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JOAQUIN BENAVIDES, L. T.
Panama, R. de P.
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COMMENTS AND PROCEDURE ON THICK BLOOD FILM TECHNIC*

JOAQUIN BENAVIDES, L. T., PANAMA, R. DE P.

THE advent of the thick-film method for the examination of dried blood films that combined laking and staining in one step was a fine advance in laboratory diagnosis. Barber and Komp's[1, 2, 3] modifications to improve the quality of the preparations and simplify the mechanical handling of the slides for mass rural survey work was another distinct advance in technic. This is an excellent time-saving method for community work and can be applied equally well to surveys of domestic and wild animals if confined to mammalian life.[2, 3] The benefits derived from the relatively new method certainly invite a much wider use than at present prevails among field, clinical and research workers. This is particularly true for those who are located in tropical regions. It permits the use of four or five times as much blood as can be made into a satisfactory thin film and reduces the searching time from about thirty minutes to three or five minutes. A vast number of large parasites such as microfilaria, trypanosomes and even the large forms of malaria parasites are carried away by the instrument used in spreading a thin film. None of the blood taken by the thick-film method need be lost by the use of a spreading or stirring instrument. Cycles in the chronic stage of a disease can be more easily followed. It is even helpful in preliminary diagnostic work in revealing the presence of leucemia, leucopenia, leucocytosis, eosinophilia and polychromatophilia. The writer recently found a case of myelogenous leucemia during the course of a survey for malaria. This man had not been under treatment and was not aware that he had a condition that warranted attention.

An experienced technician can examine with a fair degree of accuracy 125 thick films in seven hours provided that the films are well prepared and do not exceed a half inch in diameter. The writer's experience covers twelve years in the tropics and four years of that time have been very largely spent in thick, blood film work. This personal experience with the method is not given with the intention of matching it with others who may already employ the method but rather to stimulate fellow technicians in its use and to mention the latitude the technic permits in its application.

Slides.—One should be sure that all slides fit the slide boxes. Frequently, they are too long or too short or even too thick. Very thin slides offer trouble with the use of the mechanical stage. We use green tinted slides rather than the colorless or clear slides. They are capable of longer use and stand cleaning better.

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New slides should be cleaned and soaked in alcohol before use, and it is very necessary to have them perfectly dry and free of dust as well as fingerprint grease.

Used survey slides should have the cedar oil and stained blood film removed as far as possible by rubbing them with gauze moistened with xylol before they are immersed in a hot (not boiling) solution of a mild soap. The slides are then washed individually in running water and then immersed in 70 per cent alcohol overnight. They should be dried with a clean gauze cloth and placed in the standard cardboard shipping boxes lined with gauze. Dust from the cardboard will collect on them after long travel in boats, cars, or on mules unless this measure is taken.

Equipment.—The equipment required depends on whether it is intended for hospital or field work. None is needed for hospital work except that an extra slide should be taken on which to make the film. One large drop of

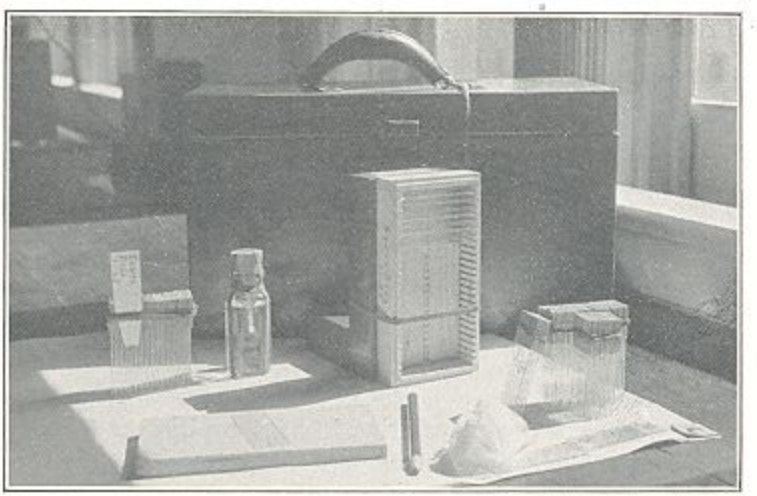

Fig. 1.—A view of the slide box and its stand, the field case, the needle bottle and a block of slides with the identification tag.

blood can be maneuvered into a circular spot about half an inch in diameter at one end of the slide. This must dry thoroughly without causing irregularity in the film margin else time is lost in examining the protrusions that form. The film must be at least a quarter of an inch away from the end of the slide to permit the mechanical stage to cover the entire film without interference from the objective. The only thing that fastens this film to the slide is thorough drying. The slide is stained standing on end to save staining solution and prevent the collection of débris on the film.

Field equipment must be assembled to meet the needs for the day. One must have some knowledge of how many people or animals are to be included in a survey in order to provide sufficient slides. In the case of people, squares of gauze and a bottle of 70 per cent alcohol are needed to clean the skin of the ear, finger, or toe from which blood is to be taken. A two-ounce bottle of alcohol with a No. 5 Hagedorn needle inserted in the cork makes a good blood-lancet. Each time the cork is replaced the needle is submerged in

alcohol thus preparing it for the next person. One should dip the blood-lancet needle up to the cork in paraffin to prevent rusting if it is not in daily use. A wax pencil is required to number the slide on the end not occupied by the film. A lead pencil is wanted to record these numbers in a field book with the name, age, etc., to follow.

Slide boxes with a capacity of 25 slides are used and on these boxes are entered the village, school or organization with the first and last numbered slides. These boxes should be held tightly closed by an elastic band and kept standing in a vertical manner until the blood films are quite dry. To do this, while a slide box is being filled, I use a right-angled stand made of blocks two inches thick. The upright block is the same height and width of a slide box. Another block 4 inches long and the same thickness and width is nailed to the bottom of the upright piece. This makes the base that rests on the table or stand. The opened slide box is then strapped to the upright by an elastic band. This keeps the box level so the films can dry without running. On removing the slide box from this stand it is placed in the same vertical position in the carrying case.

Field Case.—The use of leather cases has been abandoned, since the field cases are subjected to all manner of transportation and to heavy rains. This laboratory uses a metal case 8 by 9 by 16 inches with a leather handle similar to that of a suitcase. This case will hold all equipment needed for 400 people or animals. These cases are really carpenter's tool kits made by the Kennedy Manufacturing Co. at Van Wert, Ohio, and Chicago, Ill. They come with a tray but we discard it. These cases are very much less expensive than any made of other materials and they stand severe usage as well as being rain-proof.

Animal Surveys.—The only variation in field equipment for these surveys is the addition of a scissors to clip hair off the point of an ear, or to expose the tip of a tail in small animals. A scalpel is also added. This is drawn lightly across the point of the denuded ear and then by pressure drops of fairly clean blood can be removed by contacting the slide. Always wipe the scalpel with alcohol before use on the next animal and apply iodine to the ear wounds. In some animals like the sloth and bat, a toe nail or thumb nail must be cut off to secure blood.

Source of Blood for Film.—There is some divergence of opinion as to the most suitable place from which to draw the blood. The writer, for field work, prefers the lobe of the ear as being less sensitive, cleaner, out of sight of the patient and as having some degree of capillary stasis. Parasitized cells are believed to be more abundant in such a system of capillaries. When very young babies are to be included in a survey, it is better to use the ball of the great toe. Many use, as a routine measure, the skin near the base of a finger nail. The Daland's blood-lancet is a very dissembling little instrument that does not necessarily require sterilization. It can easily be covered up with the same gauze used for cleansing the area to be punctured and the puncture is made with a firm quick stab without the patient knowing what moment it is to occur. This is an important point in the management of

nervous people. I have used this lancet on over 6,000 patients without the occurrence of local infection. Care should always be taken to dry the skin and to clear the puncture of any alcohol since blood expressed through or over an alcohol wet surface will fix the cells, and they will not lake as thoroughly as they should. A dry skin allows discrete drops of blood to form at the site of the puncture, and these are sure to be cleaner than blood rubbed off the skin. The blood specimen is stirred or maneuvered into a circular spot about the size of a dime. It is then given its place in the slide box and dried thoroughly before the vertical position of the box is changed. The field notebook will receive the same number given the slide and identification data will be entered on the same line with the number. If care is taken to secure a large drop of blood, there will be no need to stir it into a spot the size of a dime, since it will spread to the proper size if given a level position. Some place the slides with the blood film upside down in the box and place the wax pencil number on the other side at the opposite end. I like the number and the film on the same side of the slide but, of course, at opposite ends. The blood film end of the slide should always occupy the same side of the slide box else it will be difficult to block the slides in 25 lots for the staining bath. Much time is saved and better results are obtained if sufficient blood has been spread in a uniform manner and in a discrete spot about half an inch in diameter. Search the entire film, do not depend on the *thickest focus in the film to always supply the information you seek.*

When a slide box is filled, it is covered and the lid bound on the box with a good rubber band. The necessary information is written on the side or bottom of the box with a lead pencil. For example: Santa Rosa School, slides 1 to 25 or Santa Rosa School, slides 26 to 50, etc. These boxes are kept in a vertical position in the field case until the close of the day or until the following day. It is always better to stain them within twenty-four hours, since they lake with increasing difficulty after that period of time. We have, of course, used such films even after a month or two of drying but they are not as satisfactory and the blood film is apt to crack in many lines, and segments of the film may even be lost. I have been able to use films that were six months old but they are not desirable films. Animal surveys are frequently done in barns and whether the floor is of dirt or boards it pays to wet down the floor to avoid dust settling in the blood films. It is even more necessary to avoid destruction of the films by flies, ants, etc. Flies can remove a large part of a film in a very short time. Care must be taken to guard against breakage or leakage of the alcohol and iodine bottles carried in the field case, since a whole day's collection may be ruined for the staining and examination. Stock bottles are protected by several vertical and transverse rubber bands which not only serve to keep the corks in a tight position but also protect against the jarring of the bottles against each other during transportation.

Thick blood films will dry in a half hour during the *dry season* but in the *rainy season* it is always better to dry them a half hour by placing the closed boxes in the incubator at 37° C. The lid of a box is now removed and pieces of cardboard one inch square by 1/16 of an inch thick are dropped in the spaces between the slides on the ends where the identification numbers have

been written. It is well to drop one between the box and the first slide as well as between the last slide and the box. These cardboard separators are to provide a staining space between the slides. The lid is now replaced and the box turned wrong side up on the table, the bottom of the box is raised slowly taking note that no slides are fast in their brackets. The slides are then brought compactly together by a thumb and finger and moved to the side of the lid where the separators are then pushed flush with the ends of the slides. This is best done by lightly holding the block of slides while a scalpel blade pushes all separators into good position. These separators add protection to the numbers unless too firm a grip is used while moving them into position. In such a case they smear the wax pencil numbers and make reading difficult. The block of 25 slides is then lifted up to the edge of the lid while a good elastic band is placed about the numbered ends of the slides. A second band should then be applied over the first to guard against the breaking of the one band and the unfortunate release of the block of slides. Some prefer a rubber web for this instead of pure rubber bands. Rubber bands 3½ inches long by ¼ inch wide are used in our equipment. They are long enough to allow two turns of the band and the firm pressure is desired. This makes a solid block that can be handled with ease and safety. Beneath the rubber bands, a small strip of white card is adjusted and on this card is transcribed the entry made on the slide box such as: Santa Rosa School, 1 to 25. Once the boxes have all been blocked in this manner the blocked slides are ready to be stained.

Staining Method.—Giemsa's stain is probably universally used. It is an expensive stain when purchased ready for use and after long periods of storage some of the bottles of stain deteriorate. Its preparation from the powder is so simple, the results obtained so satisfactory and its price so greatly reduced that I see no reason why the stain should not be prepared in any laboratory. We estimate it at 75 cents an ounce (stock stain) when made by us. This laboratory does not use Azur II as is the case with the old formula. I place 2.4 gm. of Azur II eosin (Grübler) in a clean Erlenmeyer flask and then add 200 c.c. of C. P. anhydrous glycerin. The flask is covered with a well-fitted cork and then placed in a water-bath at 60° C. for thirty minutes. The flask is shaken once or twice during this period. It is then removed from the bath and 200 c.c. of C. P. methylic alcohol are added and after mixing the contents the flask is again placed in the hot water-bath for another half hour, shaking it once or twice during this period. It is then removed and left in an incubator overnight at 37° C. Next day it is passed through filter paper and stored in 200 c.c. bottles that have been thoroughly cleansed, washed with methylic alcohol, and dried before use. These are well stoppered and kept in the dark. I use equal parts of glycerin and alcohol rather than the larger amount of alcohol advised by some. The liberal use of alcohol is undesirable since thorough laking of the cells is the main object in thick film work. Everything used in the preparation of a stock of Giemsa stain should be as clean as the stock bottles in which it is stored. I use a set of glassware exclusively for this work. Application of the stain requires a dilution of this Giemsa stock

in a proportion of 1 c.c. to one ounce of water. Pour this mixture back and forth about four times and then consider the solution ready for use. The blocked slides are placed in the staining dishes resting on the film ends of the slides with a quantity of stain just sufficient to cover the films. Our service uses the rectangular staining dish 34 by 59 by 82 mm. inside measurement and these hold 3½ ounces of the staining solution and will receive 50 slides. White enameled trays 1⅛ by 2⅞ by 7½ inches (inside bottom dimensions) will accommodate 150 slides and use 9 ounces of staining solution. The slides are left in the stain for one and one-half hours, and then very gently dipped in two changes of water like that used for making the staining solution. They are then dried by standing the blocks of slides on paper that absorb water rapidly such as paper towels. We usually leave them overnight but drying can be hastened if necessary. The films must be thoroughly dry before they are examined. The authors[1, 2, 3] of the modified thick-film method that this

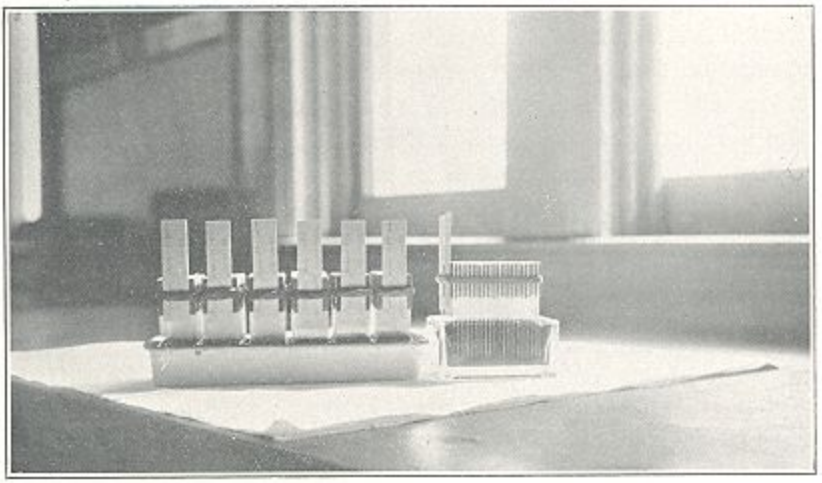

Fig. 2.—An instrument tray with 150 slides and the smaller staining dish with 50 slides in the aqueous solution of Giemsa's stain.

laboratory uses state clearly that the water used for diluting Giemsa stain should be neutral, or only slightly alkaline (pH 7.0 to pH 7.2), and must be nearly or quite free from salts. It has been our experience that clean, fresh rain water and our local city water supply all serve well without treatment for this purpose. Freshly distilled water needs to be corrected. There is always the risk that some accident or repair to the water supply system might cause the loss of a day's collection of slides but thus far it has not and I like our results as well as with water corrected to the proper values. There is considerable latitude in the character of the water to be used but one must stay within reasonable bounds. Much discussion has arisen from the formation of greenish yellow crystals that occasionally cover the film and make examination difficult. This accident will not happen if the staining dishes are kept thoroughly clean between their periods of use.

The complete examination by a trained technician of a well-stained thick film a half inch in diameter will require about three and one-fourth minutes.

There are three factors, at least, that must be taken into consideration in an efficient examination of a thick film, these are as follows: (1) one must be acquainted with the parasites for which he is searching, (2) the entire film should be searched, (3) a proper light and magnification must be employed and a sufficient amount of good immersion oil (cedar oil) to cover the search must be applied to the film.

One familar with thin films stained with an alcohol polychrome stain will find the thick film picture confusing for a time since all the red blood cells are gone. The field contains leucocytes, platelets, shadows of red cells, and parasites. It will not take much time for one who knows the parasites and is acquainted with thin film work to learn the thick film method, and for diagnostic work it is a most desirable method. There is more upon which to base an opinion than the sparse findings of a thin film. Both methods have their particular points of value but for mass survey work the thick-film method of today fills a most important need. More mixed infestations of malaria, scanty trypanosomal and spirochetal carriers are being found and in wild animal life it is uncovering many facts that are desired in the way of the incidence of filariasis,[4] trypanosomiasis,[5] spirochetosis,[6] piroplasmosis, anaplasmosis, etc. Naturally the different species of parasites vary a great deal in the number that may be found in a film. The monkey spirochete at the apex of an acute attack may show a countless number to a microscopic field while a trypanosome like *T. cruzi* may never reach a figure above 40 to a field and certain microfilaria may show but a few to the entire film. Scant infections are far more frequently found with this film technic. The writer and his fellow technicians have, during the past four years, examined from man and animals about 200,000 thick films. This diagnostic method is capable of just as wide use in veterinary medicine as it is in human medicine and affords an excellent yardstick to measure the results of sanitation and malaria control. The chief purpose of this thick film method is the diagnosis of parasites in mammalian blood. It is not intended for a study of the characteristics of these parasites or their relationship to the red blood cells.

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