Recognition of malarial parasites

Tutorial witness statement 28.05.13

The diagnosis of malaria is based on clinical examination and laboratory detection of malarial parasites in the red blood cells through an antigen test and examination of both thin and thick EDTA blood films. During a tutorial with ********* on the identification of malarial parasites, we examined multiple slides showing each of the different parasites. I learnt:

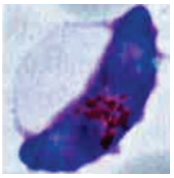
- How to produce and view the 5 films (2 thick and 3 thin) necessary for malarial identification. The thick film is important for the initial detection of parasites and the thin film for their precise identification.
- How to consider the slide as a whole when identifying malarial type e.g. size and shape of red blood cells, the cytoplasm of the cell around the parasite and the colour/pigmentation of the cell.
- How to identify the 4 different types of malaria and the main features associated with each (see below).

Stages of malaria infection seen on the blood film

- Film preparation - 1 thick film is stained with Field's stain (after a 20-30 minute drying period on the slide warmer to avoid loss of the film during staining) and 1 thin film with reverse Field's stain. The stains are pH 7.2; a slightly more alkaline pH is used to more easily identify any malarial parasites present as an acid stain may fail to show the parasites. The thin film is then stained on the automated Hematek slide stainer. 1 thick film and 1 thin film remain unstained, with a thin film stained with dilute Giemsa if malarial parasites are detected in the other films.
- Trophozoite stage - This is the most commonly seen stage on the blood film. Trophozoites have many appearances as this is the growing stage; however, the main appearance is based around the ring form.
- Gametocyte stage - The gametocyte stage is the stage that can infect the mosquito to begin the cycle again. The gametocyte numbers can also be used to monitor treatment.
- Schizont stage - Schizonts are the stage of the malaria lifecycle in which there is asexual reproduction. It is the replicating stage, where the nucleus is undergoing division to produce merozoites. The merozoites are released from the red cell when it ruptures and can enter other erythrocytes or the liver to increase the number of cells that are infected and replace cells that may be removed from the circulation.

Plasmodium fa/ciparum

- *P. fa/ciparum* is the species of malaria that most commonly causes severe disease and death. The size of the infected cell is the same as that of normal cells. *P.* fa/ciparum infected erythrocytes have an altered surface structure which enables them to stick to endothelial cells in various tissues, causing parasite sequestration and blood vessel occlusion.
- Trophozoites - The trophozoite is usually a ring form; when found upon the edge of the cell, these are known as accole forms. Maurer's clefts may be present.
- Gametocytes - The gametocytes are the infectious stage. The *P. fatciparum* gametocytes have a characteristic shape, normally being a crescent "banana" shape.

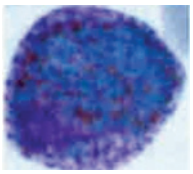


- Schizonts - In *P. fatciparum* infections it is rare to see schizonts in the peripheral blood, as they are usually sequestered in internal organs; their presence on the slide indicates advanced infection. During the tutorial, we looked at a histological section of the brain of a person with a fatal malarial infection to see how the schizonts occluded the blood vessels. There are normally 12-30 merozoites in the schizont.

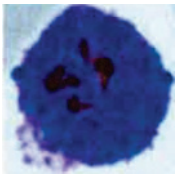
Plasmodium vivax

- *P. vivax* has been associated with severe malaria and death, but not to the same degree as *P. fatciparum* as the parasite can only infect the reticulocyte and the cells are larger than normal. *P.* vivax-infected cells also have Schuffner's dots present.
- Trophozoites - In *P. vivax* the trophozoites have various forms as the parasite matures. There is usually one chromatin dot and the ring is a similar size to that of *P. matariae,* with a diameter of a third to a half of the erythrocyte. The cells do not have smooth edges.

- Gametocytes - In *P. vivax* infections the gametocytes are round.



- Schizonts - The schizont contains clear darker regions which are the developing merozoites, along with clumps of brown malarial pigment. There are normally 12- 24 merozoites in the schizont.

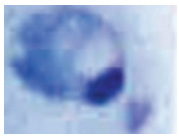


Plasmodium ovale

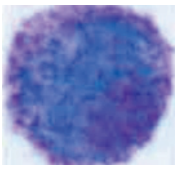
- Infection with *P. ovate* is uncommon and only affects reticulocytes, so infected cells are

larger than normal. *P. ovate* gained its name for the characteristic oval shape of the infected erythrocyte, but this occurs in less than 20% of cells. The ends of *P. ovate* infected erythrocytes are colourful (appearing to change from blue to black as the microscope focuses in and out) and may also have a fimbriated appearance (fringed, uneven, rough ends).

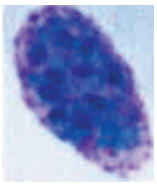
- Trophozoites - The trophozoites show a great variety of shapes from the ring form to amoeboid shapes. The cells contain fine, red James's dots.



- Gametocytes - *P. ovate* gametocytes have a circular shape and are difficult to distinguish from those of *P. vivax.*



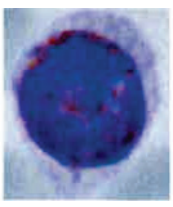
- Schizonts - *P. ovate* schizonts again have a similar appearance to those of *P. vivax* but with only 4-12 merozoites.



P/asmodium malariae

- *P. matariae* is the least common form of malaria in humans. The infection is usually benign, although chronic infection can lead to severe complications such as nephrotic syndrome. The erythrocytes are neat, colourful (with lots of yellow/brown pigment) and small, as *P. matariae* often infects older erythrocytes.
- Trophozoites - Trophozoites vary from the ring form to a band form that fills the cell and extends from one end to the other.

- Gametocytes - Malaria pigment can be clearly seen as yellow/brown pigment scattered through the parasite. The nucleus is usually very dense.



- Schizonts - Schizonts are arranged with the merozoites surrounding a central area of malaria pigment. There are normally 6-12 merozoites in the schizont.