United States Patent [19]

Trowe

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[54] **METHOD FOR AUTOMATIC DIFFERENTIAL LEUKOCYTE COUNT**

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[52] **U.S. Cl.** **250/304**; 250/362; 356/36; 356/39

[51] **Int. Cl.** **G01n 33/16**

[58] **Field of Search**..... 250/83.3 UV, 218; 356/36, 356/39, 40; 235/92 PC

[56] **References Cited**

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[57] **ABSTRACT**

A differential count of leukocytes in blood is performed automatically by passing the individual blood cells through a constricted chamber while illuminating them with sufficient ultraviolet light to cause fluorescent emissions from each cell. The respective fluorescent emissions from the different types of leukocyte cells are each characteristic of the particular type of cell and can be automatically registered using photomultiplier tubes and counters.

3 Claims, No Drawings

METHOD FOR AUTOMATIC DIFFERENTIAL LEUKOCYTE COUNT

This invention is concerned with a high speed, accurate and automated method for performing a differential analysis of white blood cells. More specifically, the present invention is concerned with determining the individual counts of the various types of white blood cells or leukocytes by registering their distinctive fluorescent emissions.

Hematology is an important department of the clinical pathology laboratory. For many years pathologists have been concerned with various hematologic procedures. Among the earliest procedures was an examination of unstained blood; later the use of specific staining methods for the identification of various kinds of white cells was attempted. Then methods of absolute blood counting were introduced together with special tests such as, supravital staining and a method of differential counting of the various types of blood cells, introduced by Victor Schilling.

Increasingly it has become important to be able to make rapid, accurate blood tests and while at least one generally accepted method has been derived to give hematrocrit, hemoglobin and total leukocyte counts in a rapid and reasonably accurate, semi-automated manner; the method for performing a differential count of the various kinds of leukocyte cells has improved only in respect to the staining techniques used. No method has heretofore been developed which would even partially automate the leukocyte differential and bring it to the level of performance of the hematrocrit, hemoglobin or total leukocyte count.

According to present techniques for making differential leukocyte counts, a slide is prepared of a drop of blood and the specimen then stained and dried. The various types of leukocytes (i.e. lymphocytes, eosinophils, basophils and monocytes) present different appearances when illuminated under a microscope and accordingly a trained technician is able to make a count. Normally, a total of 100 leukocytes are counted in this manner and the number of each type of cell counted is reported in percentage form.

Assuming that the drop of blood placed on the slide to make the smear is of a uniform size, it is imperative that the prepared blood smear be made in such a way that there is a feathering of the smear down into a one cell thickness. Care must be taken to assure that the smear is made without the blood reaching the edge of the slide. If this does occur, the large cells are concentrated at the edge of the smear and the smaller cells are concentrated in the middle of the smear. An accurate differential on a smear of this type is impossible. In the staining technique itself, there are also many points at which error can occur. One of the most common errors at this point is in using a buffer for the staining which is either too alkaline or too acid; the buffer and stain mixture must be neutral. If the buffer is either too acid or too alkaline, the possibility of determining the maturity of the leukocyte is greatly reduced because of the non-uniform staining of the nucleus.

Now in accordance with the present invention it has been found that both the total and differential count of leukocytes in blood can be quickly, efficiently, and accurately determined automatically without the need for technicians or other personnel visually observing the cells or otherwise manually making the determination

and consequently with a great reduction in the possibility of error.

More specifically, it has been found that the various types of white blood cells or leukocytes i.e. lymphocytes, monocytes, and granulocytes including neutrophils, basophils, and eosinophils each exhibit characteristic fluorescence and can be excited with ultraviolet radiation so each emits fluorescent radiation having a wave length characteristic of that particular type of cell. Further it has been found that using a spectrofluorometor, the respective fluorescent emissions from the different types of white blood cells can be recorded in such a way as to permit the mechanical counting of the number of each type of cell in a given volume.

According to the present invention, a blood sample which has been suitably diluted with about 40 – 50 parts by volume of say about a 1 percent saline solution is treated, for example with an appropriate lysing agent to destroy the erythrocytes present. Subsequently the treated specimen is introduced into a chamber or tube which narrows to a sufficiently small diameter, e.g. about 70 microns, so that the passage of cells is in single file by one or more beams of ultraviolet monochromatic light of sufficient intensity to excite the cells to fluorescent emission. These fluorescent emissions from the respective illuminated cells are segregated according to the wavelengths characteristic of each cell and counted on photomultiplier tubes. Segregation of the fluorescent emissions from the respective cells according to wavelength can be effected by means of an analyzer monochrometor having photomultiplier tubes located at slits positioned to discriminate the wavelengths of the emissions of each of the different types of white blood cells being counted. Alternatively, for example, dicroic minors can also be arranged to receive and separate the respective wavelengths emitted from the cells.

The ultraviolet illumination from the exciter monochrometor causes the white blood cells to fluoresce and the fluorescence is detected by the photomultiplier tubes at the analyzer exit slits. The output pulse from the photomultiplier tubes is passed to counters corresponding to the specific white cell being detected. In addition, the outputs of each of the photomultipliers is passed to a total white cell counter. When the total white cell counter indicates a total of 100 counts, all counters can be stopped, their counts now indicating the percentage abundance of each of the cell types.

EXAMPLE

0.1 c.c. of whole blood diluted in 4.9 c.c's of 0.9 percent aqueous sodium cloride solution was added to one drop of lysing agent to destroy the erythrocytes. After mixing thoroughly, the solution was poured into the mouth of a vessel provided with a release valve and a vacuum applied which caused the blood to pass into the testing chamber which narrowed to a 70 micron diameter orifice and then into a 70 micron tube which was placed at the exit slit of an exciter monochrometor. The cells flowed through in single file and were illuminated by a monochromatic beam of light. At right angles to the path of the exciter beam was the entrance slit to an analyzer monochrometor. At the appropriate wavelength positions for each of the different types of white blood cells to be counted, photomultiplier tubes were located.

The ultraviolet illumination from the exciter monochrometor caused the white blood cells to fluoresce

and the characteristic fluorescence of the individual cells was detected by the individual photomultiplier tubes at the analyzer exit slits. The output pulse from the photomultiplier tubes was passed to counters corresponding to the specific white cells being detected. In addition, the outputs of each of the photomultipliers was passed to a total white cell counter. When the total white cell counter indicated a total of 100 counts, all counters were stopped so that their counts indicated the actual percentage abundance of each of the cell types.

What is claimed is:

1. A method for making a differential count of leukocytes in blood which comprises the steps of:

a. preparing a dilute saline solution of the blood sample;

b. passing the dilute saline blood solution into a constricted chamber so that the individual cells are disposed in a single array;

c. subjecting the cells disposed in said constricted chamber in single file to sufficient ultraviolet radiation as the cells pass through the chamber to cause them to fluoresce;

d. segregating the fluorescent emissions from each cell according to the characteristic wavelength of the fluorescent emission of that type of cell;

e. registering the respective fluorescent emissions from each leukocyte cell according to its wavelength on a photomultiplier tube;

f. by means of counters recording the number of fluorescent emissions registered on the photomultiplier tubes from each type of leukocyte cell.

2. The method of claim **1**, wherein the leukocyte cells are lymphocytes, monocytes and granulocytes.

3. A method for making total and differential counts of the leukocytes in blood which comprises the steps of:

a. destroying the erythrocytes in a sample of the blood cells;

b. subjecting said sample to ultra-violet radiation sufficient to cause fluorescent emissions of the leukocytes therein;

c. separating the resulting emissions according to wavelengths into lymphocytes, monocytes and granulocytes emissions sets;

d. registering the number of emissions for each set and for the sum of said sets; and

e. stopping registration of said emissions when the total leukocyte count reaches 100; whereby said number of emissions for each set indicates the percentage abundance of said set relative to said total leukocyte count.

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Disclaimer

3,896,307.—*Neil M. Trowe,* Potomac, Md. METHOD FOR AUTOMATIC DIFFERENTIAL LEUKOCYTE COUNT. Patent dated July 22, 1975. Disclaimer filed Feb. 13, 1981, by the assignee, *Wheeler International, Inc.*

Hereby enters this disclaimer to claims 1-3, all the claims of said patent.

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