

A Rapid Method for Estimation of the Total Leukocyte Count

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IN THE EVENT of atomic warfare, those charged with the medical service will be confronted with large numbers of radiation victims. One problem will be to identify those whose radiation injury is severe enough to require medical care. Because of the large number of people involved and the primitive conditions in devastated areas, a relatively simple and rapid sorting procedure should be available. The leukocyte count is reported to be a fairly good index of the dose of atomic radiation received by experimental animals and total body x-irradiation received by man. For this reason the leukocyte count has been proposed as the best screening procedure presently available for the sorting of atomic casualties. However, when the cleaning and loading of pipets and counting chambers is considered, about ten minutes of a technician's time is required for each count. Under field conditions a short supply of pipets and chambers could lengthen the time required and would provide a further obstacle to rapid screening.

With these considerations as a background, a simplified leukocyte counting procedure was sought. By eliminating the pipets and counting chamber from the white cell count, much time and money could be saved. However, our attempts to estimate the white cell counts from blood smears proved unsuccessful. The degree of anemia and thickness of the smear were cumbersome variables when the red cells were used as the point of reference. However, a method of scanning rather than counting seemed feasible if it were possible to deliver a standard volume of blood onto a definite area of a microscope slide. In such a situation the concentration of leukocytes, if adequately stained, could be estimated at a glance. A number of methods of making a standard sized drop of blood were tried. In this way the method to be described was developed.

MATERIALS AND METHODS

1. Microscope capable of at least x 25 magnification.
2. Microscope slides.
3. Straight pins.
4. Staining dish.
5. Stain capable of staining leukocytes.
6. Formalin.

The stain is made up approximately of .02 per cent (w/v) in a tap water solution of formalin (5 per cent v/v) or 1 cc. Giemsa solution in 50 cc. of tap water-formalin solution.

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The blood smears for evaluation are prepared as follows: (See fig. 1A.) The head of a straight pin is immersed in the blood so that a small drop adheres to the rounded surface of the pinhead. Immediately the pinhead is touched to the surface of a microscope slide and a portion of blood forms a small round dot (fig. 2). This is repeated two or three times. With practice it becomes easy to deliver drops of uniform size. Those which are obviously too large, too small or distorted are wiped off before they dry. By measuring the hemoglobin in the drops, it was found that the volume of each drop is approximately 0.4 cu.mm. The diameter of the drops is approximately 2.5 mm.

If the drop is carefully dried, an accurate estimate of the leukocyte concentration can be attained. When the drop is allowed to dry slowly—when no heat is applied—it becomes relatively thin in the center and thick at the edge. When too much heat is applied and the drop dries too rapidly, it tends to be thick in the center and thin at the edge. The drops may be smoothly dried by placing the slides over an electric light bulb (fig. 1B) or by directing the warm air blast from a small hair dryer against the bottom of the slide. But even the drops that dry too slowly or too rapidly can be used to establish the presence of leukopenia or leukocytosis (leukocyte count of less than 4,000 per cu.mm. or greater than 12,000).

When the drop has dried the slide is immersed in the staining solution for 5 to 10 minutes. During this time the water leaches the hemoglobin out of the red cells and the drop becomes transparent. The formalin fixes the drop, and the stain is taken up by the leukocytes. The red cell and plasma proteins stain lightly, providing a pale background for the darkly stained leukocytes. The slide is then gently rinsed in a dish of tap water and dried. A drop of oil on the preparation makes the examination easier.

The slide is examined under low power. If the second lens of the low power objective is removed the field is magnified about 25 times and the entire drop is seen. After a

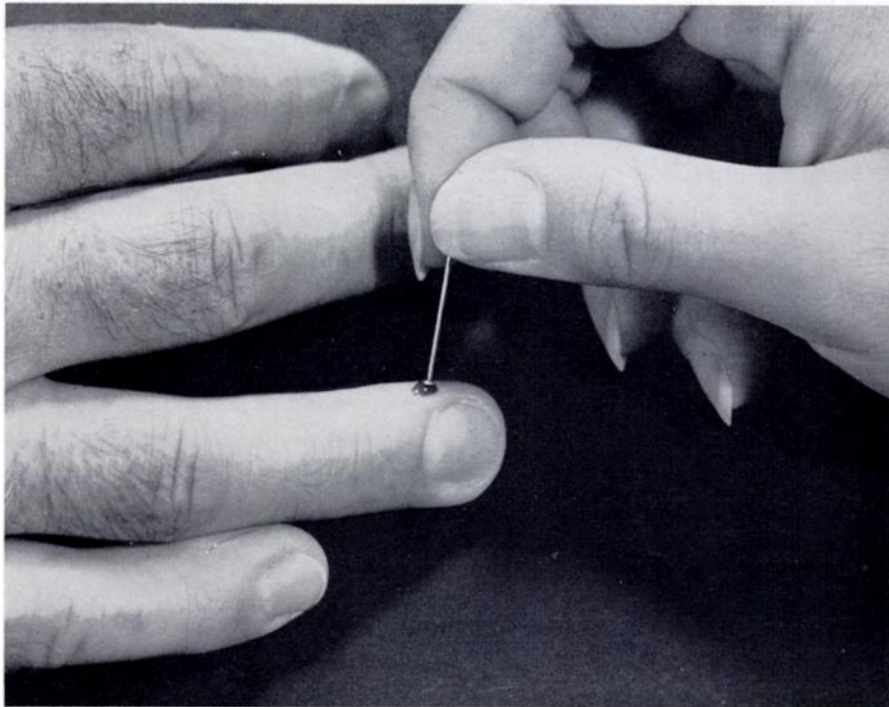


FIG. 1A.—The pinhead preparation. The head of a straight pin is immersed in a drop of blood. When the finger is pricked on the dorsum where the skin is thin, the pin point is a satisfactory lancet.

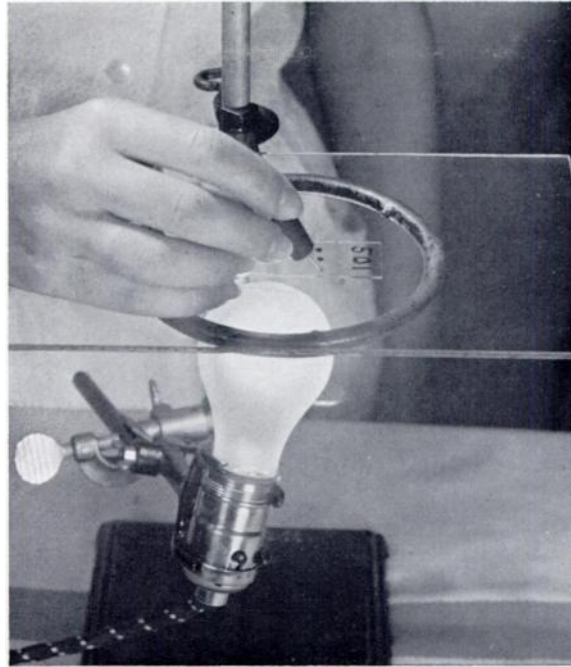


FIG. 1B.—The accuracy of the estimates is improved by rapid, but not too rapid, drying. This can be done by adjusting the distance between the slide and the electric light bulb or by using a blast of warm air from an electric hairdryer directed against the bottom of the slide.

period of training and experience the white cell count can be estimated by comparing the microscopic field with photographs of standard preparations (fig. 3). A set of training slides is a useful means of teaching the pinhead method. Slides labeled with the determined white cell count are studied and used for practice before estimates of unknown white cell counts are attempted.

A Suggested Procedure For Applying the Pinhead Method To the Screening of Mass Casualties

Where hundreds or thousands of casualties are to be screened it is recommended that teams of two technicians be established at each available microscope. The casualties should be channeled to each team at the rate of 100 to 150 per hour. The procedure may be fragmented as follows:

1. *Coding of the patient.* The patient writes his own name and address on a piece of paper.
2. *Preparation of the slides.* Each slide is given a number. Drops of blood from five patients are placed on each slide (fig. 2). This is done by the first technician who also writes on the patient's slip of paper the number of the slide and a letter indicating the patient's relative position on the slide. The slips and slides remain in series.
3. *Staining of the slides.* When a slide basket has been filled, it is immersed in the staining solution, removed from the solution and rinsed in a dish of tap water.

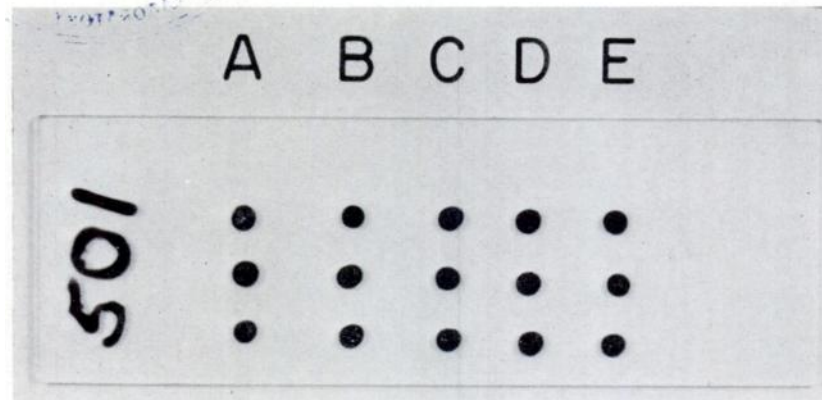


FIG. 2.—One numbered slide can be used for several patients. Position A is understood to be nearest the number.

4. *Reading the slides.* The second technician examines the stained slides, estimates the leukocyte count of each patient and writes the estimate on the patient's slip of paper.

DISCUSSION

The method for the estimation of the total leukocyte count is recommended not only by its simplicity and speed but also by its economy and flexibility of means. The most elaborate item needed—the microscope—can be found in many places besides the hospital laboratories: in doctors' offices, high schools and toy shops. Formaldehyde was selected as the fixative because of its widespread availability at drug stores, hardware stores and undertakers, in addition to pathology laboratories. Giemsa stain can be found in all laboratories. Wright's stain and other chromatin stains work equally well. Rit dyes, Script red ink, and Tintex dyes were tested and found adequate. Hair dyes proved unsuccessful. The pin itself can be used as both lancet to prick the skin (fig. 1) and as the blood carrier. A number of pins can be stuck into a board and sterilized by baking. The points remain sterile, and the pins can then be withdrawn from the board as needed. The slides can be cleaned and used many times. A technician who can use a microscope can be trained in 10 minutes to identify leukopenia, if not to make an accurate estimate of the total count.

As a further measure of its usefulness, the method was subjected to a field trial in a tent laboratory at the Nevada desert during the atomic tests of the 1957 series. Over 2500 pinhead counts were done and compared with standard chamber counts done simultaneously. At leukocyte counts of less than 15,000 per cu.mm. the agreement was better than ± 25 per cent (fig. 4). There were no developments to indicate that the method was not practicable under these relatively primitive conditions.

The refinement of controlled drying of the blood drops would be unnecessary in mass screening for serious radiation. Leukopenia is unmistakable whether the drop has dried too fast, too slow, or just right. With a carefully

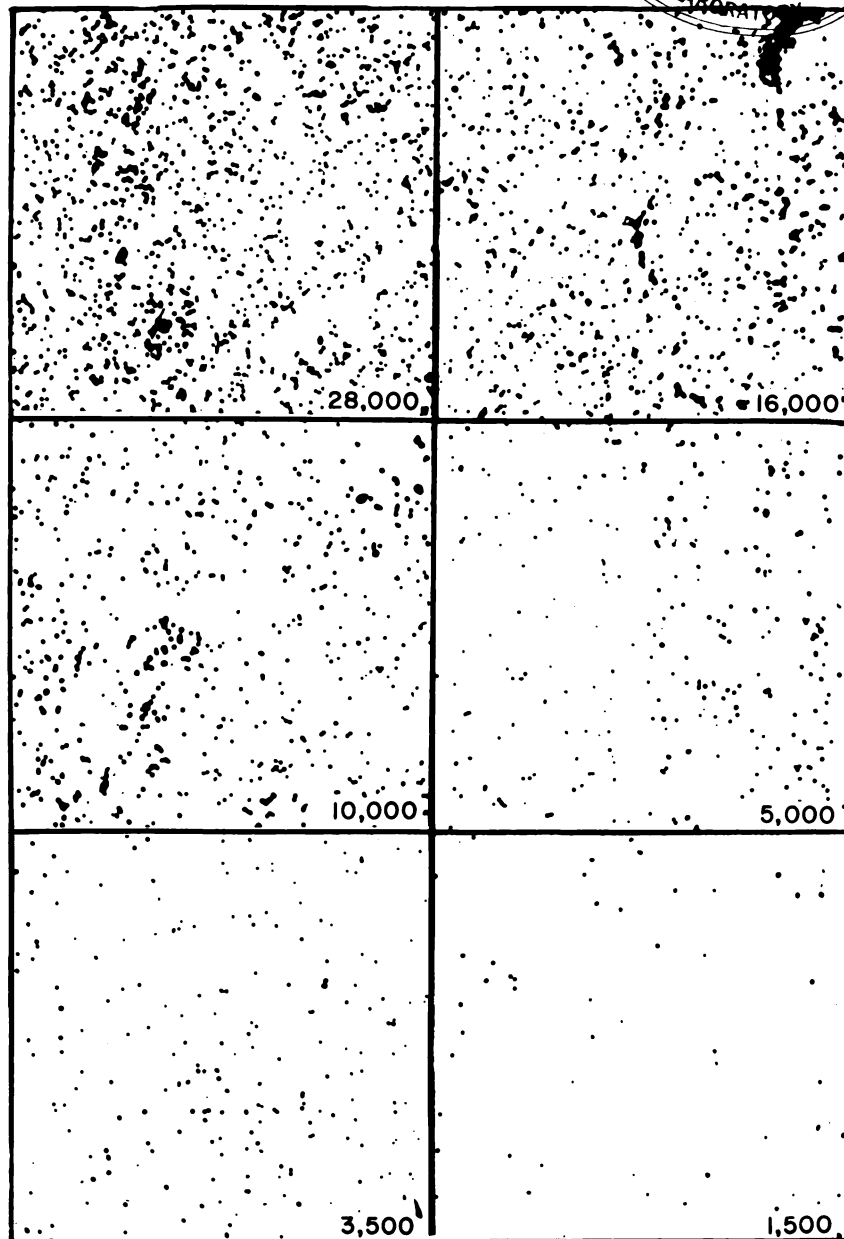


FIG. 3.—Photomicrographs (x 85) of pinhead preparations. Figures in the lower corners represent corresponding chamber counts.

dried preparation, a consistently and reasonably accurate estimate of the leukocyte count is possible. For this reason, it has been suggested that the pinhead method would be an adequate and economical screening procedure in the hematology laboratories of large hospitals. Most "routine" white cell counts are within the normal range, therefore most of the technicians' time

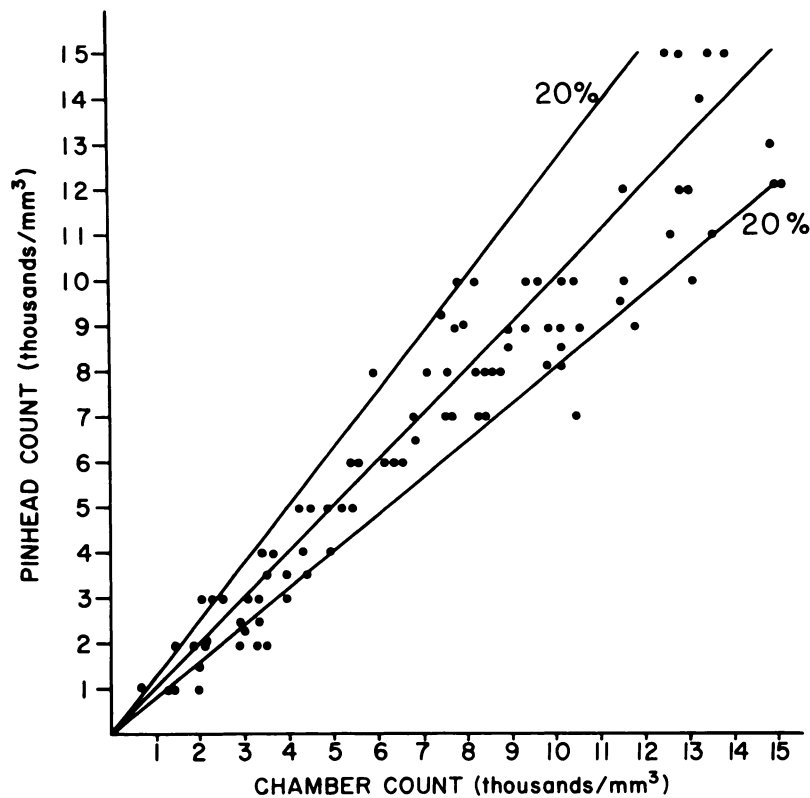


FIG. 4.—Correspondence between chamber counts of leukocytes and estimates by the pinhead method on 84 consecutive trials by three technicians. The counts were done in the field during 1957 series of weapons tests in Nevada on pigs exposed to atomic radiation. A small knife wound was made on the skin of the ear to obtain the blood. This field trial was made by SP3 Raymond S. Payne, SP3 Joe S. Evans and Pvt Benjamin Nakamura to whom I am indebted for these data.

is spent on normal counts. The pinhead method can be used to screen the leukocyte counts and a standard chamber count reserved for any blood that appears abnormal or even questionable. When a large number of counts is done the saving in time is considerable. The chamber count, when one includes filling and cleaning of the pipet, requires from 7 to 10 minutes. The pinhead method requires 60 to 90 seconds. A further saving would result from the reduced investment in pipets and counting chambers.

SUMMARY

A technic has been devised for the estimation of the white blood cell count by examination of a stained drop of blood of standard size. The drop is measured by immersing a pinhead in the blood and then touching onto a microscope slide the blood which adheres to the surface of the pinhead. The drop is dried and stained and the count is estimated by comparing its microscopic appearance with photographs or "standard" preparations. It was

developed to be used as a screening procedure in case of atomic war, but it may prove useful in epidemics or as a routine screening method in the hematology laboratories of large hospitals.

SUMMARIO IN INTERLINGUA

Esseva disveloppate un technica pro le estimate numeration de leucocytos per examinar un gutta de sanguine colorate de dimensiones standard. Le gutta es mesurate per immerger le capite de un agulia in le sanguine e per toccar con illo un lamina porta-objectos. Le gutta que adhere al lamina es siccate e colorate, e le numeration estimate es obtenite per comparar le apparentia microscopic con photographias de preparatos standardisate. Le methodo esseva disveloppate como technica de tests de detection in massa in caso de guerra atomic, sed il pare possibile que illo va provar se utile in epidemias o in le routine del laboratorios hematologic in grande hospitaes.