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DETECTION OF MALARIAL PARASITES IN BLOOD

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ABSTRACT OF THE DISCLOSURE

Pre-stained microscope slides having a surface covered with a dried mixture of Methylene Blue NN (also known as New Methylene Blue N) and Cresyl Violet Acetate, disclosed in a copending application as useful for observing normal blood constituents, are employed to observe samples of blood from patients suffering from Falciparum Malaria. Trophozoites and gametocytes are rendered readily observable and identifiable; personnel experienced in malarial-oriented hematology found the parasites in such preparations at least as readily observable as in the conventional Wright-stained preparation.

CROSS-REFERENCE TO RELATED APPLICATION

Pre-Stained Slides for Blood Tests, John A. Geating and Frederick W. Thomae, Jr., Ser. No. 222,654, filed Feb. 1, 1972, now U.S. Pat. 3,796,594, assigned to the assignee of the present application.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

This invention pertains to methods of biological diagnostic observation, and in particular to a method of identifying the presence of malarial parasites in human blood.

(2) Prior Art

U.S. Pat. 3,796,594 there is described a pre-stained microscope slide which has on one surface a dried mixture of Cresyl Violete Acetate and Methylene Blue NN in suitable proportions and surface density so that when a drop of blood is applied to the slide and spread over it by application of a cover glass, the normal components of the blood are differentially stained, producing the following characteristics:

Neutrophils contain a finely granulated cytoplasm which stains a light purple in the fresh preparation and fades to a greenish tint in the older smear. Nuclei stain a bright purple.

Eosinophils exhibit a bright purple nucleus and a cytoplasm packed with large uniform orange-staining granules which fade to a greenish orange when the preparation is about twelve hours old.

Basophils contain smaller number of uniform-size granules than eosinophils, which stain a dark purple and often completely obscure the nucleus. With focusing, an orange tinge can be observed in the granules found at the edge of the cell, an effective identifying feature.

Lymphocytes develop a purple staining nucleus surrounded by a lighter purple cytoplasm which may become as dark as the nucleus as the preparation grows old.

Monocytes have an affinity for the stain similar to lymphocytes. Distinguishing features are the larger size and the greater amount of cytoplasm of the monocytes. It has been found that the differentiation is readily learned.

Platelets stain a pink color. A platelet estimation is best performed as soon after the staining period as possible because of an accumulation of debris with increasing time.

Reticulocytes contain a reddish-purple network but do not fade even after the red cells become distorted. Nevertheless, the reticulocyte count should also be made within 4 to 5 hours after the staining period because of the fading of some of the red blood corpuscles when they die.

The published prior art, including that used for observation of blood for suspected malarial infection, involves the use of Wright's stain. A publication entitled "The Morphology of Human Blood Cells in Wright Stained Smears of Peripheral Blood and Bone Marrow," by L. W. Diggs, M.D., Dorothy Sturm, and Ann Bell, University of Tennessee College of Medicine, Department of Medicine, Section of Hematology and City of Memphis Hospitals, Memphis, Tenn., copyright 1970, published by Abbott Laboratories, North Chicago, Ill., at page 35 thereof contains illustrations of various samples stained in the conventional manner with Wright's stain.

SUMMARY OF THE INVENTION

Samples of blood are applied to the pre-stained slides described in U.S. Pat. 3,796,594, to which reference is made for details not given herein. In addition to the staining of normal blood constituents described therein, trophozoites are stained so that they are readily observed not only in erythrocytes, but also extra-erythrocytically, which latter has not previously been observed, so far as the applicant is aware. It is possible that the presumed failure to make the latter observation in the prior art reflects a failure of the conventional stains of the prior art to render them sufficiently visible to be detected, although this has not been the subject of investigation.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Samples of blood from three hospitalized patients receiving anti-malarial therapy, and from three patients suspected of having Falciparum Malaria were made into conventional thin preparations stained with Wright's stain, and samples from the same six patients were applied to pre-stained slides having dry mixed stains on their surfaces, as described in detail in U.S. Pat. 3,796,594. Briefly, a solution of 2.0 grams Methylene Blue NN in 25 milliliters of absolute methyl alcohol, and a solution of 1.0 gram Cresyl Violet Acetate in 25 milliliters of absolute methyl alcohol, are filtered separately after standing from 16 to 24 hours. One milliliter of the first named solution and 0.13 to 0.2 milliliters of the second solution are mixed and a clean microscope slide is coated with a film of the mixture and allowed to dry in air, leaving a dried film of 91 to 94 percent by weight Methylene Blue NN and 6 to 9 percent by weight of Cresyl Violet Acetate. No malarial parasites were observed in either the Wright-stained preparation or the pre-stained slide preparation of the samples from the patients receiving therapy. Parasites observations of the blood of one of the suspected patients were also negative for both the Wright-stained preparation and the pre-stained slides. Samples of blood from the remaining two suspected patients showed identifiable malarial parasites both in the conventional Wright-stained preparations and in the pre-stained slides. Several observers, including a doctor of medicine and a research assistant from a university faculty of tropical medicine, both with considerable experience in the identification of malarial parasites, confirmed all the findings described herein.

The mode of preparation of all samples applied to pre-stained slides, and the observations of such preparations in the case of samples containing malarial parasites was as follows:

A drop of blood was obtained from the finger and placed on the pre-stained slide. A 22 by 40 mm. cover slip was placed over the drop of blood which resulted in spreading of the drop of blood under the cover slip. After approximately five minutes the slides were placed on the micro-

scope and were examined under both high dry magnification and oil immersion and the following observations were made:

Comparison was made with the illustrations found on page 35 of "The Morphology of Human Blood Cells in Wright Stained Smears of Peripheral Blood and Bone Marrow," by L. W. Diggs, M.D., Dorothy Strum, and Ann Bell, University of Tennessee College of Medicine, Department of Medicine, Section of Hematology and City of Memphis Hospitals, Memphis, Tenn., copyright 1970, Abbott Laboratories, North Chicago, Ill. Of the two cases observed, one individual was found to have a light infection and the other was found to have a very heavy infection.

Trophozoites, both early and late, were observed in the smears of both individuals. The chromatin dot in the ring forms stained purple to red-purple in color as compared to the conventional staining method. The cytoplasm was slightly pink in color. In the light infection the trophozoites appeared more spherical and smaller in size as compared to the standard method in which they appeared larger and more ovoid. In the case of the heavy infection, many trophozoites appeared ovoid as in a conventional preparation but they did, however, appear smaller.

In several instances ring forms were detected extra-erythrocytically. The experienced physician previously mentioned stated that trophozoites are not known to occur extra-erythrocytically and that he had never observed it previously. Many erythrocytes contained two parasites, a condition which is allegedly pathognomonic of Falciparum Malaria. The doctor and the medical technicians who observed the slides had no difficulty in diagnosing the patient as having Falciparum Malaria. In one instance, a trophozoite was found to be present in a reticulocyte.

In the heavy infection, gametocytes were found to be present. However, they did not have the typical crescent appearance of gametocytes which appear in Falciparum Malaria. The gametocytes were mostly ovoid in shape with dense staining chromatin material at one pole. However, some of the gametocytes were dumb-bell shaped with the dense staining chromatin material in the middle.

The doctor expressed his impression that it was easier to detect the parasites with pre-stained slides than it was on the conventional thin preparation. The utilization of pre-stained slides for recognition and identification of malarial parasites has the following advantages over the existing method:

(1) Greater ease of recognition
(2) Faster method for preparing slides
(3) Slides can be prepared in the field without the need for laboratory facilities.

What is claimed is:

1. The method of identifying malarial parasites in human blood which comprises:
   (a) applying a fresh sample of blood to a microscope slide having a surface coated with a dried mixture of 91 to 94 weight percent Methylene Blue NN and 6 to 9 weight percent Cresyl Violet Acetate;
   (b) letting the slide with the blood sample so applied remain for a time sufficient to produce staining of the blood constituents;
   (c) observing the blood constituents upon the slide.

2. The method of differentially staining malarial parasites in human blood which comprises applying a sample of blood containing such parasites to a surface bearing a dried mixture of 91 to 94 weight percent Methylene Blue NN and 6 to 9 weight percent Cresyl Violet Acetate.

References Cited

Sabin: Bull., The Johns Hopkins Hospital, vol. 34, 1923, pp. 277–288.

Blecher, AJCP, vol. 19, 1949, pp. 895–896.

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U.S. Cl. X.R.

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