


P. FALCIPARUM CONTAINMENT PROGRAMME

COURSE CONTENTS OF  
PFC MEDICAL OFFICER  
RECREATION TRAINING IN  
MALARICLOS



DIRECTORATE OF  
NATIONAL MALARIA ERADICATION PROGRAMME

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## NEED, OBJECTIVES AND MECHANICS OF THE COURSE

Control of malaria is not an easy task. The different facets of antimalaria campaign are very complicated. These facets are required to be not only coordinated but also each one should exhibit high level of efficiency. Naturally, the persons entrusted with the responsibility to make the antimalaria campaign effective must be trained and experienced.

NMEP has undergone changes over the years. It is no longer a vertical programme. The peripheral activities under the epidemiological surveillance including laboratory services which at one time was unipurpose under NMEP has been decentralised at PHC level from 1977 under MPO. Under the MPW scheme epidemiological surveillance has been integrated at the peripheral level. The planning, implementation and assessment of spray programme are the responsibility of Malaria Officer from District level although PHC Medical Officer and MPW Scheme staff are involved.

The integration of malaria services with the Primary Health Care System has brought into focus the difficulties of malaria control activities in high risk areas which require undivided attention.

Systematic and well planned intervention measures are the basic necessity to reduce malaria transmission and sustain efforts are required towards this high risk areas.

SIDA assisted Plasmodium falciparum Containment Programme (PfCP covers most of the high risk areas where P.falciparum is predominant infection). It is realised that training to improve the functioning of the P.falciparum Containment Programme is require at all levels and more so in the peripheral levels. The Medical Officer of the PHC has to play an important role in anti-malaria campaign particularly in respect of epidemiological surveillance through Primary Health Care System. It is, therefore, necessary that the Medical Officers of the PHC's located in the districts covered under PfCP should be imparted a short in service training on malaria. As such short training course of four working days has been designed exclusively to expose the Medical Officers of the PHCs to some of the basic element of Anti-malaria campaign and the part they should play for control of malaria at PHC.

## STATUS OF MALARIA CONTROL/ERADICATION PROGRAMME

### Brief Background Information:

Prior to nation-wide malaria control programme in India, the disease was rampant in the country so much so that Sinton (1928) had observed that malaria affected all walks of life and that the problem of very existence was the problem of malaria. In 1952 it was estimated that about 7.5 million people suffered from the disease of which 0.5 million (8 lakhs) died as a direct effect of malaria.

### 2. Pilot Control Projects:

With the advent of synthetic insecticides such as DDT and HCH, possessing residual efficacy and availability of these for commercial purpose after the war, it became evident that the population particularly residing in rural areas could be economically protected from malaria with two or three rounds of residual insecticide spray. Several pilot projects undertaken, confirmed the feasibility of control of rural malaria within the economic resources of the country. About 30 million people received full protection by the end of 1952.

### 3. National Malaria Control Programme:

In view of the knowledge gained a nation wide campaign was launched in 1953 in a phased manner with the object of affording protection to 230 million people living in the hyper and mesoendemic areas. The yardsticks of survey supported by entomological activities.

### 4. National Malaria Eradication Programme:

The World Health Assembly recommended in 1956 that a global efforts should be launched to eradicate malaria. In view of the tremendous success of the NMCP, and the assurance of bilateral and international assistance, the country switched over from control programme to one of eradication in 1958, which envisaged total coverage of the entire country under the spraying operation from 1959. The epidemiological surveillance was run in early 1960 and became fully operative from 1961. The programme was run in phases such as attack and consolidation. After eradication was achieved the programme would have to be brought under the General Health Service in the maintenance phase.

The main operational unit had about a million population and there were about 400 such entities. The smallest operational unit was a section, covering a population of about 10,000 in the plain areas.



The impact was measured through a number of epidemiological parameters developed through epidemiological surveillance. The assessments were undertaken by Independent Appraisal Teams. The movement of the programme from one phase to the other was based on the such evaluation.

By 1966-67, malaria was eradicated from 250 million population area (233 units) out of 476 million (393 units) that is 53 per cent of the country about 34 per cent of the population area reached an advanced stage and major efforts were needed in about 13 per cent of the country. Total malaria cases registered in 1966 were about 0.1 million and *P.falciparum* 26163. Co-lateral benefits were disappearance of plague and of Kala-Azar.

5. Set back in the programme:

The programme met a major set back with the stoppage of supply of imported insecticides through bilateral agencies. At the time many of the areas under maintenance phase had reverses because of inadequacy of vigilance service through the health infrastructure, which had yet to develop. Many of the experienced malarialogists and senior professional staff had retired or were no longer available. Meanwhile, there was rapid escalation in cost of imported insecticides for which funds were not available. Besides there were other constraints. These resulted in rapid increase in malaria especially from seventies at an unprecedented rate. The problem was compounded because of loss of immunity of many communities in the wake of control/eradication of the disease. Number of cases went up to 6 million in 1976 from about 0.1 million a decade ago.

6. Steps taken to combat situation:

The Government of India reviewed the programme by setting up two committees. On the recommendation of these committees the Government of India launched a 'Modified Plan of Operation' to cope with the situation in April 1977.

The objectives of which were as under:

- (i) To prevent deaths due to malaria
- (ii) Reduction of morbidity due to malaria

- (iii) Maintenance of the status of industrial development and green revolution due to freedom from malaria and retention of the achievements made so far.

To achieve these objectives, a three pronged attack was launched.

- (a) Government's efforts - spray and surveillance operations.
- (b) Public participation - through voluntary agencies for collection of blood smear and drug distribution (RTD & DDC), co-operation in spray operations, reporting of any out-break to local authorities etc.
- (c) Intensifying Research under NMEP and ICMR + operational and fundamental.

7. P.falciparum Containment Programme:

One of the objectives of NMCP is prevention of deaths which is mainly due to P.falciparum. To prevent its spread P.falciparum Containment Programme was launched in October 1977 with the assistance of SIDA (Swedish International Development Authority). PfCP is a part of the NMCP with special inputs in the hard-core areas. The necessity for intensification has been further high-lighted due to emergence of dissemination of the same to other parts of the country. 81 districts are under the ambit of P.falciparum Containment Programme with a population of 98 million. In order to Co-ordinate and implement the various activities, four zones have been established with the Head-Quarters at Shillong, Bhubaneswar, Ranchi and Bhopal in addition to HQ Zone at Delhi. Each Zone is headed by a Senior Epid-Cum. Coordinator who is assisted by Special Epidemiologist, Senior District/Dist.Epidemiologist and other technical and administrative staff.

The teams are to assist in the programme implementation with particular reference to continuous epidemiological assessments. PfCP component is also supported by a few entomological teams to conduct specific investigations on vector bionomics. In addition to research, training of Medical Officer I/C PHC in the area covered. PfCP is an integral part of the programme. PfCP cell is located at the NMCP Directorate, with a Chief Coordinator, two senior epidemiologist, a training Co-ordinator, a reference laboratory and other ancillary staff.



### 8. Malaria Incidence in India:

Statement showing malaria cases and  
P.f. cases in India from 1961 to 1987

[illegible]

## LIFE CYCLE OF HUMAN MALARIA PARASITES

The species of human malaria parasites found in India are:-

1. Plasmodium vivax
2. Plasmodium falciparum
3. Plasmodium malariae

Malaria parasite completes its asexual cycle in man and sexual cycle in the mosquito and the same are briefly described below:-

1. Asexual cycle in man
  - (a) Exoerythrocytic tissue phase

When an infective female anopheles mosquito bites a healthy individual, it introduces a large number of sporozites in the blood where they circulate for about 30 minutes after their introduction and then they enter the hepatocyte cells of the liver. Here the young plasmodia undergo a period of growth and reproduction (cryptozoic and meta cryptozoic schizonts) which lasts for a time varying with the species until some of the resulting off spring spill into the blood stream and enter into erythrocytes. The erythrocytic cycle has now been worked out for all the human plasmodia. The duration of this stage in each of the plasmodial species is:

P.vivax 8 days, P.falciparum 5 to 6 days and P.malariae 14-15 days.

The term tissue phase includes all exoerythrocytic forms. The exoerythrocytic schizogony represents the development of the sporozoites in the hepatocytes (cryptozoic schizogony).

- (b) Erythrocytic stage

The cycle of the parasite in the blood begins with the appearance of an extremely minute form in the red blood cell. This consists of a blob of chromatin and a little cytoplasm which generally assumes a ring shape (early trophozoite).

The young form grows and especially in case of P.vivax exhibits a good deal of amoeboid activity (mature trophozoite). But eventually the parasite tends to round up and becomes less active (pre-schizont). Stained preparations show that about this time the chromatin begins to divide, and this process continues until the number of chromatin masses characteristic of the mature re-producing form which are the immediate product of segmentation are called merozoites.

In a fully developed or matured schizont the chromatin has fully divided, with formation of schizont, the stage is called as segmenter. The merozoites attach to and penetrate the red blood cells, thus initiating a new cycle, The process of asexual multiplication may continue in the host for several cycles.



### Gametocytes:

Not all the merozoites upon entering erythrocytes proceed through another cycle of schizogony. Some develop in the red blood cell into sexual gametocytes instead of asexual segmenters. Male gametocytes are called microgametocytes, female are macrogametocytes.

Gametocytes are capable of development only in the invertebrate host and play no part in the pathology of the disease in the vertebrate host; other than assisting in blocking the capillaries and also causing the eventual destruction of the host erythrocytes.

### 2. Sexual cycle in the mosquito

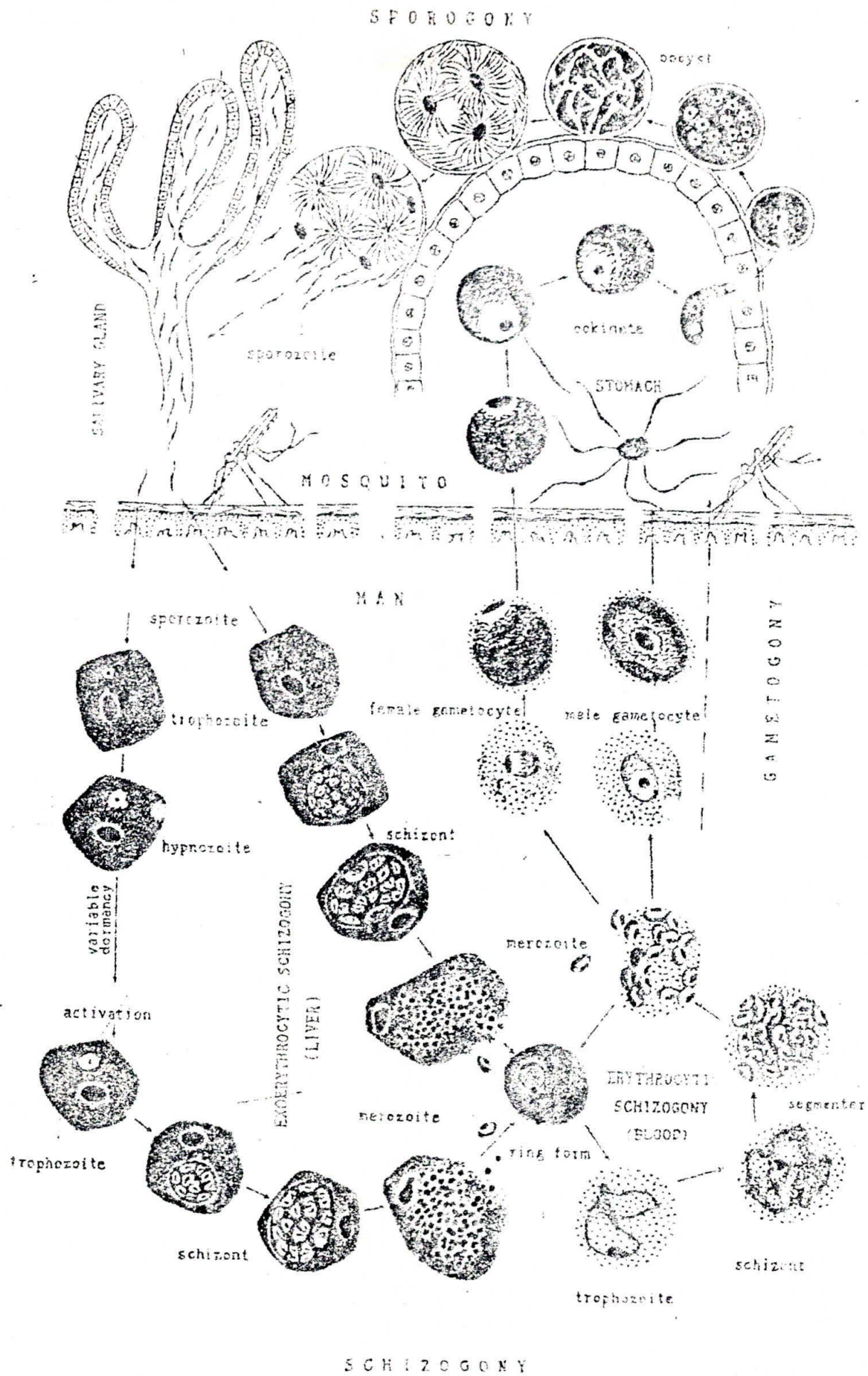
When a suitable species of mosquito (vector species) takes blood containing mature gametocytes, these develop rapidly into fullfledged gametes. The process of ex-flagellation of the male gamete may be completed within ten or fifteen minutes, fertilization ensuing soon thereafter. The change in the male cell involves leaving the host erythrocyte, and extruding about eight microgametes. These remain attached to the parent cell for a few minutes, whipping about actively until they are liberated and seeking the female gamete. There is also a maturation process which is necessary before the macrogametocyte is ripe and ready for fertilization i.e. when it becomes a macrogamete. Fertilization involves union of the nuclei of the micro and macrogametes, and formation of zygote which later on becomes a motile, elongate cell known as the ookinate or vermicule.

The ookinate penetrates the mid-gut mucosa. An oocyst then develops beneath the epithelium on the outer surface of the gut. Maturation of the oocyst takes a variable amount of time depending on the temperature, species of mosquito. Ten days to 15 days is the usual time required.

From a small body, a few microns in diameter, the oocyst grows until it may be 50 to 55 microns in diameter. The chromatin divides repeatedly until there are hundreds of minute nuclear masses. Then the cytoplasm follows suit, so that each bit of chromatin has its share of cytoplasm, and in this way a great number of spindle like sporozoites enter into the body cavity of the mosquito, from which they eventually reach the salivary glands from where they mature into infective form. When a female infective anopheles bites a person for taking a blood meal, the sporozoites are infected into the body.

### Relapses

Previously persistence of malaria parasite for many years was thought to be due to relapse cycle in liver. It is now known to be solely initiated by sporozoite. The sporozoites of relapsing malaria parasites differentiated into either hypnozoites or developing schizonts. The hypnozoites remain dormant as single nucleated intrahepatocytic round bodies. At the predetermined time for a relapse (e.g 8-10 months, in temperate zone, *P.vivax* infection) the hypnozoites start growing and undergo exoerythrocytic schizogony forming merozoites that invade blood. True relapse is caused in *P.vivax* and *P.ovale* in man. It is confirmed that persistent hypnozoites in liver do not occur in *P.malariae*.



LIFE CYCLE OF PLASMODIUM VIVAX



## DIAGNOSTIC FEATURES OF MALARIA PARASITE IN THIN AND THICK SMEARS

### 1. THIN FILM DIAGNOSIS OF MALARIA : P.VIVAX

Introduction: This parasite was discovered by Grassi and Feletti in the year 1890. The name vivax is given to the specie because it shows marked amoeboidicity. It produces a disease in man known as benign tertian malaria. It is called benign as it is really fatal and tertian because the temperature comes after every 48 hours.

Morphology: The parasite as they appear in stained thin films from peripheral blood are:

#### 1. i) Youngest ring form:

It measures about one third the diameter of a normal red blood cell. It may be located centrally or peripherally. It consists of a blue margin of cytoplasm and a rather heavy red dot of chromatin. A vacuole can be seen in the cytoplasm. After a few hours, the infected red blood cell is usually enlarged and becomes pale. Any stage after this period the stippling known as Schuffner's dots may be demonstrated in many of the parasited cells in a well stained preparations.

As development progresses the parasite may continue to show a ring like appearance in stained film with much thickened cytoplasm and enlarged, chromatin mass. However, it may very early exhibit pseudopodia, an indication of amoeboid-movement.

#### ii) Trophozoite:

It develops in about 5 or 6 hours. It may assume practically any shape within the enlarged red blood cell. The growing parasite has pseudopodia. The chromatin is also increased in size. Vacuoles may be one or more. At this stage yellowish brown pigment makes its appearance in the cytoplasm of the parasites. There are small, angular or red like and increase in number with the growth of the parasite. In the young form they frequently cannot be distinguished as separate granules or foci, but exhibit their presence by giving a yellowish tinge to the cytoplasm.

#### iii) Schizont:

At the end of about 36 to 40 hours the parasite practically fills the entire red cell, which may be twice its normal size. It has now completed its vegetative growth and is preparing for multiplication. The motility ceases and it assumes a rather compact form. It has an irregular outline with cytoplasm mottled in appearance as though unevenly massed. It still has single nucleus which is compact and usually lies near the periphery of the parasite. It would look smaller than some of the amoeboid from which have proceeded it. It stains much more on account of its compactness.

Now the division of the chromatin begins. The nucleus division into 2 to 24 masses but as division proceeds, the segments appear more regular in size and shape, smaller and more compact. The cytoplasm follows suit and breaks up. Each portion of cytoplasm adheres to each dot of chromatin. Thus forming individual parasites. These are the merozoites. During this process the pigments collect into one or two loose masses. The complete is a definite sign that segmentation in this species is complete. Frequently there is an uneven number of merozoites in the mature schizont. The entire growth from small trophozoite through



mature schizont required liberating another generation. The majority of parasites attain maturity at about same time but the process is not entirely synchronous. Hence there are often several stages of vivax parasites in the peripheral blood at the same time.

#### iv) Gametocytes:

Some of the trophozoites develop as the sexual forms. But what constitutes the stimulus for their liberation is not exactly known. They appear quite early in the blood when in the initial schizogony has continued through several generations. When a gametocyte grows, it is rounded in shape and may be distinguished as male and female. The mature microgametocyte or male gametocyte is often about the size of normal red cells. The quantity of pigment granules in the mature gametocytes of both sexes is usually greater than in schizont and the granules are evenly distributed through out the cytoplasm. The grains and rods of pigments are usually darker in colour in the female.

The microgametocyte has a densely blue staining, generally homogenous cytoplasm. The nucleus is usually compact and deep red. Around the nucleus sometimes a colourless areas called the zone of chromophila exists. The nucleus is usually situated near the periphery of the parasite.

The microgametocyte contains less cytoplasm. It stains more lightly and may be greyish blue, greenish blue, pinkish blue or at times practically colourless. There is a loose nucleus system with stellate and some times it extends in a broad spindle across the body. Practically always it is placed centrally and is light stained. An unstained reticular area around the mass of grains of stained chromatin is often encountered in microgametocyte.

#### PLASMODIUM FALCIPARUM

P. falciparum was first identified by Laveran in the year 1901. It is exclusively a human parasite and does not show predilection for any other animal host for its growth and multiplication under natural conditions.

P. falciparum is commonly known as the parasite of malignant tertian or pernicious malaria. The malignancy of the organism is attributable to two of its important characteristics viz. (a) its much greater invasiveness than any of the other species of human plasmodia and (b) its inherent of growth and multiplication in the internal organs of the host (and not in the peripheral circulation) unlike the other species of human plasmodia. As a result of the development simultaneous formation of fibrin deposits over the surfaces of the infected erythrocytes these cells tend to agglomerate to and form clumps which often occlude the lumen of the capillaries and thereby give rise to various pernicious pathological manifestations. The invasive property of the parasite is all the more manifested by the comparatively delayed development of immunity in the host than in either P. vivax or P. malaria infections.

In P. falciparum infections the commonest of sexual form of the parasite - detectable in the peripheral circulation is the ring or early trophozoite. The ring in the very early stage is of an extremely delicate texture and is generally about 1/6th to 1/5th of the diameter of the infected red blood corpuscle. The cytoplasm is of a fine hair like consistency surrounding a small clear vacuole and take a purplish blue stain. The nucleus or the chromatin is stained ruby red and implanted on one edge of the cytoplasmic ring, some times with a little blue outwards giving at a "signet-ring" appearance. Some times instead of one



chromatin dot in a ring, one comes across two chromatins lying side by side on the surface of the ring, which is a morphological characteristic of P.falciparum almost exclusively. Then again, the ring in itself although always endoglobular, often occupies a position near the surface of the infected corpuscle thus giving it an accolé or applique form. This is another characteristic of the parasite.

The multiplication of the species of the parasites is so rapid that more than one parasite in a red cell frequently occur and this is considered almost pathognomonic of P.falciparum infection.

The cytoplasmic ring of the parasite, as it grows in the uninfected red cell, becomes stouter in size and takes a deeper stain. When it occupies about 1/3 to 1/2 of the infected cell, the cell recedes into the internal circulation where further development of the parasite takes place. The infected corpuscle does not normally enlarge in volume along with the growth of the harboured parasite, except when multiple infection occurs.

Besides the ring form, no other asexual form of parasite is normally detectable in the peripheral circulation nor the infected corpuscle show the characteristic pigment. The pigment (almost black and coarsely granular) appears in the developing forms of the parasite inside the internal organs of the host. The infected red cells do not also present the "Schuffner's dots" on their surfaces unlike P.vivax infection but, they may have large irregular reddish clumps called "Maurer's dots".

When the infection of the host is of an over-whelming nature and especially in the terminal stages of such infections all kinds of a sexual developmental forms of this species of parasite are found in the peripheral circulation. Some of the growing trophozoites in such instances are seen to be of an extremely amoeboid nature, and in multiple are also of red cells the pseudopodia of parasites are also often seen to anastomose with each other. - That is why till about few years ago parasitologists used to think this stage of growth as due to infection by a different.

In the schizonts of P.falciparum the merozoites are seen to arrange themselves in rosette forms with about 8 to 32 merozoites in each. The schizonts occupy 2/3 to 3/4 of the R.B.C. coarse black pigments are seen clustered at the centres of the schizonts.

Gametogenesis in P.falciparum infections under optimum climatic condition occurs about 10 to 12 days after the sexual parasites become patent in the circulation and the gametocytes take another 2-4 days to become infection for the mosquitoes. In P.vivax infections, on the contrary, gametogenesis is almost simultaneous with the appearance of a sexual parasitaemia and that is why the time lag between the appearance of infective gametocytes in secondary cases derived from the same primary case, called the incubation interval, is shorter nearly a fortnight than in P.falciparum infections. The difference in the incubation interval of infections of the above two species of human plasmodia has an important bearing on the genesis of epidemics.

The merozoites are the precursors of gametocytes after a few generations of schizonts have been produced in the blood. Some of the merozoites have a different density. They grow more slowly produce more pigment and ultimately develop into large single nucleated organisms having no visible central vacuoles, which are known as gametocytes or the sexual forms of the parasite.



The gametocytes of P. falciparum, unlike the other species of human plasmodia are elongated in appearance with a slight convexity on one side. The convexity is more marked in the female gametocytes of P. falciparum appear in the peripheral circulation in waves at difference intervals depending upon the strain of the species and their intra corpuscular origin is evidenced by the membranes of the host corpuscles frequently showing against their venous surfaces.

The male gametocyte is called the micro-gametocyte and the female macro-gametocyte. Besides there is slight difference in shape which is not always quite discernible. The male and the macrogametocytes morphological differ from each other in their following three characteristics:

1. Staining reaction - the cytoplasm of the macrogametocyte stains more deeply than that of microgametocyte.
2. Nucleus - is relatively larger in the microgametocyte than in the macrogametocyte but it is more compact and conspicuous in macrogametocytes.
3. Distribution of pigments - is diffused over the entire cytoplasm mass in microgametocyte and is concentrated round the central nucleus in the macrogametocyte.

#### P. MALARIAE:

Introduction: The parasite was first discovered by Laveran in the year 1880, this is in fact the earliest parasite discovered. It produces quartan malaria in man in the sense that the attacks of fever occur every 72 hours or 4th day. The distribution of this species is limited.

#### MORPHOLOGY:-

Thin smears made from peripheral blood on staining exhibit the following stages:

1) Rings: The young trophozoites are ring forms and are about the size of or slightly smaller than those of P. vivax. They some times seem to have thicker circle of cytoplasm than younger vivax rings. Double chromatin dots are rare. The vacuole of the ring stage disappears very soon, after the parasite begins its growth.

2) Trophozoites: The growing stages may assume band forms stretch across the red blood cell. They may also be compact nonvacuolated forms, angular or even round to avoid in outline. The chromatin is a rounded mass. It is more frequently streaked or semicircular in form even in the rounded parasites. Very little amoeboid forms. Hence irregularities in the outline of the cytoplasm is a rare feature. Parasites becoming older may grow into a wide bandform or have rounded shapes. The pigments appears early in the growth and is a characteristic of this species. Granules are dark brown and the granules are usually arranged after along the edges opposite the nucleus.

3) Schizont: Mature forms of the parasite almost fill or completely fill normal sized red cells. They may be rounded or band shaped with a rounded or an elongated chromatin mass. The cells containing them are never enlarged. Frequently they even seem to be smaller than normal and sometime darker in the early stages. The cycle is completed in 72 hours and hence the growth of the parasite is rather slow. At this stage the nucleus divided into 6 to 12 masses (usually 8) and the cytoplasm and forms merozoites. The merozoites are sometimes arranged peripherally around the centrally clumped pigment (rosette form).



a) Gametocytes: P. malaria seems to have fewer gametocytes than the other two species. P. vivax and P. falciparum. The gametocytes are spherical or oval in shape. The sexes have same difference in staining quality was in P. vivax and P. falciparum. The pigment is more abundant, darker brown and cells containing P. malariae certain stipplings have been demonstrated known as "Ziemann's dot". With ordinary stains that are used they can hardly be demonstrated. They seem to be best brought out by intensive staining with a stain solution having a PH of 7.2.

## 2. THICK FILM DIAGNOSIS OF MALARIA

### History:

Laveran (1891) appears to have been the first to use haemolysed blood as a means of concentrating the parasites for microscopic examination. Ross in 1903 described first thick film method recognisably similar to those used today. Although his method had many defects, it was an important advancement for it showed unmistakably that thick smears of blood could be made transparent by haemolysis and the parasites concentrated and stained well enough to be recognisable. Hock in 1907 simplified Ross's method and adapted it to Romanowsky stains the combined haemolysis and staining came into use. Quick simultaneous staining of both the thick and thin smears was introduced by JSB method.

### THE THICK BLOOD FILM:

The thick film is not a thick drop. It is a smear which transmits enough light for microscopic examination when haemoglobin is partly or wholly removed. Haemolysis or dissolving of the r.b.c. is thus the first essential and staining is second. Dissolving the r.b.c. and staining are concurrent with JSB. The thick film field shows leucocytes, platelets and blood protozoa on a back ground of lightly stained remnants of the red cell.

The normal thick film field contains leucocytes and platelets with occasional bluish cloud - the remains of dissolved reticulocytes. The cytoplasm is always somewhat scattered, cytoplasmic granules are often lost except the granules of eosinophils which have a special resistance and usually shine out with great clarity. The film may contain a few degenerated white cells which are structurally and recognisable only by their size, shapes and staining.

The blood platelets, single, in small groups or occasionally in clusters of several hundreds are stained pale purple and have a wooly texture and outline which is unmistakable.

Other structures seen in the microscopic fields are probably extraneous such as dust, moulds, yeasters, vegetable spores, bacteria or deposit of stain.

### ARTIFACTS:

Structures which confuse diagnosis from their resemblance to malaria parasites are sometimes, seen no matter how carefully the film are taken and stained.

The commonest sources of error are:

- a) Solitary or small groups of platelets may be mistaken for quartan trophozoites at the early compact stage, and the later for advanced vivax trophozoites at the stage when cytoplasm has broken into a cluster of fragments while the chromatin is yet distinct. The resemblance between vivax at this stage and



small platelet groups is extra-ordinarily close, particularly when the magnification is not high or the light is not enough to show up the colour of the pigment.

b) Blocs of chromatined debris from immature red cells when associated with tags of red cells reticulum. These chromatined masses are common in anaemia. They simulate found trophozoites when they are in chance combination with blue staining materials.

c) Reticulum from immature red cells may stain deeply enough to have a vague resemblance to vivax trophozoites surrounded by thick film equivalent of Schuffner's dots.

The above artefacts cannot be avoided and they must be learned from experience.

Other artefacts which are extraneous can be avoided by scrupulous cleanliness in taking the smear and protection of slide from dust etc.

#### PARASITE MORPHOLOGY IN THICK SMEAR

The technique of staining thick-film destroys the host cell and exposes the parasites to change in shape and size. The parasites appear smaller and are less regular in outline. Parasites are seen in unfamiliar setting, no longer as in the fixed thin films neatly framed by their host red cells, but stripped and distorted on a mottled gray grounds of red cell residue. With the disappearance of the host cells, Maurer's dots in falciparum infection are not seen in thick film. Schuffner's dots may be seen in thick smears. In thick film the red stained chromatin with associated blue cytoplasm must be seen before it is pronounced as a parasite.

#### PIGMENT IN THICK FILM

Pigment is seen more clearly in thick than in thin films. A trained observer may often identify the species and the phase of the parasites by nothing shape size and distribution of the pigment granules.

#### THE CONCENTRATION FACTOR

One great advantage of thick film is concentration of the parasites. That more blood may be examined in a given time or the same amount of blood in less time is self-evident. A film thick enough not to impair microscopic examination is expected to produce an average concentration of fifteen with a range between ten and twenty.

The parasites as they appear in stained thick films from peripheral blood are:

##### 1. Ring forms:

The chromatin consists of only a single bead but somewhat larger than *P. falciparum* bead. The parasite appears in 'Ring' 'coma' 'swallow wing' and 'Exclamation mark' patterns. This stage, it may be difficult to distinguish from *P. malariae* rings of the same age and *P. falciparum* older rings. Cytoplasm appears irregular early, and is an important distinguishing feature. Other stages of the parasite may be present along with the ring forms.

ii) Early trophozoite form:

Chromatin is a single dot, but fairly isolated still, cytoplasm is irregular. It may be characteristically stretched or broken into delicate wisps and strands. Some individual forms may, however, be compact. Pigment appears as small granules. Other stages of parasite will be present.

iii) Fully grown trophozoite form:

The cytoplasm is irregular. It breaks up into a cluster of fragments around the nucleus. The chromatin is often isolated from the cytoplasmic fragments. The pigments are fine and granules and dispersed moderately. The parasites may be associated with typical stippled appearance. Ghost outline of the infected cells may sometimes, be clear.

iv) Early schizonts:

Bigger than P. falciparum early schizonts. The cytoplasm is undivided and loosely covers completely or incompletely the chromatin segments. Pigment granules are usually discrete and loosely concentrated into one or two.

v) Mature schizonts:

These are large. Individual merozoites are also large. Merozoites vary from 12 to 24 (average 16). Each chromatin particle may or may not be clothed with cytoplasm. The merozoites may have well marked vacuole. Pigments appear lightly as a close collection or they are sometimes slightly scattered. Other stages may be present.

vi) Gametocyte:

These parasites are round or oval. Chromatin is single (prominent in females and diffuse in males). The cytoplasm may be fairly uniform or somewhat loose knot and frayed at the periphery. Pigment scattered irregularly as small short redlets sometimes tending to a peripheral distribution. They may have surrounding some of schuffer's dots. Sex differentiation is not easy. Differentiation from late trophozoite stage is also not always easy. There may be associated sexual form in the same smear.



COMPARATIVE CHARACTERS OF PLASMODIA OF MAN (STAINED THIN SMEARS)

Early period	<u>Plasmodium vivax</u>	<u>Plasmodium malariae</u>	<u>Plasmodium falciparum</u>	<u>Plasmodium ovale</u>
Early trophozoite ring	Relatively large; usually one chromatin dot, sometimes two; often two rings in one cell.	Compact; one chromatin dot; double cell infections rare.	Small, delicate; sometimes two chromatin dots; multiple red cell infection common; applique forms frequent	Compact; one chromatin dot; double infection uncommon.
Large trophozoite	Large; markedly amoeboid; prominent vacuole; pigment in fine rodlets.	Small; often band-shaped; not amoeboid; vacuole inconspicuous; pigment coarse.	Medium size; usually compact, rarely amoeboid; vacuole inconspicuous; rare in peripheral blood after half grown; pigment granular.	Small; compact; not amoeboid; vacuole inconspicuous; pigment coarse.
Growing schizont	Large; somewhat amoeboid; chromatin masses numerous; pigment in fine rodlets.	Small; compact; chromatin masses few; pigment coarse.	Medium size; compact; chromatin masses numerous; pigment granular; rare in peripheral blood.	Medium size; compact; chromatin masses few; pigment coarse.
Mature schizont	Larger than normal red cells	Smaller than normal red cells; single rosette.	Smaller than normal red cells; single rosette.	Larger than <u>P. malariae</u>
Number of merozoites	6-24, usually 12-18	6-12, usually 8	8-36, usually 8-18	6-16, usually
Microgametocytes (usually smaller and less numerous than Macrogametocytes)	Spherical; compact; no vacuole; single large nucleus; diffuse coarse pigment; cytoplasm stains light blue	Similar to <u>P. vivax</u> but smaller and less numerous	Crescents usually sausage-shaped; chromatin diffuse; pigment scattered, large grains; nucleus rather large; cytoplasm stains darker blue.	Similar to <u>P. vivax</u> but somewhat smaller; never abundant.
Macrogametocytes	Spherical; compact; larger than microgametocyte; smaller nucleus.	Similar to <u>P. vivax</u> but smaller and less numerous	Crescent often larger and more slender; chromatin central; pigment more compact; nucleus compact.	Similar to <u>P. vivax</u> but somewhat smaller; never abundant.

(continue)



(concluded)

Early period	<u>Plasmodium vivax</u>	<u>Plasmodium malariae</u>	<u>Plasmodium falciparum</u>	<u>Plasmodium ovale</u>
Pigment	Short, rather delicate rodlets irregularly scattered, not much tendency to coalesce.	Seen in very young rings; granules rather than rods; tendency towards peripheral scatter.	Pigment granular; early tendency to coalesce; typical single solid mass in mature trophozoite; coarse scattered 'rice grains' in crescents.	Similar to but somewhat coarser than <u>P. vivax</u> .
Alterations in the infected red cell	Enlarged and decolourized; Schuffner's dots usually seen.	Cell may seem smaller; fine stippling occasionally seen.	Normal size but may have 'brassy' appearance; Maurer's dots (or clefts) may be seen; host cell of crescent barely seen.	Enlarged and decolourized; Schuffner's dots (or James's stippling) early and prominent at all stages; numerous oval-shaped red cells or crenated margins.
Length of asexual phase	48 hours or a little less	72 hours	36-48 hours	48 hours or a little longer.
Repatent period	Usually 13-17 days	Usually 28-37 days	Usually 8-12 days	Usually 14-16 days
Minimal	8 days	14 days	5 days	8 days
Usual incubation	8-17 days, average 14 or longer	18-40 days, average 28	9-16 days, average 12	16-18 days, average 17.
Interval between parasite patency and gametocyte appearance.	3-5 days	7-14 days, appearance irregular and numbers few	7-12 days	12-14 days; appearance irregular and numbers few.

From WHO Regional Publications, South East Asia series No.9 (1966)

Role of Mosquitoes in Malaria transmission and bionomics of Local vectors:

1. Introduction: Malaria is transmitted from an infected man to a healthy one by the bite of an infective mosquito. Among different types of mosquitoes viz. Culex, Aedes, Mansonia and Anopheles, the anopheles mosquito is responsible for transmission of Malaria.

There are 365 Anopheline species found in the world. In India 53 species have been recorded. All Anopheline species do not transmit malaria. Only a few are responsible for transmission of Malaria. The species which transmit malaria are called as vectors of malaria.

2. Vectors of Malaria in India: In India there are 10 Anopheles species regarded as vectors of malaria of which 6 are regarded as principle vectors. These are An.culicifacies, An.fluviatilis, An.minimus, An. stephensi, An.philippinensis and An.sundaicus. The rest 4 species are regarded as vectors of local importance. These are An.balabacensis, An.annularis, and An.varuna and An.jeyporiensis.

3. Essential requirements of good vector

- (i) Receptivity of pathogens
- (ii) About 80% relative humidity
- (iii) Should survive 12-14 days
- (iv) Temperature around 30°C.
- (v) Good anthropophilic index (human blood preference)
- (vi) Reservoir of infection with good number of gametocyte carriers.
- (vii) High density and efficient biting species.

In general, An.culicifacies, An.annularis, and An.philippinensis are malaria vectors in the plain area and An.sundaicus in the coastal areas of West Bengal, Orissa, Andhra Pradesh, and Andaman and Nicobar Islands. In the foothill regions An.fluviatilis and An.minimus are important vectors. In the hilly forested region An.balabacensis plays an important role in transmission of malaria.



A. Difference between Anopheles and Culex mosquitoes

Anopheles

Culex

a) Egg:

Boat shaped, laid separately forms a pattern, triangular or star shape. forming raft pattern. Spindle shaped attached to each other

b) Larva:

Flat-horizontally on the surface of water no siphon tube but spiracle openings. Hang down wards with long siphon tube upwards

c) Pupa:

Breathing trumpet or spiracle traingular with wide opening (funnel shaped). Narrower with small opening longer

d) Adult:

i) Resting position:

Head, thorax, abdomen in a straight line, rest at a 45° angle with the surface. Hunch back appearance thorax and abdomen are parallel to the resting surface.

ii) Wings:

Spotted-dark and white. Unspotted.

iii) Palpi and proboscis equal in length.

Palpi much shorter than proboscis.

iv) Scutellum:

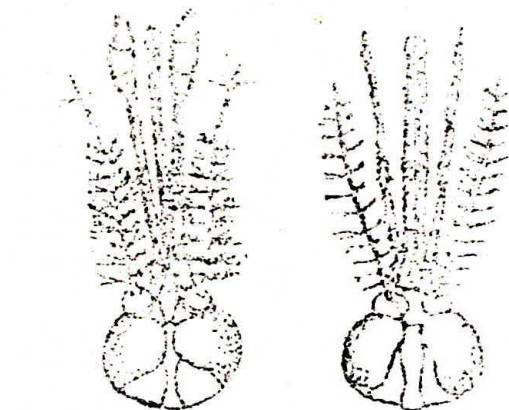
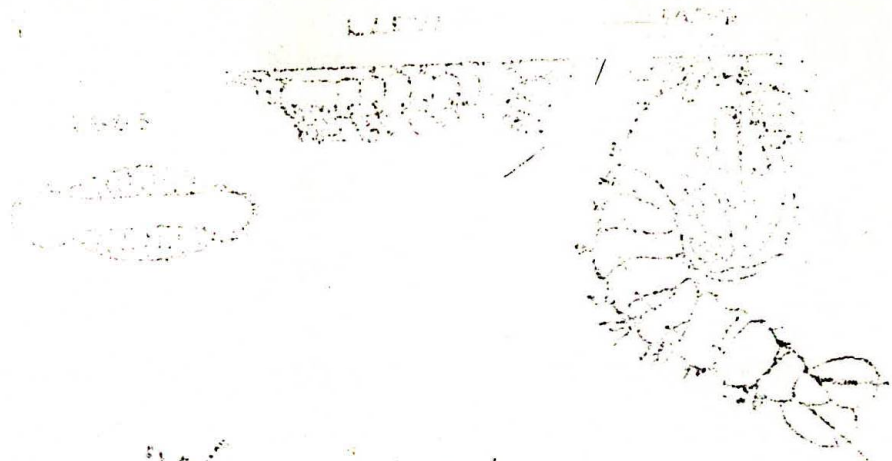
Convex or curved with regular one row of hair. Tritobed, each with one bunch of hair

v) Abdomen:

Without scales or with few scattered scales. with uniform rows of overlapping white and dark scales.

vi) Male:

Antennae Bushy Bushy.  
Pulpi - Equal in length to that of proboscis. Longer than proboscis and bent upwards



HEAD OF MALE & FEMALE  
FIG. 50



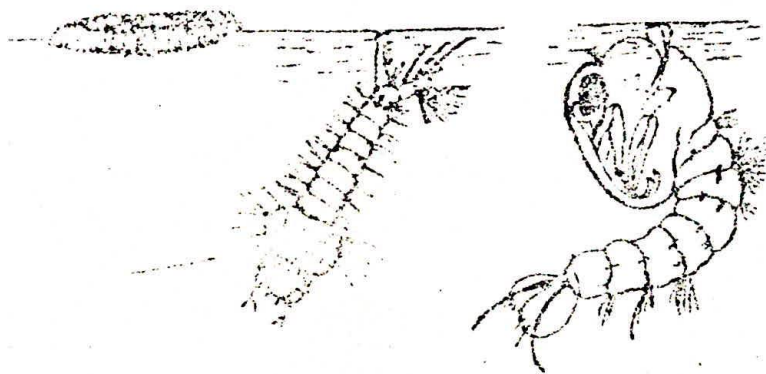
RESTING  
POSE

## CULEX MOSQUITO

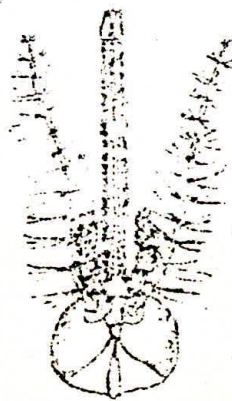
EGGS

LARVA

PUPA



HEAD OF MALE



FEMALE



RESTING  
POSE



1. CONCEPT OF EPIDEMIOLOGICAL SURVEILLANCE

2. COMPONENTS:

CASE DETECTION PROCEDURE  
DIAGNOSTIC SERVICES  
EPIDEMIOLOGICAL INVESTIGATIONS  
REMEDIAL MEASURES

3. CASE DETECTION PROCEDURE:

CONCEPT OF TOTAL COVERAGE IN SPACE AND TIME  
GEOGRAPHICAL RECONNAISSANCE  
ACTIVE CASE DETECTION (ACD)  
PASSIVE CASE DETECTION (PCD) OR INSTITUTIONAL  
VOLUNTARY/COLLABORATORS:

TOTAL COLLECTION/EXAMINATION:  
ABER: PROPORTION ACD: PCD: FTD:

4. DIAGNOSTIC: LABORATORY SERVICES:

BLOOD SMEAR EXAMINATION: OUT PUT  
CONFIDENCE IN THE SERVICES : QUALITY  
CROSS CHECKING ACTIVITIES: DISCREPANCY RATE  
TIME LAG BETWEEN: COLLECTION AND DESPATCH  
DESPATCH /ND RECEIPT  
RECEIPT AND EXAMINATION  
EXAMINATION AND REPORTING  
BACK LOG OF UNEXAMINED SLIDES: FACTORS INVOLVED  
REPORTING SYSTEM  
PROBLEMS: CONNECTED WITH LABORATORY SERVICES UNDER PHC

5. EPIDEMIOLOGICAL INVESTIGATIONS:

PRELIMINARY CLASSIFICATION  
INTENSIVE  
NOTIFICATION

6. REMEDIAL MEASURES:

RADICAL TREATMENT  
INSECTICIDAL SPRAYING  
OTHER MEASURES

7. PARAMETERS: THOUGH EPIDEMIOLOGICAL SURVEILLANCE

ABER  
TOTAL MALARIA CASES  
SPR  
P.FALCIPARUM  
SFR  
API

## SURVEILLANCE OPERATIONS IN NATIONAL ANTI-MALARIA PROGRAMME

### INTRODUCTION:

The term epidemiological surveillance was first employed in Greece in 1951 to prevent a resurgence of malaria from areas where it had apparently disappeared. In spite of the fact that the infant parasite rate was zero in most villages of Greece, the epidemiological surveillance discovered 400 malaria cases.

The Greek lesson was exported and adopted in N.M.E.P. Although, in the beginning only surveillance through Active Case Detection was introduced in Greece, this country did not find it enough and in 1957 Passive Case Detection was added through the collaboration of hospitals and rural dispensaries.

### CONCEPT OF EPIDEMIOLOGICAL SURVEILLANCE:

The concept of epidemiological surveillance in National anti-malaria programme is to ensure total coverage of the population in Space and Time. Criteria for screening a whole population might be several such as history suspicious of malaria, an enlarged spleen, or fever. The last has been chosen so that the search for cases mainly rests on detecting, first the persons to have fever or have recently had it. This criterion has the great advantage that it can also be done by non-professional persons.

As for parasites - carriers without fever, the great majority of them have had fever before and if that time the surveillance was operating they would have been detected and cured.

There are different approaches to search for cases:

- (1) Passive Case Detection (2) Active Cases Detection (3) Mass Blood Surveys (4) and investigation of persons in the neighbourhood of confirmed cases.

Even if a country develop a very effective network of passive detection covering all the malarious area there will always be a number of fever cases that would not report to the detection agency. They may be too ill to go there or so slightly ill that they do not think they had any fever at all. To fill such gaps in the passive detection system, Active case detection through periodical house to house visits for screening of fever cases is required for providing a total geographical coverage of the entire community.



COMPONENTS:

(1) PASSIVE CASE DETECTION:

FIRST LEVEL OF PASSIVE DETECTION is of compulsory notification of confirmed or suspicious cases of Malaria by all the medical profession - hospitals, health centres or dispensaries. This procedure may detect or an early stage localities where transmission persists and are liable to become "Problem areas".

"Problem area" is a defined geographical areas within which an adequate epidemiological evaluation shows that the transmission of malaria persists despite total complete regular and sufficient coverage with residual insecticides. It has been found that in most and probably all, "Problem areas" persistence of transmission was associated with operational and/or administrative short-comings.

All Medical and health units to serve as Malaria detection Agency i.e. to take blood slides, not only from clinically confirmed or suspicious cases of malaria but also from all fever cases and to confirm them and to give to all fever cases and to confirmed malaria cases appropriate treatment as indicated by N.M.E.P.

Second level of passive case detection is through voluntary collaborator. They belong and live in a community and can collaborate with NMEP in case detection, blood taking and treatment following the NMEP rules. They may appreciate the popularity and prestige conferred on them by the particular responsibility. They should possibly be persons such as school teachers, retired civil servants or Priests or Community health guides.

(2) ACTIVE CASE DETECTION:

At the outset it must be confessed that even the best detection-Active and Passive is likely to miss cases. We must therefore make sure (1) To interrupt transmission by ensuring a perfect total coverage by insecticide (2) To obtain maximum efficiency in surveillance from case detection to treatment and (3) supplementing, when in doubt the two types of detection with Mass-Blood examinations in suspicious communities during the transmission season.

Frequency and system of domiciliary visits:

In areas where reproduction rate-average number of secondary cases that might be produced by a typical primary case in the interval between the visits of surveillance worker is high before spraying the domiciliary visits should discover secondary cases within two weeks of their occurrence. Therefore the Indian Programme provides for fortnightly visits.

The system of visits should be such that on arriving in a village the worker should first pay the visit to the head man or the most influential persons, from where he may gather information of people having fever. Next he should visit the detection post if there is one, see the fever register etc. and then proceed to his visit.

3. MASS BLOOD SURVEY

These consist in the examination of blood from every persons in a community.

The most usual mass blood survey is made during the epidemiological investigation of the persons in the neighbourhood of a positive case.

Another reason for Mass blood examination is to detect all the parasite carrier when transmission is persisting to supplement ACD and/or PCD.



## SURVEILLANCE OPERATIONS - EPIDEMIOLOGICAL PARAMETERS

The surveillance operation is an unique Programme which covers - Diagnostic Services along with curative, Preventive and Epidemiological services. The Epidemiological Services includes two aspects:-

- (a) Epidemiological investigations of positive cases/foci to determine the origin of infection to facilitate appropriate remedial measures.
- (b) Measurement of malaria for assessing the malaria status of an area and also for evaluation of the progress of ongoing control measures.

The basic method for assessing malaria status in a control or Eradication programmes are:-

1. Malarionetric surveys
2. Epidemiological Surveillance - Parameters of measurement.

### MALARIONETRIC SURVEY:

A Malarionetric Survey is the examination of the population by selected age groups and in localities selected at random in order to measure the amount of malaria present at a given moment (Prevalence).

The commonly used method are expressed as:-

1. Child spleen rate =  $\frac{\text{Positive spleen} \times 100}{\text{Child: n examined.}}$
2. Child parasite rate =  $\frac{\text{Positive} \times 100}{\text{B.S. examined from Children}}$
3. Infant parasite rate =  $\frac{\text{Positive} \times 100}{\text{B.S. examined from Infants (0-12 mths)}}$

Spleen survey along with Hospital/Dispensary statistics constituted the basic principles for measurement of malaria for over a century. Furthermore, on the basis of spleen rates system of classification of endemicity was developed.

When both the spleen rate and parasite survey results, specially in children are combined, the findings on the malaria status become more accurate. Infant parasite rate is of importance because it provides information of immediate "Parasitological happenings". If done in a systematic way, Infant Parasite rates furnish informations not only about the point of time when malaria transmission has been interrupted but also about the Quantum of transmission.

Malarionetric surveys were the principal classical tool for the measurement of malaria and for the institution of malaria control measures. During the control programme and early parts of the Eradication Programme these surveys also gave adequate information on malaria status.



However, during later stages of the Eradication Programme when the malaria cases decreased rapidly, the malarionometric rates are not sensitive enough to measure the changes in the amount of malaria present in the population. In the later stages of Eradication it is necessary to measure in a continuous way and in the whole population. Malarionometric surveys do not fulfill these requirements as they are usually carried out once or at the most twice a year and as they are conducted in a sample of the localities or a selected group of population.

#### EPIDEMIOLOGICAL SURVEILLANCE - PARAMETERS OF MEASUREMENTS

Once the surveillance procedure is established adequately it is possible to measure the malaria situation in terms of "Malaria Incidence" against "Malaria Prevalence" as measured through malarionometric surveys.

Malaria Incidence means "The Number of Cases of Malaria Occurring during a given time (Usually per year). Malaria case detection in the entire population through out the year gives the basis for the measurement of malaria incidence.

The parameters commonly used are:

(1) Annual blood examination rates

$$ABER = \frac{\text{Number of blood smears examined during the year} \times 100}{\text{Population}}$$

ABER should be 10% annually with at least 1% monthly during the transmission seasons.

However, in highly malarious areas these rates may go up to 15% more.

ABER represents the index of operational efficiency and the adequacy of search for malaria cases. Because of this only the blood smears collected through ACD and FCD and voluntary organizations are to be taken into account. The slides collected through the mass surveys are not to be taken into account during calculation of ABER.

If ABER is considerably below 10% it is most likely that the population coverage is not regular in time and space.

(2) Annual Parasite Incidence

$$API = \frac{\text{Malaria Positive cases detected in one year} \times 1000}{\text{Population}}$$

API is directly proportional to the amount of malaria in the community. However, one should be cautious in interpreting API values as these figures should be based only on the results of fully adequate case detection. Although this ideal is never reached at least the case detection mechanism must attain its maximum efficiency, before the concept of API expressing the total malaria incidence is reached.



API played a significant role during the Eradication Programme for changing areas from attack to consolidation and then to maintenance phase under the MPE. Also API has been taken as the guideline for inclusion of population under spraying programme. Such operations are undertaken in areas with API 2 or above.

(3) Slide positivity rate:

$$SPR = \frac{\text{Positive S. } \times 100}{\text{Blood slide examined}}$$

SPR is directly, though usually not proportionately related to the amount of malaria. Besides amount of malaria is inversely correlated to the amount of negative blood slides collected. Therefore, when an attempt is made to increase ABER by indiscriminate screening of healthy people there is an artificial decrease of SPR. Therefore, SPR is meaningful only when ABER or API are available.

In situations when API is low and ABER is also below 10, SPR should be taken into consideration in decision making regarding spraying activities. In such areas spraying should be undertaken if SPR is 2 and above.

BER	for March
BER	for 1st Quarter (January to March)
PI	for July
PI	for 2nd Quarter (April to June)

In such calculations cases are grouped by the date of collection and not by the date of examination.

API and SPR may also be broken down specieswise.

The corresponding rates are:

Annual falciparum Incidence	= API
Annual vivax Incidence	= AVI
Slide falciparum rate	= Sfr
Slide vivax rate	= SVR

To know the efficiency of the different agencies of the case detection procedure, it is necessary to calculate the above Indices/agency-wise viz. ACD, PCD voluntary agencies etc.

Another parameter used sometimes is the P.falciparum Ratio (Pf%)

$$Pf\% = \frac{\text{Number of Pf cases detection} \times 100}{\text{Number of total positive}}$$

Pf ratio is not only directly proportional to the incidence of falciparum malaria but also negatively correlated to the incidence of other species.

Percentage of P. falciparum depends on incidence of both P. falciparum and P. vivax. If the incidence of P. falciparum is increasing faster than the incidence of P. vivax, Pf% will increase, but the same will happen if the incidence of P. falciparum is

is decreasing slower than the incidence of P. vivax.

Example:

In a fixed population of 10000, 300 cases of P. falciparum and 100 cases of P. vivax were detected during 1984. In 1985 the cases detected were 100 and 25 respectively. The APER is more or less the same both year.

Year	Pf	Pv	API	Pf%
1984	300	100	30%	75%
1985	100	25	10%	80%

(-66.67%) (-36.67%) (-73%) (-66.67%) (=6.67%)

In this example, in spite of decrease in the incidence of falciparum by 66.67% the Pf% has increased, only because the decrease in the incidence of Vivax was more rapid (75%)

Because of its ambiguity, Pf% has only a limited value

#### Measurements of dynamics of malaria indices.

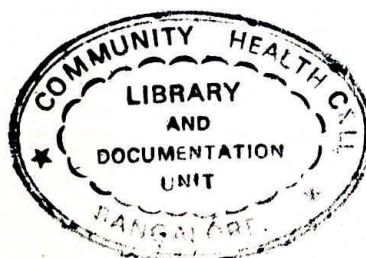
It is usually done by comparing the indices for two successive years which may be termed as year 1 and year 2.

Percentage of change (%ch) =  $\frac{\text{Index for the year-2} - \text{Index for the year-1}}{\text{Index for year-1}} \times 100$

The results may be positive (increase) or Negative (decrease)

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MORBIDITY - MORTALITY AND CLINICAL ASPECTS OF MALARIA  
DIAGNOSIS AND MANAGEMENT OF MALARIA CASES

The micro organisms causing malaria are commonly referred to as Malaria Parasites. This term is usually restricted to Plasmodium which cause the disease in man and transmits it through the anopheles mosquito host.

Malaria Morbidity Rate is the proportion of the number of cases of malaria in a unit time, in the population in which they occur. This rate is based on recorded admission or attendances at hospitals and dispensaries. Naturally in areas of high endemicity with large proportion of Asymptomatic carriers, the morbidity rate records only to clinical cause and present only a small proportion of total amount of malaria.

The true Mortality Rate from malaria is difficult to determine for similar reasons, except in conditions when the diagnosis and reporting of each case is carried out to perfection. Therefore, the often quoted average mortality rate due to malaria (1%) is not more than an estimate.

1. CLINICAL ASPECTS OF MALARIA

a. The Clinical features of malaria vary from mild to severe and complicated according to the species of parasite present, the patients state of immunity, the intensity of the infection and also the presence of concomitant conditions such as malnutrition and other diseases. The disease tends to be particularly severe in children and pregnant women.

b. The Incubation period occurs between the time of the infective bite and the onset of symptoms, during it pre-erythrocytic forms are developing in the liver. The duration of the incubation period is 10-15 days but may exceed in some strains of P. Vivax and P. malariae.

c. A typical attack of malaria has got three distinct stages. The cold stage, the hot stage and the sweat stage. These are followed by an afebrile period in which the patient feels greatly relieved. Febrile herpes is common in all malaria patients. After the Primary attack of fever, there follows an afebrile interval of 48-72 hours and then other attacks similar to the first, followed by similar afebrile period.

d. However, the history may not be so typical

In P. falciparum infections in persons with poor immunity, the irregularly or regularly spaced paroxysms are associated with marked prostration, and after 7-10 days there commonly follows a rapid deterioration in the patients condition associated with shock and other complications and in untreated infection as a result of inadequate or no treatment may suffer several weeks or months of sub-optimal health interspersed with febrile episodes, malarial anaemia and weakness. In other malarial infections the disease is rarely fatal and after 2 or 3 episodes of fever the patient's required



immunity increases, and the attacks, even in the absence of treatment, become less severe. They are followed by short term relapses or a period of sub-optimal health before a natural cure takes places.

e. Complications of P. falciparum infections

i. Cerebral Malaria : This occurs particularly when non-immune persons have remained untreated for 7-10 days after development of the primary fever. At this time patients condition deteriorates rapidly with increasing head-ache, drowsiness which merge into confusion and light coma. This may deteriorate further into deep coma with stertorous breathing. In very heavy infection, delirium and coma may develop suddenly and may even occur early during the course of febrile illness. Hyperpyrexia is not unusual. Signs of meningeal irritation are rare except in young children.

ii. Acute Renal failure: The shock like mechanism associated with severe malaria, particularly when there are no cerebral features may lead to oliguria or anuria and histologically in such cases tubular necrosis will be present. A watch should therefore, be kept on the urinary output in severely ill malarial patients. Except in very hot weather, a drop to 400 ml. or less urine per day indicates renal failure.

iii. Liver damage : Haemolytic Jaundice, more than usually enlarged liver and tender, very rarely occurs.

iv. Gastro intestinal symptoms : Diarrhoea in severe infections due to necrosis or damage to the intestinal wall. Dysentery or even cholera may be simulated.

v. Dehydration : Caused by vomiting, sweating and diarrhoea. Dehydration by increasing blood viscosity, impairs its oxygen carrying capacity and may lead to renal and cardiac failure.

vi. Collapse : The patient may suddenly collapse, possibly when the temperature is sub-normal. Peripheral circulatory failure due, in part to dehydration and in part, in some cases, to lesions in the adrenal glands is thought to be responsible for this complication which was formerly referred to as the Algid type of the infection.

vii. Anaemia : In P.falciparum infection greater number of erythrocytes become parasitized than other types of infections and there is release of more malaria antigen with consequent more marked Immuno-Haemolytic anaemia. Sometimes in pregnant women a sudden and catastrophic fall in haemoglobin may occur.

viii. Black water fever : Classical Black water fever consists of a sudden massive haemolytic episode in which the patient who has felt unwell for some time takes a dose of Quinine and within an hour or two has an attack of shivering, feels weak and collapses and the urine, which till then had been normal in colour is almost black when next passed. Marked anaemia, recurrent rigors and irregular fever follow. There is almost always history of having taken small doses of Quinine, inadequate to suppress the existing P.falciparum infection.



ix. Other complications:

- a. Petechial haemorrhages in the skin, mucus membranes and the retina.
- b. Rupture of enlarged spleen from trauma, sometimes slight.
- c. Pigment containing gallstones in chronic malaria cases.
- d. Malaria in Pregnancy :

Malaria of any form may precipitate miscarriages or abortion and may complicate pregnancy by causing severe anaemia. Pregnancy also appears to impair immunity to malaria and thus relapse may develop during pregnancy. Fever during puerperium should always be considered as possibly resulting from malaria.

e. Malaria in Children

Children commonly develop high fever even from relatively mild infections. They may develop convulsions during the malarial attack and dehydration in them develop with greater rapidity as a result of vomiting or sweating than it does in adults.

2. DIAGNOSIS

Certainty in the diagnosis of malaria depends on demonstration of the parasite in the blood, but suspicion of the diagnosis is caused by epidemiological and clinical evidence.

a. Clinico epidemiological diagnosis :

In every case of unexplained fever in person in areas where malaria is or has been endemic, or those coming from endemic areas, malaria should be considered along with other diagnosis. Under the NMEP, all fever cases are presumed to be due to malaria.

b. Laboratory diagnosis :

The finding of malaria parasites in the blood and their identification is essential for confirmation of the diagnosis of acute malaria. An initially negative result does not necessarily mean the absence of malaria, specially in persons who have received anti-malarial drugs prior to reporting. Several examinations by well trained technicians or microscopist are sometimes required in these cases. The malarial fluorescent antibody test, usually becomes positive two weeks or more after primary infection, by which time the infection may have been cured. A positive test is, therefore, not necessarily an indication of current infection. The test is of greatest value in epidemiological studies and in determining whether a person has had malaria in the past.

### 3. MANAGEMENT OF MALARIA CASE

Management of malaria case may be discussed under the following headings:

- a. Treatment of a presumed chloroquine sensitive case.
- b. Treatment of a case in chloroquine resistant areas.
- c. Treatment of complications.
- a. Treatment of a presumed chloroquine sensitive case

Presumptive treatment : To all fever cases chloroquine 600 mg. base should be given. This treatment will suppress the acute attack.

After the diagnosis is confirmed by a blood film, radical treatment should be given.

Radical Treatment : Pf cases - Chloroquine 600 mg. with primaquine 45 mg. in a single dose.

Pv cases - Chloroquine 600 mg. on 1st day and Primaquine 15 mg. daily for 5 days.

#### Treatment in areas of known chloroquine resistance

As per the present drug schedule under M.P.O.

Presumptive treatment : Amodiaquine 600 mg. by field workers  
Sulfadoxine/Sulfalene = 1000 mg.  
Plus Pyrimethamine 50 mg. in single dose by PCD centre.

#### Radical treatment:

##### Pf Cases

Sulfadoxine/Sulfalene 1000 mg. plus pyrimethamine 50 mg. with Primaquine 45 mg (Single dose)

##### PV Cases

Chloroquine/Amodiaquine 600 mg. on 1st day, and Primaquine 15 mg. daily for 5 days.

In addition, the PHCs while treating malaria cases with the above chemotherapeutic schedules need to assess the condition of the patient and other medical care may be employed when the patients condition so indicates. Thus hydrotherapy of fever, adequate rehydration, antipyretics, analgesics and attention to nutrition should be given. All patients with malaria should be treated urgently in view of the possibility that their condition may seriously and speedily deteriorate. If anti-malarials are to be administered parenterally, the intravenous route has considerable advantages over



the intramuscular. More-over most patients requiring parenteral administration are dehydrated requiring intravenous infusion which forms an ideal vehicle for the drug.

c. Treatment of complications

When a patient suffering from malaria develops high fever (above 40 C), vomiting, diarrhoea, pain in the abdomen, smoky urine, severe hypotension, oliguria, delirium disturbances of sensorium, convulsions jaundice or a bleeding tendency, that patient must be transferred to the nearest hospital as quickly as possible.

i. CEREBRAL MALARIA

For diagnosis and management of cerebral malaria cases we may refer to the booklet issued by the Directorate, NMEF, Delhi (Contents enclosed).

ii. ACUTE RENAL FAILURE

Treatment should be aimed at preventing ischaemia by electrolyte and water replacement as needed.

iii. BLACK WATER FEVER

The profound degree of haemolysis may be associated with gross anaemia and shock. Attention to fluid balance in such patients is of critical importance and for anaemia, blood transfusion is necessary as an emergency measure.

Prednisolone phosphate in doses of 40-60 mg. daily IM may be given during the period of haemolysis or until the haemoglobin concentration is maintained at over 7 gm. per 100 ml.

iv. ANAEMIA

Treatment of anaemia following single acute attack of malaria is seldom necessary, for iron and other requirement for haemoglobin production are liberated intravascularly as a result of haemolysis. These then become mostly available for resynthesis of haemoglobin. Pregnancy and

During severe acute infection particularly in childhood, blood transfusion or preferably transfusion of packed red blood cells may be required to combat anaemia.

v. DEHYDRATION AND SHOCK :

Fluid balance is important in dehydrated patients with renal or gastro-intestinal involvement or metabolic disturbances. Fluid should be replaced judiciously to avoid over hydration which may cause pulmonary oedema and probably also cerebral oedema and coma. Shock is basically non-specific and when it appears, requires immediate infusion of fluid, to restore the blood volume. Isotonic saline is commonly used and 500 ml. of it or a plasma should be given rapidly (in about  $\frac{1}{2}$  to one hour) followed by 1L of Isotonic saline or Isotonic glucose more slowly administered (at a rate of about 500 ml. every 4

hours). The total volume of fluid needed is assessed clinically.

vi. LIVER DAMAGE

Management of the hepatic failure which occurs very rarely should be carried out in the same general lines as those for liver insufficiency. Diet in the form of sips of glucose solution and vegetable soups should be substituted for solid food.

d. MALARIA IN PREGNANCY

Malaria in a pregnant woman must be regarded seriously and treated accordingly.

1. Administration of commonly used anti-malarial drugs in pregnancy is not contra-indicated. Only Primaquine should not be used for Radical Cure.
  2. Folic acid at a dosage of 5 mg. daily to prevent folate deficiency (which may be aggravated by Pyrimethamine).
  3. Iron preparations are indicated (oral or injectable forms).
  4. In severe anaemia blood transfusion may be needed as a life saving measure before the onset of labour.
  5. Proper nutrition with green leafy vegetables and protein is of great importance.
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## DIAGNOSIS AND MANAGEMENT OF CEREBRAL MALARIA

### CLINICAL PROFILE:

A patient of cerebral malaria with P. falciparum infection presents with fever and varying grades of disturbances of sensorium. There may be disorientation, delirium and even coma. In early states, sometimes there may be changes in behaviour, excitement and mania. Occasionally there is neck rigidity, focal weakness and epileptiform convulsions. Hyperpyrexia and shock may develop.

### DIFFERENTIAL DIAGNOSIS:

The condition has to be differentiated from meningitis, encephalitis, heat stroke, typhoid encephalopathy, gram negative septicæmia, uraemia with pyelonephritis, brain abscess, cerebro-vascular accident and hepatic encephalopathy. Other cause of some e.g. diabetes and narcotic poisoning when associated with fever due to secondary infection have to be distinguished from cerebral malaria.

In cerebral malaria, the coma has a rapid on-set, is accompanied with hyperpyrexia or mental changes.

In cerebro-vascular accidents, the on-set is sudden and there are signs of focal cerebral lesions such as hemiplegia and meningism if subarachnoid haemorrhage has occurred. There may also be evidence of the cause as hypertension, atherosclerosis and cardiac disorder.

In meningitis coma is of gradual on-set in most types preceded by signs of meningeal irritation and fever. The cerebrospinal fluid is turbid and contains polymorphonuclear leucocytes. Viral encephalitis as a cause of coma is not common. In heat stroke, there is a history of exposure to heat, a high body temperature and striking absence of sweating.

In uraemic and hepatic coma the history is more chronic and there are signs of the underlying disease.

In diabetic and hypoglycaemic coma, there is no rise of temperature and there is a history of diabetes and/or taking some drug for diabetes. Urine examination in the former will show sugar and ketone bodies, while there will be no sugar in the latter.

In epilepsy in children there will be previous history of such attacks.

### LABORATORY INVESTIGATIONS:

The following investigations should be carried out to confirm the diagnosis of cerebral malaria and excludes other clinical possibilities, (a) Thick and thin

Contd...

smears for malaria parasites. Sometimes peripheral blood smears may be negative, negative smears/for malaria parasites do not exclude cerebral malaria.

- (b) Total and differential leucocyte counts.
- (c) Blood culture
- (d) C.S.F. examination.
- (e) Urine examination.
- (f) X-ray skull should be done to exclude other conditions which simulate cerebral malaria.

#### TREATMENT OF CEREBRAL MALARIA AND ITS COMPLICATIONS:

##### 1. SPECIFIC TREATMENT OF CEREBRAL MALARIA:

Compounds of chloroquine and quinine are used for the treatment of cerebral malaria. Compounds of quinine should be used if parasite resistance to chloroquine is suspected or if the patient is sensitive to chloroquine.

##### 1.1 Injection of chloroquine diphosphate:

200 mg of base in 20 ml. of pyrogen free water is injected slowly intravenously using a 20 ml. syringe and a small bore needle. The intravenous injection of chloroquine diphosphate should be given very slowly and not be completed in less than 15 minutes.

1.1.1 If the patient is in a state of shock, the first dose should be administered in an intravenous drip. The total dose of chloroquine diphosphate 200 mg. should be added to 5 per cent glucose saline.

1.1.2 If there is no improvement in 8 hours, a second dose should be given. Third dose is required only in exceptional circumstances.

1.1.3 Intramuscular injection is alternative to intravenous route. This route should not be used if the patient is deeply comatose or in a stage of shock.

Chloroquine diphosphate, 200 mg. of the base, should be used in 9 ml. of sterile pyrogen free normal saline or distilled water and injected slowly and aseptically into gluteal muscle. It may be repeated at 8 hourly intervals during the first 24 hours.

1.1.4 In children, the dose of chloroquine diphosphate should not exceed 5 mg per kg. body weight.



Quinine-dihydrochloride 650 mg. dissolved in 20 ml. of sterile pyrogen free physiological saline or water to injected slowly intravenously in 15 minutes.

- 1.2.1 Dose may be repeated after 8 hours and then again 8 hours later. Total dose should not exceed 1950 mg in 24 hours.
- 1.2.2 First dose may be given by syringe, subsequent doses in intravenous saline drip. It is seldom necessary to continue this more than 24 hours. If it is considered essential, subsequent doses should not exceed 1300 mg. in 24 hours.
- 1.2.3 Intramuscular injection: Quinine hydrochloride 600 mg made in 5 ml. of physiological saline or distilled water is injected deep into the gluteal muscles.
- 1.2.4 Abscess: is more common with quinine dihydrochloride as compared to quinine hydrochloride.
- 1.2.5 In children, the drug is given intramuscularly, the dose of quinine hydrochloride is 10 mg per kg body weight. In children up to one year, the dose of quinine hydrochloride should be one-tenth of the adult dose and between 1 to 15 years it should be  $\text{age}/20 \times \text{adult dose}$ .

## 2. GENERAL TREATMENT OF CEREBRAL MALARIA IS AS FOLLOWS.

### 2.1 Treatment of shock

This must be treated as quickly as possible.

- 2.1.1 Raise the foot end of the bed.
- 2.1.2 Cortisone hemiscuccinate 300 mg. first dose and 100 mg. 8 hourly should be administered intravenously, or dexamethasone acetate 8 mg. first dose and 4 mg. 8 hourly intravenously should be administered. Dose should be gradually tapered after 24 hours. If there is marked dehydration, adequate fluid and electrolyte replacement should be ensured.
- 2.1.3 Plasma expanders like low molecular weight dextran 6-12 per cent solution 250-500 ml. should be administered intravenously.

2.1.4 Mephenteramine sulphate 30-60 mg. intravenously 6 hourly and if this does not bring up the blood pressure, 600 mg in 500 ml. of 5 per cent glucose should be administered by intravenous drip, the rate of administration being regulated by the response of blood pressure.

2.2 Treatment of convulsion / excitement:

As these appear as complications of cerebral malaria treatment should be administration of injection Diazepam 10 mg. intravenously or Amino 0.2 mg. intramuscularly.

2.3 Treatment of deep coma:

If the patient becomes deeply comatose, the following line of treatment is to be adopted.

2.3.1 Bexarilacene acetate 4 mg 6 hourly intravenously.

2.3.2 Lumbar puncture will be useful, it is also helpful in excluding other causes of coma like meningitis and encephalitis.

2.4 Treatment of hyperpyrexia:

When a patient of cerebral malaria develop hyperpyrexia. It should be treated as follows:

2.4.1 The patient should be draped in Wet sheet immersed in cold water and placed under the fan. If possible patient should be moved to an air conditioned environment.

2.4.2 Injection of paracetamol 0.5 mg. intramuscularly to be injected every 6 hours.

2.4.3 Rectal temperature should be recorded. Surface cooling should be stopped when rectal temperature comes down to 36° C.

2.4.4 If temperature is not controlled by the above measures then injection of chlorpromazine hydrochloride 50 mg. may be given intramuscularly or as slow intravenous drip. Half hourly record of blood pressure should be maintained. This should be administered with utmost caution as sometimes blood pressure is likely to fall.

2.5 Treatment of disseminated intravascular clotting:

For the recognition of intravascular clotting one should watch for bleeding at site of puncture, haematuria or bruises. This may be further supported by doing the laboratory investigations as determination of fibrinogen degradation products, platelet count and fibrinogen.

If a patient of cerebral malaria develop this clotting problem the following treatment should be administered.



2.5.1      Injection dexamethasone acetate 8 mg. first dose and  
4 mg. 6 hourly intravenously.

2.5.2      Heparin 50 units per kg. body weight in 5 per cent glucose  
solution every 6 hours.

2.5.3      Fresh and carefully matched blood transfusion.

## CHEMOTHERAPY OF MALARIA

1. Available drugs and their application according to selective action on the different stages of the malaria parasite.

No single drug available acts on all the stages of malaria parasite. In different stages different drugs have been found useful.

- (a) Causal Prophylactics: Drugs having action on the Primary tissue phase eg. pyrimethamine, primaquine.
- (b) Tissue Schizonticidal drugs: Drugs acting on the tissue Schizonts eg. Primaquine, pyrimethamine and sulfonamides (possibly some action)
- (c) Blood Schizonticidal drugs: Drugs acting on the asexual parasites in the blood eg. Quinine Amodiaquine, Chloroquine Sulfonamides, Mefloquine.
- (d) Gametocidal drugs: Drugs acting on the gametocytes in blood and destroying them eg. Primaquine.  
Quinine, Chloroquine, Amodiaquine and Mefloquine are active against P.vivax and P.malariae but not in P.falciparum
- (e) Sporontocidal drugs: Drugs acting on the gametocytes in blood but prevent development of the gametocytes in mosquitoes eg. Pyrimethamine, Primaquine.

Recrudescence: Infection comes back due to survival of the Erythrocytic forms (upto 8 weeks).

Recurrance: Infection comes back due to reactivation (Relapse) of dormant parasites in the tissue (Hypnozoites).

## II. Classification of available drugs as per their chemical constitution

- |                            |                            |
|----------------------------|----------------------------|
| 1. 4 - Aminoguanidines     | - Chloroquine/Amodiaquine  |
| 2. 8 - Aminoguanolines     | - Primaquine               |
| 3. Diamino Pyrimidines     | - Pyrimethamine (Daraprim) |
| 4. Cinchona Alkaloids      | - Quinine                  |
| 5. Sulfones & Sulfonamides | (Short and long acting)    |
| 6. Quinoline Methanols     | - Mefloquine               |

## III. Properties of available drugs

1. Chloroquine: Rapidly absorbed, stored in tissues of organ and persist for long time. Therefore, to achieve an effective concentration in plasma quickly a "loading dose" at the beginning is advisable. Slowly metabolised. Its elimination is very slow. In high dose it is toxic (deaths occurred within 2 hours of injection of 2.5 gm of Chloroquine).



Mild Toxic Symptoms : Headaches, dizziness, nausea, anorexia apatic gastrointestinal symptoms and diturbances of visual accomodation.

Severe Symptoms : Heart and nervous symptoms and death by respiratory failure. Chloroquine acts on the blood schizonts possibly inhibiting the respiratory enzymes of the parasites.

2. Amodiaquine : Same as Chloroquine. Dosage also same for single dose or 3 doses treatment. However, for suppressive therapy 400 mgm. of Amodiaquine weekly as against 300 mgm. of chloroquine should be given. It seems that in some localities Amodiaquine is more rapidly effective than chloroquine.
3. Primaquine : Primaquine diphosphate most commonly used. It is very quickly absorbed, but also rapidly eliminated within 24 hrs. Very small amounts are fixed in the tissues.

This drug shows that in some individual and particularly in Negroes, doses that were harmless for other people could cause haemolytic anaemia and Methaemoglobinamia. In a daily dose of 30 mgm. it was found to cause acute haemolytic anaemia in about 10-15% of adult American Negro males. This individual susceptibility has been shown to be a genetic characteristic, correlated with an intrinsic defect in the deficiency of an enzyme Glucose 6 Phosphate - Dihydrogenase (G6PD) which influences various functions viz Glucose Oxidation.

Methaemoglobin is formed and its reduction into haemoglobin is impaired hence methaemoglobinaemia which is recognised by the development of cyanosis in the subject and the clinical picture of 8-AQ intoxication i.e. nauses, abdominal and epigastrict pains, vomiting, darkurine that in servere cases suggest blackwater fever.

Haemolysis is self limiting as only the older RBC's are destroyed.

Primaquine sensitivity is accompanied by a sensitivity to a number of other drugs e.g. Sulphenamides, sulphenes etc.

The 8-AQ distinguish as the only drug capable of destroying P.falciparum Gametocytes. They also have action on the Secondary exoerythrocytic forms. Thirdly they have sporontocidal activities as well. Fourthly Primaquine can be used as casual Prophylaxis for Radical cure of P.vivax cases - 15 mgm. daily for 5 days found adequate with only about 5% relapses. Primaquine can be given in higher doses if the interval between them is long enough.

Primaquine inhibits parasite mitochondrial respiration and this is probably the basis of its action against the tissue schizonts and the gametocytes.



4. Pyrimethamine : Daraprim is an extremely valuable drug in malaria eradication and completes the action of the 4-AQ by acting on the sporogony and on the exoerythrocytic forms of some strains.

Absorption is rapid. Moderately stored in the organs but loading dose is not necessary. Elimination of the drug is slow after the first 72 hours. Toxicity is very low but when used at higher doses or in longer courses it may inhibit nucleoprotein synthesis in man and give rise to macrocytic anaemia. So use folic acid and folinic acid in such cases.

Daraprim acts on the malaria parasites by inhibiting nuclear division.

The sporontocidal activities of Daraprim is seen after 3-4 hours ingestion and may keep the person harmless for 3-6 weeks.

5. Quinine: With the discovery of resistant strains of P.falciparum malaria to Chloroquine, Quinine has again become a useful drug. Fortunately Quinine is second to no other drug in saving the life of a severe malaria patient and in giving quick clinical relief.

Quinine is eliminated very quickly and it appears in the urine a few minutes after ingestion. Hence the need to give daily amount in practical doses 3 or 4 times. Quinine acts on the asexual form of all species in blood and on gametocytes of P.vivax, ovale and malariae. It is inactive against exoerythrocytic forms. Radical cure can be achieved in P.falciparum cases with quinine alone at dosage of 2.g. daily for 7-10 days.

In severe cases Quinine should be injected intravenously/intramuscularly.

Sulfonamides: Sulfonamides and sulfones are highly effective against the asexual blood forms of P.falciparum but less effective against those of the other species. They produce clinical cure of falciparum malaria, but their action is too slow for them to be used alone.

They are also effective suppressive agents but should not be used for this purpose alone because of rapidity with which drug resistance can develop.

Malaria parasites like many bacteria are unable to utilize preformed folic acid and require para aminobenzoic acid as a substrate in order to synthesise it. Sulfonamides and Sulfones act as competitive antagonists of this substrate.

When administered together with Pyrimethamine, sulfonamides may potentiate the action of this drug. The potentiation may be of such a degree that the combination can be effective against strains of microorganisms that are resistant to either component used alone.

Mefloquine: This is a new antimalarial drug still being used on trial basis. Mefloquine has marked action against asexual blood forms. Against gametocytes, the drug is active against P.vivax and P.malariae but no direct action against P.falciparum.



Mefloquine is generally well tolerated, safe and has a potent blood schizonticidal activity against strains of parasites resistant to other antimalarials such as chloroquine and Pyrimethamine.

#### IV: Chemotherapy in Malaria Eradication:

A. Presumptive treatment: Given to all fever cases at the time of blood smear collection presuming that the fever is due to malaria by the active surveillance agent or the passive agencies.

1. Areas where Chloroquine resistant P.falciparum malaria is predominant

(a) ACD - Amodiaquine 600 mgm base (adult) single dose.

(b) PCD - 2 tabs. (Sulphalene or Sulphadoxine - 1000 mgm and 50 mgm of Pyrimethamine)

(c) BDO/PTD, VHG - Amodiaquine 600 mgm base (adult)

2. Areas where P.falciparum is sensitive of Chloroquine  
ACD, PCD and PTD/BDO (Chloroquine 600 mgm base (Adult dose))

B. Radical treatment: All cases found positive for malaria parasites through Blood smear examination are to be given radical treatment preferably within a week. P.falciparum cases will be given priority during the transmission season.

1. Areas with Chloroquine resistant P.falciparum malaria

(a) P.falciparum cases - Adult - Sulphalene or Sulphadoxine - 1000 + Pyrimethamine 50 mgm + Primaquine 45 mgm (single dose)  
Children - proportionately smaller doses.  
Infants & Pregnant women - No Primaquine.

(b) P.vivax, P.malariae and Mixed infection cases.

Chloroquine - 600 mgm on 1st day only

Primaquine - 15 mgm daily for 5 days.

2. Areas with Chloroquine sensitive P.falciparum malaria

(a) P.falciparum cases : Adult - Chloroquine 600 mgm + Primaquine 45 mgm

(b) P.vivax, P.malariae and mixed infection cases

1st day - 600 mgm Chloroquine + 15 mgm primaquine

2nd day - 15 mgm Primaquine daily for 5 days.

C. Mass drug administration: Recommended for smaller areas with high malaria endemicity. Chloroquine or Amodiaquine 600 mgm along with Primaquine 45 mgm (Adult dose) may be administered. MDA should always be associated with insecticidal spray in the area. The drug administration need to be repeated at monthly or two monthly interval to cover the transmission period. Total coverage of the population targetted should be ensured.

D. Drug schedule for labour population in PfCP areas.

- (a) Labourers on entry PfCP (Zone-I) areas.  
MDA with 600 mgm Chloroquine and 45 mgm Primaquine (adult dose) must be given within 10 days of their arrival.
- (b) Labourers before leaving PfCP (Zone-I) areas  
MDA with 600 mgm Chloroquine and 45 Primaquine (adult) single dose.

E. General

- (i) Use of Paracetamol - This drug will be given in the doses of 500 mgm (adult) by ACD/PCD and FTD only in current fever cases. DDC will not distribute this drug at all.
- (ii) Quinine salts are life saving in cases where other drugs fail. For acute cerebral or other serious types of malaria intravenous Preparations should be used.
- (iii) Chloroquine Injectable: Special precaution should be taken for the use of this in children, as shock may be produced. In Chloroquine resistant areas P.falciparum cases should not be treated with Chloroquine injections.

-V. Drug resistance to malaria Parasites:

Drug resistance in malaria has been defined as the "availability of a parasite strain to survive and or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of subject."

Although drug resistance embraces all aspects of malaria parasites and all acceptable dosages of blood or tissue schizontocides, gametocytocides and sporontocides, in practice it is most commonly related to the effect of blood schizontocides on falciparum malaria". Drug resistant malaria" at the present time customarily understood to refer to the 4 aminoquinolines particularly chloroquine.

Drug failure - is absence of drug action due to deficient absorption, unusual rate of metabolism or excess excretion of the drug.

Increased tolerance to a drug can be seen when the parasites disappears in increasing the dosage, say from 600 mgm to 900 mgm when parasite persists even on increasing the dosage, the strain is resistant to the drug.

Gradation of response to drugs:

WHO has standardised the "In Vivo" procedure to study sensitivity of 4 AQ drugs in Asexual parasites of P.falciparum. A system of grading the resistance of asexual P.falciparum to normally recommended doses of Chloroquine (1500 mgm) has been proposed and has proved practical and useful. As per this grading.



Response	Recommended Symbol	Evidence
Sensitivity	S	Clearance of asexual parasites is within seven days of initiation of treatment without subsequent recrudescence
Resistance	RI	Clearance of asexual parasitaemia as in sensitivity followed by recrudescence early or delay.
	RII	Marked reduction of asexual parasitaemia but no clearance
	RIII	No marked reduction of asexual parasitaemia

#### Mechanism of drug resistance

Resistance by *P. falciparum* to Chloroquine as well as by all species to Pyrimethamine is attributable to selection under drug pressure of resistant mutants, which survive by utilising alternative metabolic pathways to those blocked by the particular drug. In respect of Chloroquine, resistance is characterised by a decrease in high affinity binding sites for the drug. Once selected and provided that they escape the destructive action of host immunity the resistant parasites may be transmitted by local mosquitoes to other people in the immediate area or may be carried by a migrant host to other places.

#### Distribution of drug resistant malaria:

Drug resistant malaria has been confirmed from different countries all over the world.

In south and central America, *P. falciparum* resistance to Chloroquine was first observed in Columbia in 1960. Since then this has been reported from Brazil, Guyana, Surinam, Venezuela, Bolivia, Panama and recently Ecuador and French Guyana.

In south east Asia resistance was first suspected in Thailand in 1957. Since then it has been confirmed in Thailand, West Malaysia, Khmer republic (Cambodia), Laos, Vietnam, Philippines, Burma, East Malaysia, India, Bangladesh, Papua New Guinea and Indonesia.

In Africa Resistance has now been reported from the eastern coast viz. Tanzania, Kenya, Madagascar, Uganda, Zambia and Sudan.

The resistant strain have been continually spreading from the original focus in Thailand in an eastern south eastern direction reaching all countries.

West ward the spread has engulfed Burma, crossed into the Chittagong Hill tracts of Bangladesh and reached the North Eastern States of India. At this point there has been an onward spread down into the plains of India mainly through imported labourers from Orissa to the North Eastern State for construction projects. Resistant strains have now been confirmed from Maharashtra, Andhra Pradesh, U.P., M.P., and Karnataka.

In the North Eastern region resistant P.falciparum malaria was first detected in K.Z. districts of assam during 1973. Subsequently the resistant strain was detected in Nowgong, Kamrup, Sibsagar, Kokrajhar districts of Assam, East Khasi Hills, East Garo Hills and West Garo Hills districts of Meghalaya, Jalpaiguri and Purulia districts of West Bengal. Kohima district of Nagaland, South district of Tripura, Central District of Manipur, Aizwal and Lunglei districts of Mizoram, Tirap district of Arunachal Pradesh and Andaman and Nicobar Island.

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ANTIMALARIA DRUG POLICY FOR 1981 - DECISION  
TAKEN AT THE ANNUAL MALARIA AND FILARIA WORKERS  
CONFERENCE HELD AT CHANDIGARH FROM 25TH APRIL,  
1981.

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Chloroquine resistance problem in India was first detected in the year 1973 in Assam. In 1974 one more focus was discovered in the same state. In 1977 Directorate NMEP had taken up an applied research scheme for monitoring the chloroquine resistance status of P.falciparum in different parts of India. At present, there are six such teams working at Shillong, Bhubaneswar, Lucknow, Hyderabad, Baroda and Bangalore. Presently the number of teams raised to 12. During the last two years several in-vitro and in-vivo tests have been done and more areas have been discovered where the phenomenon of chloroquine resistance exist.

It should be mentioned here that in some areas P.falciparum constitute the major infection, in others it co-exists with P.vivax. Even in areas where P.falciparum is found to be resistant to chloroquine the entire falciparum population does not become totally resistant and in them varying degree of resistance to chloroquine is noted i.e. some are fully susceptible to chloroquine, some are having RI, RII or RIII type of resistance, Areas with RIII type of resistance are fortunately still very few.

It is worth mentioning that so far there is no authentic report about the resistance of P.vivax to chloroquine. There does not appear to be any report indicating the chloroquine susceptibility status of P.malariae in India.

In this context it appears that our policy should be clearly defined for the following four types of situations:

1. In areas where chloroquine resistant falciparum has been reported. The policy regarding presumptive treatment, radical treatment and policy for the incoming and outgoing labourers are to be defined.
  2. Similar policy decision are required in respect of areas where P.falciparum continues to be susceptible to chloroquine.
  3. The policy regarding the treatment (presumptive and radical) for areas where P.vivax dominates and P.malariae also co-exist.
  4. The drug policy for the project areas where there is a frequent influx and out-turn of labourers - Specially project areas (both in chloroquine resistant and sensitive strains).
- A. Chloroquine Resistant in P.falciparum areas  
Drug policy in areas with established chloroquine resistance in P.falciparum.

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Some studies have been carried out by six monitoring teams (under ICM) in various parts of the country. Several districts in several States/U.T.s are still to be covered and are being covered gradually. This has shown that in some States/districts the problem is of such a magnitude that there is an urgent need for changing the drug policy. The areas are listed below:

In areas where only one or two cases have been detected (cases of mild resistance types (RI) or where the foci are of doubtful nature or need of re-confirmation, it is not admissible to change the present drug policy in place of the para vis. studies carried out by the MRP. Experiments have shown the following districts with this problem & in several others, there are doubtful foci and at present it is not advisable to change the existing drug policy.

State/U.T.	District	Remarks
1. Assam	1 Karbi Anglong 2 Nowgong	Whole -do-
2. Arunachal Pradesh	1 Lohit	-do-
3. A & N Islands	-	Whole great Nicobar Little Nicobar Islands only.
4. P.H.K. Project Bastar Hill (M.P.) & (Orissa) Project		Covering the area of Madhya Pradesh & Orissa.
5. Meghalaya	All districts	-
6. Maharashtra	Chandrapur	-
7. Nagaland	All districts	-
8. Bihar Pradesh	Mirzapur	N.T.P.C. Sakti Nagar only.

(a) PRESUMPTIVE TREATMENT IN CHLOROQUINE RESISTANT P.FALCIPARUM CASES (IN CASES OF CURRENT OF WITH RESISTANCE).

1. Active case Detection (ACD) - Amodiaquine 600 mg. base (adult)
2. Passive case Detection (PCD) - 2 tablets (i.e. 1000 mg. dose of Sulphalene and 50 mg. dose of pyrimethamine).
3. DEC & FED - Amodiaquine 600 mg. base (adult)

Note:- Full dose is to be given at a time after meals. Children 0-1, 2-4, 5-8, 9-14 will receive 1/5, 1/2 and 3/4 of the adult dose respectively.

(b) Radical Treatment (P.falciparum, Chloroquine resistant areas).

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In the above districts with chloroquine resistant P.falciparum the radical treatment will consist of 1000 mg. Sulphalene and 50 mg. Pyrimethamine (given as two tablets of SLP) alongwith Primaquine 45 mg. base for the adults. The children except infants will receive the drug in proportionately small dose.

Infants and pregnant women need not be given any primaquine.

In such districts, the drug schedule for radical treatment for P.vivax, P.malariae or mixed infection (PV+PM) will remain unaltered i.e. chloroquine 600 mg base on the first day and Primaquine 15 mg. base daily for 5 days.

B. Areas where P.falciparum is sensitive to chloroquine:

- i. Presumptive treatment by ACD PCD & FTD/DDC. Use of Chloroquine will be continued as per the dose already in use (i.e. 600 mg. base for adult)
- ii. Adult R.T. - 600 mg. chloroquine + 45 mg. Primaquine (small dose)

Radical Treatment of P.vivax/P.malariae or Mixed:

1st day 600 mg. chloroquine + 15 mg primaquine  
2nd-5th day only 15 mg. primaquine daily

A. Drug scheduled or Labour population in PfCP areas

Labourers before leaving PfCP area (zone-I or any area with high incidence of Pf.

Mass drug administration with 600 mg. chloroquine and 45 mg. Primaquine (adult) single dose.

B. Labourers coming from Zone-I & on detection in Zone-II & III & other zones the regimen will be as follows:

Presumptive

Chloroquine 600 mg.  
Primaquine 45 mg.  
(adult single dose)

Radical

Chloroquine 1500 mg in three divided dosage and Primaquine 45 mg. adult single dose on 1st day. If there is no response to this regimen within three days (72 hours) 1000 mg. Sulphalene + 50 mg. Pyrimethamine with 45 mg. Primaquine (adult single) as already stated Primaquine will not be given to infants and pregnant women.

GENERAL:

- i. Use of Paraectamol - 600 mg. (adult) of this drug will be given by ACD/PCD/PTD only in current fever cases.  
DDC will not distribute this drug at all.
  - ii. Quinine sulphate/Hydrochloride - Preparation of Quinine salts are life saving in cases where other drugs fail. For acute cerebral of other serious types of malaria, intravenous preparation should be used.
  - iii. Chloroquine injectable - Special precaution should be taken for the use of this in children, as shock may be produced. In chloroquine resistant areas, P. falciparum cases should not be treated with chloroquine.
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## PRINCIPLES OF MALARIA PREVENTION AND CONTROL

Sir Ronald Ross discovered that malaria was transmitted through the bite of mosquitoes. Prior to this discovery there was no rational basis of controlling malaria was in existence and the first attempt to control malaria by mosquito reduction was put forward by Ross himself in 1899.

The measures for prevention of malaria in individuals and for larger scale control of the disease can be divided according to the classification proposed by Russell (1952).

1. Measures designed to prevent mosquitoes from feeding on man.
2. Measures designed to prevent or reduce the breeding of mosquitoes by eliminating the collections of water.
3. Measures designed to destroy the larvae of mosquitoes.
4. Measures designed to destroy adult mosquito.
5. Measures designed to eliminate the malaria parasite in the human host.

### MEASURES DESIGNED TO PREVENT MOSQUITOES FROM BITING MAN:

#### a. SOCIAL MEASURES:

Improving the economic status of the people which will result in better living conditions and less facilities for breeding of mosquitoes.

#### b. HEALTH EDUCATION

To educate the public about the necessity of controlling the disease and the simple and practical method of avoiding it.

#### c. MECHANICAL

i. Screening of building

ii. Use of mosquito bed nets.

iii. Destroying mosquito shelters.

#### d. CHEMICALS:

i. Use of mosquito repellants on clothing and skin.

ii. Dimethyl phthalate.

MOSQUITO CONTROL

(I) Against aquatic stages of mosquitoes:

(A) Measures designed to prevent the creation of man made breeding places:

- i. Special training of engineers and agronomists in water management.
- ii. Cooperation of public works, irrigation and public health authorities.

(B) Measures designed to modify breeding places:

i. Naturalistic:

- a. Altering the flora
- b. Exposing to sunlight or shading
- c. Folliating the water or changing the chemical contents.

(C) Chemical:

Use of oils and chemicals for destroying the different aquatic stages of mosquitoes.

(II) Against Adult mosquitoes:

(A) Biochemical : Augmenting natural enemies  
e.g. Bacteria, Virus, Fungi,  
Protozoa, Nematodes, Fish,  
Insect predators & Plants.

(B) Mechanical :  
i. Swatting  
ii. Collecting  
iii. Trapping

(C) Chemical :  
i. Fumigating  
ii. Fogging  
iii. Space spraying, Pyrethrum  
sprays  
iv. Residual insecticides.



## RESIDUAL INSECTICIDES, FORMULATIONS, MODE OF ACTION AND EQUIPMENT.

An insecticide is a product that kills insects. The value of an insecticide depends on the interaction of a number of factors related to the insecticidal compound, its formulations, the mode of application, the surface on which it is applied and the insects against which it is used.

The residual insecticide used under most malaria eradication programmes are chlorinated hydrocarbons (DDT, Dieldrin, EHC and Malathion.)

DDT is still the most commonly used insecticide. It is a white, creamy coloured crystalline powder possessing a fruit like odour. It is a stable compound with a melting point of  $190^{\circ}\text{C}$  and its solubility in water is less than 0.2 ppm. It is moderately soluble in petroleum and vegetable oils and readily soluble in many organic solvents such as Xylene & Benzene etc.

**Mode of Action:** DDT is primarily a contact poison acting on the nervous system of insects and causing paralysis of legs and wings un-coordinated movement, convulsion and finally death. It is also a stomach poison. It has a slow knock down effect, often takes several hours to kill.

**Formulations:** DDT may be applied in a variety of ways, the manner of application depending upon conditions under which it is to be used and against what insects it is intended. DDT is supplied commercially in a number of standard forms, although from pure 100 per cent technical grade of DDT, some of the formulations detailed below can be easily prepared in the laboratory.

1. Dry dusting powder mixed with an inert diluent such as talc, china clay or chalk.
2. As a suspension of fine DDT crystals in water (water dispersible powder).
3. Solution in Kerosene, diesel oil, used engine oil or malariol.
4. Emulsion concentrate in a special solvent with higher degree of solubility such as toluene, Xylol and turpentine.
5. As an Aerosol spray.

Water dispersible powder of DDT is used under NMEP.

**Water dispersible powder:** Water dispersible powders are preferred to other types of DDT preparations because of the storing and transport facilities and comparatively lower cost. DDT suspension in water is prepared from commercially available water dispersible powder which may contain 50% or 75% DDT. For making suspension of 75% w.d.p. for 2 lb. of the insecticide, use 3 gallons of water for a 5% suspension. Normally this is adequate for 4 houses each

having about 1500 sq.ft. sprayable surface or total of 6000 sq.ft. The code is "334".

For making suspension for 50% w.d.p. DDT for a 5% suspension use 3 lbs. of DDT in 2 gallons of water and the sample code is "334".

Dosage Schedule: The standard dose of insecticide (DDT) applied in India is 100 mg. per sq.ft. or 1 gm. per sq. meter.

Spraying Equipment: Stirrup pump, shoulder pressure sprayer or knapsack sprayer may be used. Stirrup pump with a specially adapted nozzle has been found very satisfactory. The nozzle tip used under DDDP is the flat fan nozzle tip. The correct discharge rate of the nozzle should be between 25 to 30 ounce per minute. Held at a distance of 45 cms (18 inch) from the surface to be sprayed, such a nozzle will deposit a swath of 75 cm (30 inches) wide. The biological efficacy lasts for about 10 weeks.

Benzene Hexa Chloride (BHC): BHC is a chocolate coloured powder with a persistence of odour. BHC being volatile possess a shorter residual action than DDT.

The patent name "Gammaxene" is held by the Imperial chemical industries, London who have done most of the developmental work with this product and have marketed various formulations such as 'Dusts', water dispersible powder and emulsions of this insecticide. 'Gammaxene' is the most useful formulation for public health purposes. It is a volatile powder containing 99.9% of gamma isomer and makes a homogeneous suspension when mixed with water.

For 50% w.d.p. BHC (6.5% of gamma isomer). The procedure is to mix 3 lbs. BHC in 2 gallons of water or 4.5 lbs. in 3 gallons of water.

The biological efficacy lasts for about 8 weeks.

Melathion: It is a dark brown liquid and has strong garlic odour. This insecticide is available in the form of water dispersible powder and is used in the control of household pests and other insects which have become resistant to chlorinated hydrocarbon.

Its formula may consists of emulsifiable 60-80% concentrates or of water dispersible powders containing 25% of the active compound. The duration of residual action of malathion averages about 3-4 months when the dosage of the active substance is at the standard 2 gm/m<sup>2</sup> of sprayable surface.

While most of the organophosphates are very toxic to man, the toxicity of malathion is relatively low.



## PLANNING AND ORGANISATION OF SPRAY OPERATION

The purpose of spray operation in an antimalaria campaign, is to interrupt transmission, by reducing the longevity of the vectors to less than a period necessary for the development of the malaria parasites to mature forms (Sporozoites) in mosquitoes. This is accomplished when the vectors come in contact with residual insecticide applied on all the probable resting places of the mosquitoes, in the houses e.g. walls, under surfaces of furniture etc.

In order to ensure such interruption, the spray operation must be through aiming at total coverage both in quantity and quality. So before actual commencement of operation, the basic requirements are proper planning and organisation of spray operation.

Following few points may be mentioned for planning and organisation of spray operation.

### 1. Geographical reconnaissance (G.R.):

It is an integral part of the spray operation. It serves as a base for planning, organisation, implementation and evaluation of the campaign.

G.R. is a field operation (preceded and supplemented by studies and calculation) which through census, mapping, numbering and sampling procedures determines the quantity, quality, location and means of accessibility, information and data which may be required for the success of the spray operation. Though the G.R. of all the sections are already available, continuous updating is needed to incorporate changes.

G.R. includes (i) Mapping of the district/PHC/Sector/Sections/area planned to be sprayed. Besides demarcation of different geographical boundaries, maps have to indicate locations of villages, mode of approach to each sections, streams to be crossed, project areas and other special features (ii) recording of village, houses, rooms, cattle shed and source of water in each sections, (iii) route for communication and accessibility and distance from village to village and section to section, (iv) quality of the sprayable surface (v) spraying surface area (by house measurement on sampling basis) (vi) areas subject to flooding and alternative routes (vii) population and houses enumeration to know exact number of house and population to be covered in the spray operation. Since this is done regularly every year, the same could be compared with enumeration under decennial census. Section-wise/village-wise increase or decrease of population and houses in each year would help in the planning. (viii) recording of the names of the village headmen and health guides.

### 2. Calculation of requirement of insecticide dosage and information:

#### 2.1 Scale per million (10,00,000) population is as below:

DDT	50%	150 M.Ton.	for 2 rounds
DDT	75%	100 "	for 2 rounds
BHC	50%	336 "	for 3 rounds
Malathion	2%	900 "	for 3 rounds

3. Spray schedule

In view of the biological efficacy of 2-1/2 months for DDT under normal circumstances, the local transmission period is considered and the spray timing is adjusted accordingly to have maximum effect.

Annually two rounds of DDT and three rounds of BHC are carried out (biological efficacy of BHC is less than DDT).

4. Some information and calculation related to spray operation.

1. Spray pumps

Two types of pumps are used in BHEP. (a) Stirrer pump - two men are required to operate this type of pump. Stirrer pump is used in plain areas. (b) H.C. (hand compression) pump - it can be operated by one man. This type of pump is used normally in hilly areas and in areas where isolation is required.

2. Formation of squad

A squad is formed by five field workers (spray crew) under a supervisor field worker (male or squad chief). When stirrer pumps are used two such pumps are operated by five persons - four (two in each pump) for spraying and one for preparing and supplying of suspension.

When H.C. pump is used five men would operate three pumps at a time.

3. Allocation of squad

Spray squads are allotted as per population. The approved pattern is 46 Nos. of squad for one million population for easily approachable area.

4. Dot put per pump per day

Dot put may vary from place to place depending upon the density of population and surface area of houses.



A stirrup pump can cover 45 to 50 houses per day when the density of population is high.

Experience showed that a H.C. sprayer could cover 30 houses per day but the out-put varies according to the terrain, population density and communication. The range of coverage therefore is 10 to 30 houses per day per pump.

N.B.: It is better to calculate squads on population and possible out-put per pumps.

#### Stratifications of areas on the basis of API:

This is a very vital experience in planning of spray operation. All the sections are to be stratified on the API basis year-wise. From this stratification section with population are grouped into different API grouping like below 2 API, API 2-9, 10-19, 20 and above. This will show the sections and population to be projected for spray operation. This will also help in taking up spray operation in sections with high API on priority basis. The requirement of spray squad and insecticide can also be worked out.

#### Advance spray schedule:

On the basis of all the above mentioned relevant informations, and advance spray programme shows the daily spray to be carried out by the squads. It should indicate date, section No., name of the villages and population, houses, cattle-shed, and rooms to be sprayed day to day. The names of the supervisory staff and camping place of the squads also have to be indicated.

While preparing an advance spray programme the following few points have to be kept in mind..

- (a) All the sections with API 2 and above are to be covered (Total coverage).
- (b) Sections with high API in the recent years are to be covered first in a descending order.
- (c) Sections where the falciparum rate is high or recently appeared are to get top most priority to interrupt further transmission.
- (d) Programme has to be prepared in such a way that contiguity of the sections are maintained. This would base the movement and working of the spray party and the coverage could also increase.
- (e) While preparing spray programme in areas bordering another Dist./ PHC/State the concerned officers may be consulted so that spray operations synchronise.

#### 7. Recruitment of staff:

Spray men are recruited for five months in a year Recruitment should normally be made PHC wise. The number of spray men to be recruited will be as per quota and population under spray.

#### (8) Vehicle:

Vehicle are to be kept in order before the commencement of spray operation. Vehicle are necessary for dumping of insecticide and movements of spray-men wherever required.

Contd....



### Spray pumps and accessories.

Spray pumps should be checked properly and repair/replacements may be made before the commencement of the spray operation. Spare parts like Nozzle tips, washers etc. which are often required during the spray operation should be procured and kept in stock. A provision of one spare pump for every two squads may be made for smooth running of the programme of spray operation.

R.B.: Nozzle tips to be provided 10 per pump to be changed every 15 days or when discharge rate goes up which ever is earlier.

### 0. Training related to spray operation:

- a) All the supervisory staff including SSI/MI are to be reoriented for the following:
  - i) Component parts of the spray pumps opening and reassembling.
  - ii) Determination of Nozzle tip discharge rate: The main reason why it is necessary to check the discharge rate, is to prevent over dosage thus increasing the cost of the operation or to ensure that there is no under dosage which defeats the purpose of spraying operation.
  - iii) Correct procedure of application of insecticide and the speed at which surface area to be covered.
  - iv) Actual spray operation when the inspectors are to act as spray men and superior field workers.
- b) Training and spraymen.

The spraymen are recruited only for 6 months. Of course 60% to 70% of the crew come back to work year after year. They must be given a short training on spray before sending them to the field. The training should be aimed at the following.

- i) Correct measurement of insecticide for preparation of suspension - use of standard Mug supplied.
- ii) Correct quantity of water to prepare suspension each time with standard container supplied.
- iii) Actual preparation of suspension.
- iv) Technique of spray - distance of the nozzle tip from the sprayable surface holding of the lance, movement of the spray men holding the lance, width of the stripe coming out from the nozzle tip, overlapping of the EX stripe, technique of pumping and coordination between two spray men operating a stirrup pump, number of strokes and speed etc.
- v) Total coverage with through application in every room with special importance of spraying the bed rooms, verandah, inside panels of doors and windows under surfaces of bed and furniture and not to forget the ceiling irrespective of the height (use extended lance) and E caves.
- vi) The spraymen are to be trained to rectify minor defects in the pump.
- vii) Washing and cleaning of the pumps after every day's work.
- viii) Superior field worker who is supposed to know reading and writing should be trained to measure the nozzle tip discharge rate and to maintain records of spray and consumption of insecticide.



11. Dumping of insecticides:

Dumping of insecticide at different strategic places may be done before the spray and to be notified to the S.F.W./M.P.W./SI/SMI/MI/ so that there should be no difficulty for them to find out the place. DDT holders should be reliable and insecticides to be kept in safe places.

12. Advance Notification:

Prior information regarding date of spray is very important to get full coordination of the public. It is found out by experience that best way to inform the villagers regarding the date of spray operation is through the MPW/SW during their regular domiciliary visits. The house wives may be contacted as success of spray operation rests on their cooperation. The village headman should always be informed ahead.

13. Movement of Squad:

The squads are moving from one village to another and one section to another on foot. But when they are to move to distance places transport may be provided to avoid delay in spraying.

14. Involvement of village health guide:

Since the village health guide is known to the public and his/her position is well established due to the nature of his/her job, he/she should be involved for giving advance information and during the actual spray operation which may prove to be very useful for the success of the spray operation. Health guide may be involved in imparting Health Education to the villagers in regards to spray operation.

15. Supervision:

a) Both concurrent and consecutive supervision are important. Concurrent supervision helps in understanding the training status and capability of the squad to follow the routine instructions of the technique of spraying, to check upon the condition of the pumps and accessories, acceptability of the programme by the people and to ensure the timely operation as scheduled. ON the other hand consecutive supervision refers to checking up the quality and coverage already sprayed i.e. either on the same day, previous day, week or month.

b) Malaria Inspector/Senior Malaria Inspector should be directly put incharge of spray squads. If one MI/SMI looks after more than one PHC, he may take up spray PHC wise and the advance programme may be so chalked out as to cover all PHCs under his jurisdiction within the specific schedule of spray round.

The MI/SMI should move with the squads under his charge during the spray operation and remain in the field to personally guide and supervise the spray operations. He should inspect and get the houses sprayed in his presence and correct the deficiencies in the spray operation by personal guidance. He should get at least 30 houses sprayed in his presence per day. He should maintain a record indicating date, name and the number of houses personally supervised by him. He should also sign the spray record maintained by the SFW whenever supervised.

c) It is not possible for a supervisory staff to visit every room in every house in every village. But they could take a stratified sample. Normally the sample is highest in respect of the inspectors and proportionally lower in the higher rank of officers.

d) Points to be checked during supervision:

Concurrent supervision: 25% of the supervision should be of concurrent nature. The following should be checked during such inspection.

- i) Date of advance notification and the correct maintenance of time table for spray operation.
- ii) Turn out of spray crew.
- iii) Condition of the spray pumps.
- iv) Nozzle tip discharge rate.
- v) Preparation of suspension.
- vi) Actual spraying operation including the technique speed and coverage etc.
- vii) Extent of actual refusal to accept spray and locked houses.
- viii) Maintenance of record by SFH.
- ix) Consumption of insecticide as determined by the quantity issued and stock in hand.
- x) Date and time of checking of the squad by inspectors and other supervisory personnel and remarks - if any.
- xi) Arrangements for "mopping" up.
- xii) Future programme and time schedule.

Consecutive supervision:

75 per cent of the supervision must be of consecutive nature. The following required to be checked on 10% sample basis (1 to 10 houses).

1. Evidence of insecticide deposit in every sprayable surface particularly on the ceilings "History of spray operation is written on the ceiling of houses".
2. Dispersal of the deposit.
3. Evidence of recent spray (as determined after removal of the deposit by rubbing off with finger and flashing a beam of light from a torch where deposit of recent spray shines brilliantly white).
4. Number of rooms in each house (taken as sample) sprayed satisfactorily partially and not at all.
5. Percentage of refusal and locked houses.
6. Factors responsible for not spraying any area as elicited through enquires from the residents.
7. Factors responsible for high refusal rate, if any, and action taken.
8. Attempts made for "mopping up" operation in the even of high refusal.
9. Extent of mud-plastering on the walls, if any, and other relevant matters.
10. Correlation between actual coverage and data presented.



16. "Mopping up" operation:

The experience is used for spray squads following the main team in order to ensure that the houses left unsprayed by the previous squads due to one reason or other, are attended. This is one of the most vital parts of the campaign. When the refusal rate is high or when at times large number of houses are found to be locked, as may happen during rice transplantation season, an "haat" (weekly bazar) days or during harvest, the main team may leave behind many houses unsprayed. In such cases it is essential that the team should relay back message for the "mopping up" team to visit the areas within a few days (and not after a month or two and certainly not after completion of round) to ensure coverage of the houses not sprayed earlier. Occasionally the main team may stop for the night in the particular area or leave a squad or two behind to ensure such "mopping up" the following day.

Mopping up teams are formed by making adjustment amongst seasonal staff and also from those employed on 12 months basis.

17. Health Education:

Though the programme is going on for more than two decades, a large section of people are not aware why these activities are being carried out. Objectives are not clear to them and many expect the elimination of all mosquitoes and insects. The programme has a very large number of workers and these staff members can act as health educators besides holders of FTDs, DDCs and Health guides are also motivated well towards this programme. Health education materials are being propagated through television, Radio, Postal stationery, wall posters, photographs, cinema slides and feature films.

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Paper: Technical Directives and Administrative guidance"  
by Dr. A.P.Ray.

# INSECTICIDE FORMULATION DOSAGE - ADULTICIDES

Sl. No.	Insecticide	Effectiveness	Used as and Dilution	Dosage (a.i.)	Discharge at Nozzle Tip	Coverage			Remarks
						Insecticide kg.	Ready to use spray litres	No. of houses	
1.	DDT 50% wdp	10 weeks on mud (sorptive) surface may be longer on other non-sorptive surfaces	50% suspension in water 1 Kg/10 l.	1 gm/m <sup>2</sup> (100 mg/ft <sup>2</sup> )	750-900 ml/min. Sprayed @ 5.1/100 sq.m. in 6 minutes (26-30 Oz/min. sprayed gal/1000 sq. ft. in 3 minutes)	1 (3 lbs)	10 (3 gals)	4 app. sprayable area 500m <sup>2</sup> (6000 sq.ft.)	Equipment used 1. Hand Compression sprayers stirrup pump
2.	DDT 75% wdp	-do-	5% suspension in water 2/3 Kg/10 l.	-do-	-do-	2/3 (2 lbs)	10 (3 gals)	9 -do-	-do-
3.	HCH 50% wdp (6.5% gamma isomer)	6 weeks	1% suspension in water 1.5Kg/10 l.	0.2 gm/m <sup>2</sup> (20 mg/ft <sup>2</sup> )	-do-	1.5 (4.5 lbs)	10 ( gals)	-do-	-do-
4.	Malathion 25% wdp	-do-	5% suspension in water 2 Kg/10 l.	2 gm/m <sup>2</sup> (200 mg/ft <sup>2</sup> )	1600 ml/min. sprayed @ 1680 ml/250 sq.m. in 6 mins. (1680 ml. sprayed @ 1/2 gal/1000 ft <sup>2</sup> in 3 min.)	3 (6 lbs)	10 (3 gals)	2 sprayable area 250 m <sup>2</sup> (3000 sq. ft.)	-do-



STRENGTHENING OF MALARIA CONTROL ACTIVITY THROUGH  
PRIMARY HEALTH CARE SYSTEM

Primary Health Care is defined as a system where by Essential health care is made available to all people through a means Acceptable to them, at a cost Affordable to them and at the place where they live and work. Primary health care is envisaged as the essential component of the General Health Services of which it forms the base at the Primary level. Its philosophy is catering to the Common health needs of the many; and calls for Equitable distribution of health man power, health organizations and health activities among the population. Its economy is self reliance and self sustenance. For its effectiveness and efficiency it has to have the support of Political will, Appropriate Technology, Peoples participation and Inter and Intrasectoral collaboration Content wise it addresses itself to the following eight felt-need areas, in the health realm which are:

1. Relevant Information and Education on the prevalent health problems and methods to over come it.
2. Proper Nutrition and supply of Food.
3. Safe drinking Water and basic Sanitation.
4. Maternal and Child Health services including Family Planning.
5. Immunisation and Prophylaxis against communicable and non communicable diseases.
6. Control and Elimination of prevalent Endemic Diseases.
7. Treatment and Relief of Common Ailments.
8. Provision of Essential drugs.

Malaria Containment through PHC strategy:

It can be noted that Malaria is very much an issue calling attention of every conscientious PHC worker as focused through the 6th element of PHC codified above. Items 1,3,7 and 8 of the PHC elements also indirectly have a bearing on Malaria elimination. But what is to be highlighted is the potential of PHC system as an approach to malaria elimination.

Reviewing the history of NMEP, it can be seen that an untimely and hastily attempt to build up a system based on PHC in the year around 1967, when the NMEP was not yet consolidated, had brought about a set back resulting in Malaria resurgence. It can be noted that even at the present moment, indiscrete emphasis on certain PHC elements or part thereof, as inclusive importance being given to FP or Immunization under MPW scheme, is impeding the progress of Malaria containment. Rather than decrying these essential elements, such as Family Planning or Immunization, what is essentially required is an emphasis on Malaria Containment work which can go hand in hand with such works, but could even enhance the credibility of the latter. Field staff as well as Supervisory staff engaged as Multipurpose Health Workers have to be conscientized that asking the Malaria eliciting question or making a prick for Malaria detection, or administering a Malaria curing dose of drugs, adds to their credibility as health workers, rather than adds brunt to their routine FP, immunization or such other good work.

PHC as a channel for Health Education:

PHC harps on common health problems and their solution through Community Participation. In an area where Malaria is known to be conflagrating what else could be cognised as the people's health problem? Primary Health Centres as the operational units of the concept of Primary Health Care if not sensitized to the people's health problems, what for does it exist gnawing on the purse and health of the suffering tax-payer? Malaria in a PHC area is a people's health problem and the primary responsibility for it rests with the local PHC. Training should bring this home to all health providers. But more than Training, Health Education as envisaged in element, of the PHC contents, should bring this fact to the awareness of the health beneficiary. The community should be made aware that the Malaria problem, at least to start with, was of their creation, inadvertent though it be. People have much to contribute to Malaria prevention/control/elimination by way of avoidance of mosquitogenesis, mosquito-man contact, neglect of intermittent fevers, refusal to blood screening or treatment, refusal of antimalarial measures such as insecticidal spraying



and of being part of indiscrete mass movement and congregation. Unless the people act, out of conviction generated by enlightenment, well meaning measures even by the best of official organizations will be near futile. Health Educating the public is the only sure method for community participation. Without Community Participation a colossal and spread out ~~problem~~ like Malaria in the community finds no easy way out.

No Public Health Programme can succeed without people's participation and no peoples participation can be obtained without Health Education. Unlike family welfare programme, there is no special Health Education or Extension Education component in the NMEP. Yet the success of NMEP depends entirely on people's participation sought through Health Education activities.

Health Education aimed at Malaria Containment:

The NMEP is carried on through (a) Surveillance (b) Case finding (c) Treatment and (d) Transmission control. In each of these fields of activities, people's participation is essential and we can examine how Health Education plays most vital role thereon.

Surveillance:

The Surveillance worker pays visits to the house-hold once in a fortnight, and enquires about any incidence of fever in the family. While doing so, (a) he has to first of all 'identify himself' as the Health staff for that section, in case he is not yet familiar to them. He has to tell the people that malaria is a dangerous disease, and it is a matter of life and death for the infants and children, and more than a ~~erist-~~ing diseases for the bread earning adult. The prominent symptom of malaria is fever, and he has come to enquire if there is or was in the recent past any fever case in the house. To make the surveillance effective, (b) it is necessary that the surveillance worker should become acceptable to the people and be able to elicit correct reply. It is therefore evident that through Health Education, people's cooperation should be forth coming.

Case Finding:

a) If the enquiry in the surveillance elicits the reply as 'Yes' it is necessary for him to tell the people that the only method to know whether the fever was due to malaria or not, is to examine a drop of blood from the finger tip. People may be afraid of the prick or loss of the blood. The Surveillance worker is required to explain that many people often accidentally get cut injuries and lose lot of blood. Here only a small careful prick is given to get just a drop of blood and immediately medicine is applied on the prick. He may tell the people that without their awareness mosquitoes may be taking more than a drop of blood every day; and all that he wants is a drop of blood once for all.

b) In order to prevent lot of suffering and even loss of life just a drop of blood is needed to detect if the blood is poisoned by Malaria germs. Such type of Health Education can convince the people to allow obtaining their blood smear. If the blood smear is not obtained, the case detection will not be possible and the efforts on NMEP will fail.

Treatment:

a) The people should be told that anybody having history of fever should also take a few tablets of medicine, so that in case it is Malaria, the medicine will help in treatment. The tablets may taste bitter, but in fruit is sweet. Further more if the blood examination reveals that the fever is due to malaria health worker will come again to treat him for five days.

b) The NMEP staff should also educate the people that in between the visits of Health staff if any person get fever he should (i) report to the community Health Guide who also can give medicine and collect blood slide (ii) or better they may contact the local dispensary or PHC to consult the doctor.

It is therefore evident that through such Health Education, people's participation can be obtained and treatment of all malaria patients thereby becomes possible.



Transmission Control:

The transmission of Malaria can be controlled in many ways, one of the most important measures is spraying of residual insecticides.

a) The people should be told in clear terms that the object of DDT spray is to kill the malaria carrying mosquitoes and not killing all mosquitoes. The big size mosquitoes which cause so much annoyance in the evening are not carriers of malaria germs. It is the lean and thin small mosquitoes which are responsible for malaria (b) DDT does not have much effect on insects and ugly looking fat mosquitoes, while the beautifully thin malaria carrying small mosquitoes are easily killed when they come in contact with DDT. An ugly fat woman does not care spray of dirt on her, as seen during Holi/festival while a slim fair woman is very sensitive to it.

- a) It is necessary that every room in every house should be sprayed. If some house or some rooms are left out, Mosquitoes will take shelter in those house or rooms and bite the people. It will be as ineffective as in putting up fence on three sides of a garden leaving the other side unprotected against entry of goats and cattle.
- b) After spraying walls are not to be white washed or mud plastered, else DDT, Sticking on the wall will be covered up and the malaria carrying mosquitoes will not be destroyed.
- c) Effect of DDT lasts only for 2 to 3 months. It is therefore necessary that the houses should be sprayed twice during the transmission season.
- d) Only spraying the houses is not enough. People must keep their compounds clean of bushes and jungles, as it is in such places mosquitoes take shelter and happen to bite people.
- e) Mosquitoes breed in water. Water should not be allowed to collect in drains, pot holes, and water collections etc. giving chance for mosquitoes to breed.

- f) People should sleep inside mosquito nets as far as possible to protect them from mosquitoes bites.

Finally, in adoption of all these measures for control of transmission, peoples active participation is of utmost importance. For, the spray team cannot forcefully spray the house. They can not prevent people from mud plastering the sprayed rooms. They cannot enforce use of mosquitoes nets, nor can they keep the peoples houses clean of bushes and water-logging.

The people are to be educated and constantly perused to adopt these health measures, No health programme can succeed without people's participation. They have to be made health conscious through persistant efforts of health education.

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- f) People should sleep inside mosquito nets as far as possible to protect them from mosquitoes bites.

Finally, in adoption of all these measures for control of transmission, peoples active participation is of utmost importance. For, the spray team cannot forcefully spray the house. They can not prevent people from mud plastering the sprayed rooms. They cannot enforce use of mosquitoes nets, nor can they keep the peoples houses clean of bushes and water-logging.

The people are to be educated and constantly perused to adopt these health measures, No health programme can succeed without people's participation. They have to be made health conscious through persistant efforts of health education.

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SUPPLEMENTARY NOTES

HEALTH EDUCATION AND NMEP

It is true that the NMEP has no special health education staff and that assistance should be sought from the Central Health Education Bureau and the State organisation but at the same time it should be remembered that no other organisation has so close a contact with the masses as the NMEP. Firstly during the spray season every house is approached for application of insecticide, secondly every house is visited twice in a month by the surveillance inspector. Therefore, they are the best media for propagation of health education in the country. This is more so because of the influence the surveillance staff has on the people who consider them as part of their own community. It is therefore, obvious that these personnel can assist in inducing the people to accept spray in every part of the house, in dissuading them from mud plastering the walls make them agree to submit to blood examination and accept the necessary treatment etc.

It is also obvious that these personnel can transmit all types of health messages to the people. In course of time as and when the NMEP unit areas complete the task of malaria eradication these very workers could serve the best purpose for other health activities like those under the smallpox eradication programme, campaigns, to control tuberculosis, leprosy etc. under which domiciliary visits should be one of the essential activities. It is however necessary that before they are deployed for other public health activities these personnel should receive re-orientation training.

These workers should be provided with series of talking points in simple language which could be grasped by the people easily. Under the malaria eradication programme such talking points are to be provided to every worker in the field, if not done already. The theme should be specific and must pertain to subjects relating to some of the essential activities for which the acceptance and cooperation from the beneficiaries of the programme are the basic needs.

It is useless to try to explain to the people about the intricacies and the progress of malaria eradication campaign or the manner in which malaria transmission is interrupted. Therefore, the subject for communication should be very carefully prepared. As examples some of the talking points are indicated below for guidance.

TALKING POINTS.

1. The primary object of spray operation is to prevent malaria and not just an attempt to kill mosquitoes.
2. There is no intention to kill the big mosquitoes which cause so much annoyance in the evening, nor the bed bugs cockroaches and other pests. In any case they can not be



Killed or removed by spraying the "medicine".

3. The big mosquitoes, bee bugs etc. may disappear after the first or the second application but they get gradually used to the "medicine" and come back. It is almost like opium which puts one to sleep in very small dose in the beginning, but when one gets used to it even large lamps are ineffective.
4. But the insecticides do have effect on the small mosquitoes which are dangerous as they carry malaria.
5. For adults malaria may not be so dangerous, but it is a matter of life and death for the children and infants. In order to protect them it is necessary to spray the "medicine".
6. When the spray crew still continues coming every year it should be understood that there is still risk of malaria coming back even though not many cases are noticed.
7. It is very necessary that every room of every house in every village is sprayed.
8. If some parts of a house are sprayed and the others are left out protection from malaria cannot be guaranteed. It would be just like putting up fence on three sides of a garden leaving the fourth side unprotected against the intrusion of goats, cattle etc.
9. After spraying the walls are not to be plastered with mud or else the "medicine" will not have any effect as it will be covered by mud.
10. Government spends a lot of money every year forgetting the houses sprayed. It costs about Rs.1.50 to Rs.2 to spray house. If the walls are plastered with mud after spray, it is as good as throwing the money into mud. Further if one house is not sprayed it does not mean that Rs.2 are automatically saved because "medicine" has been bought and workers have been appointed and their salaries have to be paid and so on. It is like cooking food for ten people and only six people turn up. It does not mean that money has been saved because four people did not eat. But rather food is wasted.
11. Yes, we agree that bed bugs are "eating you up" every night. But the medicine used for control of malaria cannot eliminate bed bugs. We have really no effective and yet long lasting weapon against bed bugs. It does not mean that these pests cannot be controlled or ultimately got rid of. For generations hot water has been used on beds to kill these insects, kerosene oil had also been used in the past on the cracks in the wall where bed bugs hide. Beds and clothes are to be exposed to the sun frequently. General cleanliness of the house is of help. There are lot of things which people can do themselves as our fore-fathers



did without asking the government to help. One must try to be self supporting as much as one can.

12. But malaria is difficult to control through individual efforts and hence government is extending all assistance. They are not satisfied with spraying alone. They want to know how much benefit the people have derived, how much malaria is still left. Therefore checking is being done for which workers are visiting every house once in 2 weeks to find out if any one is suffering from fever.
13. When there is any fever case, these house visitors take blood from the finger for examination incase there are any malaria germs hidden in the blood. There is no other means of detecting these dangerous organisms. Therefore, when the workers come to your house, get your blood examined if you are suffering from fever or have had fever recently.
14. Inform the house visitor if any one else is suffering from fever or had fever recently.
15. Take the "medicine" given to you after blood is taken, if germs are found the malaria worker will come and treat you for five days. Do not refuse drug after one or two days simply because you have no fever. The germs must be removed completely and hence medicine has to be taken in full doses.
16. If you or any one else in the family gets fever in between the visits of the house visitors, report to the local dispensary or doctor. If none is available request your Panchayat, school teacher or sarpanch to send information to malaria department.

if properly arranged. The inter action takes place amongst the people and they come to definite decisions regarding community action. Health Educator is an new member of public health team. The need for a specialist of this kind has arisen from the recognition that health is not primarily a problem of legislation. Its attainment depends on the interest and willingness of individuals and group to assume responsibility for the solution of their own problems on a well informed basis. People are more prone to apply acceptable health practices in their daily life if they had part in determining the changes desired in partnership with the professional health workers. This spirit of co-operation among health specialists and the people themselves, at all stages of the development of a health programme, is destined to have far reaching educational influence. It will serve to generate wide spread public goodwill and support for the total health programme.

Understanding of the laws of human behaviour comes from the social sciences. The health educator provides an essential link between the social scientists, the doctor, public health engineer and other health personnel in contact with the people of a particular area. The health educator can provide teaching aids which may be called the 'tools' of the trade. Special literature, posters, health films and film strips. Primarily however he is concerned with individual and group motivation. He assists in making effective contact between the health programme and community participation. Qualified expert of this type is essential to the fullest success of any public health programme.

Society has sought to meet health need by (1) providing health services to do things for people and by (2) educating people to do things for themselves. The former is often easy, but it is expensive and often of temporary benefits. On the other hand stimulating and guiding people to assume responsibility for themselves may take more time, but it is relatively inexpensive and its results are more lasting.



## Duties & Responsibilities of:

### I. Village Health Guides

1. The village Health Guide (VHG) will make a thin and thick smear on one glass slide from all fever cases reporting to him for treatment.
2. Administer single dose of antimalaria drugs in recommended dosage as presumptive treatment to the fever case from whom Blood Smear has been collected.
3. Keep detailed records of individual fever cases treated by him in the register as per proforma supplied to him by the PHC.
4. Maintain the accounts of antimalaria drugs supplied to him from PHC or replenished by MPW/Supervisor.
5. Report to the PHC Medical Officer if the drugs and slides are not replenished by the MPWS or the Supervisor in time and collect replenishment from PHC Medical Officer.
6. Report any death due to fever in the village to the PHC Medical Officer.
7. Administer radical treatment with the prescribed dosage of antimalaria drugs according to the schedule furnished to him by the Medical Officer, PHC and ensure that complete radical treatment is administered to the positive case by personally contacting him during the course of radical treatment. This activity will be undertaken only when fully trained in Radical treatment of malaria case.
8. Stop radical treatment administration of primaquine if toxic symptoms are observed and persuade the patient to visit PHC for further advise.
9. Assist spray teams during insecticidal spray operations in his village by motivating the community to accept insecticidal spray.
10. Impart Health education to the community on malaria and explain to them the necessity of minimising the mosquito breeding places and for observing the personal protection.

### II. Multi-Purpose Worker (Male).

Each worker shall visit all families in the section allotted to him once every fortnight according to the time and space movement schedule given by PHC Medical Officer/DMO.

2. Will enumerate the population of his section annually and enter the details of all the family members in the family register/MF-I as prescribed under the modified plan of operations.
3. Will update these records at the time of his fortnightly visits in respect of births and deaths or movement of a family member outside the area.



4. Will prepare Stencils and maintain the same as recommended under NMEP. He shall put his dated signatures during his fortnightly visit to the family on the stencil.

5. From each family, he shall enquire about

- i) Presence of any fever case;
- ii) Whether there was any fever case in the family in between his fortnightly visits;
- iii) Whether any guest had come to the family and had fever.
- iv) Whether any member of the family who had fever in between his fortnightly visit has left the village.

6. He shall collect thick and thin blood smears on one glass slide from cases having fever or giving history of fever and enter details in MF-2 and put appropriate serial number on the slide.

7. He shall give presumptive treatment for malaria after blood smear has been collected. He will follow the instructions given to him regarding administration of presumptive treatment under NMEP.

8. He shall contact the Village Health Guide during his fortnightly visit to the village and (i) collect blood smears already taken by the Village Health Guide (ii) also collect details of each case in MF-2 (iii) replenish both drugs and glass slides and look into the account of consumption of antimalarial drugs.

9. He shall despatch blood smears alongwith MF-2 collected from the Village Health Guide/Multipurpose worker (Female) of the sub-centre and also those collected during his visit in his section to the PHC Laboratory twice a week, or as instructed by the Medical Officer PHC.

10. He shall verify the radical treatment administered by the Health Guide if any during his visit.

11. He shall administer radical treatment to the positive cases as per drug schedule prescribed and as per instruction issued by the Medical Officer PHC and take laid down action if toxic manifestations are observed in a patient receiving Radical treatment with primaquine.

12. He shall visit Drug Distribution Centres and collect details of persons given treatment for malaria by the DDC and replenish antimalarial drugs during fortnightly visit.

13. He shall inform in writing his supervisor or PHC Medical Officer the reason for not having contacted the Village Guide/Fever Treatment Depot/Drug Distribution Centre during his routine fortnightly visit if any.

14. He shall intimate each house-hold in advance regarding date of spray on the basis of advance spray programme given to him and explain simultaneously the benefit of insecticidal spray to the villagers.



15. He shall contact the Village Health Guide and inform him of the spray dates and request him to motivate the community and prepare them for accepting the spray operations.

16. He shall assist the Malaria Inspector/Multipurpose Supervisor for spray supervision in his section. During this period, his routine fortnightly cycle of visit to his section/village may be disrupted but he would carry out the active surveillance and contact village Health Guide during supervision of spray activities, collect blood smears and administer presumptive treatment to the fever cases and sign on the Stencil also.

17. He shall contact Gram-Panchayat members during his visit and seek their help in implementation of the NMEP activities.

### III. Multi-purpose Worker (Female)

Multipurpose Worker (Female)- A.N.M. will assist in implementation of the NMEP in her area as follows.

i) If any fever case reports to her at the Sub-Centre, she will collect thick and thin blood smear on one glass slide and keep records as prescribed and administer single dose of presumptive treatment with antimalarials according to schedule given to her.

ii) During her routine visits to the family for MCH work, if she finds a fever case, she will collect blood smear both thick and thin and administer single dose of presumptive treatment and keep the relevant records.

iii) During her routine visit, she will impress upon the house-wives about the utility of spray operations for control of malaria and to accept the same.

### IV. Multipurpose Supervisor (Male)

Multipurpose Supervisor will supervise the work of Multipurpose Workers placed under him.

i) He will ascertain regarding the fortnightly visit of Multipurpose Worker (Male) to the village by looking at the Stencils, verifying the date of visits with the schedule and enquiring from the families.

ii) He will supervise the work of Multipurpose Worker (Male) during concurrent visit and will check whether the worker is performing his duties as laid down in the schedule.

iii) It will be desirable that he should check minimum of 10% of the houses in a village to verify the work of a Multipurpose Worker.

iv) He will put dated signature on wall-stencil during his inspection of the work of the Multipurpose worker.



v) He will visit the Village Health Guide, FTDs and DDCs as well as Sub-Centres and verify their records of Blood smear collection and stock of antimalarial drugs and microbicides and render any advice necessary.

vi) He will carry with him a kit for collection of blood smears during his visit to field and collect thick and thin smears from any fever case he comes across and he will administer presumptive treatment of prescribed dosage of antimalarial drugs.

vii) He will be responsible for prompt radical treatment to positive cases in his area. He will plan, execute and supervise the administration of radical treatment in consultation with PHC Medical Officer.

viii) If radical treatment is being given by the Multipurpose worker (Male) and Village Health Guide he will verify the radical treatment and send his observation to the PHC Medical Officer, and discuss the deficiency if any, and he will ensure that full radical treatment is given to all positive cases in his area.

ix) He would assist the Malaria Inspectors during spray operations and ensure that coverage under spray operation is satisfactory both qualitatively and quantitatively. He will carry out the work related to the malaria as per instructions of Medical Officer of PHC/DMO.

x) Multipurpose Supervisor (Male) would gather information from the Village Health Guide/Multipurpose Worker (Male) regarding migration of population in his area. If a camp of migratory population is located in his area, he will visit to ascertain the number, place or origin and enquire at the fever incidence, he will then report immediately to the Medical Officer PHC for instituting suitable remedial measures.

xi) As per instructions from the Medical Officer PHC he will be responsible for taking prompt remedial measures like Radical Treatment, Mass and contact survey, focal spray etc. round about positive cases detected in areas with API of less than two.

xii) He should also investigate initially the positive cases and any death due to fever.



# V. MALARIA INSPECTORS:

Malaria inspectors have been provided to the districts on population basis. One malaria inspector is allotted to one or more than one PHC and he will be under the Medical Officer in one of the PHCs. He is a unipurpose worker meant for planning organisation, execution and supervision of insecticidal spray in the villages. He should prepare Advance spray plan in consultation with M.O. PHC and communicate to all quarters well in advance.

2. He is primarily responsible to supervise the spray operations carried out by spray teams. He should collect details of work done daily from each spray gang and compile his records once a week. The weekly spray reports should be forwarded by him to the district malaria officer under intimation to M.O. PHC.
3. He is responsible to organise the spray teams and prepare date wise and village wise visits by the spray teams.
4. He should requisition services of the multipurpose workers and multipurpose supervisors for their assistance in supervision of spray operations in their respective areas. He should be responsible to receive the insecticides and the spray equipments.
5. He should maintain the spray equipments. He should check the discharge rate of nozzle tips periodically and maintained records of the same for changing when necessary.
6. He should keep account of insecticides received and consumed and indicate the same in the prescribed proforma to be furnished to M.O. PHC/District Malaria Officer.
7. He should also check surveillance activities and performance of FTDs, DDCs and health guides and Dispensaries during the visit to the village.
8. Undertake group meetings in the villages/schools and Panchayats and explain the objectives of NMEP operations to the public.
9. He should keep liason with other agencies like block development officers, village level workers etc.
10. He will be responsible for undertaking remedial measures like focal spray and mass blood survey in the sections with API below 2 around positive cases. He should investigate primarily all positive cases detected in areas less than API-2.

## VI- LABORATORY TECHNICIAN (Microscopist)

1. The Microscopist will be responsible for the staining and examination of all surveillance blood smears as expeditiously as possible and for the despatch of the results to the respective surveillance inspector NPS(M)/malaria inspectors.

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2. He will maintain all records of slides examined by him and must get the positive slides confirmed by the Medical Officer of PHC.

3. He will be responsible for the cleaning of all new and used blood smears and for proper distribution to the surveillance inspectors (MPS/M) and surveillance workers MPW (M).

4. The microscopist is expected to examine at least 50 blood smears a day. He will maintain section-wise MF-2 forms of blood smears indicating case number against the slides which is found positive. The MF-2 form which indicates only the distribution of chloroquine tablets received from the Panchayats/Teacher or fever treatment depots will be kept separately after necessary entries are made.

5. He will maintain section wise MF-2 forms of blood smears indicating case number against the slides which is found positive. The MF-2 form which indicates only the distribution of chloroquine tablets received from the Panchayats/Teacher or fever treatment depots will be kept separately after necessary entries are made.

6. He will maintain the following registers:

- a) Blood smears receipt and examination (MF-6)
- b) Section-wise details of positive cases and remedial measures register (MF-7).
- c) Epidemiological evaluation master register, section wise village wise and month wise (MF-9)
- d) Daily progress and output register of blood slide examination.

7. He will maintain the following charts:

- a) Master chart of active collections, examination and total positives, section-wise and monthwise.
- b) Master chart passive agencies indicating fever cases treated whether any blood slides collected and the positives detected from the passive slides (monthwise)
- c) The back log chart of pending examinations of blood slides vis-a-vis collected slides.
- d) The back log chart of pending radical treatment vis-a-vis positive cases detected.
- e) Line graph chart showing positives and blood slides collected monthwise.
- f) Chart for technique of staining with J.S.G.
- g) Map of PHC indicating section boundary and names of adjoining PHCS.

8.1 He should send prescribed percentage of negative and positive slides to State Malaria organisation/zonal office/ROH & FW for confirmation.

8.2 He will prepare the following reports to be submitted by the Medical Officer, Primary Health Centre to the District Malaria Officer with a copy to the Malaria Inspector.

- a) Weekly Epidemiological report (on every Saturday) (MF-11)

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- b) Monthly Technical Report Section-wise surveillance data and remedial measures (MP-4 & 5)
  - c) This report will be sent to the District Malaria Officer.
  - d) He will maintain the accounts of the anti-malarials used in the Primary Health Centre.
  - e) He will run the Malaria clinic at PHC.

#### VII. MEDICAL OFFICER OF PHC

- 6.1 He will be responsible for all NMCP operations in his PHC area and will be responsible for all administrative and technical matters.
- 6.2 He should be completely acquainted with all problems and difficulties regarding surveillance and spray operation in his PHC area and he responsible for immediate action whenever the necessary arises.
- 6.3 He will be responsible for the execution of the surveillance procedures as approved by the higher authorities and should be completely familiar with all aspects of the Programme.
- 6.4 He will be responsible for the proper deployment of the MPWs taking into consideration density of population, terrain communication and other factors as related to surveillance. He should ensure that the MPWs adhere to the fixed calendar of activities for fortnightly visits.
- 6.5 The medical officer will guide the multipurpose supervisor (M) on all treatment schedules, especially radical treatment with primaquine. As far as possible he should investigate all malaria cases in the area regarding their nature and origin and institute necessary measures in this connection. M.O. PHC should ensure that prompt remedial measures are carried out by MPS (M)/Malaria Inspector round about positive cases detected in areas with API less than two. He should give specific instruction to MPS (M)/Malaria Inspector in this respect, while sending the result of blood slides found positive.
- 6.6 He is responsible for proper maintenance of all record and data in this connection and their proper despatch from the PHC headquarters as expeditiously as possible.
- 6.7 He will make surprise visit for random checking of the work of the surveillance Inspector/MPS(M) and surveillance workers/MPW(M). He should also check spray operations as far as possible during his village visits.
- 6.8 He will similarly check the microscopic work of the laboratory technician and despatch prescribed percentage of such slides to the zonal organisation/Regional Office for Health & FW (Government of India) and State H.O. for cross checking as laid down from time to time.
- 6.9 He should chair the monthly meeting and ensure proper accounts



JSE STAINS BLOOD SMEAR PREPARATION  
STAINING AND EXAMINATION OF BLOOD SMEARS METHODS  
OF PREPARATION AND STAINING TECHNIQUE

1. PREPARATION OF JSE STAIN. (Jaswant Singh & Bhattacharji - 1924)

The original method and the subsequent modified method of the stain can be had from the Publication captioned "Review of JSE stain". The latest modification by which the stain can be prepared in an hours boiling, would only be described here.

J.S.E. Stains comprises of 3 solutions: J.S.E.-I, J.S.E. -II, Buffer Solution.

2. CONSTITUENTS:

a) The JSE-I

Methylene Blue (Medicinal)	0.5 gm.
Sulphuric Acid Soln. 1%	3 cc
Potassium Dichromate	0.5 gm.
Disodium Hydrogen Phosphate	3.5
Water	500 cc

b) JSE-II Eosin Yellow (Water Soluble)	1 gm.
Water	500 cc

c) Buffer or Wash Water is prepared by dissolving 0.417 gms. of Disodium hydrogen phosphate and 0.732 gms. of Potassium acid phosphate 2000 c. of distilled water.

3. TECHNIQUE OF PREPARATION OF JSE- I

The technique of preparation of JSE-I is simple. Take 1 litre flask and 500 cc. of water. Dissolve Methylene Blue. Then Sulphuric Acid should be added gradually in three stages, one cc. at each time and the solution should be stirred for proper mixing. Potassium Dichromate should be added at this stage. With the addition of Potassium dichromate; the blue colour of the mixture will change and precipitate will be formed. Now disodium hydrogen phosphate should be added. It will be evident at this stage that the precipitate will appear to get dissolved if thorough mixing is ensured. The resultant mixture is then put on the flame using a 1 metre glass tube of condenser. As soon as it starts boiling the time should be noted and left to boil for one hour. On cooling, the stain is ready for use.

4. TECHNIQUE OF PREPARATION OF BLOOD FILMS:

a) Introduction: The present practice is that both the thick and thin smears should be taken on the same slide for examination for malaria parasites. Thick film examination saves a great deal of time in the search for parasites when a large number of smears are required to be examined. The concentration of parasites in thick smears works out on an average of 10 to 15 times more than that in thin smears. Required skill to identify species of malaria parasites in thick films is only acquired by practice and experience.

The thin smear, however, is still considered valuable for the identification of species of parasites as also studying their morphology.

It is, therefore, advisable that both thick and thin smears should be taken on the same slides.



of slides and anti-malarials drugs to the surveillance inspectors MPS(M) and surveillance workers MPW (M) through the area Malaria Inspector who will also attend the meeting. The MPS (M) will also plan distribution radical treatment of positive cases under the guidance of medical officer. The Malaria Inspector should also prepare spray programme in the PHC in consultation with M.O.

6.10 He will organise passive surveillance in his area in co-operation with all medical institutions and personnel as well as other voluntary organisations and voluntary workers. For this purpose he will give necessary training to the persons concerned and direct MPS(M) to issue necessary slides and antimalarial drugs that may be required from time to time. He must contact all hospitals and dispensaries in his area for examination and administration of antimalarial drugs for the cases that may be reporting to such institutions. He should ensure that all fever cases are blood filmed.

6.11 He should check the returns from the surveillance inspectors MPW(S) and laboratory technicians will be responsible for forwarding the PHC reports to the higher authorities.

6.12 He will maintain close watch on the quality and quantity of work as carried out by the surveillance staff by periodically referring to the data maintained at the PHC level by the laboratory technician.

6.13 He should check the consumption of anti malarial drugs based on the return from surveillance inspectors MPS(M) and workers as prepared by the Malaria Inspector in the Month'y meetings.

6.14 He should organise the fever treatment depots and drug distribution centres. He should also keep watch over the availability of chloroquine to the above centre.

6.15 The publicity material and mass media equipment received from time to time will be properly distributed or affixed as per the instructions from the district organisation.

6.16 He should consult the Booklet on "Management and Treatment of Cerebral Malaria" and treat cerebral malaria cases as and when required.

6.17 He should ensure that all categories of staff in the periphery administering medical treatment to the positive cases should observe the instructions laid down in under NMEP on the subject and in case toxic effects are observed in a patient who is receiving primaquine drug is stopped by the peripheral worker and such cases are brought to his notice for follow up action/advise if any.

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### PRECAUTIONS:

- a) Scum if formed on the top of the staining solution should be removed with filter paper before use.
- b) The P.H. of wash water should be ranging from 6.2 to 6.8.

### 6. EXAMINATION OF THE THICK BLOOD FILM:

a) The thick film is not a thick drop. It is a smear which transmits enough light for microscopic examination when haemoglobin is partly or wholly removed. Haemolysis or dissolving of the red blood corpuscle is thus the first essential and staining is second. The thick film field shows leucocytes, platelets, and blood protozoa on a back ground of lightly stained remnants of the red cells.

The normal thick film field contains leucocytes and platelets with occasional bluish cloud, the remains of dissolved reticulocytes. The cytoplasm is always somewhat scattered cytoplasmic granules are often lost except the granules of eosinophils which have a special resistance and usually shine out with great clarity. The film may contain a few degenerated white cells which are structureless and recognisable only by their size, shapes and staining. The blood platelets, single, in small groups or occasionally in clusters of several hundreds are stained pale purple and have a wooly texture and outline which is unmistakable.

Other structures seen in the microscopic fields are probably extraneous such as dust, moulds, yeasts, spores, bacteria or deposit of stain.

#### b) Artefacts:

Structures which confuse diagnosis from their resemblance to malaria parasites are sometimes seen, no matter how carefully the films are taken and stained:

The commonest sources of error are:-

i) Solitary or small groups of platelets may be mistaken for Chromatin and quartan trophozoites at the early compact state, and the later for advanced vivax trophozoites at a stage when cytoplasm has broken into a cluster of fragments while the chromatin is yet intact. ii) The resemblance between vivax at this stage and small platelet groups is extraordinarily close particularly when the staining is not good and the light is inadequate.

The above facts cannot be avoided and they must be learnt from experience.

Other artefacts which are extraneous and can be avoided by scrupulous cleanliness in taking the smear, and protection of slide from dust etc.

#### c) Parasite Morphology in thick smear:

The technique of staining thick-film destroys the host cell and exposes the parasite to changes in shape and size. The parasites appear smaller and are less regular in outline. Parasites are seen in unfamiliar setting, no longer as in the fixed thin films neatly framed by their host red cells, but stripped and distorted on a mottled grey grounds of red cells residue. With the disappearance of the host cells, malarial dots in falciparum infection are not seen in thick film. Schuffner's dots may be seen in thick smears. In thick film the red stain chromatin with associated blue cytoplasm must be seen before it is pronounced as a parasite.



b) Technique: In children and adults, blood is collected from the finger tip. In infants, however, the toe is preferred for the purpose. The site from where the blood is to be collected should be cleaned thoroughly with a piece of cotton soaked in rectified spirit and allowed to dry completely. The hagedorn needle taken out from the container is also wiped dry before pricking. A gentle prik is then given on the tip of the ring finger of the left hand or the toes, as the case may be. While pricking no pressure should be exerted on the finger or the blood will flow out. The prick should be such as to get just a good sized drop of blood on gentle pressure. A clean slide is then lowered on to the drop of blood to pick it up at a distance of about an inch from the edge. Then a little more pressure is given on the pricked finger to get bigger drop of blood. This bigger drop should be collected on the same slide about 1/2" away from the edge. The first drop of blood is meant for the thin smear while the second one is for the thick smear. After the blood drops are taken the pricked finger should be attended to by the application of a piece of cotton soaked in rectified spirit over the injury.

c) The thin smear should then be drawn with the smooth edge of a second slide called the "spreader". The "spreader" should be held at an angle of 30 to 45 degrees on the slide containing the blood drops and time should be allowed for the blood drop to spread uniformly along the edge. Then the "spreader" is drawn forward so that the blood spreads behind it making an even film on the surface of the slide.

An ideal thin smear is one, which occupies the middle third of the slide on which it is drawn. It should be even and unbroken with continuous edges. The tail end of the smear should end in finger like process.

d) The other drop on the slide should then be spread into a thick circular smear with the help of corner of the "spreader". It should be 1/2" in diameter and neither too thick nor too thin. Its thickness should be such as to enable one to see the second hand of wrist watch through it.

The time for the preparation of a thick and thin smears should not be more than a few seconds. If more time is taken the blood may clot necessitating the taking of another smear.

e) As soon as smears are drawn, the slide should be moved and fro in the air for drying. The thick smear, of course, would take a little longer time than the thin one for drying. The shorter the time taken for drying the better will be the preservation of the red cells in the smears.

## 5. STAINING TECHNIQUE:

- a) Dehaemoglobinise the thick smear as described.
- b) Dip the thin smear in methyl alcohol for a second or two for fixation.
- c) Dry thoroughly in the air - in a slanting position keeping thin smears downwards.
- d) Immerse the thick and thin smears in JSB-II solution for a second or two.
- e) Wash twice or thrice in a jar containing buffer water.
- f) Immerse in JSB-I for 45 seconds.
- g) Wash several times in buffer water to remove the excess stains.
- h) Dry in air keeping the stained smears side downwards in slanting position to avoid deposit of dust.

b) Precautions:

1. No preliminary fixation of thin smear is necessary
2. Distilled water should be used in all steps
3. The undiluted or the diluted stain should on no account be allowed to dry.
4. The stain on the film should not be lowered off but should be flushed off with distilled water.

2. Giemsa Stain:

a) Technique:

- i) Dilute the concentrated stain in the proportion of 1 c.c. of the stain to 9 cc of distilled water.
- ii) Fix the thin film in methyl alcohol, Better leave the 1/4 of the head and of the film unfixed to avoid fixing of the thick smear by the vapour of alcohol. Immerse the film in a jar containing alcohol and rapidly remove it.
- iii) Allow the alcohol to dry completely
- iv) Put the slide on a staining rack with film surface upward
- v) Pour the diluted stain on the slide to cover both the thick and the thin smear
- vi) Allow the stain to work for 20 minutes.
- vii) Flush off the stain with distilled water.
- viii) Dry rapidly in the air.

b) Precautions:

- i) Dilution of the stain if required to be done only before use.
- ii) Diluted stain in the film should not be allowed to dry.
- iii) Stain on the film should not be poured off before flushing.

3. J.S.B. Stain:

a) Technique:

- i) Dip the thin smear in methyl alcohol for a second or two for fixation
- ii) Dry thoroughly in the air.
- iii) Immerse the thick and thin smears in Solution-II for a second or two.
- iv) Wash twice or thrice in a jar containing wash water.
- v) Immerse in Solution-I for 45 seconds.
- vi) Wash thrice or 4 times in wash water.
- vii) Dry in air.

Precautions:

- i) Scum if formed on the top of the staining solution should be removed with filter paper before use.
- ii) The wash water should be little acidulated with PH ranging from 6.2 to 6.8.

LABORATORY SERVICES:

1. AIM:

Under the Malaria Eradication and even in Central Programme the importance of laboratory services is top-most. Examination of all the blood smears collected



d) Pigment in thick films:

Pigment is seen more clearly in thick than in thin films. A trained observer may often identify the species and the stage of the parasites by noting the shape, size and distribution of the pigment granules.

e) Advantage of examining thick film:

- a) Less time is required for identifying the parasites.
- b) Less chance of missing <sup>the</sup> parasite - even when parasite density is low.

f) Disadvantage:

At times, it becomes difficult to identify the stage correctly. In which event, thin smear is examined for confirmation.

g) Microscopic examination of blood smears:

- i) Thick film is to be examined first
- ii) At least 100 fields are to be examined and this normally takes about 5 minutes.
- iii) If one parasite has been detected, there must be other stages or other species of malaria parasite may be present.
- iv) If there is any doubt in identifying the stage or the species, the thin smear is to be examined for confirmation.

SUPPLEMENTARY NOTES

The staining of blood films:

The following three kinds of stain are normally used for staining blood films:

- i) Leshman stain;
- ii) Geimsa stain and
- iii) J.S.B. Stain

The methods involved in staining blood smears with these different kinds of stains have been detailed below:

1. Lieshman Stain:

- a) i) Drawn a line with grease pencil across the slide between the thick and thin smears.
- ii) Place the slide on the staining rack with the smeared surface upward and the two edges of the slide on the same plain.
- iii) Pour 5 drops of stain to cover the whole surface of the thin smear.
- iv) Allow the stain to act for 1/2 minute only.
- v) Dilute the stain with 15 drops of distilled water (PH 7.2).
- vi) Draw the diluted stain across the grease pencil mark to the thick smear with a glass rod and mix thoroughly.
- vii) Allow the diluted stain to work in both thick and thin smears for 5 minutes only.
- viii) Flush off with distilled water.
- ix) Allow the water to remain on
- x) Place the slide up-right vertically for drying.

A thick film usually contains about 5 cubic mm of blood when 100 fields are examined only about 0.1-0.2 mm<sup>3</sup> of blood is usually covered. Therefore, when the parasite density is scanty, the detection depends on chance and also on excellence of staining, and careful observations. In such a situation if a parasite is seen and missed, it is solely due to the negligence of the microscopist concerned.

Compared advantages of thick film - The thick film method was introduced in 1903 by Ross for rapid detection of malaria parasites, but its merit came to be recognised only relatively recently. The thick film method depends on the fact that it is possible, by dissolving out the haemoglobin from the unfixed RBC to render the thick layer of blood on slides sufficiently transparent for examination by transmitted light.

The value of the thick film is due to its concentration and to the subsequent great saving of time in searching for parasites.

It must be emphasised that thick film calls for great care in preparation for good microscopy and for adequate practice. The margin for technical error is small. The clear outline of the leucocytes and parasites seen in thin film become distorted. Parasites lie naked on a blue-grey background. The stippling of infected Red cells is seldom seen and species diagnosis is sometimes difficult. Nevertheless the advantages of the thick film are so great that it is always worth the time and trouble to acquire proficiency in this method.

The superiority of the thick film, over thin film for the rapid detection of malaria parasite can be illustrated as below:

Thick and thin film prepared from the blood	Thin film	Thick film
Parasites in 100 fields	1	16
Time required to find first parasite	23 minutes	1 minutes
Average difference in concentration	1	20

### 3. STAINING OF BLOOD SMEARS:

Virtually all stains used in Malaria work were derived from Methylene blue processed in one of the several ways to form by oxidation the polychrome anilines and are called Romanowsky Stains, after the discovery of this principle of staining. Before Romanowsky also mixtures of Methylene blue and Eosins were used, but these mixtures always stained the nuclear chromatin blue. The oxidation product of methylene blue now used, stains the chromatin red.

The most important principle of this stain is that its selectivity for different cellular elements is maintained within a narrow range of the reaction of the diluent. The Romanowsky effect gives a range of colours ranging from red through purple to blue which are constant at a given PH, but with the change of PH the whole range is shifted.

for instance:

if too alkaline  
if too acidic

- RBC stain bluish  
- Basophilic cytoplasm of malaria parasites hardly stains at all.



through the active, passive and other voluntary agencies and their treatment in time to cut down transmission is very important. Under the Modified Plan also the spraying strategies are to be fine lised on the basis of Annual parasite incidence. From our experience it has been seen that from most of the areas the collection of the blood smears is less than 10 per cent of the population per which desired. In view of that, proper diagnosis of all the blood smears with malaria parasites to know the actual epidemiological picture of the areas is necessary.

There are certain basic conditions connected with good microscopic work at any level. Such conditions refer to the microscopist and the site of his work, to the microscope and to the material to be examined.

a) Microscopists: He should be properly selected and trained. His position should be stable. He should have reasonable security of tenure of his position and an adequate salary.

b) Site of work: The site of work of the microscopist should be such that the routine work could be done with minimum of fatigue through the provision of adequate building and furniture.

c) Microscope: A good microscope with adequate illuminating facilities should be provided to the microscopist.

d) Material to be examined: The method of collection of blood smears should be standardised. Blood smears should reach the laboratory for staining and examination as properly as possible. For good staining and examination it is necessary that blood smears are received from the field without exposure to dust or heat. Proper labelling of the blood smears is also very important.

e) The evaluation of efficiency of each microscopist should be based on the following points:

- i) Quality of the blood film
- ii) Quality of staining
- iii) Correctness of diagnosis
- iv) Daily out-put

## 2. PREPARATION OF BLOOD SMEAR

For detection of Malaria Parasites both the thin the thick smear is collected on the same slide, but only the thick film is examined as a routine.

a) Thin film is a single layer of blood. The RBCs should be contiguous than overlaping. It is better to have such film too thin than too thick.

A good thin film should:

- a) evenly spread
- b) must be free from streaks and lubs
- c) must not touch be tapered off so that the tail is
- d) the end should be tapered off so that the tail is not lost beyond the end of the slide.

b) The thick film is a drop of blood spread over an area of 1.2 cm. in diameter which may contain 15-20 layers of blood cells. But films are with several layer of RBC in the centre and has a thin edge of one cell thickness.



## LABORATORY SERVICES

### 1. AIM

Under the Malaria Eradication and even in Control Programme the importance of laboratory services is top-most. Examination of all the blood smears collected through the active, passive and other voluntary agencies and their treatment in time to cut down transmission is very important. Under the Modified Plan also the spraying strategies are to be finalized on the basis of Annual parasite incidence. From our experience it has been seen that from most of the areas the collection of the blood smears is less than 10 per cent of the population per year which is desired. In view of that, proper diagnosis of all the blood smears with malarial parasites to know the actual epidemiological picture of the areas is necessary.

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    1. Quality of the blood film
    2. Quality of staining
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- ### 2. PREPARATION OF BLOOD SMEAR

For detection of Malaria Parasites both the thin and thick smear is collected on the same slide, but only the thick film is examined as a routine.



There are many variations of the original Romanowsky stain, but the ones of interest for staining malaria parasites are Giemsa, Leishmans, Wright, Field and JSB.

- a) Thick film- 1) Dehaemoglobinisation of thick film in old new not required - like warm water 10-30 minutes.  
2) Immerse the slide in soln. II for 1-2 seconds.  
3) Dip the slide in Buffered wash water.  
4) Immerse the slide in soln. I for 10-15 seconds.  
5) Dip in Buffered water for 3-4 seconds  
6) Stand on one end to dry.
- b) Thin film- 1) Fixation of thin film by dipping once or twice in Absolute methyle alcohol (Methanol)  
2) Immerse Soln. II for 1-2 seconds.  
3) Dip in Buffered water  
4) Immerse in soln. I for 30-40 seconds.  
5) Dip in buffered water  
6) Dry.

In absence of the Buffered wash water, tap water and well or river water can be used. Whatever the source of water it must be filtered before use. The wash water does not have to be distilled.

JSB stain does not require very precise adjustment of PH in the rinsing water. The optimum is between 5.5 and 6.5. If the tap water is alkaline adjust by adding some potassium dihydrogen phosphate or a few drops of 1% Acetic Acid.

#### 4. EXAMINATION BLOOD SMEAR FOR MALARIA PARASITE

1. Set the microscope for best resolution, Plain mirror adjusted Iris diaphragm, completely open condensar at highest position.
2. Only thick film is examined as routine.
3. Lower Oil immersion lens to the film. Unless parasitised RBC is seen immediately, the slide will be gently moved until a leucocyte is found. Sometime beginners bring it into focus because of slide placed in inverted position i.e. with blood film downwards.
4. Common errors in focussing under the oil immersion.  
Lense: a) incomplete rotation of the revolving eye pieces  
b) defectively centred objectives  
c) blood smear placed upside down  
d) no blood film on the part of the slide directly under the objective  
e) the film is dirty  
f) the objective is dirty  
g) the evepiece is dirty
5. Parasite will show in its true perspective - if Chromatin is too red unlikely to find any blue cytoplasm of parasite. If RBC stains much blue, unlikely to find chromatin with much red colour. Under these conditions it is better to search for an area where leucocytes are more suitably stained.
6. The best resolution is in the centre of the field. So any object requiring careful examination to be brought to the centre.
7. Fince adjustment should be constantly turned to bring the different planes into focus.
8. Move the slide from one end to another, then vertically one field, up and then horizontally from end to end again.

### 3. STAINING OF BLOOD SMEARS

Virtually all stains used in Malaria work were derived from methylene blue processed in one of the several ways to form by oxidation the polyaniline dyes and are called Romanowsky Stains, after the discovery of this principle of staining. Before Romanowsky also mixtures of methylene blue and eosins were used, but the mixtures always stained the nuclear chromatin blue. The oxidation product of methylene blue now used, stains the chromatin red.

The most important principle of this stain is that its selectivity for different cellular elements is maintained within a narrow range of the reaction of the diluent. The Romanowsky effect gives a range of colours ranging from red through purple to blue which are constant at a given PH, but with the change of PH the whole range is shifted.

For instance :

- |                 |   |
|-----------------|---|
| If too alkaline | = RBC stain bluish  |
| If too acidic   | = Basophilic cytoplasm of malaria parasites hardly stains at all. |

There are many variations of the original Romanowsky stain, but the one of interest for staining malaria parasites are Giemsa, Leishman, Wright, Field and JSB.

For mass staining under Malaria Eradication Programme Giemsa and JSB are most suitable. In MMEP stain is mainly used J.S.B.

- (a) Thick Film :
1. Dehaemoaglobinisation of chick film in old, now not required - luke warm water 10-30 minutes.
  2. Immerse the slide in soln. II for 1-2 seconds.
  3. Dip the slide in Buffered vas water.
  4. Immerse the slide in soln. I for 10-15 seconds.
  5. Dip in Buffered water for 3-4 seconds
  6. Stand on one end to dry.

(b) Thin film

1. Fixation of thick film by dipping one or twice in Absolute Methylalcohol (Methanol)
2. Immerse Soln. II for 1-2 seconds
3. Dip in Buffered water.
4. Immerse in soln. I for 30-40 mnts.
5. Dip in Buffered water
6. Dry.



(A) Thin film is a single layer of blood. The RBCs should be contiguous than overlapping. It is better to have such film too thin, than too thick. A good thin film should:

- a. Evenly spread
- b. Must be free from streaks and lubs
- c. Must not touch the long edge of the slides
- d. The end should be tapered off so that the tail is not lost beyond the end of the slide.

(B) The thick film is a drop of blood spread over an area of 1 to 2 cm. in diameter which may contain 15-20 layers of blood cells. But films are with several layer of RBC in the centre and has a thin edge of one cell thickness. A thick film usually contains about 5 cubic m. of blood. When 100 fields are examined only about 0.2 mm<sup>3</sup> of blood is usually covered. Therefore, when the parasite density is scanty, the detection depends on chance and also on excellence of staining, and careful observation. In such a situation if a parasite is seen and missed, it is solely due to the negligence of the microscopist concerned.

Comparative advantages of thick film : The thick film method depends on the fact that it is possible, by dissolving out the haemoglobin from the unfixed RBC to render the thick layer of blood on slides sufficiently transparent for examination by transmitted light. The value of the thick film is due to its concentration and to the subsequent great saving of time in searching for parasites.

It must be emphasised that thick film calls for great care in preparation, for good microscopy and for adequate practice. The margin for technical error is small. The clear outline of the leucocytes and parasites seen in thin film become distorted. Parasites like naked on a blue-grey back-ground. The stippling of infected Red cells is seldom seen and species diagnosis is sometimes difficult. Nevertheless the advantages of the thick film are so great that it is always worth the time and trouble to acquire proficiency in this method.

The superiority of the thick film, over thin film for the rapid detection of malaria parasite can be illustrated as below:

Thick and thin film prepared from the blood	Thin film	Thick film
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Time required to find first parasite	23 minutes	1 minute
Average difference in concentration	1	20

PROFORMA FOR DETAILLED STUDY OF NEW LABORATORY

1. Name of the visiting officer
  - a. Unit/PHC - visited.
  - b. Period of visit.
  - c. Population of the unit/PHC.
2. Personal :
  - a. Existing staff
  - i. Microscopist
  - ii. Other staff attached to the laboratory
  - b. Training status
    - i. Place & Period of training
    - ii. Untrained
  - c. Posts 2. Vacant
3. Cleaning of slides
  - a. Solution in use
    - i. New
    - ii. Old
  - b. Testing of cleaned slides  
Is the present cleaning practice found to be satisfactory.
4. Drawing of blood smears  
Size - thickness - position
  - a. Thick
  - b. Thin  
Record of average No. of per 10 slides selected at random.
5. Numbering
6. Dehemoglobinisation
  - a. Arrangements of slides
  - b. Water used
  - c. Time given
  - d. Flushing



4

In absence of the buffered wash water, tap water and well or river water can be used. Whatever the source of water it must be filtered before use. The wash water does not have to be distilled. JSE stain does not require very precise adjustment of PH in the rinsing water. The optimum is between 5.5 and 6.5.

If the tap water is alkaline adjust by adding some potassium dihydrogen phosphate or a few drops of 1% Acetic Acid.

#### IV. EXAMINATION BLOOD SLIDE FOR MALARIAL PARASITE

1. Set the Microscope for best resolution. Illuminator adjusted. Iris diaphragm, completely open condenser at highest position.
2. Only thick film is examined as routine.
3. Lower Oil immersion lens to the film. Unless parasitised RBC is seen immediately, the slide will be gently moved until a leucocyte is found. Sometimes beginner bring it into focus because of slide placed in inverted position i.e. with blood film downwards.
4. Common errors in focussing under the oil immersion.

#### LIST :

- a. Incomplete rotation of the revolving eye piece
- b. Defectively centred objectives
- c. Blood smear placed upside down
- d. No blood film on the part of the slide directly under the objective.
- e. The film is dirty
- f. The objective is dirty
- g. The eyepiece is dirty
5. Parasite will show in its true perspective - If Chromatin is too red unlikely to find any blue cytoplasm of parasite. If WBC stains much blue, unlikely to find chromatin with much red colour. Under these conditions it is better to search for an area where leucocytes are more suitably stained.
6. The best resolution is in the centre of the field. So any object requiring careful examination to be brought to the centre.
7. Fine adjustment should be constantly turned to bring the different planes into focus.
8. Move the slide from one end to another, then vertically one field, up, and then horizontally from end to end again.

7. Staining
  - a. Trial & Error method
  - b. Whether only thick or both thick and thin smear stained.
  - c. Whether mass or individual
  - d. Destaining or restaining
  - e. Defects noted in staining
  - f. Filtering of the staining
8. Fixing :
  - a. Agent used
  - b. Method in practice
  - c. Whether done after or before staining the thick smear.
9. Buffered Solution:
  - a. Agent used
  - b. Water
  - c. PH indicating paper
10. Dust proof cover
11. Handling of microscope
  - a. Mirror
  - b. Position of condenser
  - c. Whether one eye or both eyes used
  - d. Oil used
  - e. Cleaning
12. Examination:
  - a. No. of fields seen
  - b. No examined by the technician (s) and the total output.
  - c. Discrepancies - RHO & State Central laboratory or any other.
13. Maintenance Record :
  - a. In ward Register
  - b. Log Book or technician
  - c. Others.
14. Maintenance of Maps, Graphs and chart
  - a. Maps of the Unit/PHC
  - b. Graphs showing - S.W. wise collection of blood smear



- c. Graphs showing No. of smear received and examined per month.
- d. Graph showing positive per month
- e. Graph showing positive case per month
- f. Back log chart.

C 15. Microscope :

- a. Number
- b. Condition

16. Spot lamp

17. Inflow of slides

- a. Active
- b. Passive
- c. Mass survey

18. Back log (on the day of visit)

19. Pigeon Holes

20. Check slides sent to RHO/STATE

- a. P.C. Sent
- b. Schedule - slide No. and result
- c. Details of disagreement - if any
- d. Packing

21. Positive recorded, species wise

22. Demonstration of positive slides

23. General conditions of laboratory

- a. Arrangements
- b. Equipment

24. Testing of technician

Examination of positive and Negative slides.

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