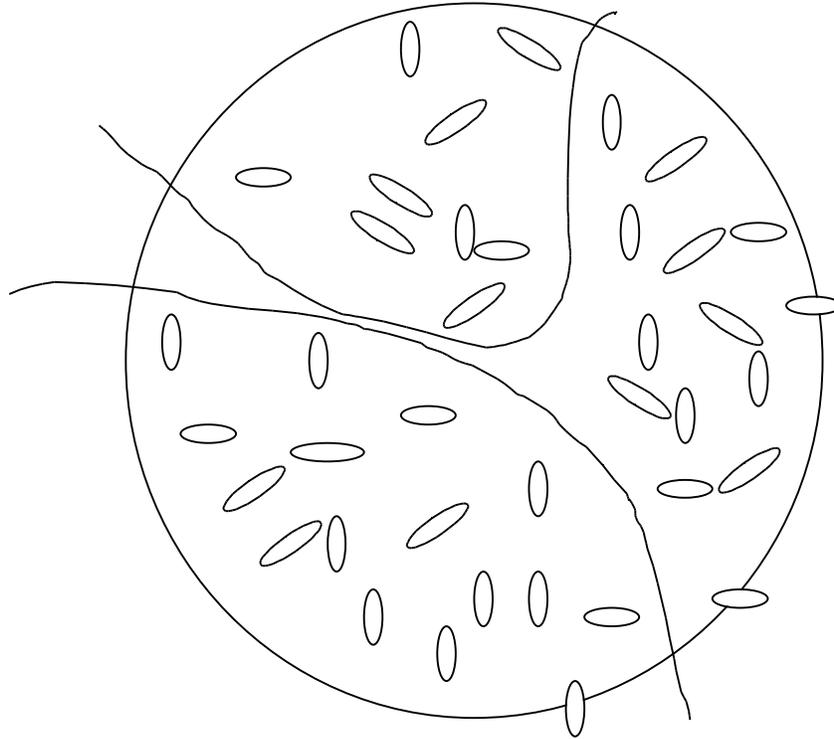


How to Scan Blood Smears, Identify, and Count Parasites

1. Scanning for *Leucocytozoon* in bird blood should be done first at 100x magnification. *Leucocytozoon* tends to be present at low densities in the blood, but are large parasites and can be spotted even under low power.
2. For *Plasmodium* and *Haemoproteus*, scanning at 1000 x is necessary. Immersion oil is placed directly on the smear and the lens lowered into the oil for scanning. (Be sure to use the immersion oil manufactured for the 1000x lens being used.) The oil will not harm the smear. After work is completed on a slide, it is placed into a plastic slide box (Carolina blue box) that holds 25 slides. The bottom of the slide box has a layer of paper+plastic benchtop paper to absorb the oil. This paper must be changed regularly. Even so, the plastic will eventually be degraded by the oil and the box will need to be replaced. After draining, the last residue of oil can be removed from the edge of the slide with a kimwipe, and the slide replaced into its normal storage box.
3. After practice, it is possible to scan smears from lizards by moving the slide continuously at a slow pace. Six minutes of such scanning will allow inspection of 10,000 erythrocytes. For bird smears, each field must be inspected because the parasite density (parasitemia) tends to be low.
4. To get a count of parasites, typically 1,000 erythrocytes are inspected. Another 1000 cells can be counted to determine the reproducibility of the counts. A field is chosen at random to inspect the cells, after doing the count for that field the slide is moved to another field. Fields should be examined in all parts of the smear because parasites are sometimes clumped in one portion of the smear. The count is made by first counting the number of erythrocytes. Cells not completely in view (at the edges) are not counted (human nature drives counting of infected cells that are partially visible, but this is an error). The number of cells are recorded using a hand counter (several versions are available, a hand-held single counter is inexpensive, those that sit on the benchtop and record several counts are hundreds of dollars...but don't provide exceptional numbers!). Next, the number of parasites of each species (if a mixed infection) and each life stage (trophozoite, schizont, male and female gametocytes) are taken.

5. A field may contain more than a hundred cells, so obtaining accurate counts takes some practice. One trick is to mentally divide the smear by landmarks, or patterns in the placement of cells. Again, with practice, such landmarks become obvious and allows very accurate counts of cells. See one illustration below.



6. Sex ratio of gametocytes can be determined, but only after examination of many smears for each species of parasite. For *Plasmodium*, male gametocytes (microgametocytes) typically stain faintly and the cell looks pale or pink. The nucleus is diffuse and adds to the overall pale pink appearance of the cell. Female gametocytes (macrogametocytes) stain blue, sometimes dark blue and the nucleus is more compact. The distribution of the malaria pigment granules also may differ between males and females, depending on the species of parasite. Immature gametocytes tend to have pointed ends (spindle shaped) and stain blue, and so all appear to be females. Thus, care must be taken not to inflate the female count by including immature gametocytes. Pictures of male and female gametocytes of a variety of *Plasmodium* species are available elsewhere on this website.

7. Identifying parasites. First the beginning investigator must learn to distinguish parasites from various kinds of junk on the slide. The best way to do this is to examine pictures of parasites (many are shown elsewhere on the website). Rather than learning the junk, learn the actual parasites. One of the most common errors is mistaking a white blood cell for a parasite; again, looking at photomicrographs will reveal the distinctive characters of the parasites.

8. *Haemoproteus* (in birds, and rarely in reptiles) and *Leucocytozoon* in birds are seen only as gametocytes in the blood. *Plasmodium*, however, undergoes asexual reproduction in blood cells, so trophozoites and schizonts are also seen. Examples of these stages are seen in the photographs of many parasites species on the website, and the entire life cycle of *P. mexicanum* is shown in the Lizard Malaria section.

9. Identifying parasites to species. The major taxa of blood parasites that are seen in birds are *Plasmodium*, *Haemoproteus*, *Leucocytozoon* (all “malaria” parasites in the phylum Apicomplexa), *Trypanosoma* (a flagellated parasite that should be in its own phylum), and microfilarial worms (larvae of a nematode worm). In reptiles, parasites seen are *Plasmodium*, rarely *Haemoproteus*, Hemogregarines of a huge number of species (also an apicomplexan group), and one probable Hemogregarine that is very common, the genus *Schellackia*. The intracellular parasites (the apicomplexans) may be difficult to distinguish. For example, *Schellackia* is often mistaken for a male gametocyte of *Plasmodium* when first learning the parasites. The best way to learn to distinguish the parasites is to study drawings and photographs in various publications, or the photographs provided elsewhere on the website.