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(54) **Determination of reverse ABO blood group**

Rückwärtsbestimmung von ABO-Blutgruppen

Détermination inverse de groupe sanguin ABO

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Description

Background of the Invention

[0001] This invention relates to the field of reverse blood group testing.

[0002] Blood group serology requires the determination of blood cell compatibility between a blood donor and a patient recipient before a transfusion or organ transplant involving the patient. Blood cell compatibility is determined by the non-occurrence of an immunological reaction between antibodies contained in the blood serum of a patient and antigens present on blood cells from the donor.

[0003] Many different blood group antigens are found on the surface of the red blood cells of every individual. These antigens, the products of inherited genes, exist in combinations that are likely to be unique between all individuals except identical twins. Blood grouping is generally the process of testing red cells to determine which antigens are present and which are absent, normally utilizing antibodies to the antigen tested for. Additionally, when a person does not have a particular red cell antigen on his or her red blood cells, his or her serum may contain an antibody to that antigen. Whether or not the antibody is present in the serum depends on whether the person's immune system has been previously challenged by, and responded to, that specific antigen or something very similar to it. For example, a person whose red blood cells are Type A, i.e., having "A" antigens on the red cells, will have anti-B antibodies in his or her serum. Thus, if such a person is given type B blood, an immunological reaction will occur with possible serious clinical consequences.

[0004] As an additional consideration, it should be noted that the human body is constantly exposed to antigens in pollens, food, bacteria and viruses. Some of these "natural" antigens are apparently so similar to human blood group antigens that they stimulate almost every susceptible person to produce antibodies. Thus, certain antibodies are expected in the serum of anyone whose red cells lack the reciprocal antigen. This is especially true of the ABO system. Accordingly, a second confirmatory test is often performed on the patient/donor sera. The test for expected antibodies of the ABO blood group system in sera is called "reverse" blood grouping.

[0005] Antibodies of the ABO blood grouping system are generally immunoglobulin M (IgM). These antibodies have ten antigen binding sites per molecule. The IgM antibody is large enough to span the distance between red blood cells, so that when they are centrifuged, the cells will be bound together in a lattice "cell-antibody-cell-antibody" and will remain clumped together in agglutinates. For example, if anti-A is added to blood group A or blood group AB cells and the mixture is centrifuged, the cells will remain in clumps when resuspended. With the same antibody, group 0 and group B cells will resuspend as individual cells. Agglutination caused by one antibody, such as an IgM antibody, is called direct agglutination.

[0006] In transfusion medicine, the most frequently performed test, for the reasons given above, is determination of the ABO blood group. The current state of the art is separately testing for A, B, and sometimes A+B together, antigens on the red cells (forward type); and confirmation (cross-check) testing for anti-A and anti-B antibodies in serum or plasma (reverse type). Thus a minimum of 4 separate tests, but as many as 7 separate tests (A, B, A+B antigens on the sample red cells; anti-A and anti-B in the sample serum/plasma using A_1, A_2, B, O reagent red cells) are routinely employed. The results from each of these typing exercises (forward and reverse types) have to agree. Thus, in the U.S. alone approximately 104 million tests are performed annually to determine the blood groups in blood centers.

[0007] Since the early 1900's, the general approach known as the "Landsteiner" method, (Landsteiner, Science 73: 405 (1931)) together with the work of Ashby, (J. Exp. Med. 29:267 (1919)) and Coombs (Brit. J. Exp. Pathol. 26:255 (1945)), has been to add a patient's red blood cells to a standard laboratory test tube containing a blood group antibody (such as Anti-A or Anti- B), mix to allow antibody/antigen binding reactions to take place, and then to centrifuge. If the antigen tested for is present, the antibody/antigen binding will have taken place resulting in agglutination of the patient's red blood cells. The test tube is manually shaken to dislodge the centrifuged button of "clumped" cells at the bottom. A subjective determination is then made as to whether or not the dislodged cells are "clumped", and to what extent.

[0008] During the mid-1900's, attempts were made to simplify this technique to minimize the subjective nature of the test and to reduce mistakes. It was recognized that a somewhat permanent record of the results of compatibility testing could be had by employing wettable, either non-absorbent or in some cases absorbent, test slides or test cards having the requisite immunochemical reagents on at least a portion of their surfaces. In this regard, U.S. Pat Nos. 2,770,572, 2,850,430, 3,074,853, 3,272,319, 3,424,558, 3,502,437 and 3,666,42 and European Patent Application #0 104 881-A2 depict select examples of such test cards and related apparati. The advantages of blood grouping in microplates include easier manipulation of large numbers of samples, objective measurement of agglutination reactions using instruments and computer interfacing for compilation and management of results. A number of expensive and dedicated systems with computer-controlled robotics and high throughput spectrophotometric readers have been introduced for blood bank automation (Chung, et al., Transfusion 33:384 (1992)).

[0009] WO98/21593 discloses a method for simultaneous reverse typing by flow cytometry.

[0010] Commercial blood typing kits have been introduced with improved detection of red cell antigen and antibody reactions in special microtubes filled with reagents in gel form. (Lapierre, et al., Transfusion 30:109 (1990)). Utility of

solid phase techniques to overcome the inherent problems in the use of hemagglutination as an end point for blood grouping was reviewed by Scott (Transfusion Med. Rev. 5:60 (1991)). Recently Growe et al. (Transfusion Med. Rev. 10:44 (1996)) reported the implementation and use of automated grouping of RBC antigen and serum antibody screening procedures applicable not only to blood centers but also for hospital transfusion laboratories. At least seven different wells with specific typing reagents have to be used in these methods for determining the blood group of a sample. All of the procedures used for large scale blood grouping essentially employed the agglutination based methods and also for the determination of only one antigen or antibody type in a single tube/well. Separately identifiable reactions using fluorescent labeled reagents have also been reported for blood typing applications in the U.S. Pat. Nos. 4,550,017 and 4,748,129.

[0011] Current procedures utilize agglutination of red cells as an endpoint. As discussed hereinabove, this is accomplished in test tubes, on slide surfaces, in microplates and in column agglutination tests. The latter 2 methods may be performed manually or by automated instrumentation. All methods require separation of serum (or plasma) from cells to perform both forward and reverse type.

[0012] A visual detection technology can be employed in the reverse blood typing contemplated herein. Such a method, using a column agglutination test (CAT), may employ a BioVue™ cassette (Ortho-Clinical Diagnostics, Inc., Rochester, NY). Such cassette contains columns to which has been added a microparticle matrix.

[0013] CAT can employ automated reader systems to interpret the agglutination result. One such reader is present in the Ortho AutoVue™ (Ortho-Clinical Diagnostics, Inc., Rochester, NY) a fully automated system to perform the CAT. The autoreader is a computerized imaging system consisting of a CCD (charged coupled device) monochrome video camera, and image processing board, and an IBM-compatible PC. The reader first acquires an image of the reaction that is digitised and processed by the image-processing software to extract the reaction features, which are then used by the reaction classification program. These features are used to separate reactions into negative and positive classes, and for translation into one of seven conventional reaction classes or grades. Discriminate analysis, a linear statistical pattern recognition tool, is used to distinguish between negative and weak reactions.

[0014] Yet another reader employed in CAT is the BioVue™ Reader 2 (Ortho-Clinical Diagnostics, Inc., Rochester, NY). This Reader has an automated loader for twelve BioVue™ cassettes and has a halogen lamp source and image analysis features permitting cell identification based on RBC wavelength thereby interpreting the agglutination result. Image acquisition is performed by a CCD camera and a digitising board.

Summary of the Invention

[0015] The present invention provides a method of simultaneous antibody testing of blood, as defined in claim 1.

[0016] The invention also contemplates a blood analysis kit as defined in claim 5.

[0017] Figure 1 is a schematic representation of visual detection of reverse testing of B serum in a CAT system. Labeled reagent A and B cells are admixed with B serum, resulting in brown agglutinates which, following centrifugation, are observed at the top of the gel column of the CAT system; the non-reacted labeled reagent B cells are observed at the bottom of the column.

Detailed Description of the Invention

[0018] In accordance with the present invention, reverse ABO blood group testing will be described. The invention may be used with the column agglutination test (CAT) reaction and separation vessels manufactured and sold in cassette form by Ortho-Clinical Diagnostics, Inc., Rochester, New York, under the trademark BIOUVUE™. Results can be determined using the AutoVue™ autoreader computerized imaging system, or the BioVue™ Reader 2, both systems described hereinabove.

[0019] Reagent RBCs typically used for reverse typing have on their surface either A_1, A_2, B or no ABO antigens (Type A_1, Type A_2, Type B, Type 0). These cells are useful for detecting preformed antibodies which will cause agglutination of the reagent RBCs.

[0020] The methods of the instant invention enable visual detection of reverse type blood group testing.

Visual Detection System

[0021] The ability to alter the color of a red cell would allow simultaneous testing of 2 cell populations to be performed visually or spectrophotometrically (by absorbance or reflectance), i.e., without the need to detect fluorescence. For example, red cells exposed to cyanide or azide would be expected to turn from bright red to brown. Red agglutinates could then be detected visually from brown agglutinates. While modifying the intrinsic color of the red cell hemoglobin would be contemplated by this embodiment, simply attaching or otherwise associating chromophores to the red cells could otherwise detectably change their spectral properties.

[0022] Figure 1 depicts a reverse testing using the column agglutination method whereby reagent A and B cells are separately labeled. Sample serum is delivered to the top of the column per manufacturer's instructions and the tube incubated and centrifuged in accordance therewith. Reagent A cells agglutinate with anti-A antibody present while the reagent B cells pass to the bottom of the column, indicating the sample serum was type B.

[0023] Reagent RBCs (Affirmgen® A_1 and B cells, Ortho-Clinical Diagnostics, Raritan, NJ) were treated with sodium azide (NaN_3) at a final concentration of 0.2%. The NaN_3 reduces the iron in the hemoglobin resulting in a color change from the typical red to a deep brown or maroon color. Untreated cells from the same Affirmagen lot served as controls. Untreated control and treated test cells were tested independently with group B sera in a BioVue™ Reverse cassette using manufacture's directions (10 μl 3-5% red cells and 40 μl of serum; then centrifuged 5 minutes in a BioVue centrifuge in accordance with manufacturer's instructions). The B sera is expected to agglutinate A_1 cells but not the B cells. No differences were observed in the agglutination of the untreated vs. treated cells, except that the untreated cells were red and the treated cells were brown. Refer to Example 1 and Table 1 herein.

[0024] Automated determination of agglutination results using the visual detection systems described may be accomplished by use of the AutoVue™ autoreader computerized imaging system, or the BioVue™ Reader 2, both systems described hereinabove.

[0025] The following example is provided for purposes of illustration only and not by way of limitation of the scope of the invention.

Example 1

Part A - Preparation of Colored Red Cells

[0026] Commercial preparations of Group A_1 and B red cells (Affirmagen®) were obtained from Ortho-Clinical Diagnostics, Inc. (Raritan, NJ). Two 1-mL aliquots of Group A_1 RBCs were removed from the vial and placed in a separate tube. One aliquot was admixed with 20 μl of 10% NaN_3 (Mallinckrodt, Paris, KY). The other aliquot was untreated and served as a control. Each tube was capped and the contents incubated for 16 hours at ambient temperature. After overnight treatment, the NaN_3-treated RBC appeared brown in color, while the untreated control cells remained red.

Part B - Reverse BioVue Column Testing - Dual Column Test (Not part of the invention.)

[0027] Sodium azide treated cells were tested in a standard BioVue™ Reverse Cassette using 10μl of A1 cells plus 40μl of group B sera in one column and 10μl of B cells plus 40μl of group B sera in a different column (columns 3 and 4 in Table 1). Control cells, untreated A1 and B cells, were tested in columns 1 and 2, respectively. Following centrifugation in accordance with manufacturer's instructions, agglutinated A1 cells, both untreated control cells (column 1) and treated test cells (column 3) were observed at the top of the bead column indicating the presence of anti-A in the group B sera. Unagglutinated B cells, both untreated control cells (column 2) and treated test cells (column 4) were observed at the bottom of the column indicating the absence of anti-B. The untreated control cells were red in appearance and the treated test cells appeared brown. These results indicated that the treatment of the A1 and B cells did not impair their ability to agglutinate in the bead column.

Part C - Reverse BioVue Column Testing - Single Column Test

[0028] In Part B, A1 and B cells were tested in individual columns to determine the reverse group of the sample. Next, in one column, we combined 10 μl of A1 untreated cells (red) and 10 μl of B treated cells (brown) with 40 μl of group B sera. Following centrifugation, 2 distinct cell populations were observed. Agglutinated (A1) cells were observed at the top of the bead column and unagglutinated brown (B) cells were observed at the bottom of the column (column 5 in table 1). The results indicated that the correct reverse group could be determined in 1 column (1 test) instead of the normal 2 columns. Reciprocally, 10 μl treated A_1 cells and 10 μl untreated B cells were tested in accordance with Part B herein and the results were able to detect agglutinated brown (A_1) cells and unagglutinated red (B) cells (column 6 in table 1).

[0029] The results from Parts B and C hereof are shown in Table 1. See Figure 1.

Table 1

Visual detection and discrimination of 2 distinct cell populations

	BioVue Reverse Column Numbrer					
	1*	2*	3*	4*	5	6
RBC (10 μl each)	A_1 untr	B untr	A_1 tr	B tr	A_1 untr + B tr	A_1 tr + B untr

(continued)

Visual detection and discrimination of 2 distinct cell populations

	BioVue Reverse Column Number					
	1*	2*	3*	4*	5	6
Reaction observed following centrifugation	4+	0	4+	0	3-4+ mixed	3-4+ mixed
Description	Red cells on top of bead column	Red cells at bottom of bead column	Brown cells at top of bead column	Brown cells at bottom of bead column	Red cells at top and brown cells at bottom of bead column. Few cells scattered in the column.	Brown cells at top and red cells at bottom of bead column. Few cells scattered in the column.

Note: all columns contained 40 μl of B sera in addition red cells indicated.
untr, untreated control cells tr, NaN_3 treated cells
* Not part of the invention.

Claims

1. A method of analyzing ABO reverse type in a reverse test, comprising:

(a) admixing a sample of blood with first reagent red blood cells bearing A antigen and with second reagent red blood cells bearing B antigen, wherein said admixing is performed in a single column, wherein one of said first or second reagent red blood cells are distinguishably stained;
(b) incubating the admixture under conditions sufficient for agglutination to occur;
(c) subjecting the incubated admixture in the single column to visual analysis to determine agglutination of said first and/or second reagent red blood cells or lack thereof; and
(d) analyzing the visual analysis of agglutination to determine ABO reverse type.

2. The method of claim 1 wherein the single column subjected to visual analysis is selected from the group consisting of tube and column agglutination technology.

3. The method of claim 2 wherein the column agglutination technology is a column agglutination test reaction and separation vessel in cassette form.

4. The method of claim 1 wherein the sample of blood is serum or plasma.

5. A blood analysis agglutination test kit for performing a reverse ABO blood type in a single column comprising:

(a) a container having therein a first population of reagent red blood cells bearing group A antigen and a second population of reagent red blood cells bearing B antigen, wherein one of the said first or second populations of reagent red blood cells is distinguishably stained;
(b) reaction means for carrying out the reverse ABO blood type, wherein said reaction means consists of a column agglutination reaction vessel; and
(c) instructions for performing the reverse ABO blood type in a single column, wherein the single column is subjected to visual analysis to detect and identify the antibody.

Patentansprüche

1. Verfahren zum Analysieren einer reversen ABO-Gruppe in einem reversen Test, umfassend:

(a) Vermischen einer Blutprobe mit einem ersten Reagenz roter Blutzellen, die A-Antigen tragen, und mit einem zweiten Reagenz roter Blutzellen, die B-Antigen tragen, wobei das Vermischen in einer einzelnen Säule durchgeführt wird, wobei eines von dem ersten oder zweiten Reagenz roter Blutzellen unterscheidbar gefärbt ist;
(b) Inkubieren der Mischung unter Bedingungen, die ausreichend sind, damit Agglutination stattfindet;
(c) Unterwerfen der inkubierten Mischung in der einzelnen Säule einer optischen Analyse, um Agglutination des ersten und/oder zweiten Reagenz roter Blutzellen oder Fehlen davon zu bestimmen; und
(d) Analysieren der optischen Analyse der Agglutination, um die reverse ABO-Gruppe zu bestimmen.

2. Verfahren nach Anspruch 1, wobei die einzelne Säule, die der optischen Analyse unterworfen wird, ausgewählt ist aus der Gruppe bestehend aus Röhren- und Säulen-Agglutinationstechnologie.

3. Verfahren nach Anspruch 2, wobei die Säulen-Agglutinationstechnologie ein Säulen-Agglutinationstest-Reaktions- und Trennungsgefäß in Kassettenform ist.

4. Verfahren nach Anspruch 1, wobei die Blutprobe Serum oder Plasma ist.

5. Blutanalyse-Agglutinationstest-Kit zum Durchführen einer reversen ABO-Blutgruppe in einer einzelnen Säule, umfassend:

(a) Einen Behälter, der darin einen ersten Bestand eines Reagenz roter Blutzellen, die Gruppe-A-Antigen tragen, und einen zweiten Bestand eines Reagenz roter Blutzellen, die Gruppe-B-Antigen tragen, aufweist, wobei eines von dem ersten oder zweiten Bestand der Reagenzien roter Blutzellen unterscheidbar gefärbt ist;
(b) Reaktionsmittel zum Durchführen der reversen ABO-Blutgruppe, wobei die Reaktionsmittel aus einem Säulen-Agglutination-Reaktionsgefäß bestehen; und
(c) Anweisungen zum Durchführen der reversen ABO-Blutgruppe in einer einzelnen Säule, wobei die einzelne Säule einer optischen Analyse zum Nachweisen und Identifizieren des Antikörpers unterworfen wird.

Revendications

1. Procédé d'analyse du typage inverse ABO dans un test inverse, comprenant :

(a) le mélange d'un échantillon sanguin avec des premiers érythrocytes réactifs portant l'antigène A et avec des seconds érythrocytes réactifs portant l'antigène B, dans lequel ledit mélange est effectué dans une seule colonne, l'une desdites première ou seconde populations d'érythrocytes réactifs étant colorée de manière distinctive ;
(b) l'incubation du mélange dans des conditions suffisantes pour qu'une agglutination se produise ;
(c) la soumission du mélange incubé dans l'unique colonne à une analyse visuelle pour déterminer l'agglutination desdits premiers et/ou seconds érythrocytes réactifs ou l'absence de celle-ci ; et
(d) l'analyse de l'analyse visuelle de l'agglutination pour déterminer le typage inverse ABO.

2. Procédé selon la revendication 1, dans lequel l'unique colonne soumise à l'analyse visuelle est choisie dans le groupe constitué des techniques d'agglutination en tube et colonne.

3. Procédé selon la revendication 2, dans lequel la technique d'agglutination en colonne est une réaction de test d'agglutination et de séparation en colonne sous forme de cassette.

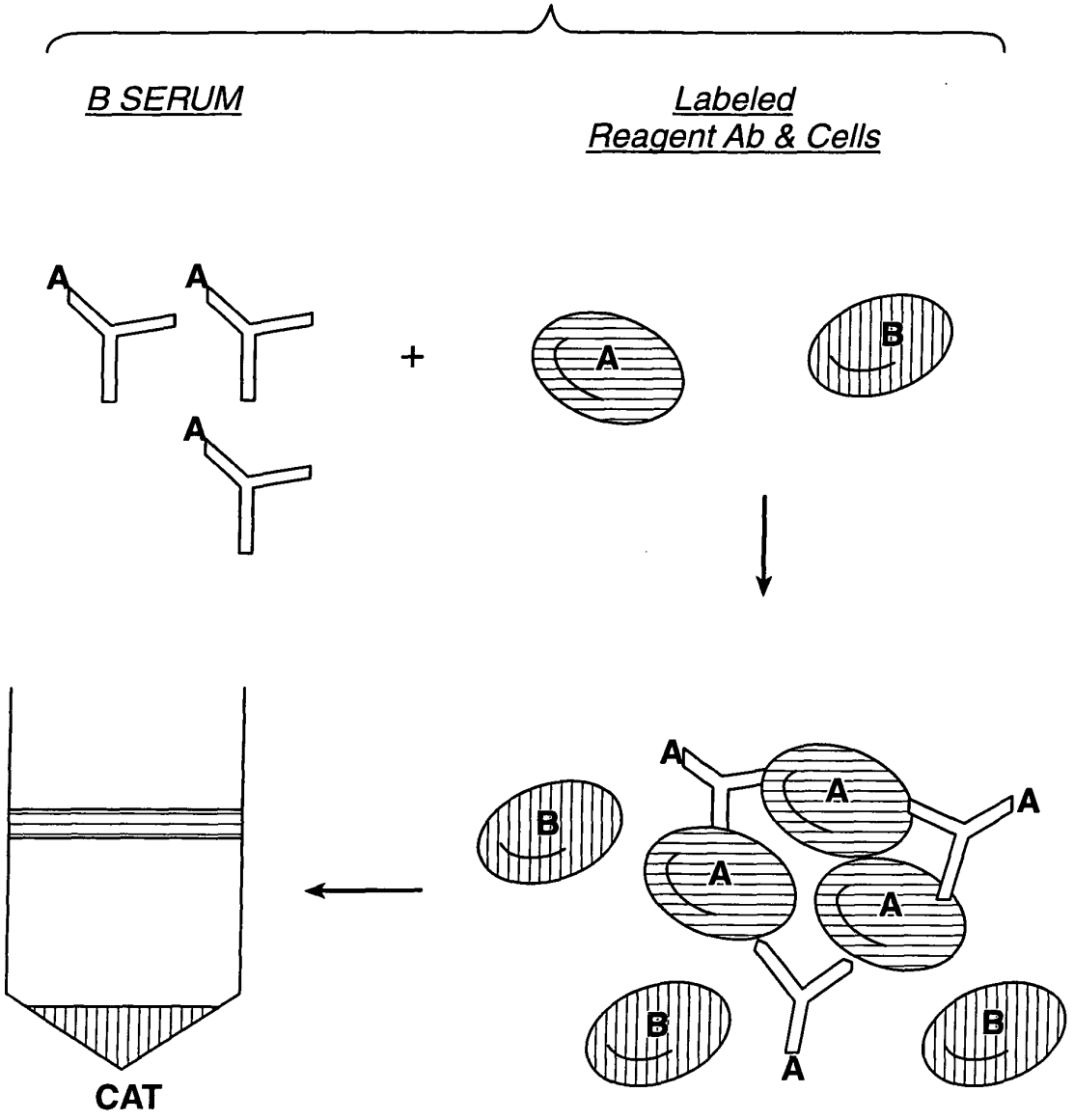
4. Procédé selon la revendication 1, dans lequel l'échantillon de sang est le sérum ou le plasma.

5. Kit de test d'agglutination pour analyse sanguine destiné à effectuer un typage du groupe sanguin ABO inverse dans une seule colonne comprenant :

(a) un récipient contenant une première population d'érythrocytes réactifs portant l'antigène du groupe A et une seconde population d'érythrocytes réactifs portant l'antigène du groupe B, dans lequel l'une desdites première ou seconde populations d'érythrocytes réactifs est colorée de manière distinctive ;
(b) un moyen de réaction pour réaliser le typage du groupe sanguin ABO inverse, dans lequel ledit moyen de réaction consiste en un réacteur d'agglutination en colonne ; et
(c) des instructions pour réaliser le typage sanguin ABO inverse dans une seule colonne, dans lequel l'unique

est soumise à une analyse visuelle pour détecter et identifier l'anticorps.

FIG. 1



REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	反向ABO血型的测定		
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摘要(译)

使用视觉检测系统和基于荧光的标记和检测系统描述了同时正向和反向血型测试。可以分别进行正向和反向测试，但可以同时检测和区分A和B凝集物。