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PROCESS FOR STABILIZING FOWL RED BLOOD CELLS

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ABSTRACT OF THE DISCLOSURE

A diagnostic composition serving as a multiple-analysis hematology reference control for red blood cell and white blood cell counting, hemoglobin content and hematocrit determination.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of the co-pending U.S. application Ser. No. 802,263, filed Feb. 25, 1969.

BACKGROUND OF THE INVENTION

This invention relates to a diagnostic hematology composition. More particularly, it relates to a stabilized and standardized suspension of blood cells that provides a reference control for a number of routine hematology determinations.

Up to now, the prior art of hematology determinations has suffered from the lack of stable reference controls. With the advent of automated devices capable of performing multiple hematological determinations, a growing need has developed for cross reference samples for manual and automated machine analyses.

The distinguishing feature of this invention resides in the preparation of a stable multiple-analysis reference control which combines in a single composition reference controls for red blood cell and white blood counting, hemoglobin content, hematocrit determination, and allows by mathematical computation, the calculation of mean corpuscular volume and mean corpuscular hemoglobin, concentration, and mean corpuscular hemoglobin.

An embodiment of this invention is the preparation of a stable cell suspension that approximates the composition of normal human blood. Oxalated or citrated whole human blood has limited stability on storage. Red blood cells slowly hemolyze and undergo changes in size and shape. Similarly, white blood cells suffer degenerative changes.

A novel feature of this invention is the preparation of a stable red blood cell suspension that serves as a reference control for human white blood cells. Human white blood cells are not suitable for control purposes, especially in the presence of red blood cells, because they are unstable, excreting a lysozyme type of enzyme that attacks red blood cells. Avian red blood cells are larger than human red blood cells, and approximate the size and shape of human white blood cells. For purposes of this invention it has been found that fowl red blood cells such as goose, chicken, duck, and preferably turkey red blood cells, closely match the size and shape of human white blood cells, and lend themselves nicely to the subject tanning process. These stabilized "simulated" white blood cells provide a satisfactory substitute for human white blood cells in our composition control product.

It has been found that fresh human red blood cells, stabilized by suspension in human plasma or serum with added metabolic preservative, satisfactorily provide the red blood cells component of the subject composition. Plasma or serum provide some of the necessary electro-

lytes and proteins necessary for red blood metabolic stability. Selected purines or pyrimidines act as additional metabolic preservatives. The untanned red blood cells are readily hemolyzed in the prior operational step for the white blood cell count and subsequent hemoglobin determination.

Suspensions of human red blood cells and "simulated" white blood cells are mixed in such proportion that the final red blood cell and white blood cell counts, hemoglobin content and hematocrit fall in the range considered normal for human blood.

The normal range in human blood for red blood cells is 4,000,000-5,000,000 cells/mm.³, and for white blood cells the count is 5,000-10,000 cells/mm.³. The normal hemoglobin value is 12-16 grams/100 ml. The term "hematocrit" is defined as the ratio of volume of packed red blood cells to the volume of whole blood. The normal ratio in humans is about 45%. The mean corpuscular volume is the ratio of the volume of packed red cells in ml. per liter of blood to red blood cells in millions per cubic millimeter. The mean corpuscular hemoglobin concentration is an index indicating the mean or average weight of hemoglobin per 100 ml. of packed red blood cell in terms of percent. The mean corpuscular hemoglobin is the ratio of hemoglobin content in grams per liter to red blood cells per cubic millimeter.

It is evident that a control product must accurately indicate on a comparative basis what a test sample of blood constitutes with regard to the above determinations. It is further evident how important it is for the control product to simulate non-treated cells. It follows, for instance, that if the control product contains cells that differ materially in size, the experimental results will be inaccurate, if not wholly meaningless. In essence, the cells treated by the method disclosed herein provides an excellent system of checks and balances so necessary in hematology determinations.

SUMMARY OF THE INVENTION

In general, this invention embodies a composition prepared by mixing a suspension of human red blood cells with a suspension of stabilized fowl red blood cells ("simulated" human white blood cells) in such proportions as to provide in a single composition a multiple-analysis reference control for red blood cell and white blood cell counting, hemoglobin content and hematocrit determination.

DETAILED DESCRIPTION OF THE INVENTION

Regarding the novel methods to be described, it is to be understood that the term "saline" throughout can be any of the following three solutions: 0.9% by weight aqueous sodium chloride solution which is designated by the term "solution A"; 0.45% by weight aqueous sodium chloride solution designated as "solution B"; or a buffered saline solution ("solution C"). In most instances, the 0.9% solution (solution A) is implied when reference is made to the term saline. Isoton (Coulter Electronic, Hialeah, Florida) is an isotonic solution of buffer salts, pH 7.2.

The preparation of red blood cells for a white blood cell reference control presents special problems. Fowl red blood cells are preferred and turkey red blood cells in particular are most preferred. However, it is to be understood that fowl cells such as goose, chicken and duck will provide effective products. The fowl red blood cells are first washed several times with 0.45% w./v. sodium chloride solution and then suspended in 0.45% saline solution prior to the stabilizing treatment. This saline concentration swells the cells so that they closely resemble human white blood cells in size and shape. The size and shape are maintained after the subsequent stabilizing treatment.

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The second step of the herein disclosed method consists of suspending fowl red blood cells in a saline suspension to provide up to 50% by volume suspension. Although a concentration as high as 50% will work, it is preferred to use a range from 5% to 10% by volume, and even more preferred to use an 8% suspension. This suspension is combined with an equal volume of 1 to 3% solution by weight of mercuric chloride in saline. Mercuric chloride was found to be clearly superior to formaline, giving a more stable red blood cell and, most importantly, the shape of the red blood cell is retained.

The third step consists of heating the resulting mixture at 37° C. for at least 12 hours, and preferably, for about 20 hours. The solid materials are recovered by centrifugation and separation, and then resuspended in saline.

The fourth step consists of combining with the aforesaid suspension, an equal volume of a 0.0025% w./v. solution of aqueous tannic acid in saline with thorough mixing, incubating the resulting mixture for about 30 to 60 minutes at about 50° C. (this temperature is critical) and recovering the solid product by centrifugation and separation. Regarding this saline solution containing the tannic acid, it is the only instance in the herein disclosed invention wherein saline solution C described earlier is utilized. It is a buffered saline solution having a pH of 7.2. Its preparation consists of combining the following reagents in the proportions indicated (per liter):

	ml.
0.9% saline	500
0.15 M disodium phosphate	385
0.15 M monopotassium phosphate	115

Further fixation of the fowl red blood cells is accomplished by treating a 0.01 to 20.0% by volume suspension of the tanned cells in a 2–10% w./v., preferably 5% w./v. solution of glutaraldehyde in Isoton. The cell suspension is incubated at 37° C. for about 16–20 hours, centrifuged, washed 3–4 times with Isoton and then reconstituted in Isoton.

The preparation of the human red blood cell suspension is carried out under sterile conditions. For this purpose, red blood cells from any blood type can be used. However, Group O blood cells are preferably used because of the ready availability of blood donors of this type. Pooled Group O blood, collected in acid citrate-dextrose anticoagulant solution, is centrifuged, the supernatant removed and set aside, and the buffy layer of white blood cells gently removed and discarded. The packed red blood cells are washed several times with Alsever's solution, reconstituted in serum diluted 1:3 with Alsever's solution and stored at 2–8° C. Alternately, the packed red blood cells are washed several times with Isoton and resuspended in undiluted defibrinated plasma.

The resuspending medium, used for the final suspension of the human red blood cells, is prepared from defibrinated plasma that is serologically compatible with the suspended human red blood cells, i.e., Group A cells with plasma from Group A, Group B cells with plasma from Group B and Group O cells with plasma from Group O. Defibrinated Group AB plasma (lacking antibodies A and B) is compatible with Group A, Group B and Group O red blood cells. These plasma supernatants are defibrinated by treatment with bovine thrombin and used directly, or diluted 1:3 with Alsever's solution.

As a further stability aid for the fresh human red blood cells component of the final cell suspension, a metabolic preservative, selected from the class of compounds comprising purines and pyrimidines, is added to the suspending medium at a final concentration of about 34–35 mg. per 100 ml. of cell suspension.

A particularly effective preservative agent is adenine (6-aminopurine or a pharmaceutically acceptable acid additon salt, Mann Research Laboratories, Inc., New York, N.Y.).

The following examples are merely illustrative and are not intended to limit the invention, the scope of which is defined by the appended claim.

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Example I

Group O blood, collected in aqueous acid citrate-dextrose anticoagulant, is centrifuged under sterile conditions at 2000 r.p.m. for 20 minutes. The supernatant is gently suctioned off, care being taken not to draw into the supernatant any of the red cells or buffy white layer at the interface of the red cells and supernatant. The supernatant is defibrinated and retained as such or after dilution with Alsever's solution used as a resuspending medium.

The buffy layer (white blood cells) is carefully removed from the top of the packed red blood cells, withdrawing a thin surface of red cells if necessary. The packed red cells are washed twice with Alsever's solution, centrifuged and stored at 2°–8° C. until subsequent mixture with stabilized "simulated" white blood cells and reconstituted with defibrinated plasma diluted 1:3 with Alsever's. Alternately, the packed red cells are washed several times with Isoton, centrifuged and resuspended in undiluted defibrinated plasma.

Example II

Fowl red blood cells, preferably turkey cells, which are collected in Alsever's solution, are washed 3 times in saline (solution B, 0.45% w./v.) 3300 r.p.m./5 minutes each washing, and after the final wash are resuspended as an 8% by volume suspension in saline (solution B). To 100 ml. of this 8% suspension fowl (turkey) red blood cells is added 100 ml. of a 1% solution by weight of mercuric chloride in saline (solution A) and the resulting suspension is thoroughly mixed. The suspension is incubated at 37° C. for 18–20 hours, without disturbing the cells, the thin murky layer resting on top of the settled cells is suctioned off. The suspension is then centrifuged, washed 4 times with distilled water (3300 r.p.m./5 minutes each washing), and resuspended in saline (solution A) as a 4% by volume suspension. To this 4% suspension is added an equal volume of a 0.0025% w./v. solution of tannic acid in buffered saline (solution C) and the resulting mixture thoroughly mixed, incubated at 50° C. for about 30 to 60 minutes, allowed to stand at room temperature for 18–20 hours, centrifuged, and washed 3 times with distilled water. A 0.01–20.0% by volume suspension of the tanned cells is a 5.0% w./v. of glutaraldehyde in Isoton is incubated at 37° C. for about 16–20 hours, centrifuged and washed 3–4 times with Isoton.

Example III

The supernatant plasma, obtained from Group O bloods collected in aqueous acid citrate-dextrose anticoagulant, is defibrinated by adding bovine thrombin (Parke-Davis & Co., Detroit, Mich.) at a concentration of 1 unit/ml., and incubated at 37° C. for 1 hour. The clotted material is shaken vigorously to break up the clot, and filtered through a No. 50 Whatman filtered pad on a Buchner funnel-suction flask set-up. Adenine sulfate is added at a concentration of 70 mg./100 ml., warming at 37° C. to hasten to dissolution, and the solution filtered successively through 0.45 and 0.22 millipore filter pads.

Example IV

The method of Example III with plasma from Group AB blood in place of Group O plasma.

Example V

The "simulated" white blood cells prepared by the method of Example II are reconstituted in the plasma suspending medium or sterile Alsever's solution to about a 24% hematocrit. This has an approximate count of 750,000–800,000 cells/mm.³. Two ml. of this suspension is further diluted by the addition of 100 ml. of the resuspending medium as prepared by the method of Example III or Example IV.

To a volume of packed human Group O blood cells (approximately 90% hematocrit) prepared by the method

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of Example I, is added an equal volume of the diluted "simulated" white blood cells suspension referred to above.

The final cell suspension product, containing approximately 34-35 mg./100 ml. of adenine, has a red blood cell count of about 4,000,000-5,000,000 cells/mm.³, white blood cell count of 5,000-10,000 cells/mm., hemoglobin, 12-16 grams/100 ml. and hematocrit, 43-47%.

Aliquots (2.5 ml.) of the final suspension product, dispensed in glass vials, are stable for approximately 4 weeks when stored at 2°-8° C.

What is claimed is:

1. The method for stabilizing the fowl red blood cells which comprises:

- (a) commingling a suspension of fowl red blood cells in 0.45% saline wherein said cells comprise about 8% by volume of said suspension, and an equal volume of a 1% solution by weight of mercuric chloride in 0.45% saline;
- (b) heating the resulting mixture at 37° C. for about 19 hours, recovering and resuspending the solid mate-

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- rials as a 4% by volume suspension in saline; and
- (c) combining therewith an equal volume of a 0.0025% weight/volume solution of tannic acid in saline, incubating the resulting mixture for about 30 to 60 minutes at about 50° C. and
- (d) incubating a 0.01-20.0% by volume suspension of the cells in a 5.0% w./v. solution of glutaraldehyde in an isotonic solution buffered at pH 7.2 at 37° C. for 16-20 hours, washing 3-4 times with said buffered isotonic solution and recovering the solid product.

References Cited

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