CHAPTER

22

Diagnostic Value of Hematology

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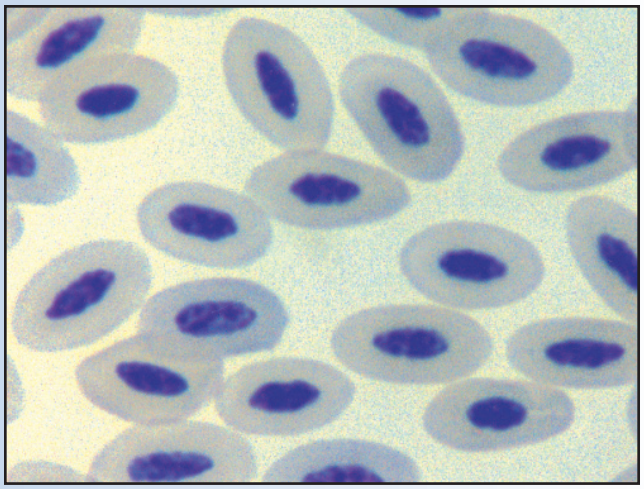


Hematology is the discipline of medical science that studies the blood and blood-forming tissues, and is currently considered an integral part of clinical laboratory diagnostics in avian medicine. Hematology assays seldom provide an etiological diagnosis, but they remain, nevertheless, indispensable diagnostic tools to evaluate health and disease in individuals, to monitor the progress of diseases, to evaluate the response to therapy and to offer a prognosis.

The routine collection and processing of blood samples allows the evaluation of the hematologic response to disease. In addition, the creation of hematology databases is important in establishing reference values for various avian species.

In the past 15 years, significant advances have been made in the use of hematology assays in the differential diagnosis of pathologic conditions in avian species. This appears to have developed parallel to other areas such as nutrition, wellness examinations, anesthesia, surgery and therapeutics.

The processing of hematology samples also has been enhanced in recent years. In the past, automatic analysis of avian blood samples was basically limited to total red cell counts using the cell counters[a] that were available and making manual adjustments of the thresholds and current aperture settings. More recently, the analysis of avian blood samples has received a significant boost with the advent of more comprehensive and accurate automatic analytical systems based on laser flow cytometry.[b] This methodology is based on the measurement of scattered laser light, which fluctuates with the size of the

Table 22.1 | Materials Needed for Blood Sample Collection

- Syringe, 1-ml or 3-ml, disposable
- Needle, 27-gauge to 23-gauge, bent, disposable
- Cotton
- Alcohol
- EDTA or heparin pediatric 0.5-ml or 1-ml blood tubes, or sodium citrate tubes

cell, the complexity of the cell (eg, overall shape, nucleus-to-cytoplasm ratio, granulation), and the size and shape of the nucleus after blood cells are exposed to a laser beam. The laser flow cytometry unit produces a graphic display containing a total optical white cell count, white cell differential count expressed in percentage, absolute values and total red cell count, hemoglobin measurement by the cyanmethemoglobin method, thrombocyte count, and white cell count by cell-lysing impedance measurement of cell nuclei.[14] However, the use of laser flow cytometric technology in avian species is not free from deficiencies.

There are certain pathological conditions in which the presence of enlarged thrombocytes (commonly referred to as megathrombocytes) in the blood film appear to be a characteristic hemoresponse. For instance, in the houbara bustard (*Chlamydotis undulata macqueenii*), the mean thrombocyte measurements in birds undergoing chronic inflammation (severe shoulder injury as a result of repeated crashing against the enclosure wall) were 9.22 ± 0.21 μm length and 8.10 ± 0.19 μm width compared with 5.47 ± 0.12 μm length and 4.96 ± 0.10 μm width in clinically normal birds.[9] The mean diameter of lymphocytes in clinically normal houbara bustards is 7.7 μm;[51] however, there are other species, such as the kori bustard (*Ardeotis kori*), in which the presence of large and small thrombocytes in the same blood film appears to be normal.[51] It is, therefore, probable that a sample containing megathrombocytes would yield a high lymphocyte count under an automatic analytical system, as it would be impossible for even a sophisticated unit to differentiate between lymphocytes and megathrombocytes. When dealing with such species, the software would require some adjustments in order to properly differentiate these cells. This would obviously imply the need to carry out extensive calibration based on repeated manual assessments on a significant number of samples.

Furthermore, in certain species it is relatively common to find large and small lymphocytes in the same blood film.[4,12,26,31,35] This phenomenon has been observed in many psittacine species. Clinically normal kori bustards, for instance, demonstrated a mean diameter for small lymphocytes of 7.2 ± 0.12 μm, whereas the mean diameter of large lymphocytes was 10.7 ± 0.16 μm.[31] Therefore, total white blood cell counts and differential white blood cell counts cannot be accepted as reliable in every clinical case and in every species if the values were estimated by laser flow cytometry. It is, therefore, highly recommended to re-evaluate these samples using manual methods. Clearly, the clinician must be fully familiar with the materials and methods of hematology analysis in order to assess and understand the results.

Blood Sample Collection

It is essential that blood samples be obtained from avian species by or under the supervision of a veterinarian who is experienced with avian venipuncture. The assistant, if one is used, also should be comfortable with restraint and handling techniques. The techniques used vary according to personal preferences and the species being handled. Materials needed for blood sample collection, ie, syringes, slides, tubes, should be labeled in advance and readily accessible (Table 22.1).

METHOD

The total blood volume in clinically normal birds is in the range of 6 to 11 ml per 100 g of body weight.[54] Thus, a bird weighing 250 g would have approximately 15 to 27.5 ml of blood, of which, in a clinically normal individual, up to 10% (1.5-2.7 ml) can be safely withdrawn without having any detrimental effect on the patient. However, 0.2 to 0.3 ml of blood is generally sufficient to carry out a comprehensive hematology examination in a bird.

In birds, blood samples are commonly collected using the right jugular vein (v. *jugularis dextra*), as this is generally larger than the left jugular vein in most avian species (Fig 22.1a). Other preferred sites include the basilic vein (Fig 22.1b) (v. *cutanea ulnaris superficialis*) and the caudal tibial vein (v. *metatarsalis plantaris superficialis*) (Fig 22.1c).

The methodology used for the collection of blood samples varies according to the species and the site selected (Fig 22.1d-h). For example, in long-legged birds such as large bustards, cranes and storks, the jugular or caudal tibial veins are very often used. In the author's opinion, blood samples should be collected from the heart or the occipital sinus only if these birds are under anesthesia and are to be euthanized. It is a poor practice to collect blood samples from clipped nails, as cell distribution and cell content is invariably affected.

The author prefers obtaining blood samples from most bird species from 200 to 4000 g using a basilic vein

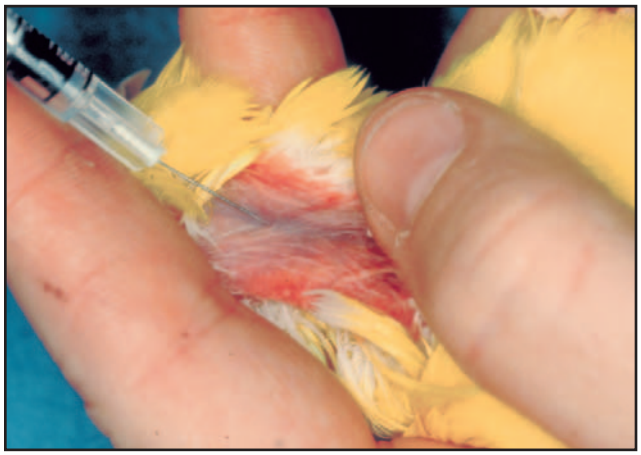


Fig 22.1a | Right jugular being blocked in a love bird and a syringe with a 27 ga needle that has been bent to allow venipuncture.

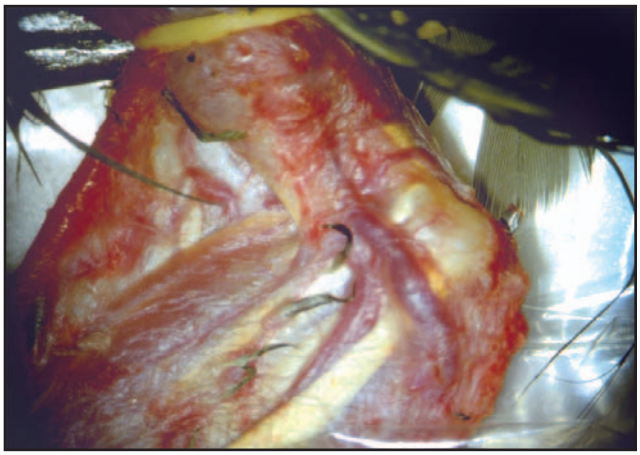


Fig 22.1b | The blocked basilic vein showing the torturous nature of the vein making cannulation with a hypodermic needle difficult at best.

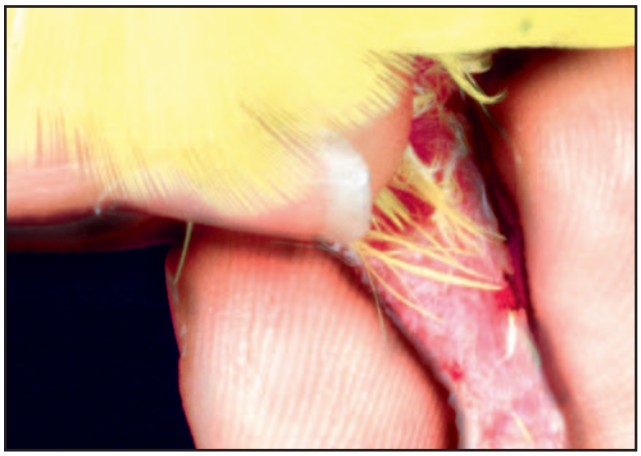


Fig 22.1c | Caudal tibial vein in a love bird.

while the bird is in dorsal recumbency, although most practitioners in the US dealing with psittacine species prefer jugular venipuncture. In most avian species, the optimal area for collecting a blood sample from a basilic vein is along the medial section of the vein. The preferred side is from the right wing if the practitioner is right-handed, while the left wing is the preferred side if the practitioner is left-handed. Venipuncture immediately above the elbow joint is not recommended, as hemostasis is difficult to achieve at this site in most cases. The application of digital pressure with the thumb at the proximal humerus would help in raising the vein, making it clearly visible running parallel to the external aspect of the humerus. After separating the feathers and preparing the site with an alcohol swab, the bent needle is gently inserted into the vein at an approximately 45° angle. The sample can now be collected, taking precaution not to exert high negative pressure while withdrawing with the syringe because this will invariably result in the collapse of the vein.

While withdrawing the sample, it is recommended to continue maintaining pressure on the proximal humerus to ensure a raised and well-defined vein. The method of approaching the basilic vein dorsally by entering under the adjacent tendon prevents hematoma formation because the underlying tissue exerts pressure on the venipuncture site when the needle is withdrawn. It is essential to avoid sudden movements that can alarm the bird and trigger a struggle, as this can easily lacerate the vein and result in a hematoma or, worse, a severe hemorrhage. After collection, a small ball of dry cotton should be placed over the venipuncture site and the wing closed to maintain pressure over the site for a few seconds. It is strongly advisable to check the venipuncture site before releasing the bird back to its enclosure to ensure no post-collection hemorrhage has occurred. Any sample that contains clots should be rejected, as the processing of such samples would invariably lead to imprecise and therefore misleading results.

STORAGE OF BLOOD SAMPLES

After collection, the needle should be removed and the blood gently deposited into a 0.5 to 1.0 ml commercially available pediatric blood storage tube containing the anticoagulant agent ethylenediamine tetra-acetic acid (EDTA) (1.5 mg/ml of blood) or lithium heparin (1.8 mg/ml of blood). Squirting the sample through the needle is a poor practice, as it may cause severe disruption to the fragile blood cells. For general hematology analysis, EDTA is the anticoagulant of choice, as it is not possible to estimate fibrinogen or to count white blood cells accurately in heparinized samples.

In some avian species, however, storing blood samples in tubes containing EDTA causes progressive red cell hemolysis and is not recommended; in these cases, it is preferable to use heparinized tubes. This is the case with some species of Corvidae such as the jackdaw

Figs 22.1d-h | Small Birds - Basilic Vein

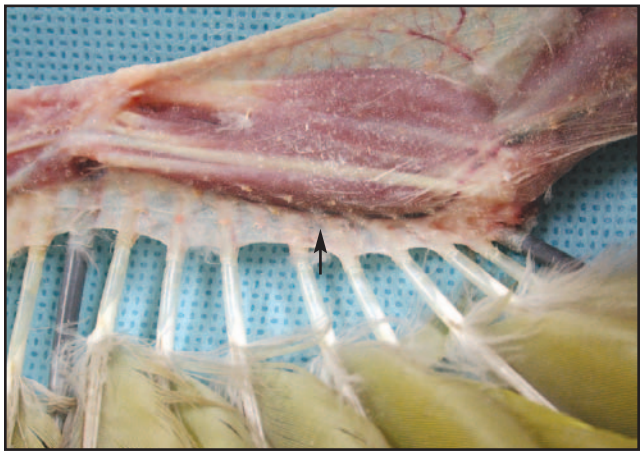


Fig 22.1d | The ventral surface of the wing of a cadaver with the covert feathers removed to show the location for superficial ulnaris vein lancing (arrow). Lancing the basilic vein in birds under 100 gms avoids subcutaneous hematomas and the possibility of death due to exsanguination. The site has a series of natural depressions over the vein that serve to allow the blood to pool. The depressions are formed by the insertion of the secondary feathers intermittently elevating and depressing the wing dermis just caudal to the border of the *flexor* carpi *ulnaris* muscle.

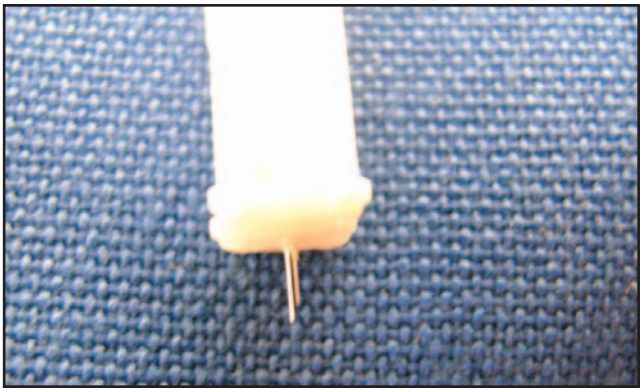


Fig 22.1f | Lancet with the cap removed and the 1 mm tip exposed.

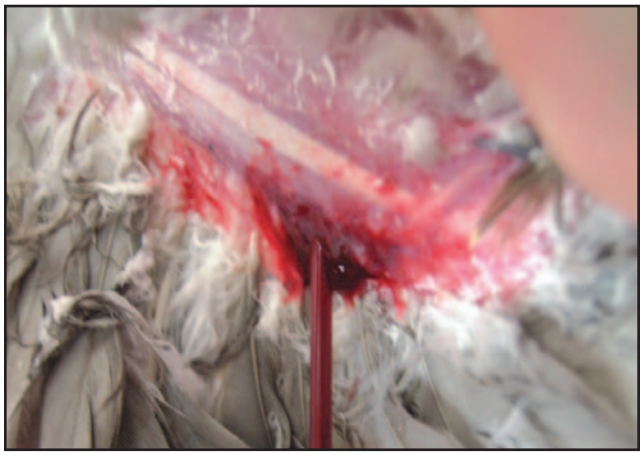


Fig 22.1h | The superficial ulnaris vein has been lanced and blood is being drawn into a micro capillary tube. A cotton ball is applied to the site until hemostasis is achieved.

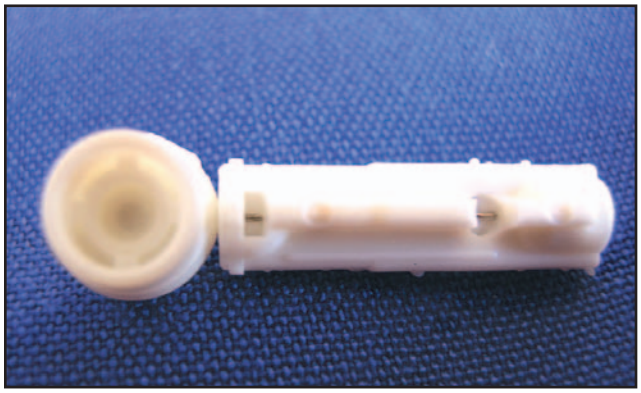


Fig 22.1e | Lancet used in a finger stick technique for blood sampling for glucose analysis in humans. The lancet still has the metal tip in place in the plastic cap.

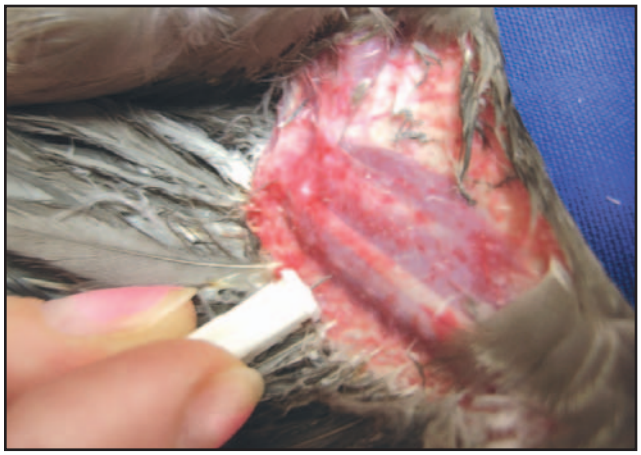


Fig 22.1g | The lancet readied at the site to lance the superficial ulnaris vein.

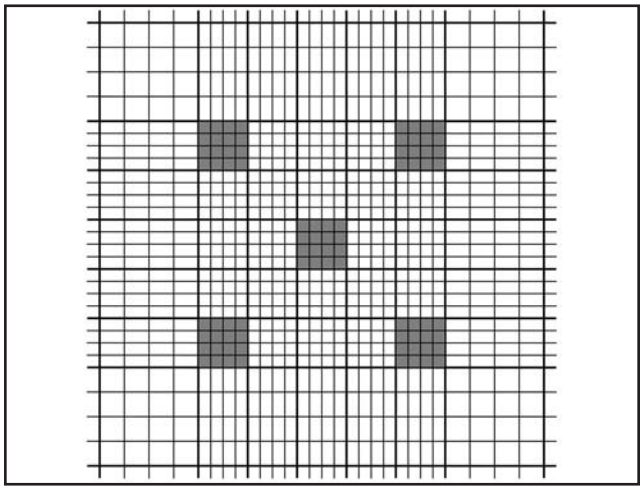


Fig 22.2a | The improved Neubauer counting chamber and the method for counting red blood cells. The total red blood cell count is performed by counting the number of cells contained in the 25 groups of 16 small squares (shaded) at the 4 corner squares and center square in the central area of the chamber. Closely ruled triple lines (illustrated in the drawing as thick lines) separate these squares.

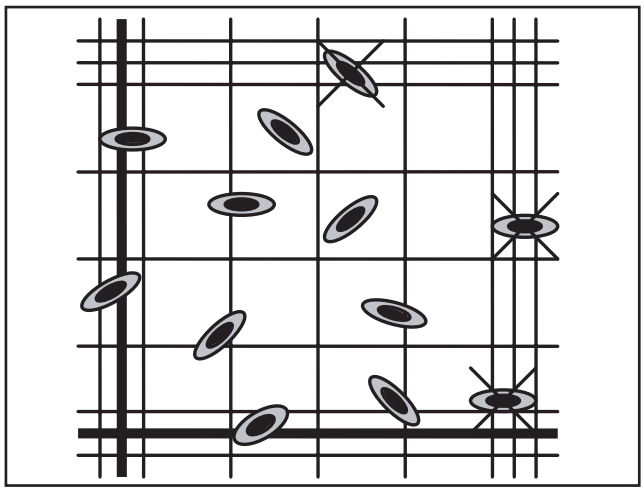


Fig 22.2b | The counting system. Count cells that touch the center triple line (seen here as a thick line) of the rules to the left and bottom; do not count cells that touch the center triple line of the rules to the right and top.

Table 22.2 | Special Considerations When Submitting Blood Samples to a Laboratory

- Seal the lid of the tube using waterproof tape in order to prevent any leakage.
- Wrap the tube using an absorbent packing material, eg, cotton, to soak up any potential leakage and to protect it from breakage. Fasten it securely with tape, preferably commercially available printed tape with the legend "Pathological specimen. Fragile handle with care" or similar tape.
- The tube should then be placed within two leak-proof plastic bags. Fasten the double wrapping securely with printed tape.
- Place submission form in a separate plastic bag.
- The package should then be placed within a postal-approved commercially available transport container made of aluminum, polystyrene, plastic or cardboard.
- Attach recipient and sender labels directly to the container using tape, or place container within a padded envelope and address it accordingly.

Table 22.3 | Priorities When Processing Hematology Samples

- Blood film (differential, white and red cell morphology, hemoparasites)
- Packed cell volume (PCV)
- White cell count (WBC)
- Hemoglobin (Hb)
- Red cell count (RBC)
- Fibrinogen

(*Corvus monedula*) and raven (*Corvus corax*); Gruidae such as the black-necked crowned crane (*Balearica pavonina*) and gray-necked crowned crane (*Balearica regulorum*); Cracidae such as the black curassow (*Crax alector*); Phasianidae such as the brush turkey (*Alectura lathami*); Bucerotidae such as the crowned hornbill (*Tockus alboterminatus*); and the ostrich (*Struthio camelus*).[3,25] Storing blood samples in sodium citrate tubes is recommended when sending samples to a commercial laboratory for processing using laser flow cytometry.[14,19]

Commercially available collection tubes usually have printed labels. A pencil or ballpoint pen is used to enter the date and identification of the bird, preferably prior to filling the tube with the collected blood sample. Always remember the rule of thumb in clinical pathology: label tubes, not lids.

TRANSPORTATION OF BLOOD SAMPLES

In avian practice, hematology samples are commonly sent to commercial laboratories for processing (Table 22.2). Therefore, it is essential to be familiar with and to submit samples in full compliance with current local mail and courier regulations.

Hematology Laboratory Analysis

Although the hematology laboratory analysis described in this chapter were developed primarily for testing human blood and are in full compliance with the recommendations of the International Committee for Standardization in Hematology,[34] these have been adapted and used successfully in avian hematology. Ideally, laboratory analysis should be carried out within 3 to 4 hours after collection. Many laboratories in the USA request that a smear be made immediately and sent along with the EDTA tube. If this is not possible, samples should be refrigerated at 8 to 12° C or within a suitable container for processing within 24 to 48 hours. Refrigerated samples are not ideal for hematology testing, as the cells invariably suffer some changes. Only an experienced hematologist would be able to differentiate these changes from true hemoresponses to particular medical disorders. Samples should not be exposed to extreme environmental conditions or excessive shaking, as this will affect the quality of the sample. Any form of mouth pipetting with a Thoma pipette or any other pipette with or without tubing is not acceptable within clinical laboratory practices.

The amount of blood available for testing from small birds (eg, <80 g) is very often limited, making it impossible to carry out a full range of analyses. The clinician should bear this in mind and request the analysis in order of priority (Table 22.3).

THE TOTAL RED BLOOD CELL COUNT (RBC X 10^{12}/L)

The total red blood cell count is in itself an important hematology assay, but it also is essential for the estimation of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Many laboratories prefer to estimate RBC using an automatic system, as this is more precise than manual methods. The materials, solutions and method described in Table 22.4, together with Fig 22.2a,b, apply to a manual technique.

HEMOGLOBIN ESTIMATION (Hb g/dl)

In avian species, estimation of hemoglobin is hampered by the presence of nuclei in the erythrocytes. Hemoglobin estimation relies on the colorimetric measurement of hemoglobin released after the lysing of the erythrocytes. Hemoglobin can be estimated using automatic methods or manual methods (Table 22.5). Commercial laboratories that estimate hemoglobin using an automatic hematology analyzer have to take into consideration the photometric interference of the free nuclei after lysing of the erythrocyte. In the manual method, it is essential to remove the nuclei from the preparation because its presence could yield unreliable results. The nuclei can be deposited by low-speed centrifugation, but because some hemoglobin remains attached to the nuclei, colorimetric readings are commonly low. This can be overcome by estimating hemoglobin as cyanmethemoglobin using alkaline Drabkin's cyanide-ferricyanide solution or as oxyhemoglobin using ammonia solution. In both cases, the estimation is carried out using a spectrophotometer at the absorbance reading of 540 nm. A calibration graph should be made using commercially available hemoglobin standards to express hemoglobin as oxyhemoglobin. Conversely, hemoglobin can be estimated directly as oxyhemoglobin using a commercially available hemoglobinometer. This is the preferred method used and recommended by the author.

PACKED CELL VOLUME ESTIMATION PCV % (HEMATOCRIT Hct L/L)

Packed cell volume (PCV) is an important hematologic assay because it provides an easy and objective way of estimating the number of erythrocytes in the sample. It also is essential for the calculation of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). In avian species, PCV is best estimated using the microhematocrit method described (Fig 22.3). The use of plain microcapillary tubes is preferable, since the same tube can be subsequently used to estimate fibrinogen.

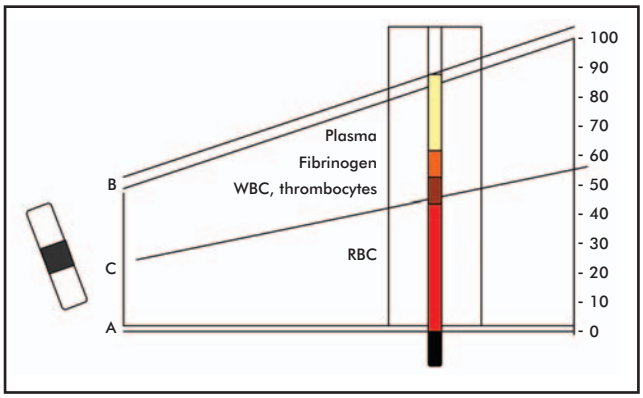


Fig 22.3 | Hematocrit reader and method for estimating packed cell volume. After centrifugation, position the capillary tube on the rack. Align tube (at the bottom, with the demarcation line between the sealing compound and the red blood cells) with line A. Slide the rack to the right or to the left, align the marginal meniscus at the top of the plasma column with line B. Position line C at the interface of the buffy layer and red cells, and read value on the scale.

Mean Corpuscular Values (Red Cell Absolute Values)

Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) is the expression of the average volume of individual erythrocytes calculated with the following formula:

MCV = (PCV x 10)/RBC = MCV femto liters (fl)

Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin (MCH) is the expression of the average hemoglobin content of a single erythrocyte calculated with the following formula:

MCH = (Hb x 10)/RBC = MCH picogram (pg)

Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) is the expression of the volume within the erythrocyte occupied by the hemoglobin and is calculated with the following formula:

MCHC = (Hb x 100)/PCV = MCHC (g/L)

TOTAL WHITE BLOOD CELL COUNT (WBC X 10^{9}/L)

The total white blood cell count (WBC) is one of the most important hematology assays in the assessment of health and disease in an individual. The WBC also is useful because it is used together with the differential white cell count to calculate the absolute number of each white blood cell within a blood sample. The materials, solutions and method described below, Table 22.6 and Fig 22.4, apply to the technique.

THE BLOOD FILM

The examination of stained blood films is the single most important assay in hematology analysis. An adequately

Table 22.4 | Manual Total Red Blood Cell (RBC) Count Hematology Test

Materials and Equipment

- Automatic dispenser, 0-50 ml
- Disposable sample tube with lid, 5 ml
- Micropipette, 20 µl and corresponding tip
- Roller mixer
- Plain capillary tubes
- Improved Neubauer hemocytometer and coverslip
- Laboratory lens tissue
- Petri dish 8.5 cm diameter
- Filter paper 8.5 cm diameter
- Toothpick
- Distilled water
- Microscope, preferably with phase contrast capability

Test Systems

The Unopette 365851 system[c] is probably the most popular method used for manual red blood cell count in avian species. It uses 10 µl of whole blood in 1.9 ml of 0.85% saline, resulting in a 1:200 dilution. The two other commonly used systems are based on using either formol citrate solution (Dacie's fluid) or Natt and Herrick's solution, depending on whether the examination is carried out with or without phase contrast microscopy. Dacie's formol citrate solution is the least known diluting fluid, but one used and recommended by the author.

Working Solutions

1. BD Unopette 365851[c] red blood count manual hematology test

2. Natt and Herrick's solution
   (for use without phase contrast microscopy)

NaCl	3.88 g
Na_2SO_4	2.5 g
Na_2HPO_4 12 H_2O	2.91 g
KH_2PO_4	0.25 g
Formaldehyde 40%	7.5 ml
Methyl violet 2B	0.1 g
Distilled water	to 1000 ml

Note: Allow solution to stand overnight. Filter before use.

3. Formol citrate solution or Dacie's fluid
   (for use with phase contrast microscopy)

Formaldehyde 10%	10 ml
Trisodium citrate	31.3 g
Distilled water	100 ml

Note: Refrigerate at 8 to 12° C.

Method[c]

- Label sample tubes using a permanent marker.
- Use an automatic dispenser to transfer 4 ml of either formol citrate solution or Natt and Herrick's solution into sample tube.
- Wait for 5 minutes to allow working solution to reach room temperature.
- Aspirate 20 µl of whole blood from storage tube using micropipette, wipe side of pipette tip carefully using tissue and dispense on the side of sample tube to make a dilution of 1:200.
- Avoid touching the distal opening of the pipette tip with the tissue, as this will cause capillary shift of blood into the tissue.
- Avoid immersing the pipette tip into the diluting fluid. This is a poor laboratory practice.
- Place sample tube in roller mixer and wait for 3 minutes.
- Clean Neubauer hemocytometer and coverslip using a dry, lint-free cloth or laboratory lens tissue.
- Place coverslip onto hemocytometer and slide gently over it, making sure Newton's rings (colored interference pattern) appear on both sides of the contact surfaces.
- Withdraw a small aliquot of the diluted sample using a plain capillary tube.
- Fill up one side of the hemocytometer by touching gently the intersection between coverslip and hemocytometer with the loaded capillary tube. Avoid air bubbles and underfilling or overfilling.
- Place filter paper at the bottom of the Petri dish. Position two toothpicks on either side of the dish. Wet filter paper lightly with distilled water. Rest hemocytometer on toothpicks. Cover Petri dish. Leave for 5 minutes for the cells to settle down.
- The hemocytometer is now ready for use.
- Count cells contained in the four corner and central squares in the mid section of the hemocytometer. Following the "L" rule: count cells that touch the center triple line of the ruling on the left and the bottom sides; do not count cells that touch the center triple line of the ruling on the right and the top sides (see Figs 22.2a,b).
- Calculate red blood cell count using:
  $N/100 = RBC \times 10^{12}/L$

Note: N = number of cells counted in 160 small squares.

Table 22.5 | Hemoglobin Estimation

Materials and Equipment

- Automatic dispenser, 0-50 ml
- Disposable sample tube with lid, 5 ml
- Micropipette, 20 µl and tip
- Roller mixer
- Toothpicks
- Cuvette, 10 mm^2
- Laboratory lens tissue
- Hemoglobinometer

Working Solution

Ammonia solution

Ammonia solution (0.88 specific gravity)	4 ml
Distilled water	to 1000 ml

Note: Refrigerate at 8 to 12° C.

Method

- Label sample tubes using a permanent marker.
- Use an automatic dispenser to transfer 4 ml of ammonia solution into sample tube.
- Wait for 5 minutes to allow working solution to reach room temperature.
- Aspirate 20 µl of whole blood from storage tube using micropipette, wipe side of pipette tip carefully using tissue and dispense on the side of sample tube.
- Avoid touching the distal opening of the pipette tip with the tissue, as this will cause capillary shift of blood into the tissue.
- Avoid immersing the pipette tip into the diluting fluid. This is a poor laboratory practice.
- Place sample tube in roller mixer and wait for 3 minutes.
- Decant approximately 3.5 ml of the diluted blood into cuvette.
- Remove cell nuclei jelly using toothpicks.
- Do not touch the clear reading walls of the cuvette with bare fingers.
- Clean clear reading walls of cuvette using laboratory lens tissue.
- Zero hemoglobinometer using ammonia solution as blank.
- Reading expressed as Hb g/dl.

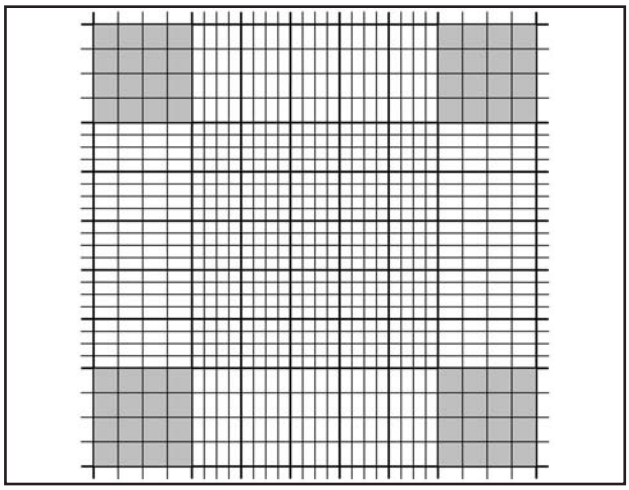


Fig 22.4 | Improved Neubauer counting chamber and the method for counting white blood cells. The total white blood cell count is performed by counting the number of cells contained in 4 groups (shaded areas) of 16 large squares at the four corner squares of the chamber. Closely ruled triple lines (illustrated in the drawing as thick lines) separate these squares.

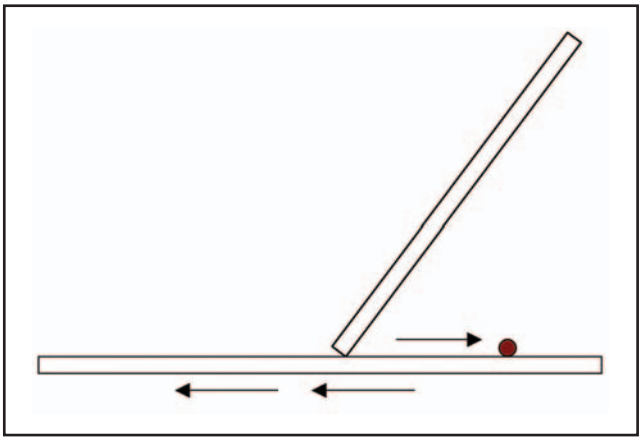


Fig 22.5 | The slide-to-slide technique. Move spreader slide backward to gently touch the drop of blood, allowing it to run across the edge of the slide. Move forward to make smear. Move slowly if blood runs slowly, move quickly if blood runs quickly.

prepared blood film provides the differential white blood cell count and absolute white blood cell count, the thrombocyte count and the hemoparasite examination.

Preparation of the Blood Film

Blood films can be made from a drop of fresh, non-anticoagulated blood directly from the tip of the syringe. Conversely, films can be made from blood stored in EDTA within 2 to 3 hours after collection. There are two generally accepted methods for the preparation of blood films in hematology: the slide-to-slide technique (Fig 22.5, Table 22.7) and the coverslip-to-slide technique (Fig 22.6, Table 22.8). A two cover-slip technique is not described here. The most popular method among avian clinicians is the coverslip-to-slide technique, as smudging of blood red cells is generally minimized.

Fixation and Staining of the Blood Film

It is commonly accepted that blood films can be prepared and be fixed and stained at a later date. This is incorrect; blood films should at least be fixed immediately after preparation, particularly if made in a hot and humid environment or under cold and freezing conditions. Blood films should not be exposed to direct sunlight, moisture of any kind or vapor from chemicals (formaldehyde in particular), as this would invariably affect cell morphology.

Table 22.6 | Total White Blood Cell (WBC) Count Hematology Test

Materials and Equipment

- Automatic dispenser, 0-50 ml
- Disposable sample tube with lid, 5 ml
- Micropipette, 100 µl and tip
- Roller mixer
- Plain capillary tubes
- Improved Neubauer hemocytometer and coverslip
- Laboratory lens tissue
- Petri dish, 8.5 cm diameter
- Filter paper, 8.5 cm diameter
- Toothpicks
- Distilled water
- Microscope, preferably with phase contrast capability

Test Systems

The Unopette 365877[d] system was originally developed for the estimation of eosinophils in human hematology, but it has proved useful for determining the total white cell count in avian species. This system uses 25 µl of whole blood into 0.775 ml of 1% Phloxine B diluent resulting in a 1:32 dilution, and is the system used by most practitioners in the USA.[3,19] The method described below is based on the use of ammonium oxalate solution, which is the method used and recommended by the author.

Working Solutions

1. BD Unopette 365877[d] eosinophil count manual hematology test[d]
2. Ammonium oxalate solution 1%

Ammonium oxalate	10 g
Distilled water	to 1000 ml

Note: Refrigerate at 8 to 12° C.

Method

- Label sample tubes using a permanent marker.
- Use an automatic dispenser to transfer 1.9 ml of 1% ammonium oxalate solution into sample tube.
- Wait for 5 minutes to allow working solution to reach room temperature.
- Aspirate 100 µl of whole blood from storage tube using micropipette, wipe side of pipette tip carefully using tissue and dispense on the side of sample tube.
- Avoid touching the distal opening of the pipette tip with the tissue, as this will cause capillary shift of blood into the tissue.
- Avoid immersing the pipette tip into the diluting fluid. This is a poor laboratory practice.
- Place sample tube in roller mixer and wait for 3 minutes.
- Clean Neubauer hemocytometer and coverslip using a laboratory lens tissue or dry, lint-free cloth.
- Place coverslip onto hemocytometer and slide gently over it, making sure Newton's rings (colored interference pattern) appear on both sides of the contact surfaces.
- Withdraw a small aliquot of the diluted sample using a plain capillary tube.
- Fill up one side of the hemocytometer by gently touching the intersection between coverslip and hemocytometer with the loaded capillary tube. Avoid air bubbles and underfilling or overfilling.
- Place filter paper at the bottom of the Petri dish. Position two toothpicks on either side of the dish. Lightly wet filter paper with distilled water. Rest hemocytometer on toothpicks. Cover Petri dish. Leave for 5 minutes for the cells to settle down.
- The hemocytometer is now ready for use.
- Count cells contained in the four outer large squares of the hemocytometer.
- Calculate total white blood cell count using:
  $N/20 = WBC \times 10^9/L$

Note: N = number of cells counted in 64 small squares.

Table 22.7 | Method for Slide-to-Slide Technique

- It is highly recommended to use one-end-frosted microscopic slides to easily note the ID of the sample on the slide using a pencil.
- Wipe slides clean with a lens tissue or lint-free cloth.
- Use a plain microcapillary tube to withdraw a small amount of fresh, non-anticoagulated blood directly from syringe tip or EDTA tube.
- Place a small drop of blood (2 µl) at one end of a slide.
- Select a spreader slide and position it in front of the drop of blood at about a 45° angle. The selected slide should be free from any indentation. To test this, pass the spreading edge over the edge of a fingernail.
- Gently move the spreader slide backward to touch the drop of blood and allow the blood to run across the edge of the slide.
- Gently drive the slide forward with a steady but firm movement to create a uniform smear.
- It is always a good practice to make two good-quality blood films.

Table 22.8 | Method for Coverslip-to-Slide Technique

The only significant difference between this method and the previous one consists of the following steps:

- Place a large rectangular coverslip over the drop of blood.
- Pull the coverslip and the slide in opposite directions in a steady but firm movement to create a uniform smear.

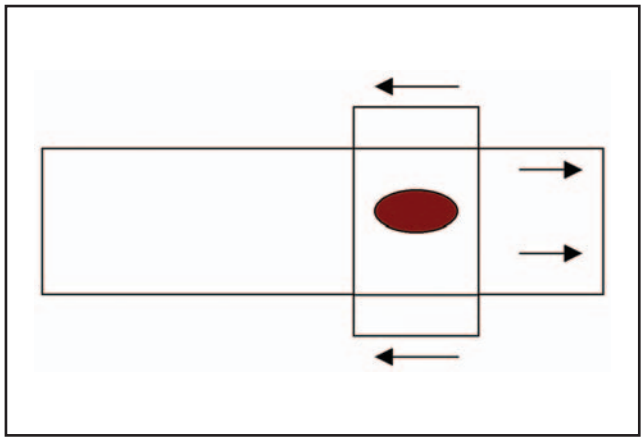


Fig 22.6 | The coverslip-to-slide technique. Place coverslip onto drop of blood. Apply gentle pressure downward. Move slide and coverslip in opposite directions to make smear.

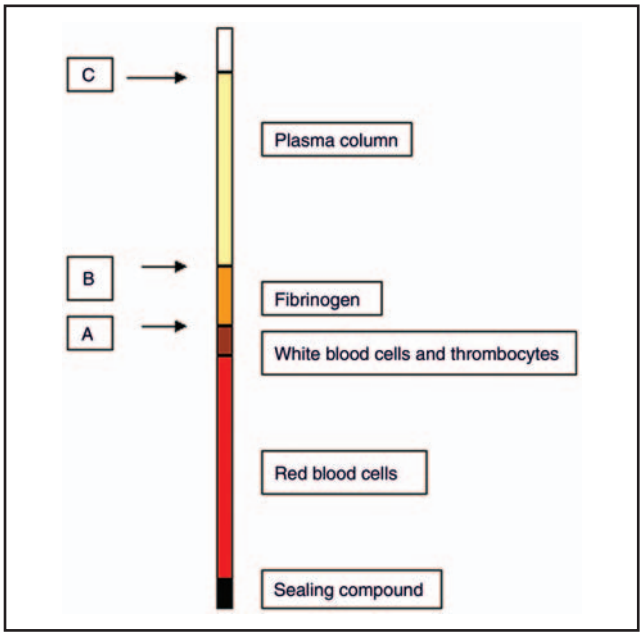


Fig 22.7 | A microcapillary tube after incubation and centrifugation, and the measurements necessary for the estimation of fibrinogen.

Fixation

In general, freshly prepared blood films should be immersed in absolute methanol within a Coplin jar for 5 to 10 minutes immediately after preparation. Fixed blood films can then be stored within commercially available slide storage boxes (eg, under field conditions) and be stained at a later date. Blood films also can be stained immediately after fixation.

The importance of adequate fixation of blood films from avian species cannot be overemphasized. The intracytoplasmic granules of the heterophils and basophils are water soluble; therefore, blood films should be adequately fixed before staining in order to preserve the integrity of these structures. A significant problem in avian hematology is the presence of smudged red cell nuclei as a consequence of hemolysis in poorly fixed blood films. This is one of the main reasons why clinicians and commercial laboratories are now inclined to use stains that are prepared in absolute methanol (eg, Wright-Giemsa stain, Leishman stain) and are used at full strength so films are fixed and stained at the same time. If absolute methanol within a Coplin jar is used for fixation in your laboratory, it must be replaced as soon as it begins showing chemical fatigue. This would depend on the number of slides fixed and the environmental conditions within the laboratory.

Staining

Most Romanovsky stains used for staining human and mammalian blood films are suitable for staining avian blood films. However, the results obtained with various stains may be slightly different, and the selection of stains is generally accepted as a matter of personal preference. Commonly used stains include Wright stain, Giemsa stain, Wright-Giemsa stain, Leishman stain, Wright-Leishman stain, May-Grünwald stain and May-Grünwald-Giemsa stain. In the author's opinion, rapid stains on their own, eg, Diff Quick and Rapid Diff, do not produce adequate quality for the differentiation of subtle blood cell structures and those of hematozoa. This is particularly important with respect to the morphological characteristics of the granulocytes.

Automatic slide stainers facilitate staining a relatively large number of blood films at the same time, producing consistent results and eliminating variations that may occur with manual techniques. However, this type of equipment is relatively expensive to purchase and maintain and is more appropriate for high-volume commercial laboratories.

It is important that clinicians or laboratory technicians recall the basic principles of hematology when staining blood films. The pH of the stains should be checked each time new stock is prepared. Some stains, particularly those prepared from powder, should be adequately filtered. Glassware should be properly washed, rinsed with distilled water and dried thoroughly before use. Many of the common artifacts on blood films are due to careless preparation and improper methodology.

The staining method currently used and recommended

Table 22.9 | Wright-Giemsa Staining Procedure

Working Stain

- 3 g Wright stain powder
- 0.3 g Giemsa stain powder
- 5 ml glycerol
- To 1000 ml absolute methanol (acetone free)
- Filter and store

Method

- Prepare thin blood smears.
- Place on staining rack.
- Flood smear with Wright-Giemsa stain, allow to stand for 3 minutes.
- Add equal amount of Sørensen's pH 6.5-6.8 buffer, depending on batch stain.
- Mix gently by blowing using a pipette until metallic green sheen forms on the surface, allow to stand for 6 minutes.
- Rinse with buffer, allowing to stand for 1 minute for differentiation.
- Wash copiously with buffer.
- Wipe the back of smear with tissue to remove excess stain.
- Prop in rack until dry.

by the author is a slightly modified technique[4] described in Table 22.9.

The placement of a coverslip using a commercially available mounting medium over the blood smear is optional. Additionally, the mounting of blood films offers several advantages such as preventing scratching during transport, protection against damage during excessive manipulation (eg, teaching material) and enhancing visualization for optimal examination and photography.

Morphologic and Staining Characteristics of Red Blood Cells, White Blood Cells and Thrombocytes

Normal red blood cells appear elliptical and have elliptical nuclei; the cytoplasm stains uniformly eosinophilic, and the nuclei is dark purple in color (modified Wright-Giemsa stain).

In general, the widely known "Romanovsky stains" contain blue azure that reacts with acid groups, including those of nucleic acids and proteins of the nucleus and cytoplasm and eosin Y, which has a particular affinity for basic groups of hemoglobin. When used in different avian species, the slight variations observed may be the result of true species diversity or simply variations in the materials and methods used from individual to individual or from laboratory to laboratory.

Adequate knowledge of the morphology and staining characteristics of the different blood cells is of the utmost importance for the differentiation and classification of those blood cells (Table 22.10 and Figs 22.8-22.43).

Table 22.10 | Morphologic and Staining Characteristics of Different Blood Cells

Blood Cell	Morphologic Characteristics	Staining Characteristics
Erythrocyte	**Mature cells**	
	Medium size, oval elongated shape, central oval elongated nucleus	Cytoplasm: uniform pale orange to red-pink; Nucleus: purple-red, condensed, clumped chromatin
	Immature cells	
	Smaller than mature cell, round to semi-oval, relatively larger nucleus	Polychromatic, cytoplasm pale to dark blue
Heterophil	Medium size, round shape, bilobed nucleus	Colorless cytoplasm, rod- to cigar-shaped brick red to pale blue granules
Eosinophil	Medium size, round shape, bilobed nucleus	Pale blue cytoplasm, round to oval brick red to pale blue granules
Basophil	Small size, round shape, unlobed nucleus	Pale blue cytoplasm, variable number of small, medium and large dark red-purple granules
Lymphocyte	Small to medium size, typically round to triangular shape, centrally positioned large round nucleus; in general, 25 cytoplasm:75 nucleus; ratio, coarsely condensed to highly condensed chromatin	Pale blue cytoplasm
Monocyte	Large size, typically round shape, eccentrically positioned kidney-shaped nucleus; in general 75 cytoplasm:25 nucleus ratio, cytoplasm lace-like appearance, often medium size vacuoles, coarsely condensed chromatin	Cytoplasm pale blue to pale gray
Thrombocyte	Small, oval to rectangular shape, nucleus oval to rectangular	Cytoplasm colorless to pale blue, large vacuoles, nucleus highly condensed dark purple-red chromatin

Differential White Blood Cell and Absolute White Blood Cell Count

For the differential white blood cell count and absolute white blood cell count, the film should be examined thoroughly under high-power magnification, under oil (1000x). The recommended topographic site is on the shoulder of the blood film. The *shoulder* is the edge of the oval-shaped end of a smear. This is the area where the blood cells are in one layer and are slightly segregated, thus facilitating examination.

In general terms, 100 white blood cells should be counted and classified according to the morphologic and staining characteristics. Counting is usually carried out using a commercially available manual or electronic differential cell counter. The differential white blood cell count is expressed as a percentage of the individual cell group. The percentage of each cell group is then converted into absolute numbers by reference to the total WBC using the following formula:

(Percentage of white blood cell counted x total WBC)/100 = absolute No. x 10^9/L

Thrombocyte Count

Thrombocytes are usually counted while performing the differential white blood cell count. Valid and reliable results cannot be obtained if there is evidence of thrombocyte clumping.

The absolute number of thrombocytes is estimated by using the following formula:

(No. of thrombocytes counted/100) x WBC = thrombocytes x 10^9/L

Figs 22.44-22.48 and Tables 22.12-22.18 are offered as references for interpreting hemotological findings.

FIBRINOGEN ESTIMATION (g/L)

Fibrinogen is a plasma protein essential for normal blood coagulation, but also is one of the acute reactive proteins that are detected in increased levels in association with medical disorders involving infection and inflammation (see Tables 22.11, 22.12 and 22.18).

Hemoparasite Examination

Hemoparasite examination is carried out on thin, good-quality blood films. Prior to a differential white cell count using high-power magnification (1000x), the blood film should be examined under low-power magnification (eg, 200x or 400x) in order to detect large extra cellular hemoparasites (eg, microfilariae), which could be missed if the film is examined only under high-power magnification. The examination under low-power magnification should concentrate on areas not commonly examined under high-power magnification, eg, head and tail of the blood smear. The blood film should be examined in full in a systematic way and following a consistent pathway.

A blood parasite quantitative assessment should be carried out, in certain cases and under certain circumstances, by examining 1000 red cells (in the case of intracytoplasmic parasitic forms) and determining the number of red cells containing hemoparasites (eg, *Haemoproteus* spp., *Babesia* spp.). The number is then expressed in percentage and this usually constitutes the degree of parasitemia.

If hemoparasites are observed during routine examination of the blood film under low- or high-power magnification, it is imperative to immediately prepare additional blood films. Ideally, a fresh blood sample should be obtained to prepare these new blood films from non-anticoagulated blood. This is of the utmost importance if a rare parasite is observed in the film. Blood films should be fixed but unstained when sending them to parasitologists, who have their own preferences for stains and staining procedures.

Table 22.11 | Fibrinogen Estimation

Materials and Equipment Needed for Fibrinogen Estimation

- Microcapillary tube rack
- Microhematocrit centrifuge
- Microhematocrit reader
- Water bath 56° C ± 1° C
- Microcapillary tube holder
- Microscope with measuring eyepiece and stage Vernier scale
- Timer

Method (following estimation of packed cell volume)

- Place microcapillary tube in tube rack.
- Place loaded rack in water bath at 56° C for 3 minutes (make sure the entire plasma column is immersed).
- Centrifuge microcapillary tubes again at 10,000 to 12,000 "g force" for 5 minutes.
- Place microcapillary tubes in tube holder and, using the microscope measuring eyepiece and the stage Vernier of the microscope, take reading at the upper and lower limits of the protein layer and at the upper limit of the plasma column (see Fig 22.7).
- Estimate fibrinogen with the following formula:
  (B - A)/(C - A) x 100 = fibrinogen g/L

Note: It is essential to perform this analysis on blood stored in EDTA because the analysis would be invalidated if performed on samples stored in heparin or on samples containing clots.

Unless specified otherwise, hematology images are from a saker falcon (*Falco cherrug*):

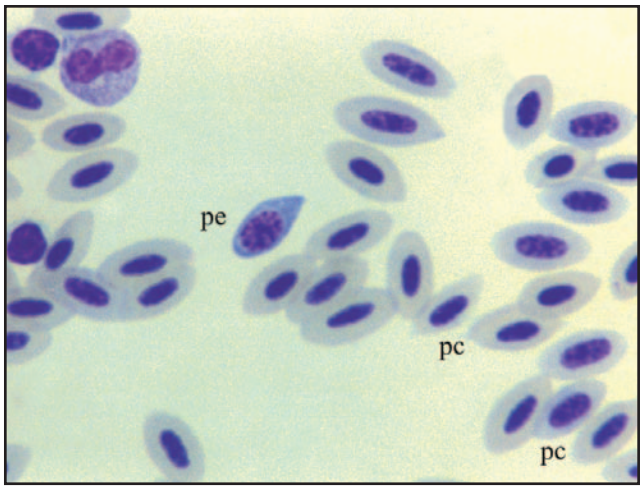


Fig 22.8 | Shown is a polychromatic erythroblast (pe) and poikilocytes (pc). The nuclear chromatin of the polychromatic erythroblast is clumped and the cytoplasm is highly basophilic (modified Wright-Giemsa stain).

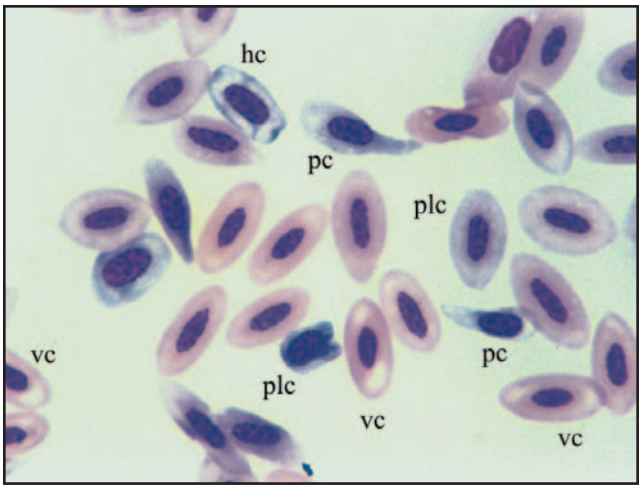


Fig 22.9 | The red cells in a bird with severe anemia show vacuolation (vc), hypochromia (hc) and polychromasia (plc). There are some poikilocytes (pc) in the smear (modified Wright-Giemsa stain).

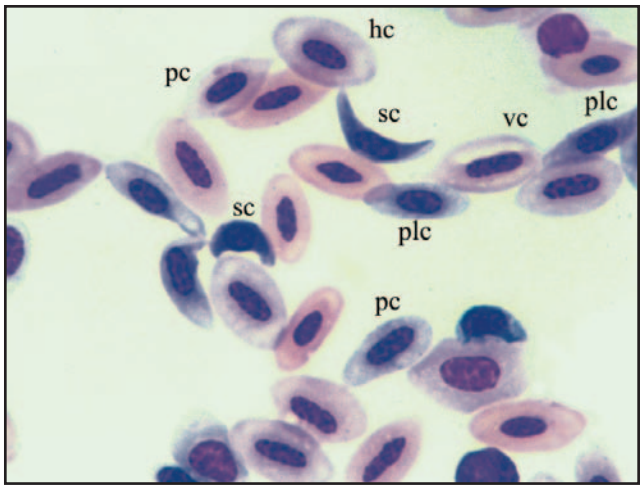


Fig 22.10 | The red cells in sickle cell anemia show sickling (sc), vacuolation (vc), hypochromia (hc) and polychromasia (plc). Some poikilocytes (pc) also are present (modified Wright-Giemsa stain).

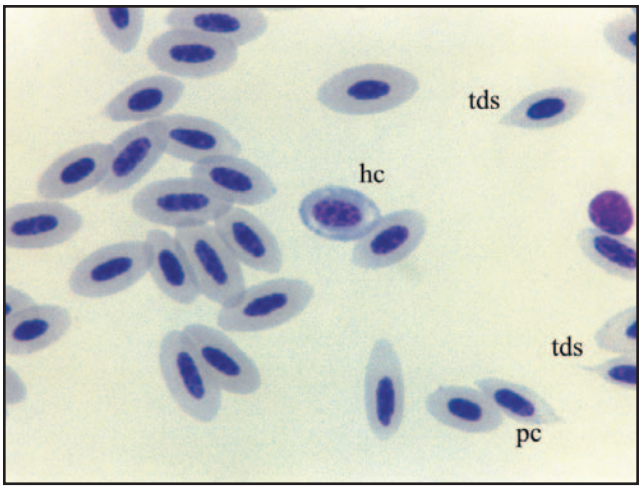


Fig 22.11 | Hypochromic (hc), teardrop-shaped red cells (tds) and poikilocytes (pc) are illustrated (modified Wright-Giemsa stain).

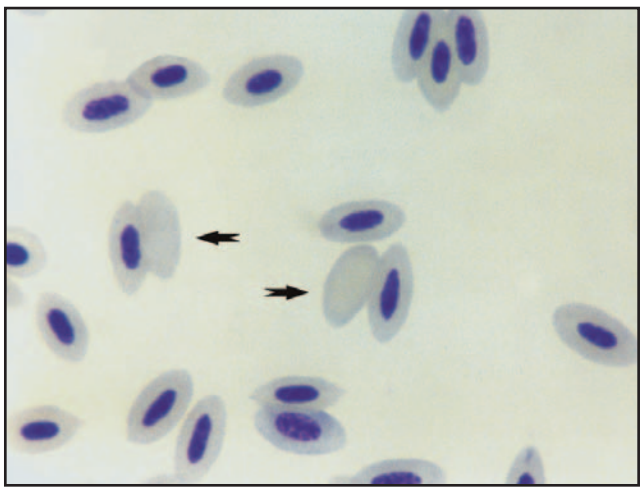


Fig 22.12 | Erythroplastid (arrows) forms (modified Wright-Giemsa stain).

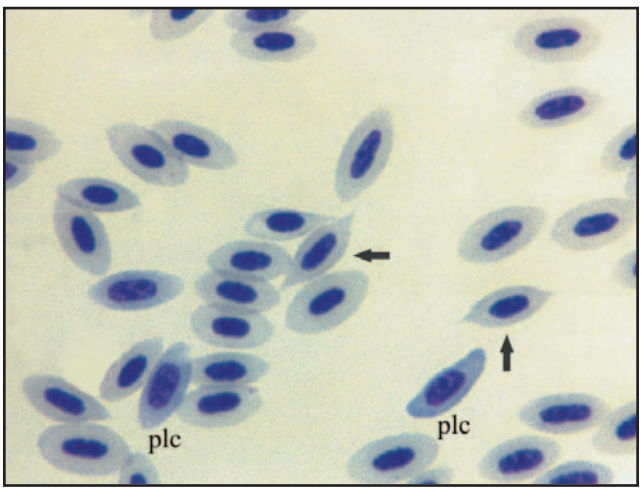


Fig 22.13 | Poikilocytes (arrows) are seen in metabolic defects and increased erythropoiesis. Polychromatic red cells (plc) are produced in response to severe blood loss. These are larger than normal cells (modified Wright-Giemsa stain).

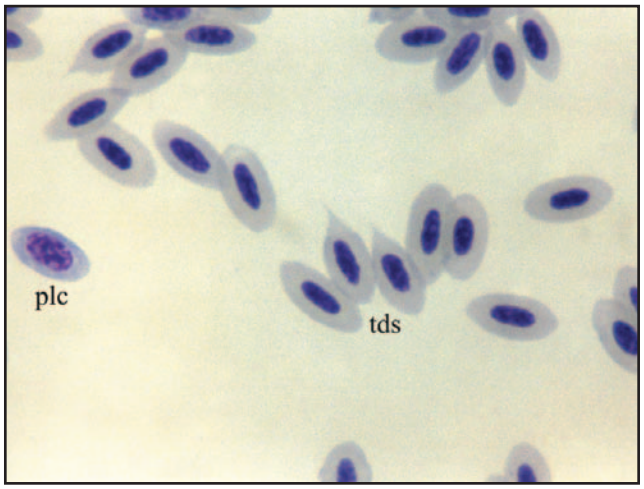


Fig 22.14 | Shown are teardrop-shaped red cells (tds) and polychromasia (plc). Teardrop-shaped cells are indications of toxicosis (May-Grünwald Giemsa stain).

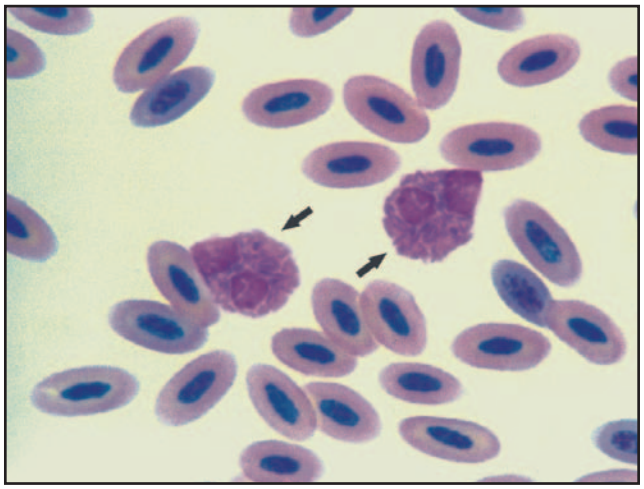


Fig 22.15 | Two normal heterophils (arrows). Heterophils are characterized by brick red, elongated intracytoplasmic granules and bilobed nuclei (modified Wright-Giemsa stain).

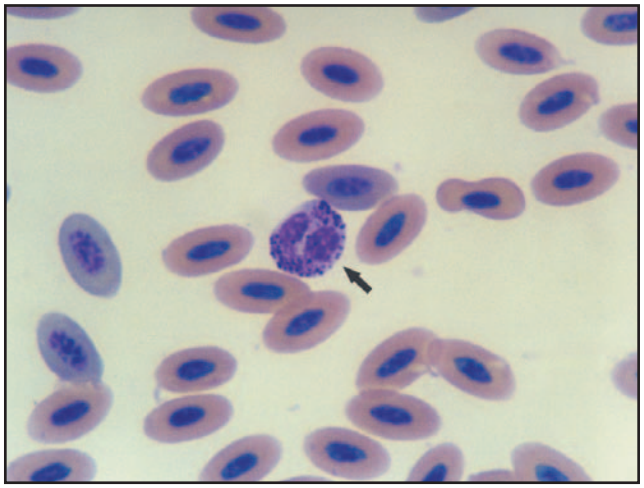


Fig 22.16 | In this eosinophil (arrow), note the numerous small and medium-sized, dark purple-colored granules located mainly in the periphery of the cytoplasm (modified Wright-Giemsa stain).

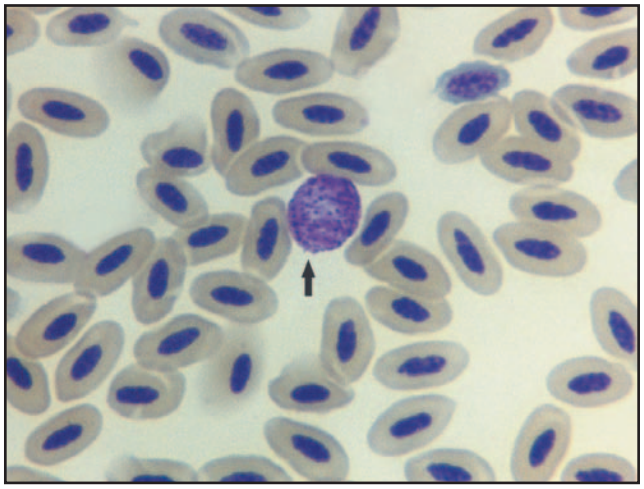


Fig 22.17 | An eosinophil (arrow) from an eclectus parrot (*Eclectus roratus*). Note the numerous small intracytoplasmic granules widespread across the cytoplasm. The granules stain dark purple in color (modified Wright-Giemsa stain).

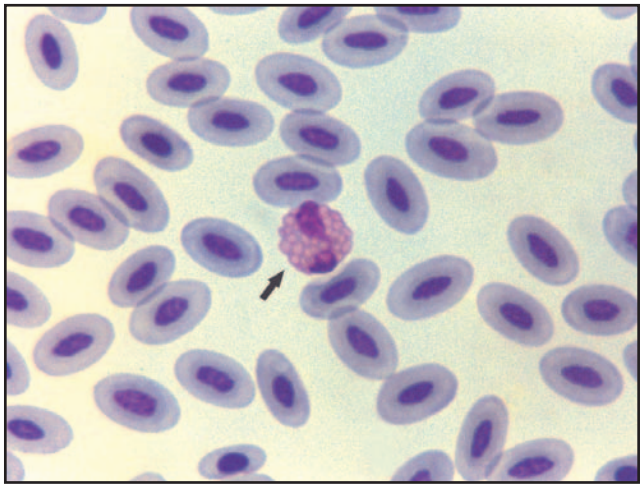


Fig 22.18 | An eosinophil (arrow) from a kori bustard (*Ardeotis kori*). Note the large, round, orange-colored granules characteristic of this species (May-Grünwald Giemsa stain).

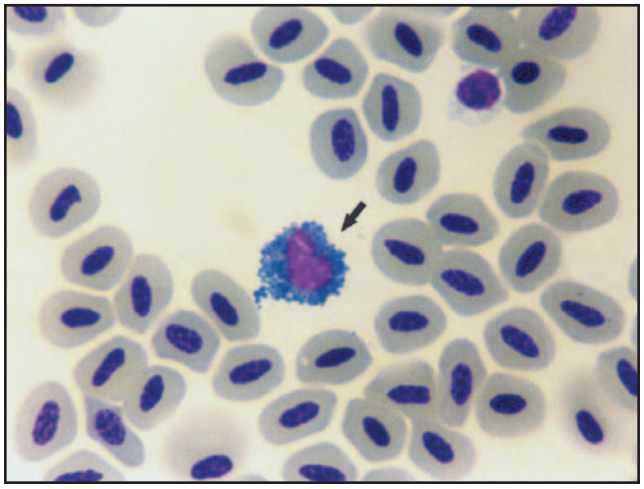


Fig 22.19 | A slightly disrupted eosinophil (arrow) from a lesser sulphur-crested cockatoo (*Cacatua sulphurea*). The medium-sized, round granules are blue in color (May-Grünwald Giemsa stain).

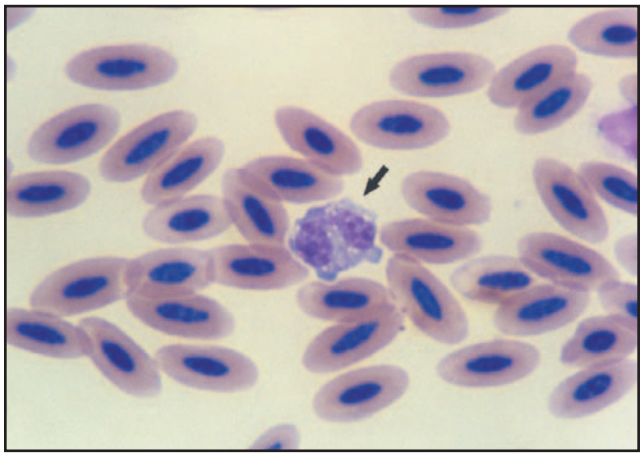


Fig 22.20 | In this eosinophil (arrow) from a saker falcon (*Falco cherrug*), the granules are not stained, giving the impression of numerous irregular vacuoles within the cytoplasm (May-Grünwald Giemsa stain).

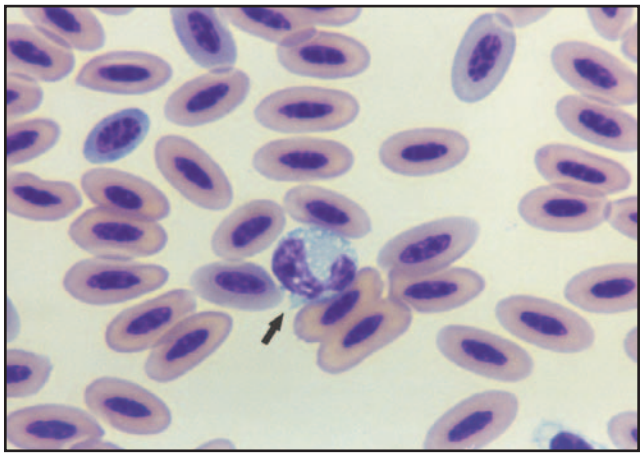


Fig 22.21 | In this eosinophil (arrow) is seen a similar-staining artifactual difference, as in the previous figure. The granules are not stained, giving the impression of numerous vacuoles within the cytoplasm (Diff Quik stain).

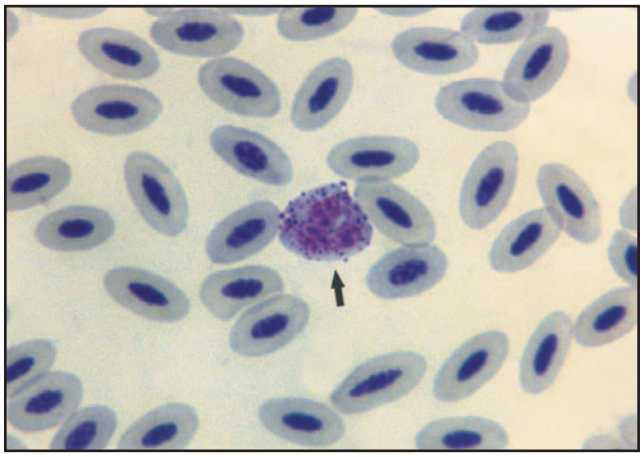


Fig 22.22 | These eosinophil (arrow) granules are well stained, irregular in shape and size, stained purple or dark purple (modified Wright-Giemsa stain). The author highly recommends the use of this stain for routine hematology.

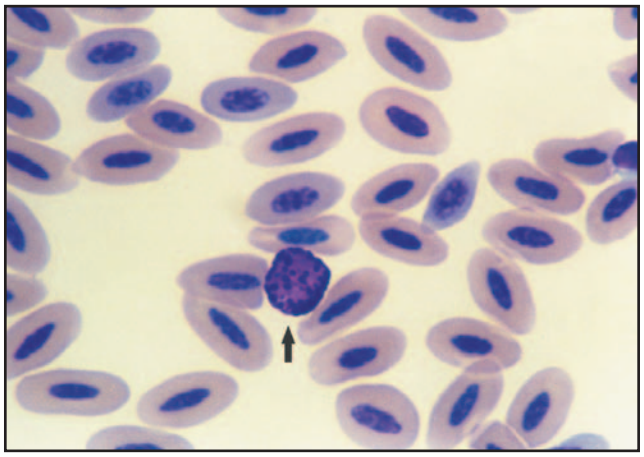


Fig 22.23 | A basophil (arrow) is characterized by the presence of large, round, dark purple granules widespread across the cytoplasm and an unlobed nucleus (modified Wright-Giemsa stain).

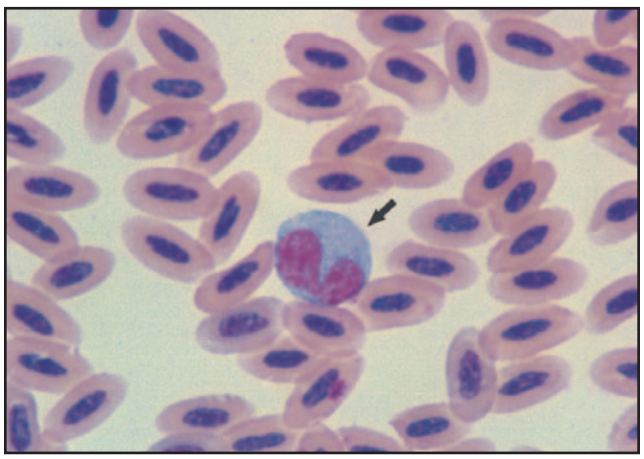


Fig 22.24 | A normal monocyte (arrow) is a relatively large cell with a kidney-shaped nucleus and abundant, slightly opaque, blue-gray, "lace-like" cytoplasm (modified Wright-Giemsa stain).

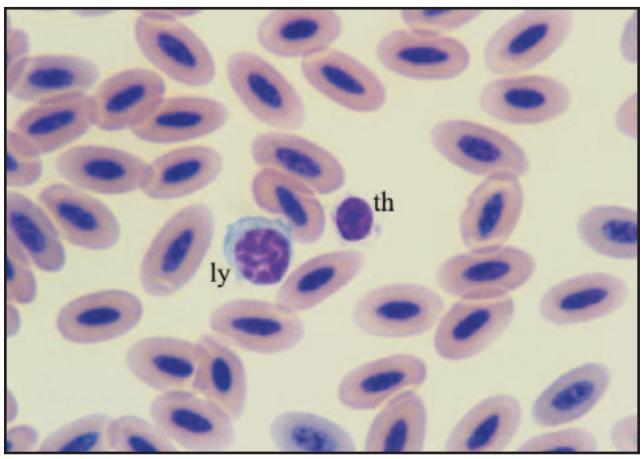


Fig 22.25 | A normal lymphocyte (ly) and a normal thrombocyte (th). Lymphocytes are regular round cells with a central or slightly eccentric nuclei, and with a varying amount of pale blue cytoplasm (modified Wright-Giemsa stain).

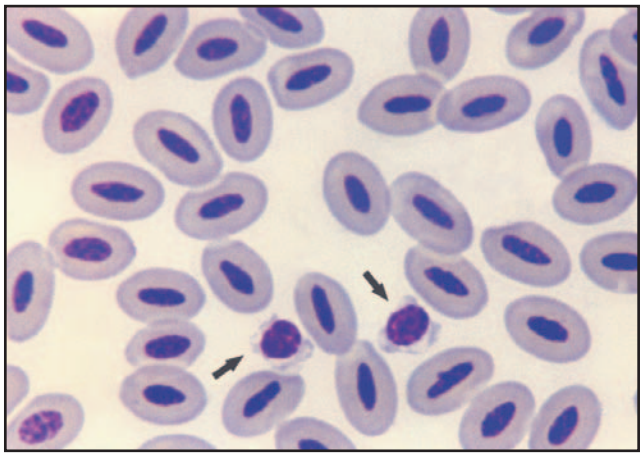


Fig 22.26 | Two normal thrombocytes (arrows) from a kori bustard (*Ardeotis kori*). Thrombocytes are round or irregular cells with completely dark purple and dense round or oval nuclei, and clear blue-gray cytoplasm. In some species, a few cytoplasmic projections can be observed. Sometimes it can be very difficult to differentiate between thrombocytes and small lymphocytes (May-Grünwald Giemsa stain).

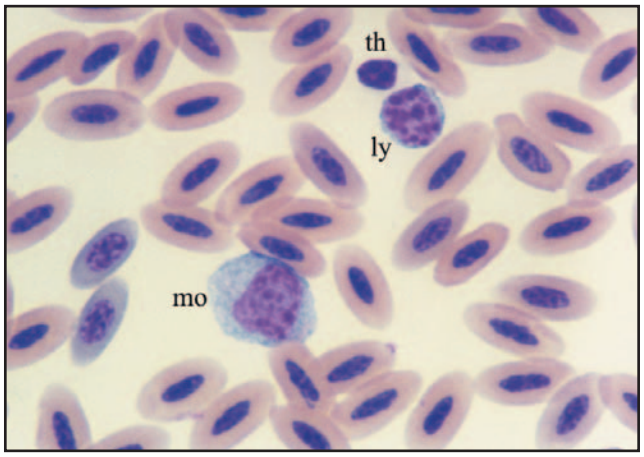


Fig 22.27 | Shown are a normal thrombocyte (th), normal lymphocyte (ly) and a normal monocyte (mo) for comparison of three different mononuclear cells. Thrombocytes and small lymphocytes can be very similar. In order to differentiate between them, the appearance of the nuclear chromatin has to be closely examined (modified Wright-Giemsa stain).

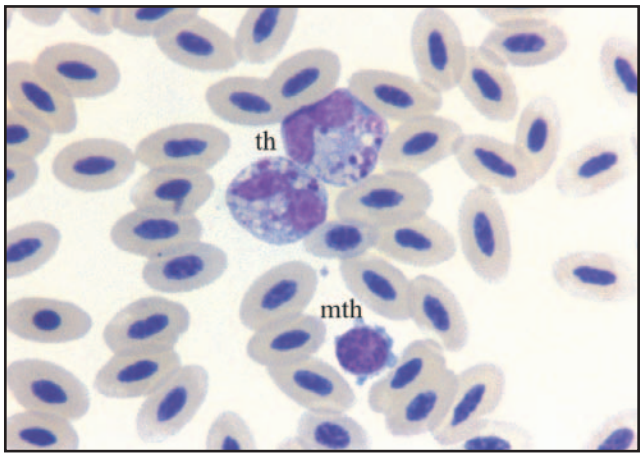


Fig 22.28 | Two toxic heterophils (th) and a megathrombocyte (mth). One of the heterophils shows a lack of lobulation of the nucleus (left shift); both show loss of granulation and the cytoplasm is stained basophilic. The megathrombocyte is significantly larger than a normal thrombocyte. The cytoplasm is basophilic, the nucleus cytoplasm ratio is increased and it has scalloped cytoplasmic margins (modified Wright-Giemsa stain).

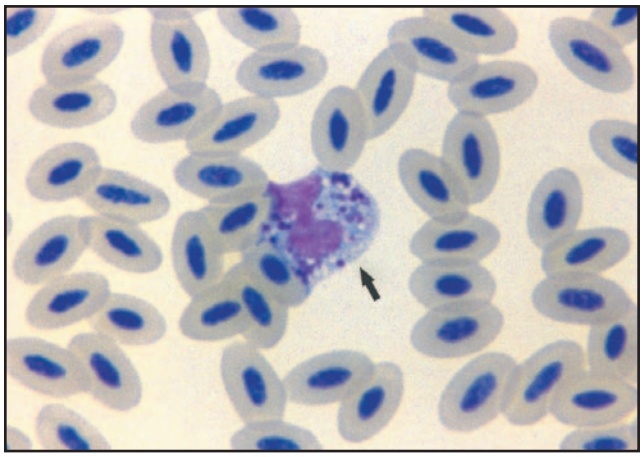


Fig 22.29 | A toxic heterophil (arrow) showing loss of nuclear lobulation (left shift) and loss of cytoplasmic granulation. The granules are round, large and stained dark purple, and the cytoplasm is basophilic (modified Wright-Giemsa stain).

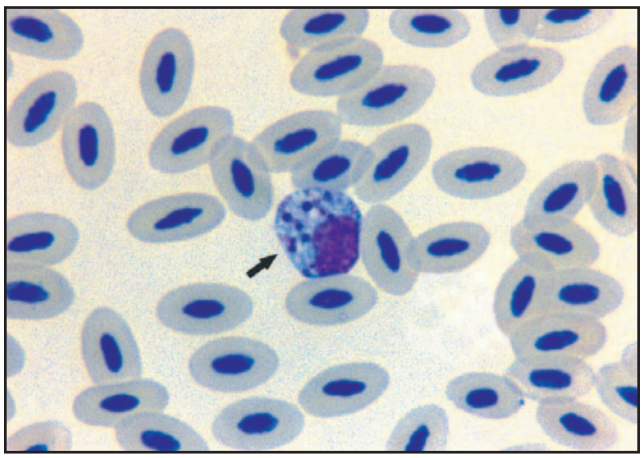


Fig 22.30 | A toxic heterophil (arrow) with a lack of nuclear lobulation (left shift) and loss of cytoplasmic granulation. Only a few large, round, dark purple granules are present and the cytoplasm is basophilic (modified Wright-Giemsa stain).

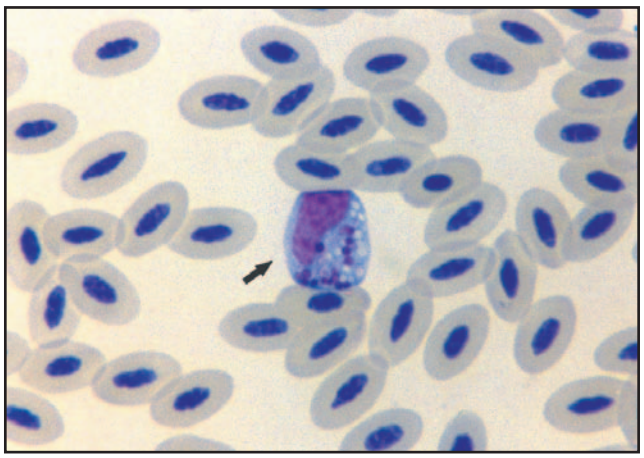


Fig 22.31 | A toxic heterophil (arrow). The heterophil shows loss of nuclear lobulation (left shift) and loss of cytoplasmic granulation. There are very few large, dark purple granules and the cytoplasm is basophilic (modified Wright-Giemsa stain).

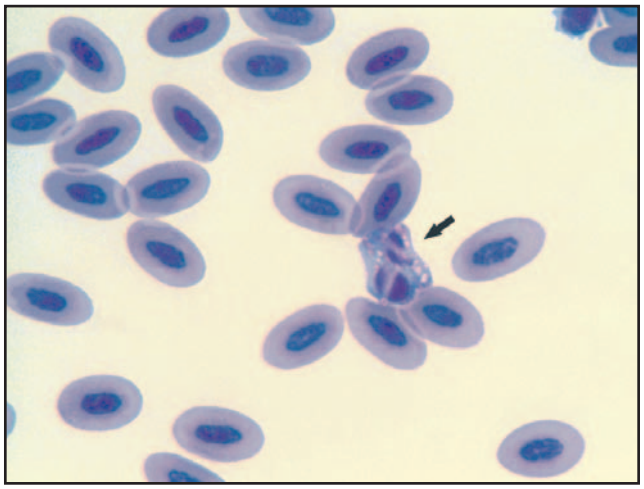


Fig 22.32 | A toxic heterophil (arrow). The nucleus is segmented into several fragments (right shift); the granules are not stained, giving the impression of numerous vacuoles within the cytoplasm (May-Grünwald Giemsa stain).

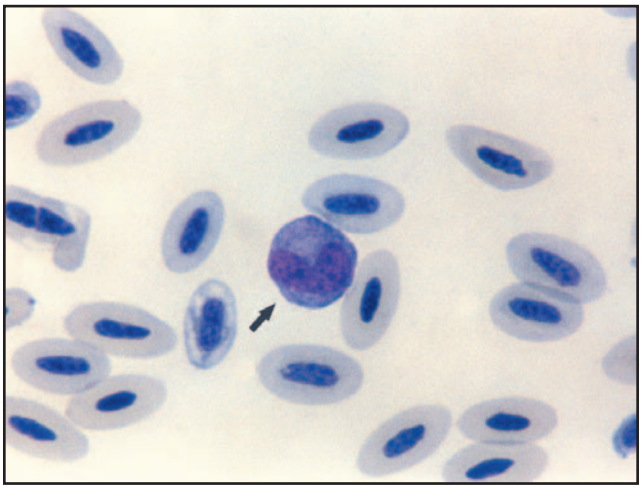


Fig 22.33 | A reactive monocyte (arrow). The cytoplasm is basophilic and the nuclear chromatin is coarse (modified Wright-Giemsa stain).

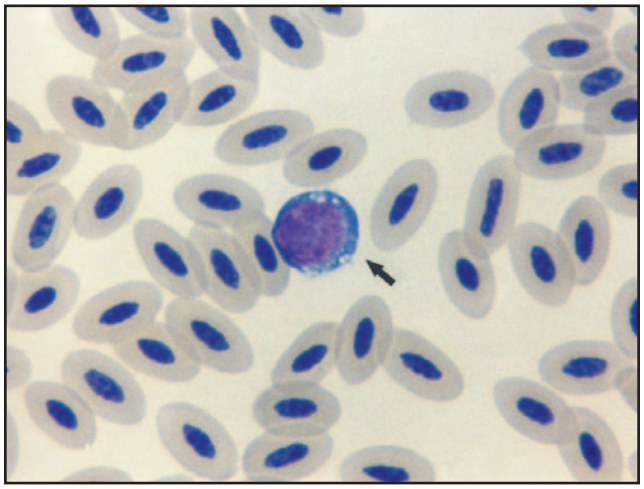


Fig 22.34 | A toxic monocyte (arrow). The nuclear/cytoplasm ratio is increased, the cytoplasm stains basophilic and there are numerous vacuoles within the cytoplasm (modified Wright-Giemsa stain).

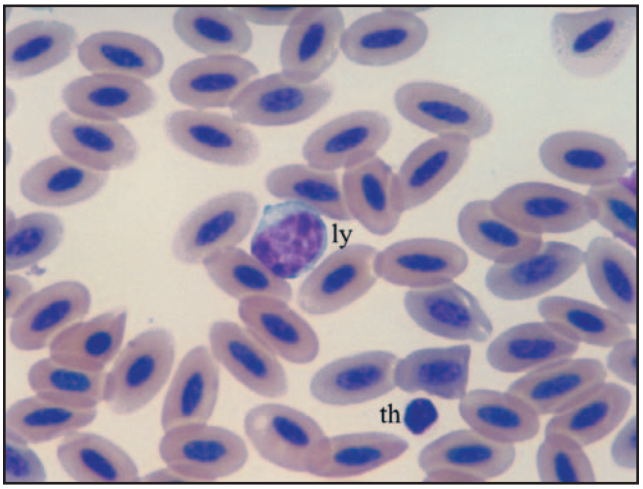


Fig 22.35 | A normal lymphocyte (ly) and a normal thrombocyte (th) (modified Wright-Giemsa stain).

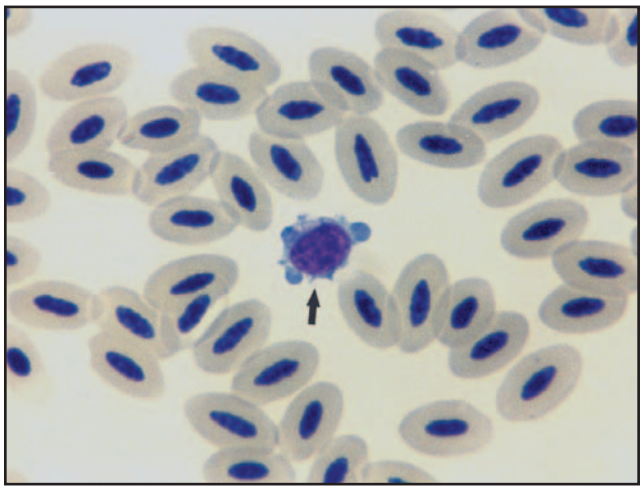


Fig 22.36 | A megathrombocyte (arrow). Megathrombocytes are larger than normal thrombocytes and can be confused with small lymphocytes. The cytoplasm of megathrombocyte stains basophilic and the nuclear chromatin is coarser (modified Wright-Giemsa stain).

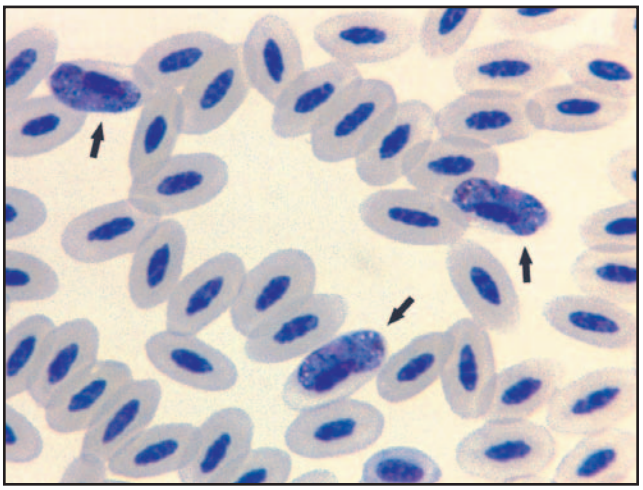


Fig 22.37 | *Haemoproteus tinnunculi* (arrows) (modified Wright-Giemsa stain).

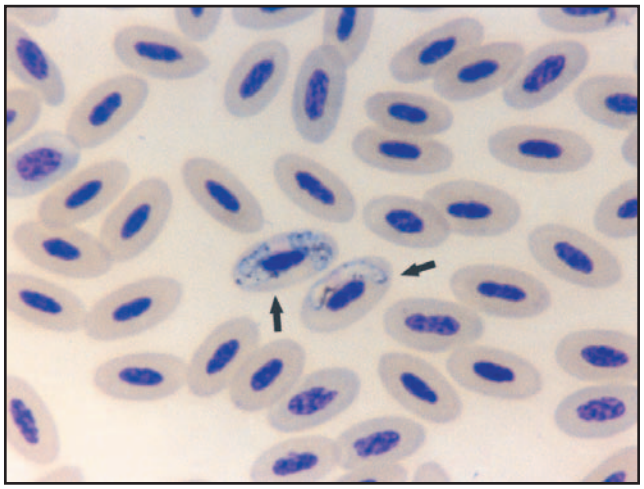


Fig 22.38 | *Haemoproteus psittaci* (arrows) from a green-winged macaw (*Ara chloroptera*).

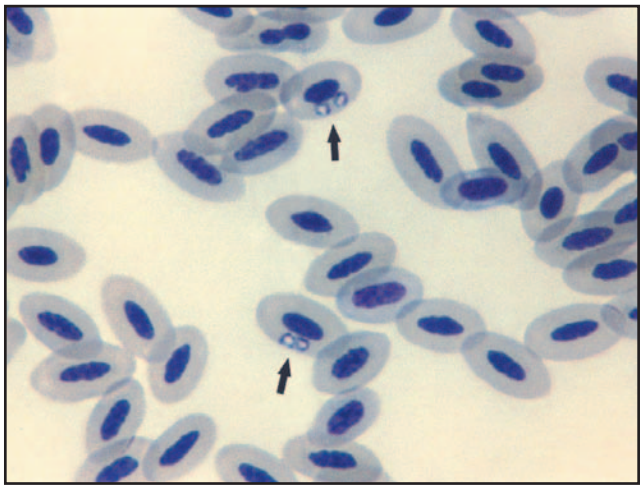


Fig 22.39 | *Babesia shortti* (arrows) (modified Wright-Giemsa stain).

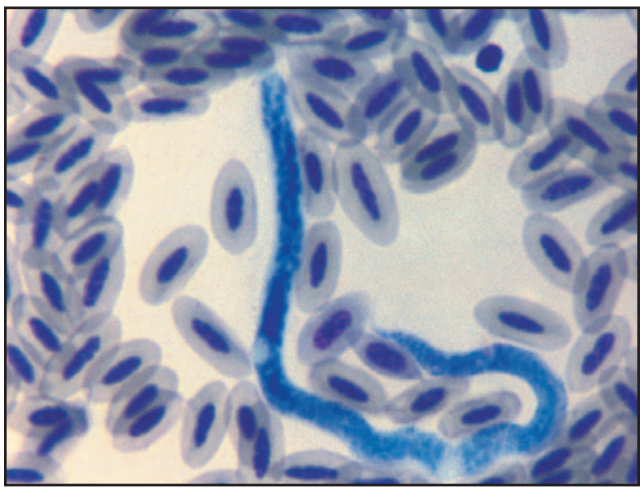


Fig 22.40 | *Microfilaria* sp. (modified Wright-Giemsa stain).

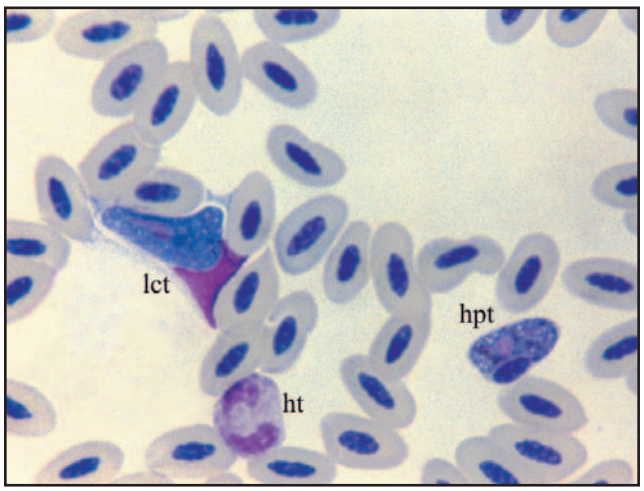
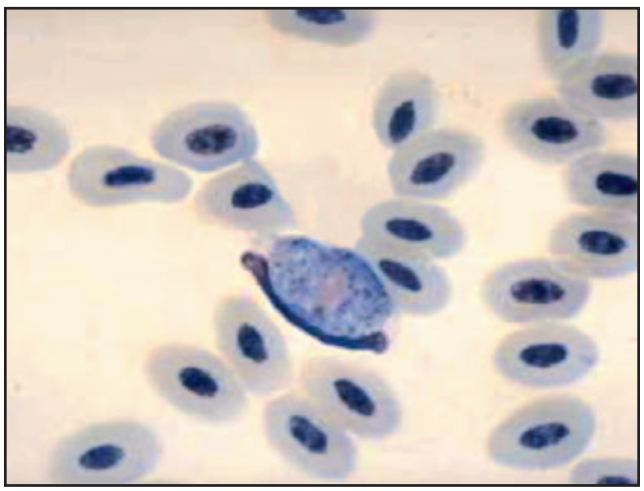
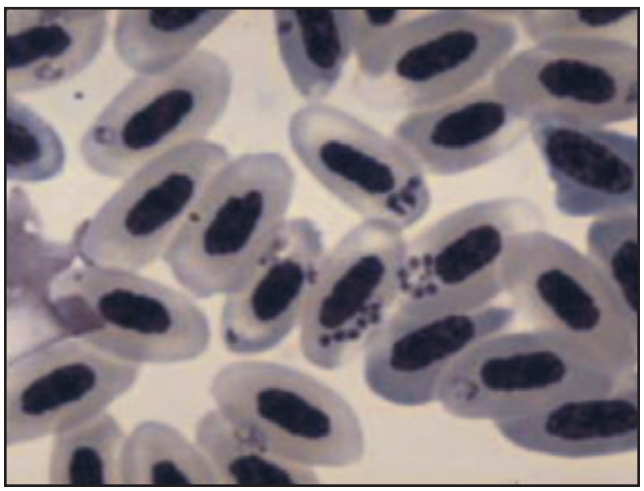


Fig 22.41 | *Leucocytozoon toddi* (lct), *Haemoproteus tinnunculi* (hpt) and normal heterophil (ht) (modified Wright-Giemsa stain).



Kendall Harr

Fig 22.42 | *Leucocytozoon simondi* from a Canada goose (*Branta canadensis*).



M. Griener

Fig 22.43 | *Plasmodium vaughani* schizont from a robin (*Turdus migratorius*).

Age-related Hematologic Changes

Age-related hematologic findings in kori bustard (*Ardeotis kori*) chicks during their growth and development are presented (Figs 22.44-22.48). Blood samples were collected from 16 clinically normal chicks at 1-month intervals. The tenth sampling was obtained at 15 months of age.

The following is a collection of hematology values and an interpretation guide for the avian veterinarian:

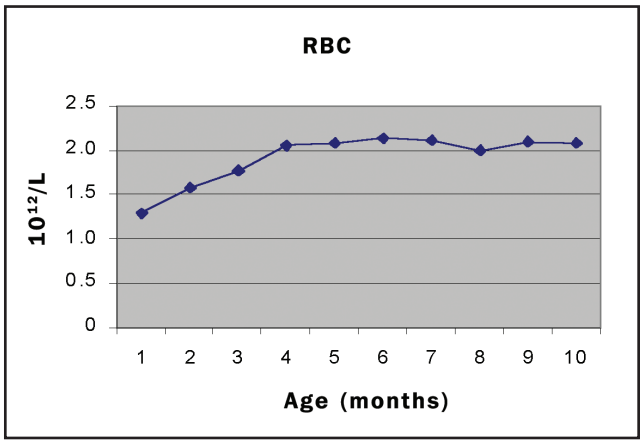


Fig 22.44 | The RBC increased steadily for the first 4 months from 1.28 ± 0.06 x 10^{12}/L at 1 month of age, increasing gradually up to the age of 4 months to 2.06 ± 0.08 x 10^{12}/L. After this time, the RBC remained fairly constant. The RBC value at the age of 12 to 15 months was 2.08 ± 0.06 x 10^{12}/L.

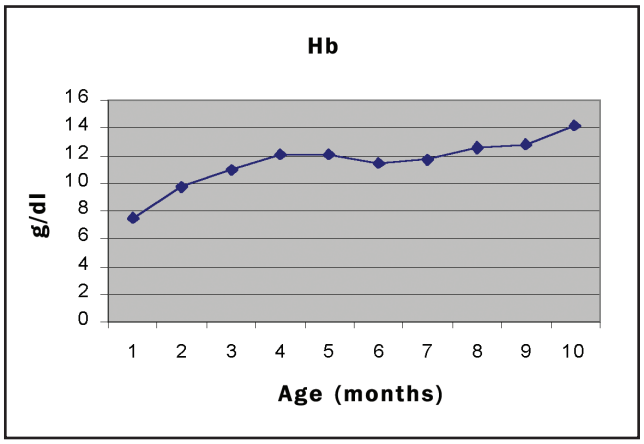


Fig 22.45 | The HB value followed a similar pattern as for RBC, with a value of 7.5 ± 0.2 g/dl at the age of 1 month, increasing to 12.1 ± 0.3 g/dl at 4 months of age. This value remained fairly constant until the age of 12 months, when it increased to 14.2 ± 0.4 g/dl.

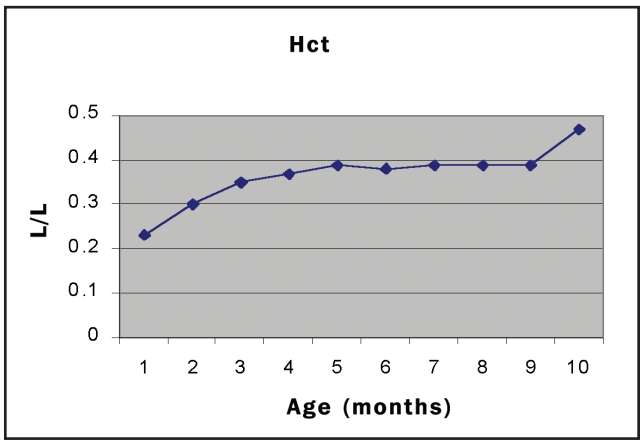


Fig 22.46 | The Hct value continued to increase steadily from 0.23 ± 0.7 L/L at 1 month of age to 0.399 ± 0.9 L/L at 5 months of age and remained fairly constant until the age of 12 to 15 months, when the value increased to 0.47 ± 0.9 L/L.

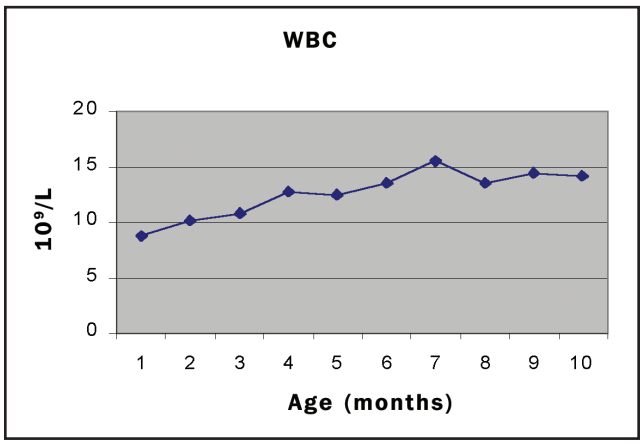


Fig 22.47 | The WBC count at 1 month of age was 8.78 ± 0.45 x 10^9/L, increasing to 15.6 ± 0.7 x 10^9/L at 7 months, then decreasing slightly to 14.5 ± 0.5 x 10^9/L at 9 months of age.

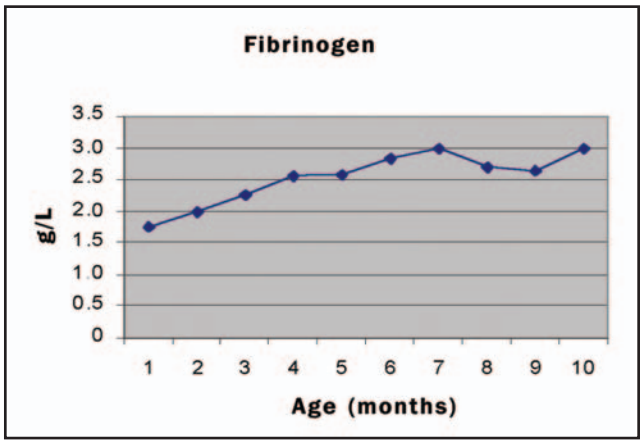


Fig 22.48 | The fibrinogen value was 1.76 ± 0.18 g/L at 1 month of age, increasing steadily to 3.0 ± 0.2 g/L at the age of 7 months.

Hemoresponses

WBC, DIFFERENTIAL WHITE BLOOD CELL COUNT

Table 22.12 | Evaluating the RBC, HB, PCV and Red Cell Indices

Hematologic Findings	Possible Causes
Polycythemia: Increased packed cell volume (PCV) or hematocrit (Hct) and red blood cell count (RBC)	**Absolute:** Primary polycythemia Polycythemia vera Secondary polycythemia, reaction to hypoxia Physiological: Adaptation to high altitudes Pathological: Chronic circulatory or respiratory disease (ie, COPD or asthma of macaws), iron storage disease, rickets Hypoxic increase in erythropoietin production Non-hypoxic, autonomous increase in erythropoietin production
	Relative: Dehydration, different etiologies
PCV >56% or Hct >0.56 L/L	Dehydration in most birds, relatively normal in small (<100 g) psittacine and passerine birds, especially cockatiels
Anemia: Decreased PCV or Hct and RBC	**Absolute:** Hemorrhage (trauma, coagulation disorders, ectoparasitism, endoparasitism); increased red cell destruction (hemoparasites, some bacterial infections, autoimmune hemolytic anemia); decreased red cell production (nutritional deficiencies, chronic infection, chronic renal disease, avian leukoses, toxicosis)
	Relative: Overhydration
Low hemoglobin (Hb) value: (eg, <11.0 g/dl)	Anemia in adult birds
Low mean corpuscular hemoglobin concentration (MCHC) value: (eg, <29.0 g/dl)	Possible iron and other element deficiency

Table 22.13 | Evaluating the Leukocytosis/Toxic Heterophilia with Left Shift

Monocyte Count	Possible Causes
Normal	**Infectious** *Acute:* Gram-negative septicemias. Tuberculosis (granulomas in Falconiformes and Galliformes, but not in Psittaciformes, in these species exclusive accumulation of epithelioid cells [Gerlach, personal communication, 2002]), coligranulomatosis, salmonellosis, yersiniosis and pasteurellosis
Monocytosis	Fungal: Aspergillosis, severe candidiasis Parasitic: Trichomonaisis, capillariaiasis, maggot infestations Miscellaneous: Foreign body inhalation pneumonia, focal peritonitis, chronic ulcerative lesions, old open wounds *Acute and chronic:* Pox and herpesvirus infections, chlamydophilosis *Chronic:* Granulomatous or purulent infections/ infestations
Monocytopenia	**Non-infectious** Maggot infestation, burns, lead/smoke intoxication, egg yolk peritonitis

Table 22.14 | Evaluating the Leukocytosis/Toxic Heterophilia with Left Shift (cont)

WBC and Differential	Monocyte Count	Humoral Cellular Response to Continued Presence of Pathogen	Prognosis
Normal WBC/toxic heterophils and/or reactive lymphocytes	Normal monocyte count - acute	Additional abnormalities-immature cells (left shift), anemia, bone marrow damage, excessive demand	Poor
	Monocytosis - chronic anemia due to bone marrow damage = depression/aplastic anemia	No additional abnormalities	Excellent

Note: Causes for bone marrow suppression and anemia include infections such as viral, bacterial endotoxins in gram-negative septicemia, neoplastic, toxic such as lead toxicosis, metabolic such as high estrogen levels, emaciation.

Table 22.15 | Evaluating Leukocytosis/Heterophilia/ Normal Heterophils

Monocyte Count	Serial Sampling	Possible Causes of Hemogram Changes
Monocytosis	WBC reduced	Healing of soft tissue damage or bone fractures without the complications of severe infection or necrosis, relative cell numbers depend on the degree of chronicity
Normal monocyte count	WBC stays elevated	Acute or chronic inflammation without severe tissue necrosis
	Return to normal within 24-72 hours in the absence of stressor	Stress

Note: Monocytes are slow-reacting cells of the immune system, still changing when other values are already close to normal levels.

Table 22.16 | Evaluating Lymphocytosis with Reactive Lymphocytes (seen rarely in species with a strong heterophilic leukogram)

Hematologic Findings	Possible Causes
Premature lymphoid cells, mitotic figures, anemia	Lymphoid leukosis; marked lymphocytosis with or without immature cells indicates lymphocytic leukemia, while marked lymphocytosis with predominantly mature, small lymphocytes with scalloped cell margins indicates lymphoid neoplasia
Blood parasites with or without lymphocytosis and/or anemia	*Haemoproteus* and *Leucocytozoon* spp. usually without manifesting clinical signs with the exception of young birds; *Babesia, Plasmodium* spp. may cause life-threatening condition with severe anemia and in some cases lymphocytosis
Monocytosis	Strong chronic stimulation of the immune system, eg, chronic inflammation, chronic viremias (leukopenia, lymphocytosis in chronic viral antigen exposure), chronic aspergillosis, immune-mediated diseases

Table 22.17 | Evaluating Leukopenia

Hematologic Findings	Possible Causes
Toxic heterophils, immature cells, anemia, monocytosis, reactive lymphocytes	Final stage of immune response, severe bone marrow damage, gram-negative septicemia, infected wounds, circovirus in psittacines, marked loss of skin, eg, burns, marked tissue necrosis, smoke intoxication. Grave prognosis.
Normal morphology, relative heterophilia, absolute lymphopenia	Initial stage of stress
Reactive lymphocytes, relative lymphocytosis, progressive or intermittent leukopenia	Acute viral infection (toxic heterophils in pox and herpesvirus infections)

Table 22.18 | Hematological Reference Values for Selected Avian Species

Hematology Assay	Egyptian Vulture[25] *(Neophron percnopterus)* n = 4	Common Buzzard[25] *(Buteo buteo)* n = 6	Golden Eagle[25] *(Aquila chrysaetos)* n = 4	Saker Falcon[50] *(Falco cherrug)* n = 25	Barn Owl[25] *(Tyto alba)* n = 10
RBC x 10^{12}/L	2.3 (1.9-2.6)	2.4 (2.2-2.7)	2.4 (1.9-2.7)	2.65 (2.0-3.9)	2.7 (2.2-3.0)
Hb g/dl	14.8 (13.3-16.5)	12.9 (11.6-14.6)	13.8 (12.1-15.2)	15.3 (13.3-21.2)	14.2 (12.7-16.4)
PCV %	43 (37-46)	38 (34-42)	41 (35-47)	47 (42-53)	46 (42-51)
MCV	190 (183-206)	159 (151-171)	174 (160-184)	183.1 (135.8-219.5)	176 (145-216)
MCH	67.7 (65.2-72.9)	53.8 (48.8-57.5)	58.9 (56.3-62.7)	60.7 (50.6-78.9)	51.1 (44.9-60.7)
MCHC	35.2 (35.0-35.5)	33.9 (31.4-36.0)	34.0 (32.3-35.9)	60.7 (50.6-78.9)	31.8 (28.9-34.9)
WBC x 10^9/L	7.6 (4.7-10.6)	9.1 (4.6-13.9)	13.1 (11.7-14.7)	33.2 (28.3-40)	16.6 (11.5-22.3)
Heterophils x 10^9/L	4.0 (1.2-5.5)	5.5 (2.3-8.8)	10.4 (9.5-12.7)	4.1 (2.1-5.9)	8.9 (5.2-12.5)
Eosinophils x 10^9/L	0.3-1.4	0.1-3.1	0.2-0.6	0	0
Basophils x 10^9/L	0	0.0-0.6	0.0-0.2	0	0
Lymphocytes x 10^9/L	2.5 (1.5-3.4)	1.7 (1.1-2.4)	2.2 (1.6-3.2)	1.3 (0.5-2.2)	5.0 (2.5-7.5)
Monocytes x 10^9/L	0.0-0.4	0	0	0.2 (0-0.6)	0
Thrombocytes x 10^9/L	13 (6-15)	27 (18-36)	14 (4-21)	0.41 (0.17-0.76)	33 (14-58)
Fibrinogen g/L	1.6 (1.0-1.9)	2.3 (1.3-3.3)	2.9 (2.0-4.1)	2.8 (1.7-4.7)	2.7 (1.9-3.3)

Hematology Assay	Crowned Crane[25] *(Balearica regulorum)* n = 33	Greater Flamingo[25] *(Phoenicopterus ruber)* n = 9	Rosy Flamingo[43] *(Phoenicopterus ruber ruber)* n = 25	White Stork[25] *(Ciconia ciconia)* n = 16	Kori Bustard[31] *(Ardeotis kori)* n = 28
RBC x 10^{12}/L	2.8 (2.4-3.1)	2.6 (2.3-2.8)	1.4 (1.1-1.8)	2.4 (2.1-2.7)	2.3 (1.74-2.95)
Hb g/dl	15.6 (11.9-18.8)	17.3 (15.9-19.6)	13.4 (9.2-17.6)	15.8 (14.4-17.7)	14.1 (11.9-15.9)
PCV %	47 (44-52)	50 (47-57)	47.8 (37.9-57.8)	45 (41-48)	47 (39.5-52.5)
MCV	171 (156-182)	193 (170-207)	326.6 (234.3-419.0)	189 (172-195)	208.5 (161.9-275.4)
MCH	64.3 (59.8-70.2)	66.2 (57.6-70.0)	91.5 (57.8-125.3)	67.2 (60.2-69.9)	62.4 (48-84.6)
MCHC	36.2 (34.5-39.2)	34.4 (33.5-35.2)	28.1 (20.4-35.8)	35.3 (31-36.9)	30.0 (29.7-34.9)
WBC x 10^9/L	11.1 (6.3-15.6)	2.4 (0.9-3.4)	8.7 (1.5-15.8)	10.8 (7-14.3)	7.3 (3.0-12.8)
Heterophils x 10^9/L	8.2 (4.1-13.3)	1.2 (0.2-3.0)	3.9 (1.0-11.4)	9.2 (5.1-14.9)	3.9 (0.9-9.25)
Eosinophils x 10^9/L	(0.0-1.3)	(0.0-0.4)	(0.0-0.3)	(0.0-0.7)	0.3 (0.0-1.1)
Basophils x 10^9/L	(0.1-0.8)	(0.0-0.4)	(0.0-0.8)	(0.0-0.5)	0.2 (0.0-0.8)
Lymphocytes x 10^9/L	1.6 (0.6-2.7)	0.9 (0.4-1.6)	5.2 (0.8-9.6)	0.8 (0.2-1.6)	2.2 (0.41-5.4)
Monocytes x 10^9/L	(0.0-0.3)	(0.0-0.2)	0.5 (0-1.8)	(0.0-0.3)	0.6 (0.0-1.5)
Thrombocytes x 10^9/L	3.6 (5-18)	4 (2-7)	—	19 (8-32)	5.5 (1.49-18.0)
Fibrinogen g/L	—	—	—	2.3 (1.7-3.2)	2.42 (1.42-4.5)

Table 22.18 | Hematological Reference Values for Selected Avian Species (continued)

Hematology Assay	Black-footed Penguin[25] (*Spheniscus demersus*) n = 57	Humboldt Penguin[55] (*Spheniscus humboldti*) n = 14	African Grey Parrot[25] (*Psittacus erithacus*) n = 11	Greater Sulphur-crested Cockatoo[25] (*Cacatua galerita*) n = 25	Scarlet Macaw[25] (*Ara macao*) n = 7
RBC x 10^{12}/L	1.74 (1.32-2.12)	1.8	3.3 (3.0-3.6)	2.7 (2.4-3.0)	3 (2.7-3.5)
Hb g/dl	16.8 (13.4-19.5)	15.0	15.5 (14.2-17.0)	15.7 (13.8-17.1)	16.8 (14.8-18.9)
PCV %	44 (36-51)	43	48 (43-51)	45 (41-49)	48 (46-52)
MCV	254 (232-273)	238.1	145 (137-155)	165.0 (145-187)	160 (143-175)
MCH	95.1 (87.2-104.3)	83.3	47.2 (41.9-52.8)	57.6 (53.8-60.6)	57.6 (51.1-64.2)
MCHC	37.8 (35.4-40)	34.8	32.5 (28.9-34)	34.9 (33.3-37.6)	35.9 (32.6-38.5)
WBC x 10^9/L	9.3 (3.5-16.3)	13.0	7.0 (3.3-10.3)	6.4 (1.4-10.7)	10.2 (6.4-15.4)
Heterophils x 10^9/L	8.1 (5.0-12.3)	8.0	4.9 (1.8-7.3)	3.7 (1-6.6)	8.0 (4.9-12.8)
Eosinophils x 10^9/L	(0.0-0.2)	1.1	0	(0.0-0.2)	0
Basophils x 10^9/L	(0.0-0.3)	0	(0.0-0.8)	(0.0-0.9)	(0.0-0.8)
Lymphocytes x 10^9/L	3.1 (0.8-5.2)	2.8	1.4 (0.7-2.1)	1.9 (1.0-3.6)	1.6 (1.2-2.2)
Monocytes x 10^9/L	0	0.6	(0.0-0.3)	(0.0-0.2)	0
Thrombocytes x 10^9/L	11 (5-19)	18.3	22.0 (11-42)	13.0 (7-24)	22 (17-30)
Fibrinogen g/L	2.9 (2.2-3.7)	—	2.2 (1.5-2.8)	1.4 (0.9-2.0)	1.7 (1.0-2.2)

Hematology Assay	Kea[25] (*Nestor notabilis*) n = 8	Fisher's Lovebird[250] (*Agapornis fischeri*)	Nicobar Pigeon[47] (*Caloenas nicobarica*) n = 16	Common Crowned Pigeon[47] (*Goura cristata*) n = 9	Brown Pelican[25] (*Pelecanus occidentalis*) n = 5
RBC x 10^{12}/L	2.6 (2.3-3.1)	4.5 (3.8-5.3)	3.4 (2.6-4.3)	2.31 (1.95-2.6)	2.7 (2.6-2.8)
Hb g/dl	13.4 (10.6-16.9)	15.3 (13.0-17.7)	17 (12.7-19.7)	12.3 (10.6-14.7)	14.5 (14.3-14.8)
PCV %	40 (34-46)	53 (45-61)	50.7 (45-56)	37.6 (33.8-42)	46 (43-49)
MCV	154 (137-186)	124.5 (108-141)	149.8 (127.6-168.5)	158.7 (142.9-175.0)	168 (166-173)
MCH	51.2 (41.6-68.1)	34.5 (29.3-39.8)	50 (41.3-57.6)	50.8 (44.2-57.3)	53.4 (51.2-56.8)
MCHC	33.2 (30.4-37.0)	29 (25.7-32.3)	33.5 (28.3-36.1)	31.9 (27.9-38)	31.7 (30.4-32.9)
WBC x 10^9/L	16 (12.1-22.6)	3.5 (0.6-6.4)	4.23 (2-8.2)	17.7 (11.7-25.1)	11.9 (6.6-19.4)
Heterophils x 10^9/L	13.8 (9.4-20.1)	2.5 (0.1-4.9)	5.2 (4.2-7.1)	6.6 (5.5-7.8)	6.7 (4.0-9.5)
Eosinophils x 10^9/L	(0.0-0.5)	0.15 (0.0-0.3)	3.7 (2.7-5.1)	0.2 (0.1-0.5)	(0.0-0.2)
Basophils x 10^9/L	(0.0-0.6)	0.2 (0.0-0.4)	0	(0.0-0.1)	(0.0-.0.2)
Lymphocytes x 10^9/L	1.9 (1.1-2.7)	2.3 (0.6-4.1)	3.7 (2.7-5.1)	3.0 (1.8-4.0)	4.0 (2.5-7.0)
Monocytes x 10^9/L	0	0.2 (0.0-0.3)	2.1 (1-5)	(0.0-0.02)	(0.0-0.2)
Thrombocytes x 10^9/L	16 (11-24)	15 (5-25)	—	—	27.5 (17-38)
Fibrinogen g/L	1.5 (1.1-1.8)	2.45 (0.9-4.0)	—	—	2.9 (2.6-3.1)

Hematology Assay	Ostrich[52] (*Struthio camelus*)	Domestic Fowl[20] (*Gallus domesticus*)	Wood Duck[45] (*Aix sponsa*) n = 15	Bar-Headed Goose[20] (*Anser indicus*)	Stone Curlew[52] (*Burhinus oedicnemus*) n = 18
RBC x 10^{12}/L	1.7	3.2 (2.5-3.9)	2.79	(2.5-3.2)	2.86 (2.59-3.27)
Hb g/dl	12.2	12.6 (10.2-15.1)	14.95	(12.2-17.2)	14.4 (12.2-16.6)
PCV %	32	39.5 (30-49)	45.5	(43-56)	47 (44-58)
MCV	174	119.5 (104-135)	164.2	(155-187)	167.3 (149.9-196.2)
MCH	61	37.9 (32.0-43.9)	54.0	(47.8-60.7)	50.7 (43.7-57.1)
MCHC	33	33.2 (30.2-36.2)	32.9	(28.5-33.9)	30.3 (27.7-35.5)
WBC x 10^9/L	5.5	5.7 (1.9-9.5)	2.3	(3.1-12.0)	7.88 (2.45-12.6)
Heterophils x 10^9/L	6.2	4.0 (0.5-7.6)	8.4	(0.8-8.3)	5.99 (0.9-11.5)
Eosinophils x 10^9/L	0	0.9 (0.0-1.8)	0.5	(0.0-0.5)	0.6 (0.0-2.7)
Basophils x 10^9/L	0	0.5 (0.0-1.0)	0.4	(0.0-0.8)	0.19 (0.0-0.8)
Lymphocytes x 10^9/L	3.4	2.7 (1.2-4.2)	13.2	(0.5-4.2)	0.5 (0.2-1.3)
Monocytes x 10^9/L	0.2	0.5 (0.0-1.0)	1.0	(0.0-1.2)	0.4 (0.0-0.9)
Thrombocytes x 10^9/L	—	18 (3-33)	—	(8-29)	8.9 (3.4-18.2)
Fibrinogen g/L	—	2.7 (1.3-4.1)	—	(1.9-4.8)	3.3 (2.1-4.1)

Products Mentioned in the Text

a. Coulter Counter ZF, Beckman Coulter Inc, Fullerton, CA, USA www.beckmancoulter.com
b. Cell Dyn 3500, Abbott Laboratories, Abbott Park, IL, USA www.abbottdiagnostics.com
c. BD Unopette 365851 red blood count manual hematology test, Becton Dickinson Co, Franklin Lakes, NJ, USA www.bd.com
d. BD Unopette 365877 eosinophil count manual hematology test, Becton Dickinson Co, Franklin Lakes, NJ, USA

Dedication

This chapter is dedicated to Dr. Christine M. Hawkey.

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