescent intensities were within the desired range. The fluorescent intensities of each bead was analyzed to determine the RPI. The (RPI represents the fluorescence intensity generated by the sample DNA hybridized to the bead. In this example, the RP1 represents the true fluorescence intensity of the PE labeled to the sample DNA. This value indicates that the sample DNA has hybridized to the SSO probe conjugated to Bead 37.

[0092] For Bead 37, 574 of the events had an RPI < 216, and were considered negative, i.e. the sample DNA did not hybridize to the SSO probe. In addition, there were 151 positive events (RPI > 5650) which indicates that the sample DNA hybridized to the SSO probe. The ratio of positive to negative events was 151:574, which is about 1:4. Therefore one out of the 5 probes were positive for the sample DNA. The mean RPI for the positive beads was 8152. This value is a stronger signal and is a enhance signal that is greater than true value that the mean RPI calculated with the conventional method (1707).

[0093] The DNA sample used in this experiment was known to be positive for only DRB2*0814 (hybridizes to probe OLR4916; see Table 1). Analysis of only 20% of the positive events indicated that Bead 37 was positive and the mean signal detected (8152) had an intensity similar to that generated by carrying out the conventional method (1 bead per 1 probe). This experiment demonstrates that the selection step of the methods of the invention enhanced a positive signal. In addition, this example demonstrates that more targets may be screened using the same number or fewer beads and the signal generated will be similar to the signal generated using the conventional method.

EXAMPLE 2

Additional Comparison of Conventional Method and the Method of the Invention

[0094] An additional comparison of conventional methods of DNA based tissue-typing for HLA allelic antigens and the improved methods of the invention was carried out as described in Example 1. The sample DNA used in the methods contained amplified HLA antigen allele DRB1*0416.

[0095] The hybridization signal was detected using a Luminex 100 Flow Analyzer as described in Example 1. Resulting MFI for each probe used in a test was used to calculate relative hybridization signals using the MFI from appropriate positive control probes. Positive control probes that recognize a non-polymorphic region on all alleles that can be amplified by a specific primer set were also used.

Conventional Method

[0096] For the conventional DNA-based tissue-typing method, 100 different fluorescently labeled beads were each conjugated with a different HLA specific SSO probe as described above. The beads were contacted with the sample DNA. Hybridization of the sample DNA to any of the beads was measured using a Luminex 100 Flow Analyzer.

[0097] The flow cytometry data was statistically analyzed using the Luminex 100 Flow Analyzer software. The bead of interest (Bead 73) had a trimmed mean value of fluorescent intensity of 400.

Analysis Using the Method of the Invention

[0098] Using the method of DNA-based tissue typing of the invention, a number of Bead 73 (838 beads) were conjugated with one of five HLA specific SSO probes that are set out in Table 2. The beads were contacted with the sample DNA. Hybridization of the sample DNA to any of the beads is measured using Luminex 100 Flow Analyzer. As 5 probes were tested, 20% of the positive bead were further analyzed.

Table 2

Bead ID	Probe ID	Probe Specificity
73	DR148	DRB1*0416
73	DR263	DRB1*1522
73	DR252	DRB1*1133, DRB1*1135
73	DR278	DRB1*1205, DRB1*1215, DRB1*1364, DRB1*1441
73	DR275	DRB1*1351

[0099] The flow cytometry data was statistically analyzed using the Luminex 100 Flow Analyzer software as described in Example 1. For Bead 73, 672 of the events had an RPI < 48 (mean = 16), and were considered negative, i.e. the sample DNA did not hybridize to the SSO probe. In addition, there were 166 positive events (RPI > 1457) which indicates that the sample DNA hybridized to the SSO probe. The ratio of positive to negative events was 166:672, which is about 1:4. Therefore one out of

the 5 probes were positive for the sample DNA. The mean RPI for the positive beads was 2510. This value is a stronger signal and is a more true value that the mean RPI calculated with the conventional method (400).

[0100] The DNA sample used in this experiment was known to be positive for only DRB1*0416 (hybridizes to probe DR148; see Table 2). Analysis of only 20% of the positive events indicated that Bead 73 was positive and the mean signal detected (2510) had an intensity similar to that generated by carrying out the conventional method (mean is 400 for conventional method). This example substantiates the analysis described in Example 1.

Claims

1. A method of screening for a binding interaction comprising the steps of:

   preparing multiple sets of uniquely identifiable microparticles wherein the microparticles within a set have the same unique identifiable characteristic and further, wherein one or more sets comprise two or more subsets of microparticles, each of said subsets presenting at least one unique probe that acts as a binding partner for a target molecule within a biological sample, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1;
   contacting said multiple sets of microparticles with a biological sample under conditions in which a target molecule within the biological sample binds to the probe,
   detecting the binding of a target molecule within the biological sample and the probe on said microparticles by generation of a signal indicative of the binding interaction between the target molecule and the probe,
   measuring the signal indicative of the binding interaction for said microparticles,
   selecting a proportion of less than 100% of microparticles exhibiting the greatest signal indicative of the binding interaction for one or more sets of uniquely identifiable microparticles,
   determining for said set(s) of uniquely identifiable microparticles whether the signal indicative of the binding interaction for the selected proportion is greater than a selected threshold indicative of the presence of one or more target molecules in said biological sample;
   and
   determining the number of uniquely identifiable microparticle sets indicating the binding interaction.

2. The method of claim 1, wherein the probe is selected from the group consisting of a sequence specific oligonucleotide (SSO) probe, SSO primer, peptide antigen or protein antigen.

3. The method of claim 2, wherein the biological sample is a sample DNA and the binding interaction is hybridization of the sample DNA and the SSO probe.

4. The method of claim 1, wherein the selected proportion of a set of microparticles is less than or equal to the inverse of the number of subsets of microparticles presenting unique probes for said set of microparticles.

5. A method of DNA-based tissue-typing comprising the steps of:

   preparing multiple sets of uniquely identifiable microparticles wherein the microparticles within a set have the same unique identifiable characteristic and further, wherein one or more sets comprise two or more subsets of microparticles, each of said subsets presenting at least one unique sequence specific oligonucleotide (SSO) selected to hybridize with tissue-typing antigen alleles, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1,
   contacting said multiple sets of microparticles with a sample DNA under hybridizing conditions,
   detecting the hybridization of sample DNA and the SSOs on said microparticles by generation of a signal indicative of the hybridization of sample DNA with the SSOs,
   measuring the signal indicative of the hybridization of sample DNA and the SSOs for said microparticles,
   selecting a proportion of less than 100% of microparticles exhibiting the greatest signal indicative of DNA/SSO hybridization for at least one set of uniquely identifiable microparticles,
   determining for said set(s) of uniquely identifiable microparticles whether the signal indicative of DNA/SSO hybridization for the selected proportion is greater than a selected threshold indicative of the presence of one or more tissue typing antigens in said biological sample; and
   determining the number of uniquely identifiable microparticle sets indicating DNA/SSO hybridization.

6. The method of claim 5, wherein the fixed numerical ratio other than about 1 to 1 will determine the selected proportion of the set of microparticles.

7. The method of any one of claims 1-6, wherein the probe or tissue-typing antigen is selected from the group consisting of HLA antigens, HLA alleles, HNA antigens, blood grouping antigens, KIL antigens and TCR antigens.

8. The method of any one of claims 5 to 7, further comprising the step of reporting a signal for positive hybridization.

9. The method of any one of claims 5 to 8 wherein the selected proportion of a set of microparticles is less than or equal to the inverse of the number of subsets of microparticles presenting unique SSOs for said set of microparticles.

10. A kit for conducting a method of measuring a binding interaction comprising:

multiple sets of uniquely identifiable microparticles wherein the microparticles within a set have the same unique identifiable characteristic, and wherein one or more sets comprise two or more subsets of microparticles, each of said subsets presenting at least one unique probe that binds to a target molecule within a biological sample, wherein the number of probes varies between the subsets and at least two of the subsets of microparticles within at least one of the sets of uniquely identifiable microparticles are present in a fixed numerical ratio other than about 1 to 1.

11. The kit of claim 10, wherein the target molecule is selected from the group consisting of HLA antigen, HLA allele, HNA antigen, HNA allele blood grouping antigen, TCR or KIL.

12. The kit of claim 10 or 11, wherein the probe is selected from the group consisting of a sequence specific oligonucleotide (SSO), primer, peptide antigen and protein antigen.

13. The kit of claim 11 or 12, wherein the kit is for conducting a method of DNA-based tissue typing, and wherein the probe is a sequence specific oligonucleotide (SSO) selected to hybridize with a tissue-typing antigen allele.

Patentansprüche

1. Verfahren zum Screenen auf eine Bindungswechselwirkung, umfassend die Schritte:

Herstellen mehrfacher Sätze eindeutig identifizierbarer Mikropartikel, wobei die Mikropartikel innerhalb eines Satzes dasselbe eindeutige identifizierbare Kennzeichen besitzen und wobei ferner ein oder mehr Sätze zwei oder mehr Mikropartikel-Teilsätze umfassen, wobei jeder der Teilsätze mindestens eine eindeutige Sonde präsentiert, die als Bindungspartner für ein Zielmolekül innerhalb einer biologischen Probe fungiert, wobei die Anzahl der Sonden zwischen den Teilsätzen variiert und mindestens zwei der Mikropartikel-Teilsätze innerhalb mindestens eines der Sätze eindeutig identifizierbarer Mikropartikel in einem festen Zahlenverhältnis vorliegen, das anders als etwa 1 zu 1 ist,
In-Kontakt-Bringen der mehrfachen Mikropartikel-Sätze mit einer biologischen Probe unter Bedingungen, in denen ein Zielmolekül innerhalb der biologischen Probe an die Sonde bindet,
Nachweisen der Bindung eines Zielmoleküls innerhalb der biologischen Probe und der Sonde auf den Mikropartikeln durch Erzeugung eines die Bindungswechselwirkung zwischen dem Zielmolekül und der Sonde anzeigenden Signals,
Messen des die Bindungswechselwirkung anzeigenden Signals für die Mikropartikel,
Auswählen eines Anteils von weniger als 100% der Mikropartikel, die das höchste die Bindungswechselwirkung anzeigende Signal zeigen, für einen oder mehr Sätze eindeutig identifizierbarer Mikropartikel,
Bestimmen für den Satz (die Sätze) eindeutig identifizierbarer Mikropartikel, ob das die Bindungswechselwirkung anzeigende Signal für den ausgewählten Anteil höher ist als eine ausgewählte, die Anwesenheit von einem oder mehr Zielmolekülen in der biologischen Probe anzeigende Schwelle, und
Bestimmen der Anzahl eindeutig identifizierbarer, die Bindungswechselwirkung anzeigender Mikropartikel-Sätze.

2. Verfahren nach Anspruch 1, wobei die Sonde aus der Gruppe ausgewählt ist, bestehend aus einer sequenzspezifischen Oligonucleotid (SSO)-Sonde, einem SSO-Primer, Peptidantigen oder Proteinantigen.

3. Verfahren nach Anspruch 2, wobei es sich bei der biologischen Probe um eine Proben-DNA handelt und es sich bei der Bindungswechselwirkung um Hybridisierung der Proben-DNA und der SSO-Sonde handelt.

4. Verfahren nach Anspruch 1, wobei der ausgewählte Anteil eines Mikropartikel-Satzes kleiner als oder gleich dem Kehrwert der Anzahl der Mikropartikel-Teilsätze ist, die eindeutige Sonden für den Mikropartikel-Satz präsentieren.

5. Verfahren zum DNA-basierten Gewebetypisieren,

umfassend die Schritte:

Herstellen mehrfacher Sätze eindeutig identifizierbarer Mikropartikel, wobei die Mikropartikel innerhalb eines Satzes dasselbe eindeutige identifizierbare Kennzeichen besitzen und wobei ferner ein oder mehr Sätze zwei oder mehr Mikropartikel-Teilsätze umfassen, wobei jeder der Teilsätze mindestens ein eindeutiges sequenzspezifisches Oligonucleotid (SSO) präsentiert, das ausgewählt ist, um mit gewebetypisierenden Antigenallelen zu hybridisieren, wobei die Anzahl der Sonden zwischen den Teilsätzen variiert und mindestens zwei der Mikropartikel-Teilsätze innerhalb mindestens eines der Sätze eindeutig identifizierbarer Mikropartikel in einem festen Zahlenverhältnis vorliegen, das anders als etwa 1 zu 1 ist,
In-Kontakt-Bringen der mehrfachen Mikropartikel-Sätze mit einer Proben-DNA unter Hybridisierungsbedingungen,
Nachweisen der Hybridisierung von Proben-DNA und den SSOs auf den Mikropartikeln durch Erzeugung eines die Hybridisierung von Proben-DNA mit den SSOs anzeigenden Signals,
Messen des die Hybridisierung von Proben-DNA und den SSOs anzeigenden Signals für die Mikropartikel,
Auswählen eines Anteils von weniger als 100% der Mikropartikel, die das höchste die DNA/SSO-Hybridisierung anzeigende Signal aufweisen, für mindestens einen Satz eindeutig identifizierbarer Mikropartikel,
Bestimmen für den Satz (die Sätze) eindeutig identifizierbarer Mikropartikel, ob das die DNA/SSO-Hybridisierung anzeigende Signal für den ausgewählten Anteil höher ist als eine ausgewählte, die Anwesenheit von einem oder mehr gewebetypisierenden Antigenen in der biologischen Probe anzeigende Schwelle,
und
Bestimmen der Anzahl eindeutig identifizierbarer, die DNA/SSO-Hybridisierung anzeigender Mikropartikelsätze.

6. Verfahren nach Anspruch 5, wobei das feste Zahlenverhältnis, das anders als etwa 1 zu 1 ist, den ausgewählten Anteil des Mikropartikel-Satzes bestimmt.

7. Verfahren nach einem der Ansprüche 1 - 6, wobei die Sonde oder das gewebetypisierende Antigen aus der Gruppe ausgewählt ist, bestehend aus HLA-Antigenen, HLA-Allelen, HNA-Antigenen, Blutgruppenantigenen, KIL-Antigenen und TCR-Antigenen.

8. Verfahren nach einem der Ansprüche 5 bis 7, ferner umfassend den Schritt, bestehend im Melden eines Signals für positive Hybridisierung.

9. Verfahren nach einem der Ansprüche 5 bis 8, wobei der ausgewählte Anteil eines Mikropartikel-Satzes kleiner als oder gleich dem Kehrwert der Anzahl der Mikropartikel-Teilsätze ist, die eindeutige SSOs für den Mikropartikel-Satz präsentieren.

10. Kit zum Ausführen eines Verfahrens zum Messen einer Bindungswechselwirkung, umfassend:

mehrfache Sätze eindeutig identifizierbarer Mikropartikel, wobei die Mikropartikel innerhalb eines Satzes dasselbe eindeutige identifizierbare Kennzeichen besitzen und wobei ein oder mehr Sätze zwei oder mehr Mikropartikel-Teilsätze umfassen, wobei jeder der Teilsätze mindestens eine eindeutige Sonde präsentiert, die an ein Zielmolekül innerhalb einer biologischen Probe bindet, wobei die Anzahl der Sonden zwischen den Teilsätzen variiert und mindestens zwei der Mikropartikel-Teilsätze innerhalb mindestens eines der Sätze eindeutig identifizierbarer Mikropartikel in einem festen Zahlenverhältnis vorliegen, das anders als etwa 1 zu 1 ist.

11. Kit nach Anspruch 10, wobei das Zielmolekül aus der Gruppe ausgewählt ist, bestehend aus HLA-Antigen, HLA-Allel, HNA-Antigen, HNA-Allel-Blutgruppenantigen, TCR oder KIL.

12. Kit nach Anspruch 10 oder 11, wobei die Sonde aus der Gruppe ausgewählt ist, bestehend aus einem sequenzspezifischen Oligonucleotid (SSO), Primer, Peptidantigen und Proteinantigen.

13. Kit nach Anspruch 11 oder 12, wobei der Kit zum Ausführen eines Verfahrens zum DNA-basierten Gewebetypisieren bestimmt ist und wobei es sich bei der Sonde um ein sequenzspezifisches Oligonucleotid (SSO) handelt, das ausgewählt ist, um mit einem gewebetypisierenden Antigenallel zu hybridisieren.

Revendications

1. Procédé de criblage d'interactions de liaison comprenant les étapes suivantes :

préparation de multiples ensembles de microparticules identifiables de manière unique dans lequel les microparticules au sein d'un ensemble ont la même caractéristique identifiable de manière unique, et dans lequel en outre un ou plusieurs ensembles comprennent deux ou plusieurs sous-ensembles de microparticules, cha-

cun desdits sous-ensembles présentant au moins une sonde unique qui agit comme partenaire de liaison pour une molécule cible dans un échantillon biologique, dans lequel le nombre de sondes varie entre les sous-ensembles, et au moins deux des sous-ensembles de microparticules au sein d'au moins l'un des ensembles de microparticules identifiables de manière unique sont présents selon un ratio numérique fixe autre qu'environ 1 pour 1 ;
mise en contact desdits multiples ensembles de microparticules avec un échantillon biologique sous des conditions dans lesquelles une molécule cible au sein de l'échantillon biologique se lie à la sonde,
détection de la liaison d'une molécule cible au sein de l'échantillon biologique à la sonde sur lesdites microparticules par génération d'un signal indicatif de l'interaction de liaison entre la molécule cible et la sonde,
mesure du signal indicatif de l'interaction de liaison pour lesdites microparticules,
sélection d'une proportion inférieure à 100 % de microparticules présentant le plus grand signal indicatif de l'interaction de liaison pour un ou plusieurs ensembles de microparticules identifiables de manière unique,
détermination pour lesdits un ou plusieurs ensembles de microparticules identifiables de manière unique du fait que le signal indicatif de l'interaction de liaison pour la proportion sélectionnée est ou non supérieur à un seuil sélectionné indicatif de la présence d'une ou plusieurs molécules cible dans ledit échantillon biologique ; et
détermination du nombre d'ensembles de microparticules identifiables de manière unique indiquant l'interaction de liaison.

2. Procédé selon la revendication 1, dans lequel la sonde est sélectionnée parmi le groupe constitué par une sonde à oligonucléotide spécifique d'une séquence (SSO, soit Sequence Specific Oligonucleotide), une amorce SSO, un antigène peptidique et un antigène protéique.

3. Procédé selon la revendication 2, dans lequel l'échantillon biologique est un échantillon d'ADN et l'interaction de liaison est une hybridation de l'échantillon d'ADN avec la sonde de SSO.

4. Procédé selon la revendication 1, dans lequel la proportion sélectionnée d'un ensemble de microparticules est inférieure ou égale à l'inverse du nombre de sous-ensembles de microparticules présentant des sondes uniques pour ledit ensemble de microparticules.

5. Procédé de typage tissulaire sur base d'ADN comprenant les étapes suivantes :

préparation de multiples ensembles de microparticules identifiables de manière unique dans lequel les microparticules au sein d'un ensemble ont la même caractéristique identifiable de manière unique, et dans lequel en outre un ou plusieurs ensembles comprennent deux ou plusieurs sous-ensembles de microparticules, chacun desdits sous-ensembles présentant au moins un oligonucléotide spécifique d'une séquence (SSO) unique sélectionné pour s'hybrider avec des allèles d'antigène de typage tissulaire, dans lequel le nombre de sondes varie entre les sous-ensembles, et au moins deux des sous-ensembles de microparticules au sein d'au moins l'un des ensembles de microparticules identifiables de manière unique sont présents selon un ratio numérique fixe autre qu'environ 1 pour 1,
mise en contact desdits multiples ensembles de microparticules avec un échantillon d'ADN sous conditions d'hybridation,
détection de l'hybridation d'échantillons d'ADN avec les SSO sur lesdites microparticules par génération d'un signal indicatif de l'hybridation d'échantillons d'ADN avec les SSO,
mesure du signal indicatif de l'hybridation d'échantillons d'ADN avec les SSO pour lesdites microparticules,
sélection d'une proportion inférieure à 100 % de microparticules présentant le plus grand signal indicatif de l'hybridation ADN/SSO pour au moins un ensemble de microparticules identifiables de manière unique,
détermination pour lesdits un ou plusieurs ensembles de microparticules identifiables de manière unique du fait que le signal indicatif de l'hybridation ADN/SSO pour la proportion sélectionnée est ou non supérieur à un seuil sélectionné indicatif de la présence d'un ou plusieurs antigènes de typage tissulaire dans ledit échantillon biologique ; et
détermination du nombre d'ensembles de microparticules identifiables de manière unique indiquant une hybridation ADN/SSO.

6. Procédé selon la revendication 5, dans lequel le ratio numérique fixe autre qu'environ 1 pour 1 déterminera la proportion sélectionnée de l'ensemble de microparticules.

7. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel la sonde ou l'antigène de typage tissulaire est sélectionné parmi le groupe constitué par les antigènes des leucocytes humains HLA, soit Human Leukocyte Antigen, les allèles HLA, les an-

tigènes de groupe sanguin, les antigènes de type immunoglobuline de cellules tueuses naturelles KIL, soit Killer cell Immunoglobulin-Like, et les antigènes de récepteur des cellules T ou TCR, soit T-Cell Receptor.

8. Procédé selon l'une quelconque des revendications 5 à 7, comprenant en outre l'étape de rapportage d'un signal d'hybridation positive.

9. Procédé selon l'une quelconque des revendications 5 à 8, dans lequel la proportion sélectionnée d'un ensemble de microparticules est inférieure ou égale à l'inverse du nombre de sous-ensembles de microparticules présentant des SSO uniques pour ledit ensemble de microparticules.

10. Kit pour mettre en oeuvre un procédé de mesure d'une interaction de liaison comprenant :

de multiples ensembles de microparticules identifiables de manière unique, dans lequel les microparticules au sein d'un ensemble ont la même caractéristique identifiable de manière unique, et dans lequel un ou plusieurs ensembles comprennent deux ou plusieurs sous-ensembles de microparticules, chacun desdits sous-ensembles présentant au moins une sonde unique qui se lie à une molécule cible dans un échantillon biologique, dans lequel le nombre de sondes varie entre les sous-ensembles, et au moins deux des sous-ensembles de microparticules au sein d'au moins l'un des ensembles de microparticules identifiables de manière unique sont présents selon un ratio numérique fixe autre qu'environ 1 pour 1.

11. Kit selon la revendication 10, dans lequel le molécule cible est sélectionnée parmi le groupe constitué par un antigène HLA, un allèle HLA, un antigène HNA, un antigène de groupe sanguin à allèle HNA, un antigène TCR et un antigène KIL.

12. Kit selon la revendication 10 ou 11, dans lequel la sonde est sélectionnée parmi le groupe constitué par un oligonucléotide spécifique d'une séquence (SSO), une amorce, un antigène peptidique et un antigène protéique.

13. Kit selon la revendication 11 ou 12, dans lequel le kit sert à mettre en oeuvre un procédé de typage tissulaire sur base d'ADN, et dans lequel la sonde est un oligonucléotide spécifique d'une séquence (SSO) sélectionné pour s'hybrider avec un allèle d'antigène de typage tissulaire.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5595890 A, Newton **[0005]**
- US 6207379 B **[0006] [0007]**
- US 20030165925 A1 **[0008]**
- US 6514714 B1 **[0009]**
- US 20070037195 A **[0054]**

Non-patent literature cited in the description

- *Hum. Immunol.,* vol. 62 (4), 419-68 **[0003]**
- **BUGAWAN et al.** *Tissue Antigens,* 1994, vol. 44, 137-147 **[0004]**
- **PEI R et al.** Flow cytometric detection of HLA antibodies using a spectrum of microbeads. *HUMAN IMMUNOLOGY,* December 1999, vol. 60 (12), 1293-1302 **[0010]**
- **DRAGO F et al.** Genotyping of the Kidd blood group with allele-specific oligodeoxynucleotides coupled to fluorescent microspheres. *TRANSFUSION MEDICINE,* December 2005, vol. 15 (6), 499-501 **[0011]**
- **ITOH Y et al.** Four-digit allele genotyping of the HLA-A and HLA-B genes in Japanese patients with Behcet's disease by a PCR-SSOP-Luminex method. *TISSUE ANTIGENS,* May 2006, vol. 67 (5), 390-394 **[0012]**
- **XU HONGXIA et al.** Multiplexed SNP genotyping using the Qbead system: a quantum dot-encoded microsphere-based assay. *NUCLEIC ACIDS RESEARCH,* 15 April 2003, vol. 31 (8 **[0013]**
- **NAM et al.** *Science,* 2003, vol. 301, 1884-1886 **[0056]**
- **ELGHANIAN et al.** *Science,* 1997, vol. 277, 1078-1080 **[0056]**
- **TATON et al.** *Science,* 2000, vol. 289, 1757-1760 **[0056]**
- **TATON et al.** *J. Am. Chem. Soc.,* 2001, vol. 123, 5164-5165 **[0056]**
- **PARK et al.** *Science,* 2002, vol. 295, 1503-1506 **[0056]**
- **CAO et al.** *J. Am. Chem. Soc.,* 2003 **[0056]**
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. cold Springs Harbor Laboratories, 1989 **[0058]**
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, 1989 **[0064]**
- **ANDERSON et al.** Nucleic Acid Hybridisation: A Practical Approach. IRL Press **[0066]**
- **BUX et al.** *Transfus. Med.,* 1992, vol. 2 (2), 143-9 **[0078]**
- **STRONCEK.** *ASHI Quarterly,* 2004 **[0079]**

专利名称(译)	使用多组微粒和独特探针筛选结合相互作用的方法		
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申请(专利权)人(译)	一LAMBDA INC.		
当前申请(专利权)人(译)	一LAMBDA INC.		
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摘要(译)

本发明涉及使用多组微粒筛选结合相互作用的方法，其中所述组具有相同的可识别特征，并且其中一组或多组包含微粒子集，并且所述子集呈现至少一种充当结合配偶体的独特探针。对于生物样品中的靶分子。特别地，本发明提供了使用这些多组微粒检测供体组织或受体组织中的组织分型抗原的方法。

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(54) METHODS OF SCREENING FOR BINDING INTERACTION USING SETS OF MICROPARTICLES AND UNIQUE PROBES

SCREENING-VERFAHREN FÜR BINDUNGSWECHSELWIRKUNG UNTER VERWENDUNG VON MIKROPARTIKELSETS UND EINZIGARTIGEN SONDEN

PROCEDES DE CRIBLAGE D'INTERACTIONS DE LIAISONS UTILISANT DES ENSEMBLES DE MICROPARTICULES ET DES SONDES UNIQUES

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(56) References cited:
US-A1- 2006 240 411
US-A1- 2006 292 601
US-B1- 6 514 714
US-B2- 6 551 784
- PEI R ET AL: "Flow cytometric detection of HLA antibodies using a spectrum of microbeads." HUMAN IMMUNOLOGY DEC 1999 LNKD-PUBMED:10626745, vol. 60, no. 12, December 1999 (1999-12), pages 1293-1302, XP002586995 ISSN: 0198-8859
- DRAGO F ET AL: "Genotyping of the Kidd blood group with allele-specific oligodeoxynucleotides coupled to fluorescent microspheres" TRANSFUSION MEDICINE 200512 GB LNKD-DOI:10.1111/J.1365-3148.2005.00632.X, vol. 15, no. 6, December 2005 (2005-12), pages 499-501, XP002586997
- ITOH Y ET AL: "Four-digit allele genotyping of the HLA-A and HLA-B genes in Japanese patients with Behcet's disease by a PCR-SSOP-Luminex method" TISSUE ANTIGENS, vol. 67, no. 5, May 2006 (2006-05), pages 390-394, XP002586999 ISSN: 0001-2815
- XU HONGXIA ET AL: "Multiplexed SNP genotyping using the Qbead system: a quantum dot-encoded microsphere-based assay." NUCLEIC ACIDS RESEARCH 15 APR 2003 LNKD-PUBMED:12682378, vol. 31, no. 8, 15 April 2003 (2003-04-15) , page E43, XP002587000 ISSN: 1362-4962

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