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THE LABORATORY ORGANIZATION AND TECHNICAL PROCEDURES FOR BLOOD
EXAMINATION OF THE MALARIA ERADICATION PROGRAMME IN CEYLON

by

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A laboratory for parasitological and entomological examinations is an integral part of any malaria eradication organization. Such a laboratory had existed from the inception of the Anti-Malaria Campaign in Ceylon. Routine and special investigations in respect of malaria parasites, and anopheline species with particular reference to the local malaria vector, were undertaken. The study of the epidemiology and methods of control was pursued for evaluation of results.

A combined laboratory which was already in existence in Colombo, the headquarters of the Anti-Malaria Campaign, was divided into separate laboratories for the intensification of parasitological and entomological work following the inauguration of the malaria eradication programme in 1958. The parasitology section which had grown in magnitude and assumed greater importance remained in Colombo as a self-contained laboratory under the immediate control and direction of the chief of the malaria eradication programme and his technical assistants. The entomology section was moved to a second laboratory set up at Kurunegala which is 58 miles (93 km) away, this being a more suitable place for entomological investigations and experiments. This laboratory has been placed under the direction and control of the entomologist of the campaign. The present paper deals only with the parasitology laboratory.

1. Parasitology laboratory

A central laboratory has manifold advantages over multi-located laboratories in organizing and equipping, effective supervision and efficient functioning, and for uniformity and better co-ordination of field operations with laboratory findings.

However the prime factor that should influence the decision to centralize the laboratory is the availability of facilities for quick transport of specimens from the field and outstation institutions for laboratory examination and immediate reporting of results. The postal services of Ceylon are very well organized; this facilitates the daily dispatch, and receipt of letters and parcels within 24 hours at the central laboratory from any part of the country.

The staff that had functioned during the time of the national malaria control programme which began in 1946, was considerably augmented with the implementation of the eradication programme in November 1958.

The present staff consists of seven technicians, 99 microscopists and six minor grade employees.

The chief technician in charge is an experienced senior medical laboratory technologist. He is assisted by other medical laboratory technologists and laboratory sub-assistants. The medical laboratory technologists are trained at locally conducted training courses by the laboratory services of the department of health in all aspects of medical laboratory work. The microscopists are specially recruited by selection and trained, from candidates who have the senior school certificate including a pass in at least one science subject. The microscopists undergo a three-month course of training before commencing independent examination of blood slides which are gradually increased in number until the standard daily quota of 65 slides is reached. All persons in the laboratory except the minor grade staff have been trained in staining and microscopic examination of blood slides for malaria parasites.

Taking of blood slides and their dispatch to the laboratory. Blood slides are taken from fever cases as a routine activity, on the principle of sampling every fever case by the following grades of officers of the Anti-Malaria Campaign: 218 active surveillance agents, 125 passive surveillance agents attached to medical institutions, 33 surveillance unit officers and 26 supervisors of active surveillance agents. The field officers carry out mass blood surveys in epidemiological investigations of positive cases and at six monthly intervals for two years in areas where positive cases were detected.

They also carry out follow-up slide-taking from all positive cases for two years. Routine taking of blood slides from fever cases is carried out by the staff of 221 medical institutions in the endemic area and 53 medical institutions in the epidemic area. The staff of the 40 remaining institutions in the epidemic area and 225 medical institutions in the non-malarious area, take blood slides only from clinical and suspected cases of malaria.

In large medical institutions with laboratories attached, not less than five blood slides are taken from clinically positive cases in addition to the routine slide-taking from all fever cases. One set of smears is examined at the local laboratory in order to check the microscopic examination at the central laboratory. All other blood slides taken throughout the country are dispatched by post on the same day or the following day to the central laboratory of the Anti-Malaria Campaign.

2. Organization of the laboratory

Extension and structural alterations were made to an existing building to make it suitable and accommodate the augmented staff and the additional equipment to match the increased work-load. Spacious windows were provided over three feet (90 cm) high walls on all sides in order to ensure adequate natural light for microscopic examination and good ventilation for the comfort of the staff. A sufficient number of ceiling fans was installed to contribute to the comfort which is essential for diligent microscopic examination involving long hours of a monotonous type of work. A diagrammatic sketch of the parasitology laboratory and the table arrangements is given in Figure 1.

Distribution of duties. The technician in charge is responsible for assignment of duties to the staff according to the directions of the supervising medical officer and for the supervision of work (checking for missed positive blood slides ^{or} back log of unexamined slides). He maintains the concentration and interest on microscopic examination by periodical surreptitious introduction of positive slides. This keeps the microscopists on their toes as commendations are received by those who detect positives and disciplinary action awaits those who miss them. He is also responsible for the preparation of indents for laboratory equipment and other requirements, and for the training of the laboratory staff and maintenance of discipline and order. He personally checks all positive

blood slides and with the assistance of senior technicians ensures that not less than 10% of the negative blood slides are re-examined. He sees that all records are properly maintained up to date and positives are immediately notified to the field staff for prompt action. He is assisted by a senior technician in his duties, who deputises for him in his absence.

The main duties of senior technicians are the checking of blood slides examined by the microscopists and the training of new recruits. Senior technicians also examine certain blood slides which are received in registered parcels which indicates that they warrant priority.

The laboratory sub-assistants are responsible for the preparation of stains and staining of blood slides.

Each microscopist examines 65 blood slides per day, devoting two-and-a-half hours in the morning and three hours in the afternoon to microscopic work. One hundred microscopic fields of the thick film are examined; this takes four to five minutes on each slide. The thin film is stained and examined only if the thick film is positive for malaria parasites. Records of blood slides examined are kept by the microscopists on a half hourly basis for checking the progress of work. The assistance of senior technicians is sought to clarify doubts and to confirm results of positive blood smears. Microscopists are subject to disciplinary action for missing positive blood slides and receive commendation for detecting malaria parasites. The number of positives detected, regularity of attendance and punctuality are given due consideration in assessing the merits of microscopists. All clerical work in the laboratory including typing is attended to by microscopists who work in rotation to keep in touch with microscopic work.

Six lower grade employees attend to minor duties such as boiling and cleaning of slides, washing and cleaning of equipment, packing and unpacking of parcels, dusting of furniture and sweeping, preparation of distilled water and any other such duties they may be called upon to perform.

Suitability of female microscopists. When the number of microscopists was considerably increased with the switch over to the malaria eradication programme, maintenance of discipline became difficult and at one stage even threatened to disrupt laboratory work. To meet this situation, a decision was taken to recruit female microscopists and reduce the proportion of males in the laboratory. This change had salutary effect and at present there are 35 male and 64 female microscopists. The males and females work separately in different wings of the laboratory.

From two years' experience, it could be said that both groups are equally capable and efficient but both require supervision to get work done satisfactorily. The females are more amenable to discipline and show greater patience to sit at one place continuously and concentrate on microscopic work. Both groups require periodic introduction of positive slides to maintain diligence in the examination of slides.

In general it should be said that females are better suited for microscopic work particularly in large laboratories with large staff, but having both sexes working together produces a sense of competition which is conducive to better turnover of work and discipline.

Technique of preparation and staining of blood slides. Thick and thin blood films are taken on the same glass slide using one slide for each person. The serial numbers are written on the thin films by a sharp pencil and correspond to the serial numbers in the blood slide record form in which are also entered the name, age, sex, and correct address (to enable the tracing of the cases without difficulty), fever history, drugs administered, whether blood slides have been taken before etc., of all persons whose blood slides are taken. The name of the officer taking blood slides and the unit or institution and date are also entered. The blood slides are wrapped in the corresponding blood slide record form and dispatched to the central laboratory. One set of serial numbers is used by each officer for blood slides taken during each month.

Blood slides taken in epidemiological surveys of positive cases, follow-up cases and from individuals and groups likely to harbour infection are dispatched on the day of taking, by registered parcel post to ensure safe delivery and immediate attention on receipt. Empty milk food tins and other suitable containers are collected from the nearby medical institutions for dispatching blood slides and cleaned slides.

Staining of blood slides. When received in the laboratory the thick and thin films are separated by marking a bold line across the slide by a glass marking pencil. The thin film is fixed by dipping that portion of the slide for 10 to 20 seconds in methyl alcohol which is in a shallow glass jar just deep enough to cover the thin smear only. The slide is then placed on the staining rack for the evaporation of the methyl alcohol.

Staining of thick film by Giemsa. Only the thick film is stained by Giemsa solution which dehaemoglobinizes at the same time. The solution is prepared by adding 150 drops of Gurr's Giemsa concentrate to 100 ml of buffered distilled water of pH 7.2. The blood slides already dipped in methyl alcohol are arranged lying flat side by side in rows on staining racks placed on a long table. The Giemsa solution is poured rapidly from a dropping bottle by moving it over each slide so as to just cover the thick films only. After 40 minutes, the stain is gently washed off by running water from a rubber tubing connected to a water tap and moved over the slides in the same order and speed at which the staining was done. The slides are then kept in a slanting position until they are dry. Five hundred blood slides can be stained at a time on the staining table.

Staining of thin film by Giemsa. The thin film which has already been fixed is stained only if the thick film was found positive for malaria parasites, in order to identify the developing stages of the parasites. The blood slides are placed on the staining rack and individually stained by pouring a few drops of Giemsa solution in the same concentration as for the thick films just to cover the thin film. After 40 minutes, the stain is rinsed off gently by running water from the tube connected to the water tap. Then the stained slides are left in a slanting position to dry before they are microscopically examined.

3. Daily routine

Letters and parcels dispatched from all parts of the country by ordinary post are delivered once in the morning and again in the afternoon. Registered parcels and letters are delivered once a day. The letters are sorted, supervised by the administrative officer, and those that have to be seen by the medical officers of the different regions are submitted to them, who direct the letters to the laboratory with instructions endorsed to the chief technician, while other routine letters not requiring the perusal of medical officers are sent directly to the chief technician.

All parcels are delivered directly at the laboratory. The registered parcels and other parcels are unpacked separately at table one (see plan of laboratory) and the blood slides wrapped in the record forms are passed to table four where the serial numbers on the slides are checked with the numbers entered in the corresponding blood film record forms and each batch of blood slides is given a new number which is written in the respective blood slide record form and in a blank glass slide, and after logging in a register, passed to the staining table (five). The officers who stain, arrange the blood slides on the staining racks in batches according to the new numbers given separating each batch by the blank glass slide on which the new number is written. Particular care is taken not to mix the slides and lose their identity. After staining, the slides are again wrapped in batches in the appropriate blood slide record forms which can be distinguished by the new numbers written on them.

The blood slides received in registered parcels are processed and stained immediately and passed to the chief technician who distributes them to the senior microscopists for immediate examination. The microscopic examination of these blood smears is checked by senior technicians and the chief technician himself.

The other blood slides received in ordinary parcels are similarly processed and wrapped in the appropriate blood slide record form after staining and passed on to table six from where they are distributed to the microscopists for examination.

Each microscopist maintains a notebook to enter daily the particulars of their work such as the batch number, the serial numbers on the blood slides examined and the time at half hourly intervals in order to check the rate of examination. The results of examination are entered on the respective blood film record forms. Senior technicians who are responsible for supervision of certain microscopists in weekly rotation, check all positives and 10% of the negative slides picked at random.

The microscopists pass the blood slide record forms with the results entered to the table 13. The following entries are made in the appropriate registers: the name of the institution or field unit that sent the blood slides, the date of slide-taking, date of examination, the batch number, serial numbers and the results of examination.

At table 13 the results from the blood slide record forms are entered in loose-leaf files maintained monthly according to districts, by medical institutions, active surveillance agents and surveillance unit officers. These forms provide for entering on each day during the month, the number of blood slides taken and sent to the laboratory and the results by parasite species. The days on which no blood slides have been taken are checked with the weekly progress reports of work done, and appropriate action taken against field staff who have failed to take blood slides or have taken defective slides.

Results of positive blood slides are immediately notified by telephone or telegraph to the surveillance unit officer, the regional officer and the officer who sent the blood slides, thus initiating a series of activities that follows the detection of a positive case. The surveillance unit officer will deviate from his routine work and contact the case on the same day or the following morning.

An average of about 3750 blood slides are stained and examined daily at present. About 250 blood slide parcels are received and 200 parcels of materials such as cleaned slides, cotton-wool, surgical spirits etc., and empty containers for sending blood slides are dispatched daily from the laboratory.

Only monthly requirements of glass slides based on the blood slides received are dispatched in order to prevent waste.

4. Some technical points

Care and use of microscopes. Out of 95 microscopes in stock, 70 are in daily use, a few binocular and others monocular. Fifty more microscopes are under order. The majority are with inclined ocular tube, others are inclinable models which suit short microscopists better. All are provided with 5x, 6x or 7x eye pieces and three objective lenses of 10, 40 and 100 times magnification.

The microscopes are issued to regular microscopists as one of the inventory items for which they are responsible. At the end of the day's work they have to devote some time to clean the objective lenses with special lens paper and the microscope generally by soft cloth. Then the lower power objective is brought to observative position and the body lowered gently to the lowest stop position and locked in the box. The keys are kept in a common cupboard by the chief technician who issues them in the morning.

Synthetic immersion oil is preferred to the traditional cedar-wood oil which tends to thicken with time and causes difficulty in removing it from the front lens of the objective and the stage. Liquid paraffin is preferable when synthetic immersion oil is not available. Use of xylene to wipe the front lens is avoided as it tends to loosen the mount.

A trained technician visits the laboratory once a month to attend to adjustments and minor repairs. For major repairs which commonly concern the objective lenses, they are sent to the manufacturers for attention. As this is expensive and takes time we prefer to purchase two oil immersion lenses of 100x magnification for each microscope sometimes in place of one of the low power objectives.

Storage and cleaning of glass slides. As thousands of imported glass slides are required for daily use, it is essential that they should be carefully stored taking all necessary precautions and used as long as possible.

It is important for tropical countries to insist on tropical packing when ordering large quantities of slides which have to be kept in store. Surface "frosting" occurs due to bad storage for long periods. The effective method of preventing "frosting" is to store slides in acid dichromate solution prepared from potassium dichromate 100 g, concentrated sulfuric acid 250 ml and water 1000 ml. The glass slides when taken into use after storage are thoroughly and repeatedly rinsed in tap water until all traces of acid have gone and then cleaned and dried.

Used glass slides are first boiled in soap solution for about two-and-a-half hours and then washed in running tap water. They are then cleaned, and after discarding unserviceable slides, methyl alcohol is sprinkled over, after which they are dried, wrapped in packets of ten and issued for use. Generally glass slides are used about six to eight times for taking blood slides, before they are found unsuitable and discarded. Scratch marks, chipping of edges and "frosting" are the common causes for which used slides are discarded as unserviceable.

Common errors in slide examination. Failure to number a blood slide by oversight is a mistake, occasionally detected when blood slides are received at the laboratory. It is easy to detect the missing number from the blood slide record form, in which the blood slides are wrapped, and enter the missing number on the thin film using sharp lead pencil. Failure to number slides should be avoided at the source by numbering as soon as the thin film dries.

When broken blood slides are received, the names and addresses of persons from whom the blood smears were taken, are obtained from the corresponding blood slide record form and sent to the officer who took the blood slide, or active surveillance agents with a request to take another set of slides.

Disciplinary action for missing and commendations for detecting positives may sometimes tempt staff to introduce positive slides to earn commendations. It is not difficult to detect such introduced slides from the running series of films by comparing the blood films on slides of the same batch. There are differences in staining noticeable in old stained slides introduced and epidemiological investigation of the false positive cases thus reported is revealing.

Missing positives in scanty fields of parasites in blood slides taken from areas of disappearing malaria, is not altogether unexpected and every effort is made to prevent this by devoting five minutes for each slide examination and intensifying the checking of blood slide examination. The tendency to rush through the daily quota of blood slides when positives are extremely rare and detected once in several days, is curbed by requesting microscopists to keep a time record of the number of blood slides examined each half-an-hour.

False positives and negatives. In taking thousands of blood slides daily, mass staining and mass examining them for parasites, false positives and false negatives are not a rare occurrence. Microscopists during training are taught theoretically and visually, over the microscopes, the identifying characteristics of thick and thin film specimens of parasites, in the developmental stages. The fundamental objective is to spot the parasite and identify the species in the thick film by the chromatin and the cytoplasm disposition. The presence of red stained chromatin with pale blue stained cytoplasm around or attached to it, should arouse suspicion of a parasite. Examination

of more microscopic fields is sure to bring into the ken more such objects with better defined characteristics of a parasite. Yellow brown coloured pigment will be seen associated with the older trophozoite stages onwards. Differentiating characteristics of the rings, older trophozoites, schizonts, number of merozoites, gametocytes, size of the parasites, the quality and quantity of pigment should enable a trained microscopist to identify the species in a thick film and discern the different developmental stages by the thin film examination. Characteristics such as multiple rings, more than one chromatin dot, rarity of intermediate and matured stages, unmistakable shapes of parasites etc. will help the identification.

Artefacts in thick film which simulate the appearance of malaria parasites can confuse inexperienced microscopists. Dust particles, bacteria from skin, vegetable spores, fungi, pollen, particles from the markings on slides by grease pencil, protozoa and other contaminants from prepared stains are commonly encountered artefacts. Many of these can be prevented by strict observance of the common rules, to clean the slides properly, not to touch their surfaces with fingers, wipe the finger-tip clean with spirit before taking blood film, prevent exposure of blood slide and contamination during the preparation of stains. Artefacts can be identified by their higher refractivity and different optical plane and difference from the characteristics of malaria parasites.

In a thick film which has several layers of erythrocytes, it is not uncommon to see parasites distorted or folded, making them difficult to identify. The doubts have to be cleared by identifying other well-defined forms in the microscopic field.

Blood platelets which also stain red can be a source of error to an inexperienced microscopist. A few platelets can look like parasites and a mass of platelets can be mistaken for separate merozoites.

All these errors can be overcome by looking for more typical parasites when in doubt and consulting experienced technicians in all instances of doubt. Similarly a positive parasite should be confirmed by consulting the more experienced technicians.

5. Advantages of central laboratory

A central laboratory to handle a large number of blood slides has a number of advantages over several decentralized small laboratories. It is easier to concentrate attention on one laboratory, equip it well, appoint all the available trained staff to it, and run it efficiently, than pay divided attention to many small laboratory units. The other advantages are, on-the-spot over-all supervision and personal attention to remedy shortcomings immediately, prompt issue of orders for co-ordinated field action on laboratory findings and economy in running one laboratory than several at different places. It will also be difficult to find suitable building accommodation at many places to run temporary laboratories for the duration of an eradication programme and persuade trained staff to go to decentralized stations which may not be to their liking.

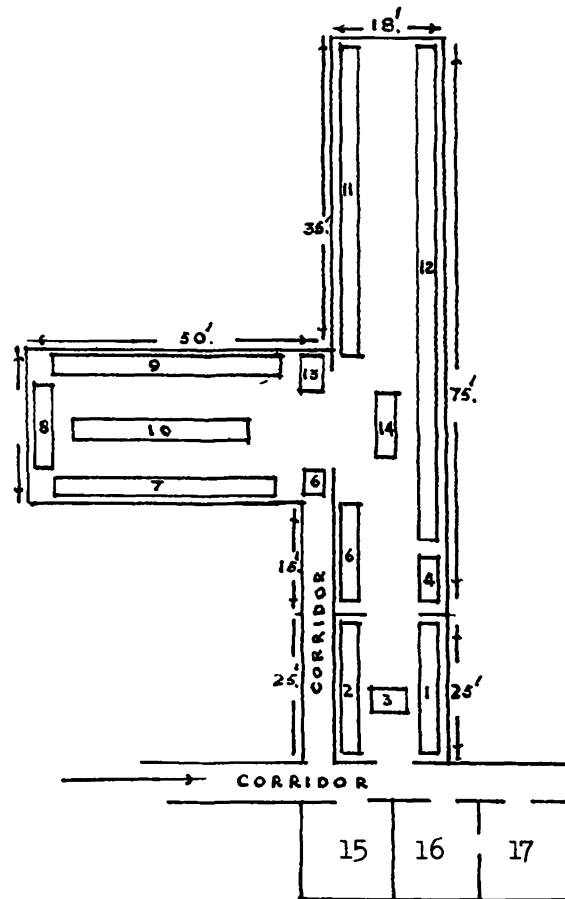
Mass handling of slides, of course, requires sound organization, efficient supervision and a foolproof system of processing blood slides from the time they are received up to the time the results of microscopic examination are reported, so that there is no chance of mixing up of or loss of blood slides, breakages are prevented and no mistakes made in entries.

Experience has shown that the staining on mass scale can be done more cleanly and efficiently with individual slides on large slide racks without devoting much extra time than by the method of bulk staining in troughs which has shortcomings such as transfer of parasites from slide to slide and deposit of particles from floating scum in staining solution, on blood films.

A well-organized and efficiently functioning central laboratory is more productive and easier to manage than a number of small laboratories, some of which have to be housed in improvised buildings, and vary in organization and efficiency according to the capabilities of different officers placed in charge.

[However, many of the advantages of a central laboratory would be outweighed by inefficient and slow transmission of slides from the periphery as mentioned earlier in the article. Editor's note.]

FIGURE 1. PLAN OF THE CENTRAL LABORATORY OF THE MALARIA ERADICATION PROGRAMME IN COLOMBO, CEYLON



1. Table for unpacking blood slide parcels received and packing glass slides and empty containers for dispatch.
2. Table for cleaning and wrapping slides in bundles.
3. Table for supervisor of work done at 1 and 2, issuing and receiving articles in store and keeping of related records.
4. Table for logging-in blood slides received and issuing for staining.
5. Table for staining racks.
6. Table for keeping stained slides and issuing to microscopists for examination.
- 7,8,9,10,11,12. Tables and rattan top high stools for microscopic examination.
13. Table for recording of blood slide examination results and notification.
14. Table for chief technician.
15. Room of the medical officer, who supervises the laboratory.
16. Store room.
17. Store room.

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- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

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