(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 766 045 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
01.12.2010 Bulletin 2010/48

(21) Application number: **05745792.1**

(22) Date of filing: **04.05.2005**

(51) Int Cl.:
G01N 31/00 *(2006.01)* ***G01N 33/53*** *(2006.01)*

(86) International application number:
PCT/US2005/015452

(87) International publication number:
WO 2005/113795 (01.12.2005 Gazette 2005/48)

(54) **HEMATOLOGY REFERENCE CONTROL CONTAINING AN IMMATURE GRANULOCYTE COMPONENT**

HÄMATOLOGIEREFERENZKONTROLLE MIT EINER UNREIFEN GRANULOZYTENKOMPONENTE

CONTROLE DE REFERENCE HEMATOLOGIQUE CONTENANT UN COMPOSANT DE GRANULOCYTES IMMATURES

(84) Designated Contracting States:
DE FR GB

(30) Priority: **13.05.2004 US 845557**

(43) Date of publication of application:
28.03.2007 Bulletin 2007/13

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EP 1 766 045 B1

Printed by Jouve, 75001 PARIS (FR)

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a hematology reference control composition containing an immature granulocyte component and the method of making and using the hematology reference control composition for determination of immature granulocytes of a blood sample on a blood analyzer.

BACKGROUND OF THE INVENTION

[0002] The presence of immature granulocytes (IG) in peripheral blood is potentially important information which indicates enhanced bone marrow activation. Besides the obvious significance of blasts for the diagnosis of leukaemia, the promyelocyte, myelocyte and metamyelocyte stages of myeloid maturation may indicate systemic inflammatory stress or leukaemic reactions. The determination of immature granulocytes is routinely done by visual microscopy, which requires manual review of each blood sample smear, and is a labor intensive and time consuming task.

[0003] Currently, several high end hematology analyzers which utilize optical, fluorescence and impedance measurements to provide automated determination of immature granulocytes of the blood samples. U.S. Patent No. 5,958,776 (to Sysmex) teaches a lytic reagent and a method of measuring immature granulocytes using light scatter and fluorescence measurements. However, these instruments and their detection systems are expensive, and not suitable for low cost analyzers. Therefore, there is a need for automated and inexpensive determination of immature granulocytes and reduction of manual review rate.

[0004] On the other hand, quality control has long been a necessary and routine procedure in clinical hematology. Accuracy in the counting of various types of blood cells is dependent, in part, upon the use of adequate control products and methods of using the control products. With the numerous types of equipment for particle counting now available, quality control by the use of control products is necessary, since the possibility of an instrument malfunctioning is ever present. The traditional method of maintaining a quality control program for automatic particle counting equipment has consisted of providing fresh human blood as a whole blood standard. However, this fresh blood is usable for only one day, therefore, various manufactured control products which have longer product lifetime have been developed.

[0005] Commonly used particles in a control product simulate or approximate the types of particles or cells that are intended to undergo analysis. Consequently, these particles have been frequently referred to as analog particles. The analog particles should be selected or designed so that they have certain characteristics that are similar to those of the particles or cells to be analyzed in the instruments. Exemplary characteristics and parameters include similarities in size, volume, surface characteristics, granularity properties, light scattering properties and fluorescence properties.

[0006] Various commercial reference control products are now available, which use various processed or fixed human or animal blood cells as analogs of human blood cells. U.S. Patent No. 4,704,364 and US 5 380 664 A (to Carver et al) teaches a hematology control comprising three white blood cell analogs made of fixed animal red blood cells for differential analysis of white blood cells into three subpopulations using DC impedance measurement. U.S. Patent No. 5,512,485 (to Young et al) teaches a hematology control comprising several white blood cell analogs made of processed and fixed animal red blood cells for differential analysis of white blood cells into five subpopulations using light scatter, radio frequency and DC impedance measurements, commonly referred to as the VCS method.

[0007] However, currently no hematology reference control provides an immature granulocyte component, which enables quality control of the immature granulocyte measurement.

[0008] Based on the foregoing, there exists a need for a hematology reference control which comprises an immature granulocyte component for quality control of the immature granulocyte measurement.

SUMMARY OF THE INVENTION

[0009] The present invention provides a hematology reference control containing an immature granulocyte component as defined in the claims, which comprises an immature granulocyte component made of processed non-human blood cells for simulating human immature granulocytes and a suspension medium suitable for delivering the component to a blood analyzer for measurement of immature granulocytes. The immature granulocyte component is in a size range from about 2% to about 85% larger than a high end of the size range of human granulocytes when measured by a blood analyzer. The immature granulocyte component can be made of processed avian, reptile; or fish red blood cells, such as emu, ostrich, alligator, or shark red blood cells.

[0010] The hematology reference control can further include a nucleated red blood cell component. Moreover, the hematology reference control also includes a mature white blood cell component which can comprise white blood cell sub-components for simulating white blood cell subpopulations. Additionally, the hematology reference control can further include a red blood cell component, a platelet component, and a reticulocyte component.

[0011] In addition to the processed avian, reptile, or fish red blood cells, processed human immature granulocytes can also be used as the immature granulocyte component in the reference control. The human immature granulocytes can be grown in vitro by a cell line.

[0012] In another embodiment, the present invention provides a hematology reference control containing an immature granulocyte component, which comprises a first processed red blood cell from a first species as a lymphoid component; a second processed red blood cell from a second species as a myeloid component; a third processed red blood cell from a third species as an immature granulocyte component; and a suspension medium suitable for delivering the components to a blood analyzer for measurement of immature granulocytes as defined in the claims.

[0013] In a yet further embodiment, the present invention is directed to a method of using the hematology reference control containing an immature granulocyte component as defined in claim 13. The method includes the steps of providing a reference control containing an immature granulocyte component; providing a blood analyzer adapted for analyzing immature granulocytes; passing the reference control through the blood analyzer for detection of the immature granulocyte component; and reporting the immature granulocyte component in the reference control. The measurement of immature granulocytes can be performed using impedance measurement, and optical measurement including light scatter measurement and axial light loss measurement.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Fig. 1 shows a DC histogram of a normal whole blood sample analyzed according to the procedure described in Example 4.

[0015] Figs. 2 and 2A show DC histograms of two clinical samples containing immature granulocytes analyzed according to the procedure described in Example 4.

[0016] Fig. 3 shows a DC histogram of the reference control composition A of Example 3, which contained only white blood cell sub-components.

[0017] Fig. 4 shows a DC histogram of the reference control composition B of Example 3, which contained white blood cell sub-components and an immature granulocyte component made of emu red blood cells.

[0018] Fig. 5 shows a DC histogram of a normal whole blood sample analyzed according to the procedure described in Example 4, and measured with a larger dynamic range.

[0019] Fig. 6 shows a DC histogram of a clinical blood sample analyzed according to the procedure described in Example 4, and measured with the same dynamic range used in Fig. 5.

[0020] Fig. 7 shows a DC histogram of the reference control composition C of Example 3, which contained white blood cell sub-components and an immature granulocyte component made of alligator red blood cells, measured with the same dynamic range used in Fig. 5.

DETAILED DESCRIPTION OF THE INVENTION

[0021] In one embodiment, the present invention provides a hematology reference control composition that contains an immature granulocyte component as defined in the claims. More specifically, the reference control composition comprises an immature granulocyte component made of processed non-human blood cells for simulating human immature granulocytes, and a suspension medium suitable for delivering the component to a blood analyzer for measurement of immature granulocytes.

The immature granulocytes referred herein include myelocytes, promyelocytes, metamyelocytes, myeloblasts and promyeloblasts. For the purpose of the present invention, the immature granulocyte component, as well as other cell type components, are also referred to as analogs, for example, immature granulocyte (IG) analog.

[0022] Suitable examples of non-human blood cells suitable for simulating human immature granulocytes include various animal red blood cells including, but not limited to, avian, reptile and fish red blood cells. More specifically, emu, ostrich, alligator, and shark red blood cells can be used. In general, immature granulocytes are larger in size than mature white blood cells, more particularly, their average cell volume is larger than that of granulocytes. The analog should simulate the properties of human immature granulocytes under a sample preparation condition and by the measurement method used for the white blood cell analysis. In general, the immature granulocyte analog is in a size range from 2-85% larger than the high end of the size range of human granulocytes when measured by a blood analyzer.

[0023] In a further embodiment, the immature granulocyte component can be made of fixed human immature granulocytes which are grown in vitro by a immature granulocyte cell line.

[0024] In one embodiment, emu red blood cells were used for making the immature granulocyte analog. To prepare the immature granulocyte analog, the emu red blood cells in a quality of emu whole blood is separated first from other blood components including white blood cells, platelets and plasma by centrifugation. The emu red blood cells are washed by an isotonic wash solution. The washed emu red blood cells are then processed by a processing medium, by incubating the cells in the processing medium with slow mixing for a period of time, preferably from about 8 to about 28

hours. The processed emu red blood cells are washed again and suspended in a suspension medium for storage and use on a blood analyzer.

[0025] One suitable wash solution is the phosphate buffered saline solution (PBS). Other wash solution known to those skilled in the art can also be used. The processing medium comprises a fixative and an osmolarity adjustment agent for providing appropriate osmolarity of the processing medium, depending on the source of the blood cells and required property of the analog. Suitable examples of the osmolarity adjustment agents include, but are not limited to, alkaline metal phosphate, alkaline metal chloride and alkaline metal sulfate. Suitable examples of the fixative include, but not limited to, formaldehyde, glutaraldehyde and paraformaldehyde. The concentration of the fixative is in a range from about 0.5% to about 1.5%.

[0026] One suitable example of the suspension medium includes phosphate buffered saline solution and an aqueous solution of a plasma substance. As defined herein, an aqueous solution of a plasma substance comprises an aqueous solution of a serum substance, serum substance in combination with a plasma protein and mixtures thereof. As further defined herein, plasma protein comprises one or more of the proteins contained in plasma. Preferably, such plasma proteins comprise albumin, lipoproteins, globulins, fibrinogens and mixtures thereof. These media may contain other ingredients known to those skilled in the art to confer long term stability. Other examples of suitable medium are more fully described in U.S. Pat. Nos. 4,213,876, 4,299,726, 4,358,394, 3,873,467, 4,704,364, 5,320,964, 5,512,485 and 6,569,682.

[0027] Example 1 illustrates an exemplary process of preparing an immature granulocyte analog using emu red blood cells. Example 2 illustrates an exemplary process of preparing an immature granulocyte analog using alligator red blood cells. The immature granulocyte analogs made of emu red blood cells and alligator red blood cells have different sizes, which can be used for the measurement methods having different detection dynamic ranges.

[0028] In an alternative embodiment, the immature granulocyte analog can also be made of more than one type of processed blood cells. Immature granulocytes in the clinical samples have a broad size distribution, and they can extend from the high end of granulocytes to a size about twice that of the granulocytes. To simulate such a broad size distribution, a mixture of two different processed blood cells which have an overlap in size distribution can be used.

[0029] In a further embodiment, the hematology reference control composition further comprises a mature white blood cell component for measurement of white blood cells (WBC) and immature granulocytes. Moreover, the mature white blood cell component can further comprise sub-components for simulating subpopulations of white blood cells, such as lymphoid cells, myeloid cells (the sum of monocytes and granulocytes), or further into lymphocytes, monocytes, neutrophils, eosinophils and basophils, which can be utilized as a reference control for differential analysis of white blood cells.

[0030] Suitable examples of white blood cell analogs include stabilized and fixed mammalian white blood cells, and processed and/or fixed human and animal red blood cells, as known in the art. In one embodiment, the white blood cell analogs can be made from processed avian and human red blood cells for differential analysis using an impedance measurement, as taught in U.S. Patent No. 4,704,364. In a further embodiment, the white blood cell analogs can be made from fixed mammalian white blood cells. The mammalian white blood cells are fixed prior to lysing the red blood cells in the whole blood during the preparation of the white blood cell analogs. In another embodiment, the white blood cell analogs can be made from processed goose and alligator red blood cells for differential analysis using a combination of impedance and light scatter measurement, as taught in U.S. Patent Nos. 5,320,964 and 5,512,485.

[0031] Optionally, the mammalian white blood cells and the human and animal red blood cells can be further processed by contacting with a lipoprotein during the process of preparing the white blood cell analogs. The contact with lipoprotein can occur prior to fixing the white or red blood cells, it can also occur after fixing and during storage in the suspension medium, as taught in U.S. Patent Nos. 5,320,964, 5,512,485, 6,406,915, 6,403,377, 6,399,388, 6,221,668, and 6,200,500.

[0032] Example 3 illustrates an exemplary process of preparing reference control compositions which contained white blood cell sub-components and an immature granulocyte component. Three reference control compositions, A, B and C were prepared. The reference control composition A contained 30% lymphoid analog made of processed human red blood cells and 70% myeloid analog made of processed goose red blood cells. The processes of making lymphoid and myeloid analogs are described in detail in Example 3. The reference control composition B contained 30% lymphoid analog made of processed human red blood cells, 49% myeloid analog made of processed goose red blood cells, and 21% immature granulocyte analog made of processed emu red blood cells of Example 1. The reference control composition C contained 25% lymphoid analog made of processed human red blood cells, 58% myeloid analog made of processed emu red blood cells of Example 1, and 17% immature granulocyte analog made of processed alligator red blood cells of Example 2.

[0033] It is noted that the analog made of processed emu red blood cells was used for two different purposes, one as an immature granulocyte analog in reference control composition B, and the other as a myeloid analog in reference control composition C. Reference control compositions B and C have different cell size ranges, and can be used for the measurement having different dynamic ranges. The former can be used for the measurement using a smaller dynamic range, which has a higher resolution for the blood cells measured, more suitable for a concurrent measurement of measurement of immature granulocytes and nucleated red blood cells. The latter can be used for the measurement

having a larger dynamic range, which allows the measurement of extremely large immature granulocytes.

[0034] These reference control compositions were utilized for measurement of white blood cells and immature granulocytes using a DC impedance measurement as shown in Example 4. The measurement method and instrumentation used for the measurement were described in co-pending patent application Serial No. 10/770,193, filed on February 2, 2004, entitled "Method for Measurement of Immature Granulocytes".

Moreover, the instrumentation and the reagents used are described in detail in Example 4.

[0035] Fig. 1 shows a DC histogram of a normal whole blood sample analyzed on the experimental hematology analyzer. For a normal blood sample, the white blood cells had a bi-modal distribution, with the lymphoid subpopulation on the left and the myeloid subpopulation on the right. No cell population located on the right side of the myeloid subpopulations. Fig. 2 shows the DC histogram of a clinical sample containing about 12% of immature granulocytes (IG), including metamyelocytes, myelocytes and promyelocytes. As shown, immature granulocytes showed on the right side of the myeloid subpopulation. Fig. 2A shows the DC histogram of another clinical sample containing about 6% of immature granulocytes including metamyelocytes and myelocytes, which were indicated by the large cells extending into the right-most region of the histogram. This sample also contained 5 NRBC per 100 WBC, which located on the left side of the lymphoid population.

[0036] Figs. 3 and 4 show DC histograms of reference control compositions A and B analyzed on the same instrument. As shown, the histogram of the reference control composition A resembles the cell distribution of the normal blood sample, and the histogram of the reference control composition B resembles the cell distribution of the clinical sample containing immature granulocytes. Using the reference control, one can determine the presence of immature granulocytes, hence, providing a quality assurance for the instrument and the detection method.

[0037] Figs. 5 and 6 show DC histograms of a normal whole blood sample and a clinical sample containing immature granulocytes analyzed on the experimental hematology analyzer, but with a larger dynamic range of the measurement. With the larger dynamic range, normal white blood cells distributed in approximately only half of the histogram. The manual reference reported the clinical sample having about 24% of immature granulocytes (IG), including metamyelocytes, myelocytes and promyelocytes. As shown, some of the immature granulocytes were very large and had a cell size close to double of the granulocytes which constitute the majority of the myeloid population.

[0038] Fig. 7 shows the DC histogram of the reference control composition C analyzed on the experimental hematology analyzer with the same dynamic range shown in Fig. 5. As shown, the histogram of the reference control composition C resembles the cell distribution of the clinical sample containing immature granulocytes measured under the same condition.

[0039] It should be understood that although the reference control containing an immature granulocyte component as described above is analyzed by a DC impedance measurement, it can also be used for radio frequency (RF) impedance measurement, and optical measurement. It is known that the forward light scatter or low angle light scatter measurement reflects the size of cells. Furthermore, axial light loss measurement, which measures the light loss due to absorption and scattering of a cell passing through a light beam, also reflects the size of cells. Therefore, the immature granulocyte analog prepared using the method of the present invention can also be used with these measurement methods.

[0040] In another embodiment, the reference control containing an immature granulocyte component and sub-components of white blood cells, as described previously, can also be used for differential analysis of white blood cells into two, three or five subpopulations.

[0041] In a yet further embodiment, the reference control composition can further comprise a nucleated red blood cell component for simulating nucleated red blood cells of a blood sample on a blood analyzer. Nucleated red blood cells are immature red blood cells present in clinical samples due to certain clinical conditions, which are usually detected together with white blood cells, since both are nucleated cells. The nucleated red blood cells are reported either as the numbers of NRBC per 100 WBC, or absolute concentration. The methods of making a nucleated red blood cell analog have been described in the co-pending patent applications Serial No. 10/689,245 and 60/560,236.

[0042] Example 5 illustrates a preparation of a reference control composition containing an immature granulocyte component, a white blood cell component that includes two sub-components as described above, and a nucleated red blood cell component. This reference control can be used for measurement of white blood cells, immature granulocytes and nucleated red blood cells using a DC impedance measurement.

[0043] Depending on the reaction condition and detection method, the reference control composition containing both an immature granulocyte component and a nucleated red blood cell component can be either used for a simultaneous measurement of both immature granulocytes and nucleated red blood cells, or for two separate measurements, one for each cell population. The latter is commonly utilized on automated hematology analyzers, which aspirate a blood sample and segment it into several aliquots for separate sample preparations and analyses by the analyzer.

[0044] In another embodiment, the reference control composition further comprises a red blood cell component and a platelet component in the suspension medium. The red blood cell component can be stabilized human or animal red blood cells, preferably, stabilized human red blood cells. The process of making red blood cell component has been described in details in U.S. Patent Nos. 4,299,726 and 4,358,394. The platelet component can be stabilized human or

animal platelets, or platelet analogs made from other cell types. One suitable example is processed goat red blood cells as the platelet analog, as disclosed in U.S. Patent Nos. 4,264,470, 4,389,490 and 4,405,719.

[0045] The red blood cells of a blood sample or the stabilized human red blood cells in the reference control composition are lysed under lysing conditions normally used for preparing a blood sample for the measurement of white blood cells, and should not be detected in the measurement if the analyzer operates properly. The platelets of a blood sample under the lysing conditions are reduced in size and they are either below the detection threshold for the measurement of white blood cells or nucleated red blood cells, or are separated from the nucleated blood cells. The platelet analog described above simulates the response of the platelets of a blood sample under the lysing condition. Therefore, the red blood cell component and platelet component in the reference control composition further reflect the response of the control composition to the lysing reagent, as well as the reaction conditions on the instrument. Hence, the hematology reference control composition containing red blood cell and platelet components can provide further information related to instrument operating conditions.

[0046] Furthermore, the reference control composition can further comprise a reticulocyte component for simulating reticulocytes of a blood sample on a blood analyzer. Moreover, the hematology reference control composition containing red blood cell component, platelet component and reticulocyte component can also be used for the red blood cell and platelet measurements, which are commonly performed together with the measurement of the white blood cells on an automated hematology analyzer.

[0047] Example 6 illustrates an exemplary process of preparing a reference control composition containing an immature granulocyte component, a white blood cell component that includes two sub-components, i.e., a lymphoid component and a myeloid component, a red blood cell component and a platelet component.

[0048] The following examples are illustrative of the invention and are in no way to be interpreted as limiting the scope of the invention, as defined in the claims. It will be understood that various other ingredients and proportions may be employed, in accordance with the proceeding disclosure.

Example 1

[0049] Immature Granulocyte Component Made of Emu Red Blood Cells

Phosphate Buffered Saline Solution (PBS)

Sodium dihydrogenphosphate:	0.2g
Disodium hydrogenphosphate $7H_2O$:	2.0g
Sodium azide:	0.1g
Sodium chloride:	9.4g
Qs to 1 liter with distilled water:	pH approximately 7.4 osmolarity 315 to 345 mOsm/kg H_2O

Analog Processing Medium 1

Disodium hydrogenphosphate $7H_2O$:	2.0 g
Sodium dihydrogenphosphate:	0.2 g
Sodium chloride	24.5 g
Glutaraldehyde (25%)	40 ml
Qs to 1 liter with distilled water:	pH approximately 7.4 osmolarity 900 mOsm/kg H_2O

Suspension Medium 1

Component	Range (g/liter)	Preferred (g/liter)
Xanthine compound	1-10	2-7
Adenosine monophosphate	0.1-1.0	0.2-0.8
Inosine	0.1-1.0	0.2-0.8
pH adjusting agents sufficient to obtain	pH 5.8-6.8	pH 6.0-6.5

(continued)

Component	Range (g/liter)	Preferred (g/liter)
Osmolarity adjusters sufficient to obtain	200-400 mOsm	250-350
Preservative	effective amount	2.0-6.0
Qs to 1 liter with distilled water		

Suspension Medium 2

	Preferred (g or ml/liter)
Propyl paraben	0.3 to 1.0 g
Methyl paraben	0.5 to 1.0 g
Procaine hydrochloride	0.1 to 0.5 g
Deoxycholic acid	0.1 to 0.9 g
Lactose	10.0 to 50.0 g
Actidione	0.1 to 0.6 g
Trisodium citrate dehydrate	3.0 to 8.0 g
Citric acid monohydrate	0.3 to 0.9 g
Sodium dihydrogenphosphate monohydrate	0.8 to 2.5 mg
Phenergan hydrochloride	0.1 to 1.0 g
Colistimethate, sodium	0.2 to 0.9 g
Penicillin G., sodium	0.5×10^6 to 3×10^6 units
Kanamycin sulfate	0.2 to 0.8 g
Neomycin sulfate	0.2 to 1.0 g
5'-AMP	0.4 to 1.0 g
Adenine	0.2 to 0.8 g
Inosine	0.4 to 1.0 g
Dihydrostreptomycin sulfate	0.2 to 1.0 g
Tetracycline hydrochloride	0.2 to 1.0 g
30% Bovine albumin	100 to 350 ml
Qs to 1 liter with distilled water	

Process steps for preparing immature granulocyte analog using emu red blood cells:

[0050] 1. 50 ml of emu whole blood was collected in an anticoagulant containing container. The emu whole blood was centrifuged and the top layer (including white blood cells, platelets and plasma) was removed.

[0051] 2. The packed emu red blood cells was washed three times with the phosphate buffered saline solution (PBS), and the washed packed cells was re-suspend in the residual PBS. The cell washing steps were a series of centrifugations (1000 rpm/5 minutes), followed by removal of the supernatant and re-suspension of the packed cells with PBS.

[0052] 3. 1 ml of packed emu red blood cells was added into a test tube containing 49 ml of the Analog Processing Medium 1, and mixed by inversion to form a cell processing suspension.

[0053] 4. The test tube was placed on a roller and mixed at a slow speed overnight at room temperature.

[0054] 5. The processed cells were then washed three times with PBS, as described in step (1).

[0055] 6. The processed cells was re-suspended in the Suspension Medium 1 or 2 to form an immature granulocyte reference control for analysis on a blood analyzer.

Example 2

Immature Granulocyte Component Made of Alligator Red Blood Cells

[0056] An amount of alligator whole blood was collected and processed with the same process steps described in

Example 1, except that in step 3 the Analog Processing Medium 2 shown below was used. The processed alligator red blood cells were resuspended in Suspension Medium 1 to form another immature granulocyte reference control.

Analog Processing Medium 2

Sodium chloride	31.7 g
Glutaraldehyde (25%)	40 ml
Qs to 1 liter with distilled water:	pH approximately 6.5
	osmolarity 1000 mOsm/kg H_2O

[0057] As shown in Example 3 hereinafter, the immature granulocyte analog made of alligator red blood cells are larger in size than that made of emu red blood cells, and can be used to simulate human immature granulocytes in a larger dynamic range of the measurement.

Example 3

[0058] Hematology Reference Control Composition Containing an Immature Granulocyte Component and White Blood Cell Sub-Components

1. Lymphoid analog

[0059] The lymphoid analog was made of fixed human red blood cells. The process has been described in detail in U.S. Patent No. 4,704,364.

2. Myeloid analog

[0060] The myeloid analog was prepared by processing goose red blood cells using the process described in Example 1, except that in step 3 the Analog Processing Medium 3 shown below was used.

Analog Processing Medium 3

Disodium hydrogenphosphate $7H_2O$:	2.0 g
Sodium dihydrogenphosphate:	0.2 g
Sodium chloride	16.5 g
Glutaraldehyde (25%)	40 ml
Qs to 1 liter with distilled water:	pH approximately 7.4
	osmolarity 550 mOsm/kg H_2O

3. Prepare the reference control composition

Procedure:

[0061] 1. Provide a predetermined volume of the Suspension Medium 1 described in Example 1.
[0062] 2. Add predetermined amounts of lymphoid and myeloid analogs in the suspension medium.
[0063] 3. Add a predetermined amount of immature granulocyte analog in the suspension medium.
[0064] The proportion of the three analogs resembles the white blood subpopulations and immature granulocytes in normal and abnormal human whole blood. As a reference control resembling a normal human blood, the control composition does not contain immature granulocyte analog. As a reference control resembling a clinical sample containing immature granulocytes, the control composition can contain a certain amount of immature granulocyte analog, for example, about 10% to 50% of the total white blood cells.
[0065] Following reference control compositions were made using the process described above:

(1) Reference control composition A, containing:

[0066] 30% lymphoid analog made of processed human red blood cells of Example 3; and
70% myeloid analog made of processed goose red blood cells of Example 3.

(2) Reference control composition B, containing:

[0067] 30% lymphoid analog made of processed human red blood cells of Example 3;
49% myeloid analog made of processed goose red blood cells of Example 3; and
21% immature granulocyte analog made of processed emu red blood cells of Example 1.

(3) Reference control composition C, containing:

[0068] 25% lymphoid analog made of processed human red blood cells of Example 3;
58% myeloid analog made of processed emu red blood cells of Example 1; and
17% immature granulocyte analog made of processed alligator red blood cells of Example 2.

[0069] Reference control compositions B and C have different size ranges, and can be used for the measurements having different dynamic ranges, as described hereinafter in Example 4. As shown in the compositions, the analog made of processed emu red blood cells can be used as an immature granulocyte analog, as in reference control composition B, and can also be used as a myeloid analog, as in reference control composition C.

Example 4

[0070] Use of the Hematology Reference Control on a Hematology Analyzer for Measurement of Immature Granulocytes Using a DC Impedance Measurement

[0071] The reference control compositions described in Example 3 were analyzed on an experimental hematology analyzer. In the analysis, an aliquot of 28 l of the reference control or a blood sample was diluted with 6 ml of Coulter LH Series Diluent (Beckman Coulter, Inc., Miami, Florida) in a WBC bath, then mixed with 1 ml of a lytic reagent composition to lyse red blood cells. The lytic reagent contained 25.0 g/L of tetradecyltrimethylammonium bromide, 15.0 g/L of Igepal SS-837 (an ethoxylated phenol from RhÔne-Poulenc), 4.0 g/L of Plurofac A38 prill surfactant (from BASF Corp.), and had a pH of 6.2. The experimental hematology analyzer was a modified LH750 (product of Beckman Coulter, Inc., Miami, Florida), which was equipped with non-focused apertures of a length of 100 and a width of 80 for measuring the prepared sample mixture as described above. The sample mixture was drawn through a set of three apertures (arranged in parallel) by a constant vacuum. The white blood cells were counted by a DC impedance measurement, and a histogram of the blood cells, after pulse editing, was also produced (averaged from the measurements of three apertures).

[0072] Fig. 1 shows a DC histogram of a normal whole blood sample analyzed on the experimental hematology analyzer as described above. As shown for a normal blood sample, the white blood cells had a bi-module distribution, with the lymphoid subpopulation on the left and the myeloid subpopulation on the right. No cell population located on the right side of the myeloid subpopulation. Fig. 2 shows a DC histogram of a clinical sample containing immature granulocytes analyzed according to the procedure described above. The manual reference reported about 12% of immature granulocytes (IG), including metamyelocytes, myelocytes and promyelocytes, which showed on the right side of the myeloid subpopulation. This sample had only 7% lymphocytes, and the majority of the white blood cells were myeloid population. Fig. 2A shows the DC histogram of another clinical sample containing about 6% of immature granulocytes including metamyelocytes and myelocytes, which were indicated by the large cells extending into the rightmost region of the histogram. The manual reference also reported 5 NRBC per 100 WBC in this sample. As shown, NRBCs located on the left side of the lymphoid population, which was differentiated from the white blood cells.

[0073] Figs. 3 and 4 show DC histograms of reference control compositions A and B analyzed using the same process as described above on the same instrument. As shown, the histogram of the reference control composition A resembles the cell distribution of the normal blood sample, and the histogram of the reference control composition B resembles the cell distribution of the clinical sample containing immature granulocytes.

[0074] Figs. 5 and 6 show DC histograms of a normal whole blood sample and a clinical sample containing immature granulocytes analyzed on the same experimental hematology analyzer as described above, but with a larger dynamic range of the measurement. With the larger dynamic range, normal white blood cells distributed in approximately only half of the histogram. As shown, some of the immature granulocytes in this example were very large, and extended to the extreme right of the histogram.

[0075] Fig. 7 shows the DC histogram of reference control composition C analyzed on the experimental hematology analyzer with the same dynamic range shown in Fig. 5, which resembles the cell distribution of the clinical sample containing immature granulocytes.

Example 5

[0076] Hematology Reference Control Composition Containing an Immature Granulocyte Component and Nucleated

Red Blood Cell Component

1. NRBC analog

[0077] 1. Select alligator whole blood having a red blood cell mean cell volume range from about 380 to about 460 fl. Centrifuge the alligator whole blood and remove the top layer (including white blood cells, platelets and plasma).
[0078] 2. Wash the packed alligator red blood cells three times with the phosphate buffered saline solution (PBS).
[0079] 3. Re-suspend the washed alligator red blood cells in one of the above-described suspension media. Preferably, the cell count is in a range from about 0.4 $x10^6$ to about 0.6 $x10^6$. The stabilized alligator red blood cells can be stored for a time period in excess of 45 days.

2. Prepare the reference control composition

Procedure:

[0080] 1. Provide a predetermined volume of the Suspension Medium 1 described in Example 1.
[0081] 2. Add predetermined amounts of lymphoid and myeloid analogs of Example 3 in the suspension medium.
[0082] 3. Add a predetermined amount of immature granulocyte analog of Example 1 in the suspension medium.
[0083] 4. Add a predetermined amount of the NRBC analog in the suspension medium.
[0084] The cell concentrations of the white blood cell sub-components are prepared to simulate the corresponding cell concentrations of a human whole blood sample. The cell concentration of the immature granulocyte component is prepared to simulate a clinical sample containing a certain level of immature granulocytes, preferably in a range of 5% to 50% of total white blood cells. The cell concentration of the NRBC component is prepared to simulate a clinical sample containing a certain level of NRBCs, preferably in a range of 2 to 50 NRBC per 100 WBC.
[0085] This reference control composition can be used for the method of measuring immature granulocytes using a DC impedance measurement as described in Example 4, and can also be used for simultaneously measuring NRBC as described in co-pending patent application Serial No. 10/770,193.

Example 6

[0086] Hematology Reference Control Composition Containing an Immature Granulocyte Component, Mature White Blood Cell Sub-components, and Red Blood Cell and Platelet Components

Procedure:

[0087] 1. Provide a predetermined volume of the first suspension medium described in Example 1.
[0088] 2. Add a predetermined amount of stabilized human red blood cells in the medium. The stabilized human red blood cells were processed following the procedure described in U.S. Patent Nos. 4,299,726 and 4,358,394.
[0089] 3. Add a predetermined amount of platelet analog in the suspension medium containing the stabilized human red blood cells. The platelet analog is made of fixed goat red blood cells following the procedure described in U.S. Patent Nos. 4,264,470, 4,389,490 and 4,405,719.
[0090] 4. Add predetermined amounts of lymphoid and myeloid analogs into the suspension medium containing the stabilized human red blood cells and platelet analog.
[0091] 5. Add a predetermined amount of immature granulocyte analog into the suspension medium containing the stabilized human red blood cells, platelet analog and white blood cell component to form a reference control composition.
[0092] 6. Mixing the reference control composition. The cell concentrations of the red blood cell, white blood cell and platelet components are prepared to simulate the corresponding cell concentrations of a human whole blood sample. The cell concentration of the immature granulocyte component is prepared to simulate a clinical sample containing a certain level of immature granulocytes, preferably in a range of 5% to 50% of total white blood cells.
[0093] While the present invention has been described in detail and pictorially shown in the accompanying drawings, these should not be construed as limitations on the scope of the present invention, but rather as an exemplification of preferred embodiments thereof.

Claims

1. A hematology reference control containing an immature granulocyte component, the reference control comprising:

(a) a mature white blood cell component simulating human mature white blood cells on a blood analyzer;
(b) an immature granulocyte component for simulating human immature granulocytes; and
(c) a suspension medium suitable for delivering said components to said blood analyzer for measurement of immature granulocytes;
wherein said immature granulocyte component is in a size from 2% to about 85% larger than a high end of a size range of human granulocytes when measured by a blood analyzer, wherein the immature granulocyte component is made of processed non-human blood cells or processed human immature granulocytes.

2. The hematology reference control of claim 1, wherein the immature granulocyte component is made of processed non-human blood cells.

3. The hematology reference control of claim 1, further comprising:

(d) a first processed red blood cell from a first species as a lymphoid component; and
(e) a second processed red blood cell from a second species as a myeloid component;
wherein the immature granulocyte component is a third processed red blood cell from a third species.

4. The hematology reference control according to claim 1 or claim 2, wherein said immature granulocyte component is made of avian, reptile, or fish red blood cells, preferably of emu, ostrich, alligator or shark red blood cells.

5. The hematology reference control according to any one of claims 1 to 4, further comprising a red blood cell component and a platelet component.

6. The hematology reference control according to any one of claims 1 to 5, further comprising a nucleated red blood cell component.

7. The hematology reference control according to any one of claims 1, 2 and 4 to 6, wherein said immature granulocyte component is a mixture of said red blood cells of two different species.

8. The hematology reference control according to any one of claims 1 and 3 to 7, wherein said immature granulocyte component is made of processed human immature granulocytes grown *in vitro.*

9. The hematology reference control according to any one of claims 1 and 4 to 8, further comprising a reticulocyte component.

10. The hematology reference control according to any one of claims 3, 5, 6 and 8, wherein said first processed red blood cell from a first species is a processed human red blood cell.

11. The hematology reference control according to any one of claims 3, 5, 6, 8 and 10, wherein said second processed red blood cell from a second species is a processed avian red blood cell, preferably a processed goose or emu red blood cell.

12. The hematology reference control according to any one of claims 3, 5, 6, 8, 10 and 11, wherein said third processed red blood cell from a third species is a processed avian, reptile or fish red blood cell, preferably a processed emu, ostrich, alligator or shark red blood cell.

13. A method of using a hematology reference control containing a mature white blood cell component and an immature granulocyte component, the method comprising the steps of:

(a) providing a reference control containing a mature white blood cell component and an immature granulocyte component;
(b) providing a blood analyzer adapted for analyzing immature granulocytes;
(c) passing said reference control through said blood analyzer for detection of said immature granulocyte component; and
(d) reporting said immature granulocyte component in said reference control; wherein said immature granulocyte component is in a size from 2% to about 85% larger than a high end of a size range of human granulocytes when measured by a blood analyzer, wherein the immature granulocyte component is made of processed non-human blood cells or processed human immature granulocytes.

14. The method according to claim 13, wherein in step (b) said analyzing immature granulocytes is performed by an impedance measurement, or by an optical measurement.

15. The method according to claim 14, wherein said optical measurement is a light scatter measurement, or axial light loss measurement.

Patentansprüche

1. Hämatologie-Vergleichskontrolle, enthaltend einen unreife Granulozyten-Bestandteil, wobei die Vergleichskontrolle umfaßt:

   (a) einen reife weiße Blutzellen-Bestandteil, der humane reife weiße Blutzellen in einem Blutanalysegerät simuliert,
   (b) einen unreife Granulozyten-Bestandteil für das Simulieren von humanen unreifen Granulozyten, und
   (c) ein Suspensionsmedium, das geeignet ist, die Bestandteile an das Blutanalysegerät für die Messung von unreifen Granulozyten zu leiten, wobei der unreife Granulozyten-Bestandteil eine Größe von 2% bis etwa 85% größer als das hohe Ende eines Größenbereichs humaner Granulozyten, wenn diese von einem Blutanalysegerät gemessen werden, aufweist, wobei der unreife Granulozyten-Bestandteil aus verarbeiteten nicht-humanen Blutzellen oder verarbeiteten humanen unreifen Granulozyten hergestellt ist.

2. Hämatologie-Vergleichskontrolle nach Anspruch 1, wobei der unreife Granulozyten-Bestandteil aus verarbeiteten nicht-humanen Blutzellen hergestellt ist.

3. Hämatologie-Vergleichskontrolle nach Anspruch 1, weiter umfassend:

   (d) ein erstes verarbeitetes rotes Blutkörperchen aus einer ersten Spezies als einen lymphoiden Bestandteil, und
   (e) ein zweites verarbeitetes rotes Blutkörperchen aus einer zweiten Spezies als einen myeloiden Bestandteil, wobei der unreife Granulozyten-Bestandteil ein drittes verarbeitetes rotes Blutkörperchen aus einer dritten Spezies ist.

4. Hämatologie-Vergleichskontrolle nach Anspruch 1 oder Anspruch 2, wobei der unreife Granulozyten-Bestandteil aus roten Blutkörperchen von Vögeln, Reptilien oder Fischen hergestellt ist, vorzugsweise aus roten Blutkörperchen von Emu, Strauß, Alligator oder Hai.

5. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 1 bis 4, weiter umfassend einen rote Blutkörperchen-Bestandteil und einen Blutplättchen-Bestandteil.

6. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 1 bis 5, weiter umfassend eine Erythroblasten (*nucleated red blood cell*)-Bestandteil.

7. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 1, 2 und 4 bis 6, wobei der unreife Granulozyten-Bestandteil eine Mischung der roten Blutkörperchen von zwei verschiedenen Spezies ist.

8. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 1 und 3 bis 7, wobei der unreife Granulozyten-Bestandteil aus verarbeiteten humanen unreifen Granulozyten, die *in vitro* gewachsen sind, hergestellt ist.

9. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 1 und 4 bis 8, weiter umfassend einen Retikulozyten-Bestandteil.

10. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 3, 5, 6 und 8, wobei das erste verarbeitete rote Blutkörperchen aus einer ersten Spezies ein verarbeitetes humanes rotes Blutkörperchen ist.

11. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 3, 5, 6, 8 und 10, wobei das zweite verarbeitete rote Blutkörperchen aus einer zweiten Spezies ein verarbeitetes rotes Blutkörperchen aus Vögeln, vorzugsweise ein verarbeitetes rotes Blutkörperchen aus Gans oder Emu, ist.

12. Hämatologie-Vergleichskontrolle nach einem der Ansprüche 3, 5, 6, 8, 10 und 11, wobei das dritte verarbeitete rote

Blutkörperchen aus einer dritten Spezies ein verarbeitetes rotes Blutkörperchen aus Vögeln, Reptilien oder Fischen, vorzugsweise ein verarbeitetes rotes Blutkörperchen aus Emu, Strauß, Alligator oder Hai, ist.

13. Verfahren zur Verwendung einer Hämatologie-Vergleichskontrolle, enthaltend einen reife weiße Blutzellen-Bestandteil und einen unreife Granulozyten-Bestandteil, wobei das Verfahren die Schritte umfaßt:

(a) Bereitstellen einer Vergleichskontrolle, enthaltend einen reife weiße Blutzellen-Bestandteil und einen unreife Granulozyten-Bestandteil,
(b) Bereitstellen eines Blutanalysegeräts, das für die Analyse unreifer Granulozyten angepaßt ist,
(c) Hindurchleiten der Vergleichskontrolle durch das Blutanalysegerät für den Nachweis des unreife Granulozyten-Bestandteils, und
(d) Anzeigen des unreife Granulozyten-Bestandteils in der Vergleichskontrolle,
wobei der unreife Granulozyten-Bestandteil eine Größe von 2% bis etwa 85% größer als das hohe Ende eines Größenbereichs humaner Granulozyten, wenn diese von einem Blutanalysegerät gemessen werden, aufweist, wobei der unreife Granulozyten-Bestandteil aus verarbeiteten nicht-humanen Blutzellen oder verarbeiteten humanen unreifen Granulozyten hergestellt ist.

14. Verfahren nach Anspruch 13, wobei in Schritt (b) das Analysieren der unreifen Granulozyten durch Impedanzmessung oder durch eine optische Messung durchgeführt wird.

15. Verfahren nach Anspruch 14, wobei die optische Messung eine Messung der Lichtstreuung oder eine Messung des axialen Lichtverlusts ist.

Revendications

1. Témoin de référence hématologique contenant un composant de granulocyte immature, le témoin de référence comprenant :

(a) un composant de globule blanc mature simulant des globules blancs matures humains dans un analyseur de sang ;
(b) un composant de granulocyte immature servant à simuler des granulocytes immatures humains ; et
(c) un milieu de suspension convenant pour délivrer lesdits composants audit analyseur de sang pour la mesure des granulocytes immatures ;
dans lequel ledit composant de granulocyte immature présente une taille de 2 % à environ 85 % plus importante qu'une extrémité supérieure d'une plage de tailles de granulocytes humains, lorsque mesurée par un analyseur de sang, dans lequel le composant de granulocyte immature est constitué de globules rouges non humains traités ou de granulocytes immatures humains traités.

2. Témoin de référence hématologique selon la revendication 1, dans lequel le composant de granulocyte immature est constitué de globules rouges non humains traités.

3. Témoin de référence hématologique selon la revendication 1, comprenant en outre :

(d) un premier globule rouge traité provenant d'une première espèce en tant que composant lymphoïde ; et
(e) un deuxième globule rouge traité provenant d'une deuxième espèce en tant que composant myéloïde ;
dans lequel le composant de granulocyte immature est un troisième globule rouge traité provenant d'une troisième espèce.

4. Témoin de référence hématologique selon la revendication 1 ou la revendication 2, dans lequel ledit composant de granulocyte immature est constitué de globules rouges d'oiseaux, de reptiles ou de poissons, de préférence des globules rouges d'émeu, d'autruche, d'alligator ou de requin.

5. Témoin de référence hématologique selon l'une quelconque des revendications 1 à 4, comprenant en outre un composant de globule rouge et un composant de plaquette.

6. Témoin de référence hématologique selon l'une quelconque des revendications 1 à 5, comprenant en outre un composant de globule rouge nucléé.

7. Témoin de référence hématologique selon l'une quelconque des revendications 1, 2 et 4 à 6, dans lequel ledit composant de granulocyte immature est un mélange desdits globules rouges de deux espèces différentes.

8. Témoin de référence hématologique selon l'une quelconque des revendications 1 et 3 à 7, dans lequel ledit composant de granulocyte immature est constitué de granulocytes immatures humains traités mis à croître *in vitro.*

9. Témoin de référence hématologique selon l'une quelconque des revendications 1 et 4 à 8, comprenant en outre un composant de réticulocyte.

10. Témoin de référence hématologique selon l'une quelconque des revendications 3, 5, 6 et 8, dans lequel ledit premier globule rouge traité provenant d'une première espèce est un globule rouge humain traité.

11. Témoin de référence hématologique selon l'une quelconque des revendications 3, 5, 6 et 8, dans lequel ledit deuxième globule rouge traité provenant d'une deuxième espèce est un globule rouge d'oiseau traité, de préférence un globule rouge d'oie ou d'émeu traité.

12. Témoin de référence hématologique selon l'une quelconque des revendications 3, 5, 6, 8, 10 et 11, dans lequel ledit troisième globule rouge traité provenant d'une troisième espèce est un globule rouge d'oiseau, de reptile ou de poisson traité, de préférence un globule rouge d'émeu, d'autruche, d'alligator ou de requin traité.

13. Procédé d'utilisation d'un témoin de référence hématologique contenant un composant de globule blanc mature et un composant de granulocyte immature, le procédé comprenant les étapes consistant à :

(a) pourvoir un témoin de référence contenant un composant de globule blanc mature et un composant de granulocyte immature ;
(b) prévoir un analyseur de sang adapté pour analyser les granulocytes immatures ;
(c) faire passer ledit témoin de référence dans ledit analyseur de sang pour la détection dudit composant de granulocyte immature ; et
(d) rapporter ledit composant de granulocyte immature dans ledit témoin de référence ;
dans lequel ledit composant de granulocyte immature présente une taille de 2 % à environ 85 % plus grande qu'une extrémité supérieure d'une plage de tailles de granulocytes humains, lorsque mesurée par un analyseur de sang, dans lequel le composant de granulocyte immature est constitué de globules rouges non humains traités ou de granulocytes immatures humains traités.

14. Procédé selon la revendication 13, dans lequel dans l'étape (b), ladite analyse de granulocytes immatures est réalisée par une mesure d'impédance, ou par une mesure optique.

15. Procédé selon la revendication 14, dans lequel ladite mesure optique est une mesure par dispersion de lumière, ou une mesure par perte de lumière axiale.

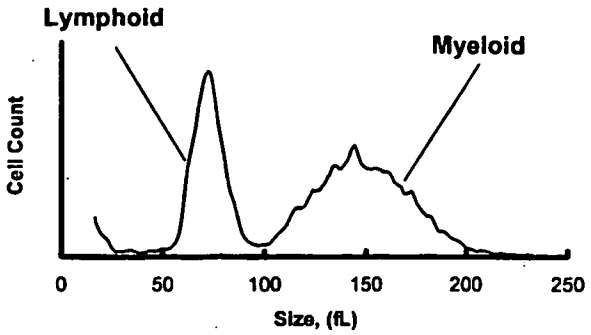


Fig. 1

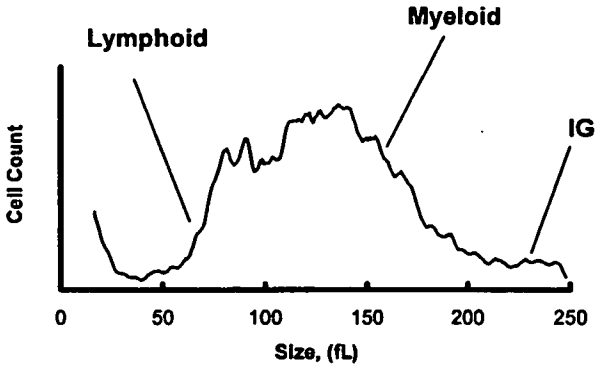


Fig. 2

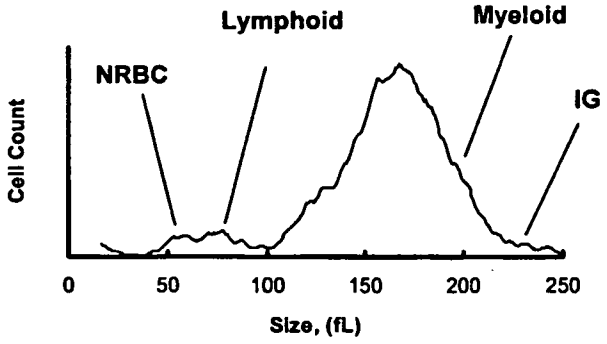


Fig. 2A

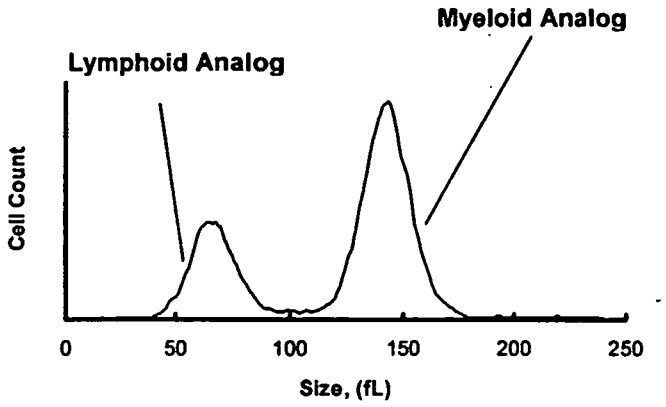
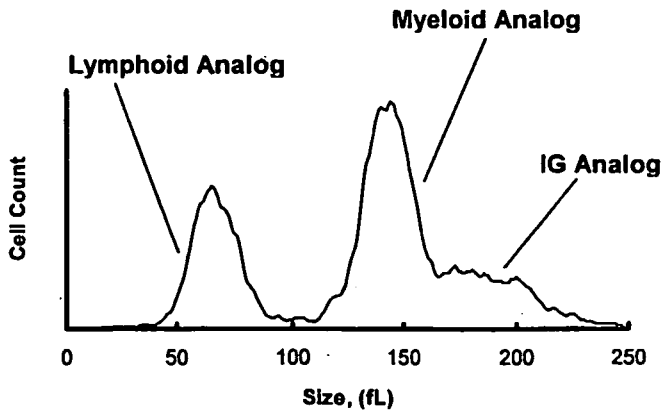

Myeloid Analog
Lymphoid Analog
Cell Count
0
50
100
150
200
250
Size, (fL)

Fig. 3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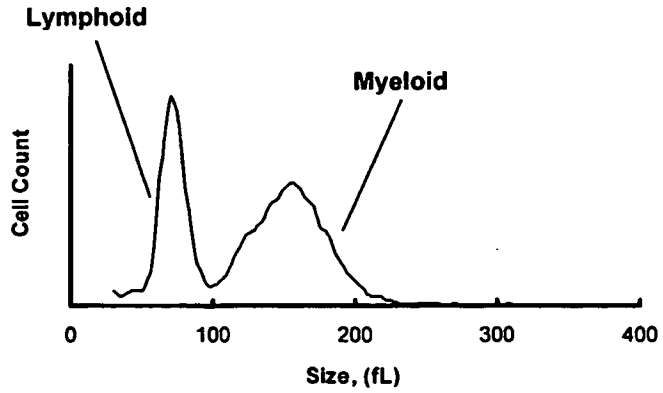
Lymphoid Analog
IG Analog
Cell Count
0
50
100
150
200
250
Size, (fL)

Fig. 4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Myeloid
Cell Count
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100
200
300
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Size, (fL)

Fig. 5

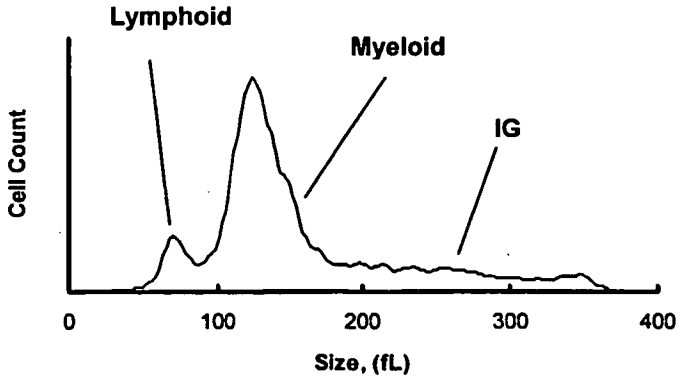
Lymphoid
Myeloid
IG
Cell Count
0
100
200
300
400
Size, (fL)

Fig. 6

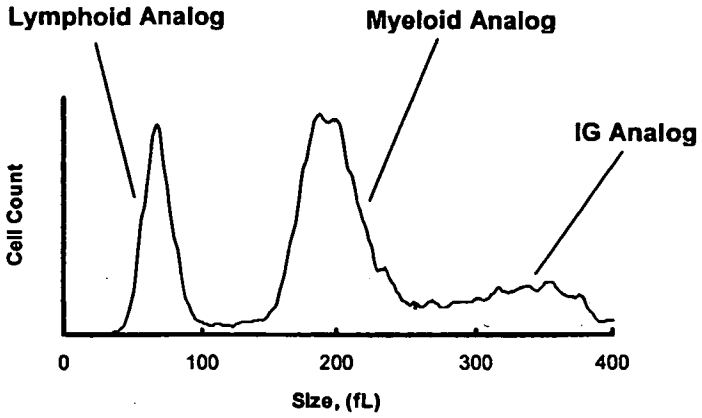
Lymphoid Analog
Myeloid Analog
IG Analog
Cell Count
0
100
200
300
400
Size, (fL)

Fig. 7

REFERENCES CITED IN THE DESCRIPTION

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- US 4405719 A **[0044] [0089]**

专利名称(译)	含有未成熟粒细胞成分的血液学参考对照		
公开(公告)号	EP1766045A4	公开(公告)日	2009-06-03
申请号	EP2005745792	申请日	2005-05-04
[标]申请(专利权)人(译)	贝克曼考尔特公司		
申请(专利权)人(译)	BECKMAN COULTER，INC.		
当前申请(专利权)人(译)	BECKMAN COULTER，INC.		
[标]发明人	ORTIZ NERY GERULA THEODORE J LI YI		
发明人	ORTIZ, NERY GERULA, THEODORE, J. LI, YI		
IPC分类号	C12Q1/02 G01N31/00 C12Q1/00 C12Q1/56 G01N33/53 G01N33/96		
CPC分类号	G01N33/96 G01N15/12 G01N2015/1006 Y10S435/967 Y10T436/10 Y10T436/101666 Y10T436/106664		
优先权	10/845557 2004-05-13 US		
其他公开文献	EP1766045A2 EP1766045B1		
外部链接	Espacenet		

摘要(译)

公开了一种使用参考对照测量血液分析仪上的未成熟粒细胞的方法。该方法包括分析参考对照，该参照对照包含用于模拟未成熟粒细胞的由处理过的红细胞制成的未成熟粒细胞成分和用于在适于测量未成熟粒细胞的血液分析仪上模拟血液样本的白细胞的成熟白细胞成分，并报告参考对照的未成熟粒细胞成分和成熟白细胞成分。

Granulocyte Component Made of Emu Red Blood Cells

Phosphate Buffered Saline Solution (PBS)

Sodium dihydrogenphosphate:	0.2g
Disodium hydrogenphosphate $7H_2O$:	2.0g
Sodium azide:	0.1g
Sodium chloride:	9.4g
Qs to 1 liter with distilled water:	pH approximately 7.4
	osmolarity 315 to 345 mOsm/kg H_2O