EVALUATION OF AUTOMATED DIFFERENTIAL LEUKOCYTE COUNTING SYSTEMS

THESIS

SUBMITTED FOR THE PARTIAL FULFILMENT

OF MASTER DEGREE IN CLINICAL PATHOLOGY

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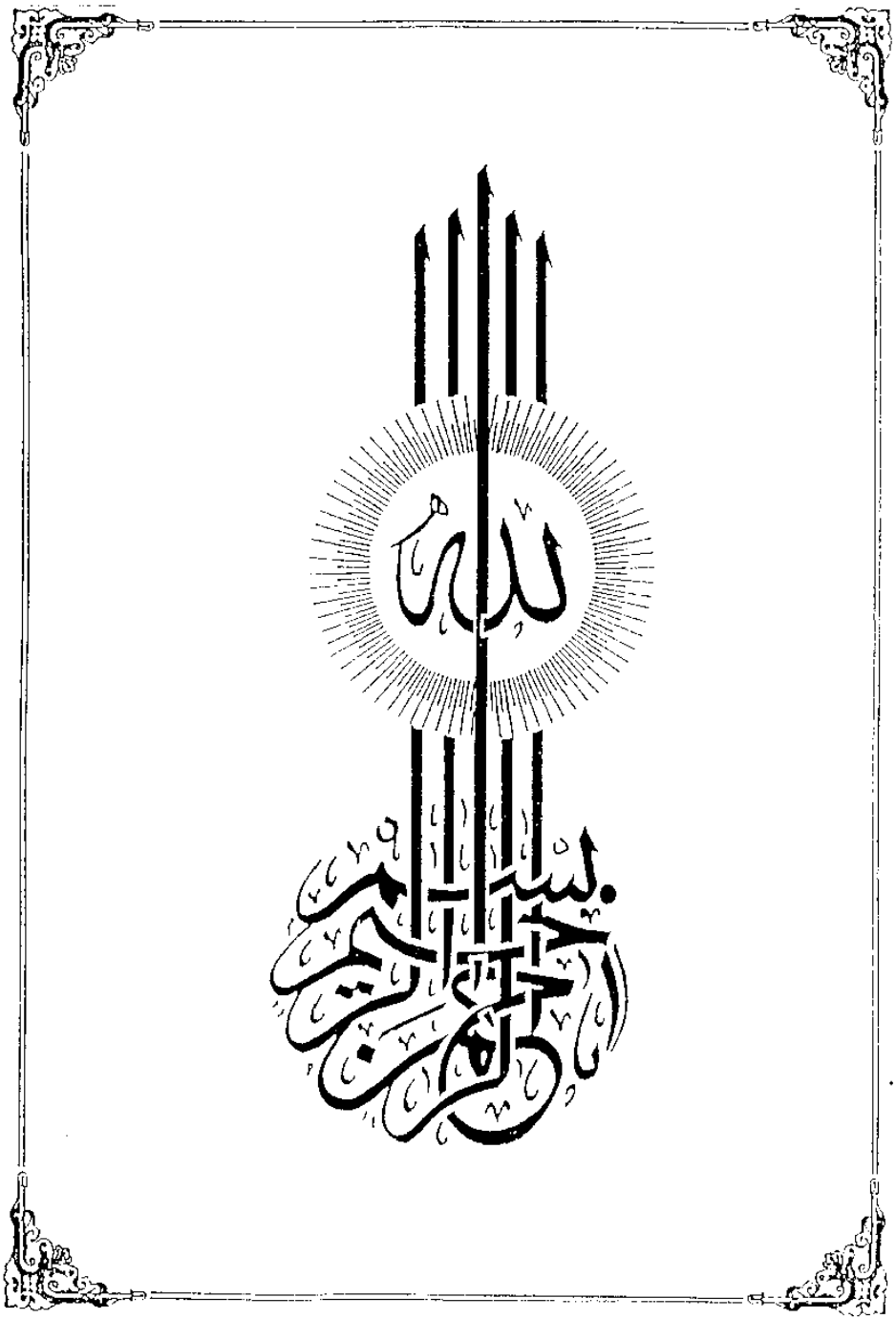
1988



ACKNOWLEDGEMENT

I would like to express my deepest thanks and gratitude to Prof. Dr. **WAGEEH NAGUIB IBRAHIM,** for his generous advice,sincere help, valuable suggestions and consistent supervision during the progress of this work.

I would like to offer my warmest thanks to Prof. Dr. **SAWSAN FAYAD,** for her fruitful guidance, valuable suggestions, generous advice, sympathatic help and her encouragement.

I feel greatful to Dr. **NEVINE AHMED KASSIM** for her guidance in helping me through this work, for her kind supervision and her constant assistance during the preparation of this thesis.

I would like to thank my husband for his continuous help and his continuous support

INTRODUCTION AND AIM OF THE WORK

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The medical usefulness of the differential leukocyte count is unquestioned, and it is one of the most frequent laboratory test (Ross et al., 1981).

Each day, world wide, many millions of peripheral blood smears are examined. Ingram and Preston (1970) estimated that more than one million peripheral blood smears were scrutinized daily in the United States alone.

In manual differential count, quite apart from preparation time, the operator who performs the examination is required to observe morphology and colour of red cells, check platelet number and differentially categorize 100 leukocytes. The time required for this exercise is variously quoted as 90 seconds (Bull and Korpman, 1981) to 5 minutes (Preston, 1976).

Automation of the differential count may help to eleminate some of the detractions. ideally , requirements for the automated differential leukocyte counting system should include the followings:

I. The distribution of cells analyzed should be identical with that in the blood.

(2) All leukocytes, usually found in blood diseases should be accurately identified or detected and "marked" in some way.

(3) The speed of the process should enable a large number of cells to be counted in order to minimize statistical error.

(4) The instrument should be cost effective (Bentley and Lewis, 1977).

In selecting a comprehensive automated system, it is important to assess how it will fulfill the requirements of the laboratory. Accounts must be taken of the rate at which the equipement can operate, the cost of the reagents and other materials must be ascertained, the availability of services, water, compressed air, drainage has to be considered. Also the training required, the extent to which this is provided by the manufacturer or agent (Dacie and Lewis, 1984).

The purpose of this study is to write a comprehensive review of automated differential leukocyte counting system to choose an instrument suitable for the needs of an individual

laboratory and also to evaluate its performance, reproducibility, cost and clinical use.

MANUAL DIFFERENTIAL LEUKOCYTE COUNTING

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In recent years the manual white cell differential count has attracted a lot of attention. It is one of the most commonly ordered haematological tests. It is also one of the most labor intensive procedures in the entire clinical laboratory (Ross et al., 1981).

The purpose of the differential count is to evaluate the distribution of various white cells in the blood stream by identifying a limited number of these cells in the blood film and by reporting their percentage distribution . Also , allowing the morphologic study of red and white blood cells and platelets (Bauer , 1980).

The routine morphologic examination entails an evaluation of fixed blood films, stained with one of Romanowsky's dyes. To prepare satisfactory thin blood films, three aspects of the procedure must be considered :

(1) Preparation of blood smear.
(2) Staining blood film
(3) Microscopic examination of cellular elements.

(I) Preparation of Blood Smear :

Blood sources for blood film examination are capillary, peripheral or venous blood.

Three methods of making films are described the slide or wedge method , the coverglass method, and the spinner method (Nelson and Morris, 1984).

a. Slide technique (Koepke, 1980) :

Slide technique has the advantages of being easily handled, easily stained as well as being the most familiar method for haematologist. But it has the difficulty of obtaining an even distribution of leukocytes in the film. For an unexperienced technician there are difficulties in spreading including the blood drop being too large or too small, the spreader pushed across the slide in a jerky manner, failure of maintaining the angle between the spreader and the slide and failure to push the spreader completely across the slide (Undritz, 1973).

Optimal blood films may be difficult to prepare in patients with severe anaemia or polycythemia, as well as in patient with abnormal plasma proteins resulting from diseases such as myeloma or cold agglutinin disease (Koepke, 1980).

Also there is a disproportion distribution of different types of white cell in wedge slide (Rogers, 1973) .

b. Cover glass method (Wintrobe et all., 1981).

Cover glass method has the advantage of being easily handled and easily stained. the distribution of the different white cells in coverglass method is presumably random (Rogers, 1973).

On the other hand many disadvantages are encountered with this method. As soon as the drop of blood is placed on the cover glass, there should be no delay in bringing together the two cover glasses, otherwise clumping of platelets and white cells and rouleaux formation of red cell will occur. Also coverglass is too thin and so easily broken.

c. Spinner method (Fig. 1).

A special type of centrifuge known as spinners (Rogers, 1973) is used as a mechanical spreaders which automate the manual technique. A platen holds the slide in a horizontal phase perpendicular to the rotor. About 1-2 drops of blood placed in the centre of a glass slide (on the platen). Closing the cover of the centrifuge activates the motor. The motor spins the rotor, rapidly accelerates to about 5000 r.p.m., and quickly

stops after a spinning time of a few seconds. The blood spreads on the slide in a monolayer (Nelson and Morris, 1984).

The more sophisticated spinners contain an optical system. During spinning a beam of light passes up through the glass slide onto a sensor. When the cells have separated the proper amount, the sensor detects this and the platen automatically

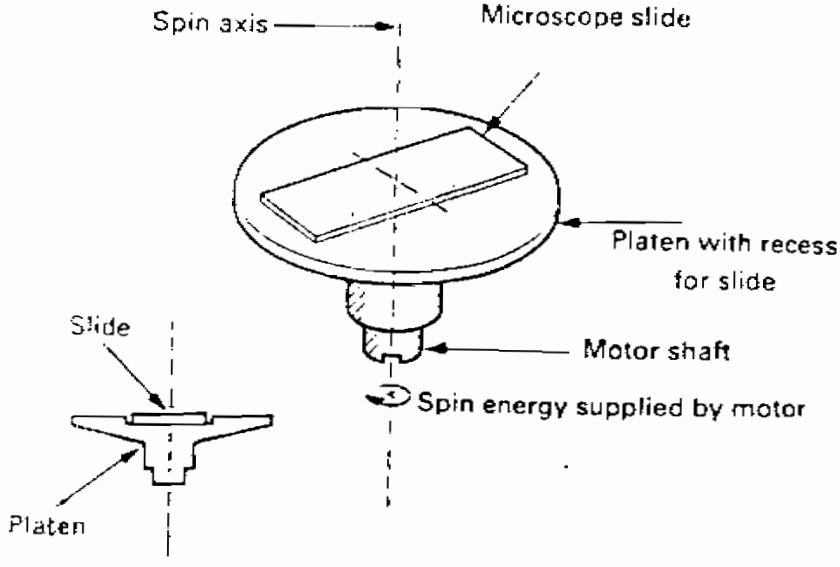

Fig. (1) A centrifugal spinner for the preparation of peripheral blood films showing the platen and microscope slide arrangement (Bacus, 1974) .

stops spinning. It is used in several of the automatic white cell differential systems available (Nelson and Morris, 1984). By this method leukocytes and platelets are distributed uniformly and free of distortion (Nourbakhsh et al., 1978). Also it is easily handled and useful specially with large number of blood samples. A minor disadvantage of spinner slide is the tendency of red cells to have eccentric central pallor , mimicking the appearance of spheroidocytes (Nelson and Morris, 1984).

(2) Staining Blood Film

Romanowsky stains are universally employed for staining blood films as a routine (Dacie and Lewis, 1984).

Polychrome methylene blue and eosin stains are the outgrowth of the original time-consuming Romanowsky method and are widely used. They stain differentially most normal and abnormal structures in the blood (Nelson and Morris, 1984). The different preparations vary in the proportions of the two compounds and the method by which methylene blue is converted to its active forms, methylene azures. When mixed with eosin, these stains are designated as " polychromes" because they impart metachromatic qualities to cell constituents (Wintrobe et al., 1981).

The active forms of methylene blue are basic dye which impart a violet blue colour to acid components of the cell. In contrast, eosin is an acidic compound and thus will react with basic components of the cell. The Romanowsky stains are of particular value because they possess the ability to differentially colour leukocyte granules (Wintrobe et al., 1981).

The typical colouration of certain cell components is produced by the combined action of the dyes at the appropriate pH (6.4 - 7.0) (Koepke, 1980). Romanowsky-type stains include: Wright, Giemsa, Leishman, Jenner, May-Grünwald, MacNeal, and various combinations (Nelson and Morris, 1984).

Staining techniques:

Staining of previously prepared blood films is done by one of two techniques: the first is the " on slide technique" widely used in Egypt, and the second is " the automatic staining". Staining machines of the " Hema Tek" types (Ames Co.) follow the procedure of " on slide staining " i.e. each slide is individually automatically processed. The process is not affected by the possibility of gradual " exhaustion" of premixed solution (Rogers, 1973).