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CALIBRATING FLUID FOR AUTOMATED HEMATOLOGY INSTRUMENTS

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ABSTRACT OF THE DISCLOSURE

A calibrating fluid for automated instruments for blood cell counting and hemoglobin determination comprising an admixture of nigrosin dye and a carbon black dispersion in dilute aqueous solution which exhibits absorbance linearity in the visible and ultraviolet range having suspended therein synthetic latex particles with a 5 to 15 micron particle size range.

This invention relates to a calibrating fluid for automated blood cell counting instruments.

The use of automated blood cell counting instruments in hematology is well known. In general, these instruments employ one or the other of two types of particle size analyses. In one type, each particle is counted and its discrimination property is measured directly. In the other type, the particles are measured in bulk and particle behavior is recorded through a series of measurements of the magnitude of the bulk, in terms of the count, combined surface area or combined mass. The type of measurement used then determines the basis of the size distribution.

Optical and electrical properties are two of the most prevalent types of size discriminating properties employed in these particle size analysis instruments. The optical equipment generally employs imaging, spectral transmission, scattering and diffraction mechanisms, while the electrical equipment usually employs resistance, capacitance, and charge mechanisms.

In addition to the counting of blood cells, the more sophisticated automated blood cell counting instruments provide determinations of other blood values such as hematocrit and hemoglobin values. Because of its characteristic red color, hemoglobin lends itself to colorimetric determinations. This gives rise to the use of a colorimeter unit on various blood cell counting instruments.

Several methods of hemoglobin determination which are generally employed in these automated instruments are the cyanomethemoglobin technique and the oxyhemoglobin method. In the former technique, the blood specimen is diluted with a reagent containing ferricyanide and cyanide, which converts both reduced hemoglobin and oxyhemoglobin to the cyanomethemoglobin form. The absorbance of the cyanomethemoglobin at 540 mμ is then used for quantitation. In the latter method, the blood specimen is diluted with an aqueous solution of tetrasodium salt of EDTA and mixed with air to convert reduced hemoglobin to oxyhemoglobin. The absorbance of oxyhemoglobin at 540 mμ is then measured.

Notwithstanding the general reliability of commercially available automated blood cell counting instruments, test values obtained with these instruments are only as accurate as the operater's technique and understanding of the instrument. In the absence of stringent control programs, together with regular recalibration of the instruments, one cannot always be assured of proper results. Experience has shown that quality control in the application and use of these automated blood cell counting instruments is a necessity for good hematological practice.

A unique advance in the quality control of hematological determinations made with automated blood cell counting instruments is disclosed in U.S. Pat. 3,558,522. Said patent describes a hematological control standard containing washed red blood cells and synthetic latex particles suspended in a serum albumin medium.

While the control standard of that patent has outstanding utility, it does not have the advantage of a completely synthetic or artificial product since it contains components prepared from naturally-occurring material. Some laboratories and clinics prefer to use completely synthetic or artificial control products since they are subject to less variability than natural products from one sample to the next sample. Moreover, in the calibration of blood cell counting instruments which also make hemoglobin determinations, it is frequently desired to employ a fluid which does not contain any red cells since these cells will gradually break down or hemolyze over a period of time.

The need for a color standard in clinical hemoglobinometry to establish the relation between instrument readings and concentration of hemoglobin in unknown samples is further set forth in various publications, for example, the paper by Cannan, Amer. J. of Clin. Path., vol. 30, pp. 211–215 (1958).

Accordingly, it is an object of this invention to provide an improved calibrating fluid for automated blood cell counting instruments.

It is another object of this invention to provide a completely synthetic calibrating fluid for automated blood cell counting instruments.

It is a further object of this invention to provide an artificial calibrating fluid for automated instruments used for blood cell counting and hemoglobin determinations.

Other objects and advantages of the invention will be apparent to the person skilled in the art after reading the present specification and the appended claims.

In the co-pending application Ser. No. 132,562, of Allan L. Louderback, filed on or about Apr. 8, 1971, entitled "Neutral Density Solution" and assigned to a common assignee, there is disclosed a neutral density solution for periodically checking the accuracy of spectrophotometric and colorimetric equipment. Said neutral density solution comprises an admixture of nigrosin dye and a carbon black dispersion in dilute aqueous solution which exhibits absorbance linearity in the visible and ultraviolet range.

It has now been found that when suitable synthetic latex particles are suspended in said neutral density solution, a unique calibrating fluid for automated instruments for blood cell counting and hemoglobin determination is provided.

In brief, the calibrating fluid of this invention comprises an admixture of nigrosin dye and a carbon black dispersion in dilute aqueous solution which exhibits absorbance linearity in the visible and ultraviolet range having suspended therein synthetic latex particles with a particle size ranging from about 5 to 15 microns and in an amount of from about 10,000 to about 22,000 particles per cubic millimeter.

The nigrosin dye employed in this invention is nigrosin, water soluble, as certified by the Biological Stain Commission (C. I. No. 50420) and described by Conn. Biological Stains, The Williams and Wilkins Co., at pp. 125, 294–5 (7th ed. 1961). To be certified, the nigrosin dye must appear distinctly black and not have a bluish hue to the eye. Any mixture of blue-black or violet indulin with a yellow dye in such a proportion that the resulting blend appears black is suitable. A preferred example of a commercially available certified product is identified as "Nigrosin (Water Soluble)" by the National Aniline Division of Allied Chemical Company.

The carbon black employed in this invention is an amorphous quasi-graphitic form of carbon of small parti-

cle size such as, for example, gas black, furnace black, channel black and thermal black. These carbon blacks are generally obtained by the incomplete combustion of gaseous hydrocarbons, for example, natural gas.

A preferred carbon black is lampblack. Lampblack is made by burning substances rich in carbon such as, for example, turpentine, petroleum, tar, acetylene, and similar such oils, fats and resins in a limited supply of air.

The carbon black employed in this invention preferably has a particle size in the range of from about 100 A. to 4000 A. in diameter and preferably is dispersed in aqueous solution at a concentration of from about 10% to 25% solids.

A suitable example of a carbon black dispersion is India ink, which generally consists of lampblack or other carbon black and a stabilizer such as, for example, gelatin, dextrin, or gum arabic in aqueous suspension. A preferred India ink is commercially available as "Pelikan-Gunther Wagner-Auszieh Tusche." This is an India ink imported into the United States from Germany. Another suitable commercially available India ink is the product identified as "A. W. Faber-Castell Higgins 4415" by the Higgins Ink Co., Inc., Newark, N.J.

It has been found that admixture of nigrosin dye and a carbon black dispersion or India ink in suitable proportions in dilute aqueous solution provides a grey solution which exhibits absorbance linearity in the visible and ultraviolet range.

In general, the proportions of these substances employed in the present invention are from about 0.2 to 2 mg. of the nigrosin dye and about 0.002 to 0.02 ml. of the carbon black dispersion or India ink per liter of water. The neutral density solution can be conveniently prepared by admixing from about 1 to 10 ml. of a stock solution of 200 mg. nigrosin dye in one liter of water with from about 1 to 10 ml. of India ink or other said carbon black dispersion in one liter of water followed by adding the mixture to 100 ml. of water. Preferably, about 2 to 4 ml. of the aforesaid nigrosin dye stock solution is conveniently admixed with about 2 to 4 ml. of the aforesaid India ink stock solution and added to 100 ml. of distilled water. After admixture, the solution is preferably filtered to remove extraneous or undissolved particulate matter.

The synthetic latex particles used in this invention are generally spherical in shape, they have a relatively uniform size of from about 5 to about 15 microns, which approximates the relative size of the normal leukocytes, or white cells, and are preferably employed in the calibrating fluid at a concentration of from about 10,000 to about 22,000 particles per cubic millimeter, which is above the approximate count in normal blood. In the calibration of blood cell counting apparatus, it is preferred to use these elevated levels of particles which simulate the white cells to provide greater accuracy at the lower end of the curve for average white cell counts. The most preferred average particle count is about 16,000 particles per cubic millimeter.

These latex particles can comprise polystyrene, polyvinyltoluene and/or styrene-divinylbenzene copolymer latex and the like synthetic polymeric latex materials of suitable particle size.

The sytrene-divinylbenzene copolymer latex particles are preferred for use in this invention. These latex particles are visible under the microscope under conventional magnifications at 10× and 40×, they are inert to the usual red cell lysing agents, such as acetic acid and various detergent substances, and otherwise provide suitable simulation of the white blood cells in the calibrating fluid of this invention.

Various commercially available instruments are suitable for calibration with the calibrating fluid of this invention. One specific example is the "Coulter Electronic Blood Cell Counter" and similar such equipment as described, for example, in U.S. Pats. Nos. 2,656,508, 2,869,078, 2,985,830 and 3,340,470. This particular instrument discriminates among particles by how they affect the electrical resistance of the fluid medium containing the particles in suspension as they pass through an orifice.

Other examples of apparatus which can be calibrated by the calibrating fluid of this invention are the "Technicon Instruments' Auto Analyzers" and similar such equipment as described, for example, in U.S. application Ser. No. 427,593, filed Jan. 25, 1965. This apparatus provides a support for a plurality of whole blood samples which are sequentially diluted, a flow cell of small cross-section through which the volume of diluted blood is passed, illuminated optical means coupled to the flow cell for detecting the passage of individual cells therethrough and providing an output pulse signal in response thereto, and electronic means for receiving and totaling the number of pulses per unit of time and providing an output signal.

Another example of a blood cell counting device with a colorimeter unit for hemoglobin determination to which the calibarting fluid of this invention is adapted is described in U.S. Pat. No. 3,427,135. In that device, the colorimeter is continuously coupled to a log-ratio single-ended output amplifier to continuously provide thereto an analogue voltage which is responsive to the precent transmittance of the increment of blood in a flow cell which is responsive to its hemoglobin concentration, and an analogue reference voltage. The log-ratio amplifier continuously provides an analogue voltage which is directly proportional to the hemoglobin concentration.

Various other conventional types of particle counting instruments which employ the metering of a sample of the particle-containing suspension past a scanning point in the detecting system will be apparent to the person skilled in the art.

In the use of the calibrating fluid of this invention, the fluid suspension is preferably well mixed prior to its use to ensure good dispersion and prevent the particle sizes from being indicated too large and size distribution too wide. The calibrating fluid preferably is used for blood cell counting instruments after "true" standardization with fresh blood. For example, with the "Coulter S" instrument, following standardization with fresh blood, the calibrating fluid is placed in the red blood cell chamber and is aspirated into the white blood cell chamber. The electronic outputs of this fluid are then used to set values (all parameters of the instrument) electrically on the timing generator card. In practice, the blood cell counting instruments should be calibrated at least daily with the calibrating fluid of this invention. Once the instrument is calibrated daily with the calibrating fluid of ths invention, it is preferred to maintain control of the instrument by employing a blood control product which simulates natural blood, such as the blood control standard described in U.S. Pat. No. 3,558,522. The latter product is employed in place of an unknown blood sample at predetermined periodic and regular intervals, for example, at every fifth sample.

The following example will further illustrate the invention although the invention is not limited to this specific example:

EXAMPLE 1

A calibrating fluid for automated instruments for blood cell counting and hemoglobin determination is prepared as follows:

A neutral density solution is first prepared by adding 100 milligrams of "Nigrosin (Water Soluble)," National Aniline Division, Allied Chemical, C.I. No. 50420, Biological Stain Commission, to 500 milliliters of distilled water and thoroughly mixing to form Solution A. Two milliliters of "Pelikan-Gunther Wagner-Auszieh Tusche" India ink is added to one liter of distilled water and thoroughly mixed to form Solution B. A grey Solution C is then made by mixing 3.2 ml. of Solution A with 2.6

ml. of Solution B and adding the mixture to 100 ml. of distilled water. The optical density of Solution C is then determined on a Beckman "DU Spectrophotometer" at wavelengths ranging from 340 mμ to 650 mμ. A substantially linear absorbance curve is obtained in this entire wavelength range with optical density readings of 0.25±10% at each interval of 10 mμ.

To the foregoing neutral density solution are added latex particles of styrene-divinylbenzene copolymer having a particle size range of 6 to 14 microns and an average particle size of 7.6 microns and in an amount of about 16,000 particles per cubic millimeter. This final product is eminently suitable for calibrating automated instruments for blood cell counting and hemoglobin determination as described hereinbefore.

Preferably, the calibrating fluid of this invention will also contain a small but effective amount of a preservative or biocidal agent such as, for example, sodium azide, thimerosal, and similar such agents.

Various other examples and modifications and adaptations of the foregoing examples will be apparent to those skilled in the art after reading the foregoing specification and the appended claims without departing from the spirit and scope of the invention. All such further examples, modifications and adaptations thereof are included within the scope of the invention.

What is claimed is:

1. A calibrating fluid for automated instruments for blood cell counting and hemoglobin determination comprising an admixture of nigrosin dye C.I. No. 50420 as certified by the Biological Stain Commission and a carbon black dispersion in dilute aqueous solution which exhibits absorbance linearity in the visible and ultraviolet range having suspended therein synthetic latex particles selected from the group consisting of polystyrene, polyvinyltoluene and styrene-divinylbenzene copolymer with a particle size ranging from about 5 to about 15 microns and in an amount of from about 10,000 to about 22,000 particles per cubic millimeter and in which the proportions of the nigrosin dye and carbon black dispersion are from about 0.2 to about 2 mg. of the nigrosin dye and from about 0.002 to about 0.02 ml. of the carbon black dispersion per liter, said carbon black dispersion having a solids content of from about 10% to about 25%.

2. The calibrating fluid of claim **1** in which the carbon black dispersion is India ink containing a stabilizer selected from the group consisting of gelatin, dextrin and gum arabic.

3. The method of making a calibrating fluid for automated instruments for blood cell counting and hemoglobin determination comprising admixing nigrosin dye C. I. No. 50420 as certified by the Biological Stain Commission and a carbon black dispersion in dilute aqueous solution in proportions of from about 0.2 to about 2 mg. of said nigrosin dye and from about 0.002 to about 0.02 ml. of the carbon black dispersion per liter of water, said carbon black dispersion having a solids content of from about 10% to about 25%, and suspending therein synthetic latex particles selected from the group consisting of polystyrene, polyvinyltoluene and styrene-divinylbenzene copolymer with a particle size ranging from about 5 to about 15 microns and in an amount of from about 10,000 to about 22,000 particles per cubic millimeter.

4. The method of claim **3** in which the carbon black dispersion is India ink containing a stabilizer selected from the group consisting of gelatin, dextrin and gum arabic.

References Cited

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