Malaria

Environment and Disease Laboratory 1
Fall 2002

Introduction:

Malaria is an extremely debilitating, often fatal disease that is endemic in tropical and subtropical areas of the world. It is caused by protozoans of the genus *Plasmodiu*m, which infect the bloodstreams of people and other animals. The protozoans are carried from one host to another by mosquitoes of the genus *Anopheles*.

The complicated life cycle of *Plasmodium* is depicted on the "Malaria Life Cycle" Bioreview Sheet (No. 4170; Burlington NC: Carolina Biological Supply Co.) following. Sporozoites are injected into the bloodstream of the human host by an infected female *Anopheles*. Finding their way to the liver, the parasites become cryptozoites (1) and reproduce inside liver cells for ten days before releasing a burst of merozoites (2). The merozoites released can re-infect liver cells to repeat the pre-erythrocytic phase of growth, or they can infect erythrocytes (red blood cells) to begin the erythrocytic phase. In the latter, the parasites develop into trophozoites (3,4), reproduce inside the erythrocytes for two days to produce schizonts (5,6), and emerge as a burst of merozoites (7), which re-infect erythrocytes. The periodic bursts of merozoites bring about the periodic fever characteristic of malaria patients. Occasionally older trophozoites (4) differentiate into microgametocytes (8) or macrogametocytes (9), and these must be picked up by *Anopheles* vectors for the cycle to continue. In the gut of the mosquito the micro- and macrogametocytes differentiate into microgametes (10) and macrogametes (11), fuse to form zygotes (12), and differentiate into ookinetes (13). The ookinetes invade the gut wall of the mosquito, form oocysts (14) inside mosquito gut cells, reproduce and differentiate into sporozoites (15), and ultimate emerge into the mosquito's hemolymph as mature sporozoites (16). These find their way to the salivary glands and thence into the saliva of the mosquito, and thus are poised for injection into a suitable human host.
Today you will prepare microscope slides of your own blood, stained with the Wright stain, and you will compare these to slides of blood infected with the malaria parasite. You will also examine slides of the *Anopheles* mosquito to get a good look at the injection apparatus of the female.

Procedure:[1]

1. A small drop of blood from a finger tip should be placed on one end of a clean glass slide. A spreader slide should be touched to the drop of blood, and while held at an angle of about 30° it should be pushed forward over the surface of the slide (see Fig. 1). The end of the smear should have an irregular edge where the smear is at its thinnest. The thinnest area is often the best area to search to find plasmodia or to observe blood cells. Let the slide dry in a closed Petri dish.

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[1] Adapted from CLARK, GEORGE, ed. *Staining Procedures Used by the Biological Stain Commission.* 3rd ed.
Baltimore: Williams & Wilkins, 1973, pp. 123-124.

PLEASE DISCARD ANYTHING YOUR BLOOD TOUCHES IN THE BIOHAZARD BAG, WHICH WILL BE AUTOCLAVED. ASSUME YOU HAVE AIDS.

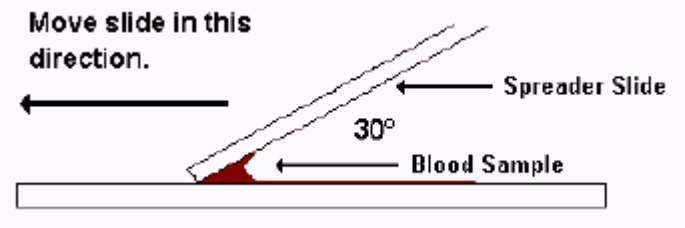


Figure 1. Preparation of a thin blood film.

2. Quickly fix your film by immersing it in absolute methanol for 2–3 minutes.

3. Immerse the slide in the Wright stain for 5 minutes; it's hard to predict the rapidity of staining of a given batch of dye.

4. Wash the slide by dipping in deionized water, until "properly differentiated," which usually means until stain stops obviously running out of the smear. The thin part of the smear should be differentiated in about one or two dips in the buffer; the thick part of the smear may need decolorization for 3–5 minutes.

5. Air dry; do not heat, do not blot. A warm lamp or small fan can be used to hasten drying.

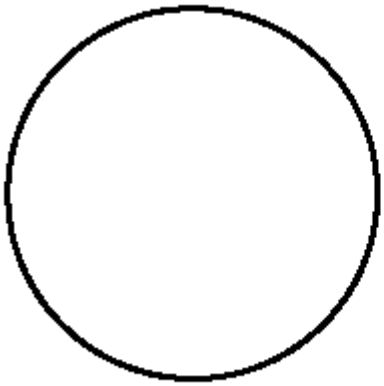
6. The slide may be observed directly, without a cover slip. You will probably want to use your oil immersion lens. If you happen to have malaria, the cytoplasm of the protozoa should be stained blue; nuclear structures should be stained red; erythrocytes should be stained pink.

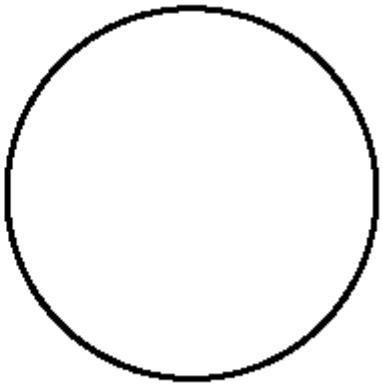
7. Compare your blood with that of blood infected with *Plasmodium falciparum* (on the commercial slide).

8. Draw representative fields of each slide in the areas below, indicating which stages of the life cycle you think you have seen.

YOUR BLOOD
MAGNIFICATION: _____X
FIELD DIAMETER: _____ mm

INFECTED BLOOD
MAGNIFICATION: _____X
FIELD DIAMETER: _____ mm





9. Draw the heads of male and female *Anopheles*. In both sexes of this species the two segmented palps should be roughly as long as the unsegmented proboscis, but the male's two antennae are very bushy compared to the female's.

MALE HEAD
MAGNIFICATION: _____X
FIELD DIAMETER: _____ mm

FEMALE HEAD
MAGNIFICATION: _____X
FIELD DIAMETER: _____ mm