

# THE FELINE BLOOD FILM

## 1. Techniques and erythrocyte morphology

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### Blood film examination – we have a hematology analyzer so why the need?

Many private veterinary practices have quality automated hematology instruments in-house that rapidly provide complete blood count (CBC) results. Some analyzers provide automated leukocyte differential results, but others require a manual differential count to be performed using a stained blood film. Regardless of the analyzer used, there are important additional hematology findings that can only be determined by microscopic examination of stained blood films.<sup>1–3</sup> These include:

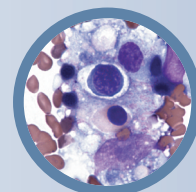
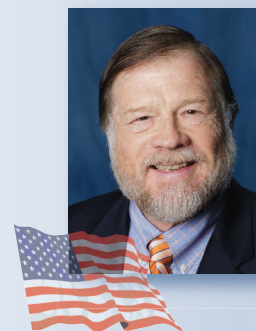
- ❖ **Erythrocyte abnormalities** – abnormal shapes, inclusions and parasites;
- ❖ **Leukocyte abnormalities** – neutrophilic left shifts and hypersegmentation, neutrophil cytoplasmic toxicity, abnormal granules, inclusions and infectious agents;
- ❖ **Platelet abnormalities** – megaplatelets and platelet aggregates;
- ❖ **Miscellaneous cells** – for example, presence of nucleated erythrocytes, neoplastic cells and mast cells;

#### Blood film examination for quality control

Differential leukocyte counts have been considered unreliable in first-generation in-house veterinary hematology analyzers,<sup>4</sup> although the reliability of the automated differential count is better in at least one newer instrument.<sup>5</sup> Basophils are especially difficult to identify with most automated instruments. They are generally missed and other cells are sometimes counted as basophils.<sup>6</sup> Platelet aggregation, which is common in cat blood samples,<sup>7</sup> can result in an abnormally low platelet count. Consequently, it is important to verify that a thrombocytopenia reported by an analyzer is true, by examining a stained blood film. Similarly abnormalities in other parameters can be verified by blood film examination; for example, if an analyzer reports a high reticulocyte count, there should be evidence of polychromasia in the blood film, or if the red cell distribution width is increased, increased anisocytosis should be visible.

❖ **Platelet numbers** – most automated hematology analyzers cannot perform platelet counts in cats because there is an overlap in size between feline platelets and erythrocytes. Consequently, platelet numbers must generally be estimated from a stained blood film, or a manual count must be performed.

The scanning of stained blood films also provides a measure of quality control for the hematology analyzer (see box). For this, and all of the above reasons, a quick blood film review should be performed alongside each automated CBC.



**Practical relevance:** Many veterinary practices have invested in quality automated hematology instruments for use in-house. However, regardless of the specific choice of analyzer, there are important hematology findings that can only be determined by microscopic examination of stained blood films. For this reason, and also for the purpose of quality control for the analyzer, a quick blood film review should be performed alongside every automated complete blood count. Even those practices that submit their blood samples to outside diagnostic laboratories for evaluation, still require the capability to examine stained blood films in emergency situations.

**Series outline:** This is the first of a two-part article series that aims to familiarize the practitioner with normal findings on feline blood films, with a particular focus on unique features in the cat, as well as to assist with interpretation of common abnormalities. Part 1 focuses on how to prepare and examine blood films in order to maximize the reliability of the information they convey, and describes the morphology of feline erythrocytes in health and disease.

**Evidence base:** The information and guidance offered is based on the published literature and the author's own extensive clinical pathology research.



Some veterinary practices submit their blood samples to outside diagnostic laboratories for evaluation, but these practices nevertheless require the capability to examine stained blood films in emergency situations. Hematocrits can be determined by microhematocrit centrifugation, and leukocyte and platelet counts can be estimated from blood films.

**Sample collection and handling**

Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant for CBC and blood film evaluation in cats. Cat platelets are prone to activation and clumping when blood samples are collected, making it difficult to obtain accurate platelet counts. The collection of blood directly into a vacuum tube generally results in less platelet activation than occurs when blood is collected by syringe and then transferred to a vacuum tube.

**Blood film preparation**

Blood films should be prepared as soon as possible after blood sample collection to minimize artefactual changes that can distort blood cell morphology.

The most common method of preparation is the wedge method, which requires two clean glass slides (Figure 1).<sup>1</sup> The blood sample is mixed either using a blood tube rotator or by hand for at least 1 min, and a microhematocrit tube is used to collect blood from the vacuum tube by capillary action. A glass slide is placed

on a flat surface and a small drop of blood from the hematocrit tube is placed on one end of the slide. This slide is held in place with one hand and a second glass slide (spreader slide) is placed on the first slide and held between the thumb and forefinger with the other hand at about a 30–45° angle in front of the drop of blood. The spreader slide is then backed into the drop of blood, and as soon as the blood flows along the back edge of the spreader slide, the spreader slide is rapidly pushed forward.

The thickness of the smear depends on a number of factors, including the size of the drop of blood, the viscosity of the sample (which is greater as the hematocrit increases), the angle of the spreader slide and the speed of spreading. A greater angle and faster speed of spreading results in thicker and shorter blood films.<sup>8</sup> The drop of blood should be of sufficient size to prepare a smear that occupies at least half the length of the slide. Large cells and clumps of cells tend to be pushed to the front (feathered) end of the smear. If the drop of blood is too large, some of the blood will be pushed off the end of the slide, and large cells and clumps of cells will not be available for examination.

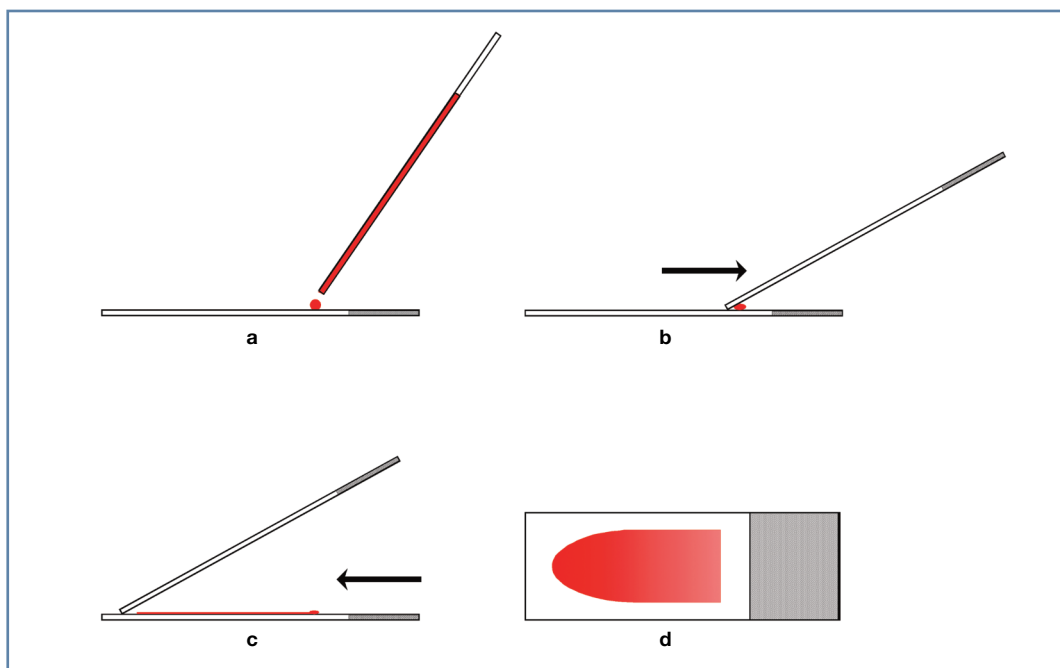
Once prepared, the slide is immediately dried by waving it in the air or by holding it in front of a hair dryer set on a warm (not hot) setting.



**Blood films should be prepared as soon as possible after blood sample collection to minimize artefactual changes that can distort blood cell morphology.**

**Figure 1** Blood film preparation. (a) A glass slide is placed on a flat surface and a small drop of well-mixed blood is placed on one end of the slide using a microhematocrit tube. (b) A second glass slide (spreader slide) is placed on the first slide at about a 30–45° angle in front of the drop of blood. The spreader slide is then backed into the drop of blood. (c) As soon as the blood flows along the back edge of the spreader slide, the spreader slide is rapidly pushed forward. (d) The blood film produced is thick at the back of the slide, where the drop of blood was placed, and thin at the front (feathered) edge of the slide.

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## Blood film staining

**Romanowsky-type stains**

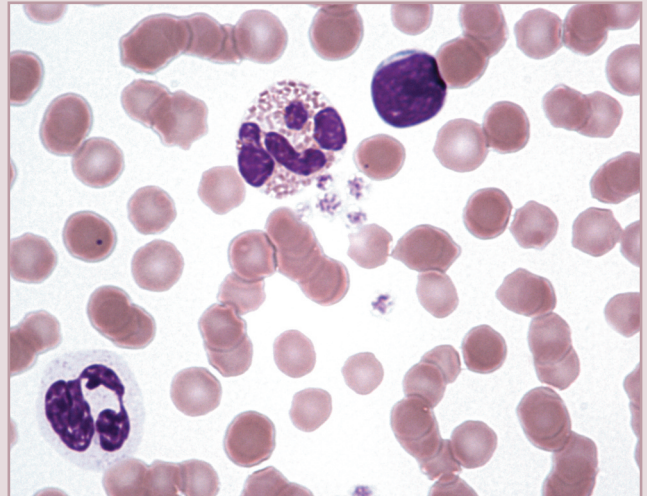
Blood films should be fixed in water-free methanol within a few hours of preparation. They are typically stained with Romanowsky-type stains, including Wright, Wright-Giemsa and Diff-Quik (Figure 2). The appearance of stained blood films depends on the quality of the fixative and stains, the staining and rinse times, and the pH of the stains and rinse water. Morphologic artefacts, including loss of cellular detail and vacuolation, may be present if the methanol fixative contains more than 3% water.<sup>8</sup> Low pH, inadequate staining time, degraded stains or over-washing can result in excessively pink-staining blood films. High pH, prolonged staining or insufficient washing can result in excessively blue-staining blood films. Blood films may also have an overall blue tint if stored unfixed for long periods before staining, or if exposed while unfixed to formalin vapors (as might occur during shipping to a laboratory in a package that also contains formalin-fixed tissue). Blood films have an overall magenta tint when blood is collected with heparin as the anticoagulant.

Drying or fixation problems can result in variably shaped refractile inclusions in the erythrocytes. Precipitated stain may be present because the stain(s) needed to be filtered, the staining procedure was too long, or the washing was not sufficient.<sup>1</sup>

Various rapid stains, such as Diff-Quik (Dade Behring), Hema 3 (Fisher Scientific) and the stain used in the automated stainer Aerospray 7120 (Wescor), are commonly used. These rapid stains are classified as aqueous stains, even though the fixation is done in methanol, because the component stains were prepared in water. The quality of blood films stained with aqueous stains is generally somewhat lower than that obtained by longer methanolic staining procedures, but staining quality is improved by allowing the blood film to remain in the fixative for several minutes. A significant limitation of aqueous stains is that they generally do not stain basophil, mast cell or large granular leukocyte granules well.<sup>9</sup>

**Reticulocyte stains**

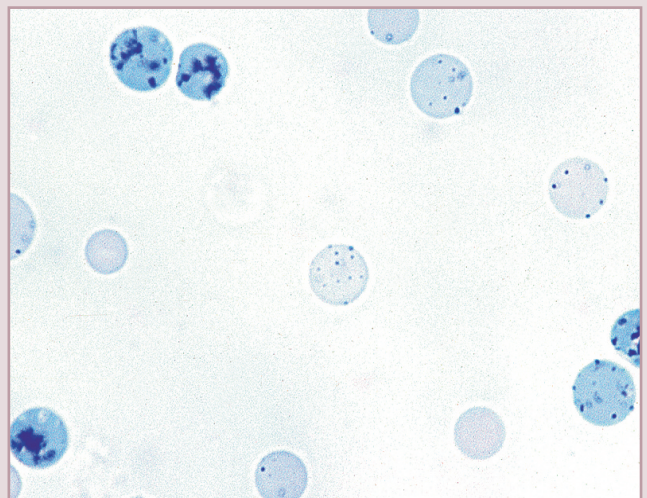
Reticulocyte stains are commercially available (new methylene blue, N Brecher, Harleco, EMD Chemicals). Blood and stain are mixed together in equal volumes in a test tube and incubated at room temperature for 10–20 mins. Blood films are made following the incubation. Reticulocyte stains are used to visualize Heinz bodies, as well as reticulocytes. The blue-staining material in reticulocytes results from precipitation of ribosomal ribonucleic acid (rRNA) during staining. rRNA is present in reticulocytes but not in mature erythrocytes. Cats are unique in that two types of reticulocyte are identified. Reticulocytes formed at the time of nuclear extrusion contain substantial aggregates of precipitated rRNA and



**Figure 2** Neutrophil (bottom left), eosinophil (red granules) and a lymphocyte (round nucleus) are present in this blood film from a cat. Erythrocytes exhibit rouleaux, which is normal in cats. Wright-Giemsa stain

are called aggregate reticulocytes (Figure 3). As a reticulocyte matures, the number of ribosomes decreases until only small dot-like inclusions are observed when stained with the reticulocyte stain. These cells are called punctate reticulocytes (Figure 3).

The number of punctate reticulocytes in the blood of normal cats, as well as cats with regenerative anemia, is much greater than present in the blood of other species. This apparently occurs because the maturation (loss of ribosomes) of reticulocytes in cats is slower than in other species. Based on composite results from several authors, normal cats generally have from 0–0.5% aggregate and 1–10% punctate reticulocytes when determined using reticulocyte stains. The stages of reticulocytes are not differentiated in species other than the cat because few punctate reticulocytes are present. Automated hematology instruments generally only identify aggregate reticulocytes in cats.



**Figure 3** Reticulocytes in cat blood. Three whole aggregate reticulocytes (containing blue-staining aggregates of RNA) and one half of an aggregate reticulocyte (far right) in blood from a cat with a markedly regenerative anemia. The majority of the remaining cells are punctate reticulocytes containing discrete dot-like inclusions. New methylene blue reticulocyte stain. Reproduced from Harvey (2012),<sup>1</sup> with permission

**Trouble-shooting**

- ❖ Stain too blue: high (basic) pH – water or stain
- ❖ Stain too blue: prolonged length of time stained
- ❖ Stain too red: low (acidic) pH – water or stain
- ❖ Stain too red: over-washed
- ❖ Artefact in/on erythrocytes – precipitated stain, water contamination usually in the fixative
- ❖ Blue tint to slide: delayed staining
- ❖ Magenta tint to slide: heparin anticoagulant

## Method to examine stained blood films

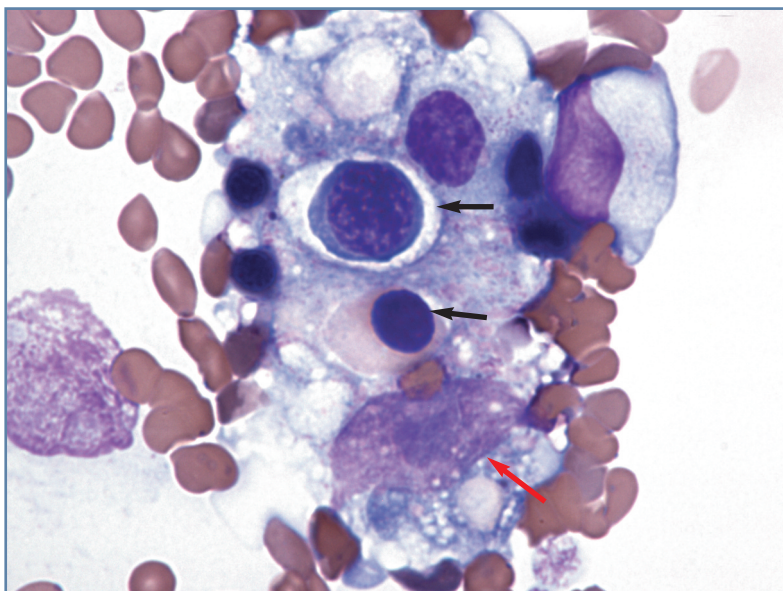
❖ **Initial low-power survey** Stained blood films should first be scanned using a low-power objective to look for the presence of erythrocyte autoagglutination, leukocyte aggregates, platelet aggregates, microfilaria and abnormal cells that might be missed during the differential leukocyte count. It is particularly important that the feathered end of blood films made on glass slides is examined because large cells and aggregates of cells (especially platelets) may be concentrated in this area (Figure 4).

### Examination of blood cell morphology

When examining a glass slide blood film, the blood film will be too thick to evaluate blood cell morphology at the back of the slide and too thin at the feathered edge where cells, especially erythrocytes, are flattened. The optimal area for evaluation is generally in the front half of the smear behind the feathered edge. This area should appear as a well-stained monolayer (a field in which erythrocytes are close together, with approximately one half of the cells touching each other).

❖ **Leukocyte numbers** As a quality control measure if using a hematology analyzer, the number of leukocytes present should be estimated to assure that the number present on the slide is consistent with the total leukocyte count measured. If a x 10 ocular and a x 10 objective are used (x 100 magnification), the total leukocyte count in blood (cells/ $\mu$ l) may be estimated by determining the average number of leukocytes present per field and multiplying by 100. If a x 20 objective is used, the total leukocyte count may be estimated by multiplying the average number of leukocytes per field by 400. The correction factor used may vary, depending on the microscope used. Manual differential leukocyte counts are inherently imprecise, so it is also important to scan the blood film to gain an appreciation of the relative distribution of leukocyte types present prior to performing the differential leukocyte count.

❖ **Differential leukocyte count** A differential leukocyte count is performed by identifying 200 consecutive leukocytes using a x 40 or x 50 objective. Because monocytes and other large leukocytes are pushed to the sides and ends of wedge-prepared blood films, differential counts are performed by examining cells in a pattern that evaluates both the edges and the center of the smear.<sup>8</sup> After the count is complete, the percentage of each leukocyte type present is calculated and multiplied by



**Figure 4** Aggregate of cells, which appears to contain a macrophage with phagocytized nucleated erythrocytes (black arrows), located at the feathered end of a blood film from a cat with *Mycoplasma haemofelis* infection. The large oval magenta structure near the bottom of the image (red arrow) is the nucleus of the macrophage. The nucleus contains a blue-staining nucleolus. Wright-Giemsa stain

the total leukocyte count to determine the absolute number of each cell type present per microliter of blood. It is the absolute number of each leukocyte type that is important. Relative values (percentages) can be misleading when the total leukocyte count is abnormal.

❖ **Nucleated red blood cells** Nucleated red blood cells (NRBCs) are counted along with the leukocytes by most hematology analyzers. When encountered during blood film examination, the number of NRBCs per 100 leukocytes should be tabulated and the total leukocyte count should be corrected for the number of NRBCs present, unless the instrument can differentiate between NRBCs and lymphocytes, and exclude NRBCs from the total leukocyte count.

**Table 1** Semiquantitative evaluation of toxic changes in the cytoplasm of neutrophils

Neutrophils with toxic change	
Few	5–10%
Moderate	11–30%
Many	>30%
Severity of toxic change in cytoplasm	
Döhle bodies*	1+
Mildly basophilic	1+
Moderately basophilic with Döhle bodies	2+
Moderately basophilic and foamy	2+
Dark blue–gray and foamy <sup>†</sup>	3+
Basophilic with toxic granules <sup>†</sup> (not reported in cats)	3+

\*One or two Döhle bodies are sometimes seen in a few neutrophils from cats that do not exhibit signs of illness

<sup>†</sup>May also contain Döhle bodies  
From Harvey (2012),<sup>1</sup> with permission

❖ **Leukocyte morphology** The presence of abnormal leukocyte morphology, such as toxic cytoplasm in neutrophils or increased (eg, >5%) reactive lymphocytes, should be recorded on the hematology report form. The frequency of toxic neutrophils is reported as few (5–10%), moderate (11–30%) or many (>30%), and the severity of toxic change is recorded as 1+ to 3+ (Table 1).<sup>1,10</sup>

❖ **Erythrocyte morphology** Erythrocyte morphology should be examined and recorded as either normal or abnormal. Observations regarding erythrocyte morphology, such as the degree of polychromasia (presence of polychromatophilic erythrocytes), anisocytosis (variation in size) and poikilocytosis (abnormal shape), should also be made. Abnormal erythrocyte shapes should be classified as specifically as possible, because specific shape abnormalities may help to determine the nature of a disorder that is present. The number of abnormal cells should be reported in a semiquantitative fashion, using a system such as that shown in Table 2.<sup>10</sup>

❖ **Platelet numbers** Platelet numbers should be estimated as being low, normal or increased. Blood smears from cats normally average between 10 and 30 platelets per high power field when examined using a x 10 ocular and x 100 objective (x 1000 magnification). An estimate of the number of platelets/μl of blood may be obtained by multiplying the average number per field by 15,000 to 20,000. If a thrombocytopenia is suspected, it should be confirmed with a platelet count. The presence of platelet aggregates and abnormal platelet morphology (large or hypogranular platelets) should also be recorded on the hematology form.

**Table 2** Semiquantitative evaluation of erythrocyte morphology based on average number of abnormal cells per x 1000 microscopic monolayer field\*

	1+	2+	3+	4+
Anisocytosis	5–8	9–15	16–20	>20
Polychromasia	1–2	3–8	9–15	>15
Hypochromasia	1–10	11–50	51–200	>200
Poikilocytosis	3–10	11–50	51–200	>200

\*A monolayer field is defined as a field in which erythrocytes are close together, with approximately one-half touching each other. In severely anemic animals, such monolayers may not be present. When erythrocytes are generally not touching (eg, tend to be separated by the distance of one cell diameter), then the number of erythrocytes with morphologic abnormalities is counted for two fields. From Weiss (1984),<sup>10</sup> with permission

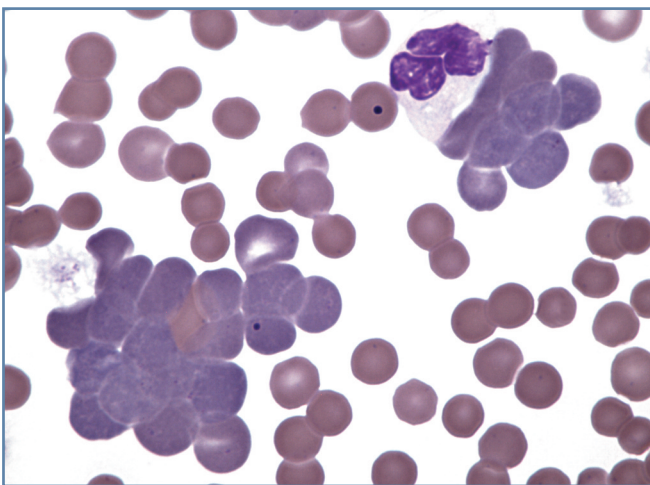
## Erythrocyte morphology – normal and abnormal

### Rouleaux

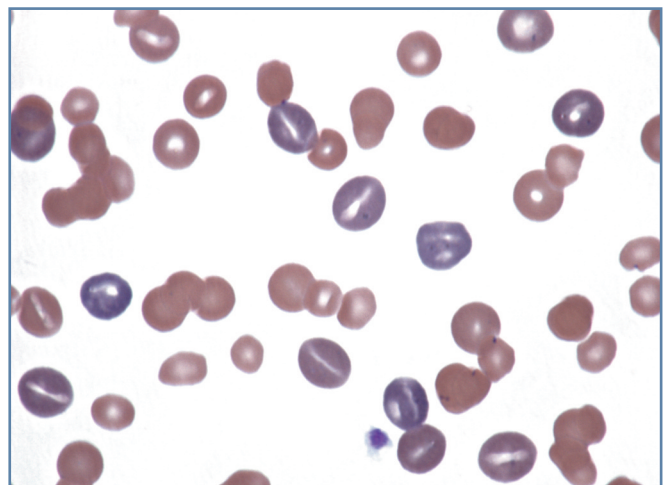
Like horses, blood films from healthy cats often exhibit rouleaux (aggregations of erythrocytes resembling stacks of coins; Figure 2).<sup>1</sup> Rouleaux formation depends on both the nature of the erythrocytes and the composition of plasma. Although difficult to evaluate in cats, rouleaux formation is further increased when plasma globulins are increased, as occurs with inflammation and plasma cell neoplasia. Prominent rouleaux formation results in rapid erythrocyte sedimentation in whole blood samples allowed to stand undisturbed. Consequently, cat blood must be well mixed before hematology measurements are made.



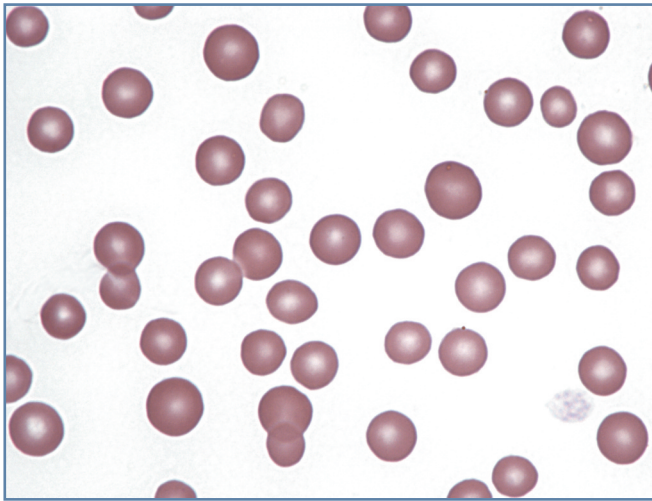
**Abnormal erythrocyte shapes should be classified as specifically as possible, and may help to determine the nature of a disorder that is present.**



**Figure 5** Agglutination of polychromatophilic erythrocytes (aggregate reticulocytes) in blood from a cat with immune-mediated hemolytic anemia. Many mature erythrocytes appear to be spherocytes. Wright-Giemsa stain



**Figure 6** Increased polychromasia and anisocytosis in a *Mycoplasma haemofelis*-infected cat with a regenerative anemia. Most polychromatophilic erythrocytes have a stomatocyte appearance. Wright-Giemsa stain



**Figure 7** Increased anisocytosis without polychromasia in blood from a feline immunodeficiency virus-infected cat with a non-regenerative anemia. Wright-Giemsa stain

### Agglutination

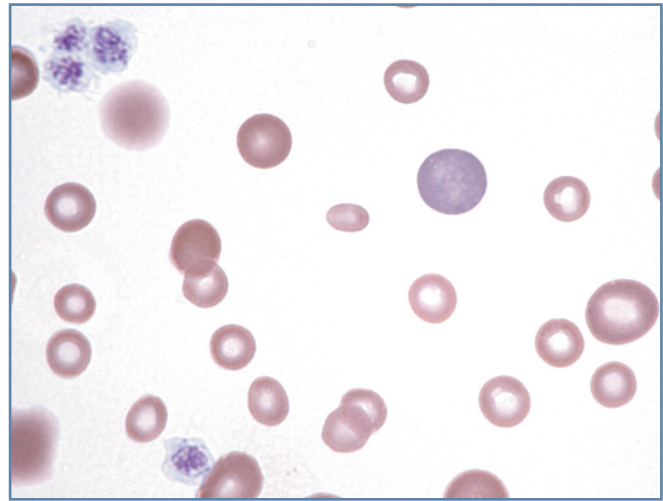
The aggregation of erythrocytes together in clusters (not in stacks of coins as in rouleaux) is termed agglutination (Figure 5). Agglutination is caused by the presence of immunoglobulins bound to erythrocyte surfaces. It may be observed in association with both primary and secondary (eg, *Mycoplasma haemofelis* infection) immune-mediated hemolytic anemia.<sup>1</sup>

### Polychromasia

Aggregate reticulocytes in cats appear as polychromatophilic (bluish-red) erythrocytes in routinely stained blood films (Figures 5 and 6).<sup>11</sup> This coloration results from the presence of blue-staining RNA in cells that are synthesizing red-staining hemoglobin. Slight polychromasia may be present in normal cats, but normal cats may also exhibit no polychromasia in stained blood films. Punctate reticulocytes in cats do not contain sufficient RNA for these cells to appear bluish. The presence of increased polychromasia in cat blood films indicates that an aggregate reticulocytosis is present, as expected in response to hemorrhage or increased erythrocyte destruction (hemolysis).

### Anisocytosis

Anisocytosis refers to the variation in erythrocyte diameters in stained blood films. Increased anisocytosis may be observed when substantial numbers of larger than normal cells are present, as occurs when increased numbers of reticulocytes are produced. Consequently, increased anisocytosis is often a feature of regenerative anemia (Figure 6). However, large erythrocytes may also be produced during dyserythropoiesis in some cats with non-regenerative anemia (Figure 7).

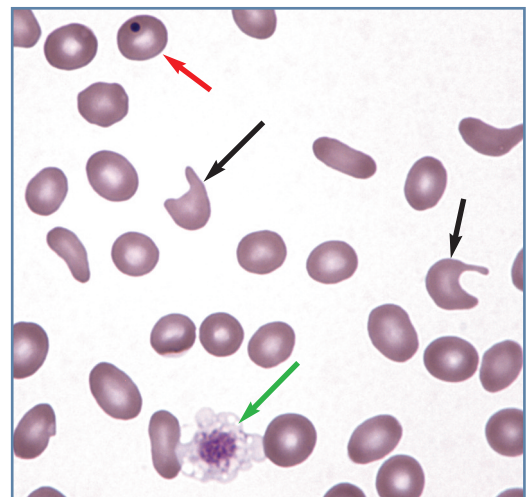


**Figure 8** Marked anisocytosis in blood from a 6-week-old kitten after a blood transfusion. The kitten presented with severe iron deficiency anemia and marked lipemia. The small, hypochromic erythrocytes are from the kitten, and the larger erythrocytes are from the blood donor cat. A large polychromatophilic erythrocyte and two lysed erythrocytes (red smudges on the left side of the image) are present. The lipemia promoted erythrocyte lysis during blood film preparation. Wright-Giemsa stain

Increased anisocytosis may also be seen when substantial numbers of smaller than normal cells are present, as commonly occurs in iron deficiency anemia in dogs secondarily to chronic blood loss. With the exception of nursing kittens, iron deficiency anemia is rarely recognized in cats (Figure 8).<sup>1</sup>

### Hypochromasia

Hypochromasia refers to the presence of erythrocytes with decreased hemoglobin concentration and increased central pallor. Increased hypochromasia is commonly observed in dogs with chronic iron deficiency anemia. With the exception of severe iron deficiency anemia in kittens (Figure 8), hypochromasia is generally not apparent in iron-deficient cats.<sup>1</sup>



**Figure 9** Poikilocytosis in blood from an anemic cat with a portosystemic shunt. Multiple elliptocytes (ovalocytes) and two keratocytes with ruptured vacuoles (black arrows) are present. A discocyte containing a Howell-Jolly body (red arrow) and a large platelet (green arrow) are also present. Wright-Giemsa stain

### Erythrocyte shapes

Cat erythrocytes are anucleated biconcave discs (discocytes) that are smaller than dog erythrocytes, and similar in size to horse erythrocytes. The biconcavity of feline erythrocytes is less pronounced than that of their canine counterparts, resulting in less central pallor of erythrocytes in feline blood films compared with canine blood films.

### Poikilocytosis

The general term used to describe the presence of abnormally shaped erythrocytes is poikilocytosis (Figure 9). Although specific terminology is used for certain abnormal shapes, it may be less important to quantify each type of shape change than it is to determine the cause of the shape change. Poikilocyte formation occurs in various disorders associated with erythrocyte fragmentation including disseminated intravascular coagulation (DIC), liver disease, cardiomyopathy, myeloid neoplasms, renal failure and doxorubicin toxicity.<sup>1,12–14</sup> Poikilocytes can also form when oxidant injury results in Heinz body formation and/or membrane injury (eccentrocytes).

### Echinocytes

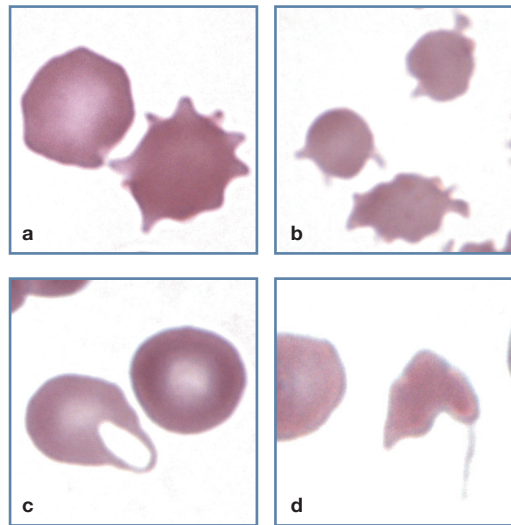
Spiculated erythrocytes in which the spicules are relatively evenly spaced and of similar size are classified as echinocytes. When observed in stained blood films, echinocytes are usually an artefact resulting from excess EDTA, improper blood film preparation, or prolonged sample storage before blood film preparation. Echinocytosis (previously referred to as crenation) has been reported in uremic cats (Figure 10a).<sup>15</sup>

### Acanthocytes

When spiculated erythrocytes have irregularly spaced, variably sized spicules they are classified as acanthocytes (Figure 10b). They have been recognized in cats with liver disease and in cats with disorders that result in erythrocyte fragmentation such as DIC and congestive heart failure.<sup>12,14,16</sup>

### Keratocytes

Erythrocytes containing what appear to be one or more intact or ruptured vesicles are called kera-



**Figure 10** Abnormal erythrocyte shapes. (a) A normal discocyte (left) and an echinocyte (right) in blood from a cat with uremia. (b) Three acanthocytes in blood from a cat with hepatic lipidosis. (c) A keratocyte with intact vacuole (left) and a discocyte (right) in blood from a cat with a portosystemic shunt. (d) A schistocyte in blood from a cat with diabetes mellitus, interstitial lung disease and erythrocyte fragmentation. Wright-Giemsa stain

toocytes (Figure 10c). The rupture of a vesicle results in the formation of one or two projections. Keratocytes have been observed in various disorders in cats, including liver disease, portosystemic shunts, DIC, heart disease and doxorubicin toxicity.<sup>12,13,16,17</sup>

### Schistocytes

Erythrocyte fragments with pointed extremities are called schistocytes (or schizocytes) (Figure 10d). They are smaller than normal discocytes. Schistocytes have been described in cats with liver disease, DIC, heart disease and doxorubicin toxicity.<sup>12,13,16,18</sup>

### Spherocytes

Spherical erythrocytes that result from cell swelling and/or loss of cell membrane are referred to as spherocytes (Figure 11a). Spherocytes lack central pallor and have smaller diameters than normal on stained blood films. By comparison, flattened erythrocytes present in the feathered end of blood films lack central pallor, but have large diameters.

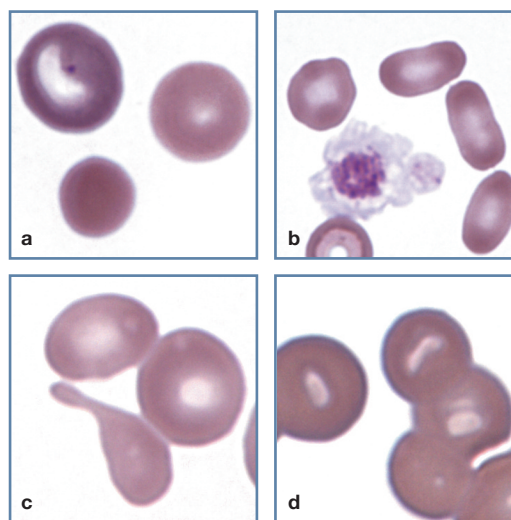
Spherocytes are difficult to recognize with certainty in cats because of the small size and limited central pallor of feline erythrocytes. Consequently, a description of spherocytosis should be assigned cautiously in cats, particularly when being used as a criterion for diagnosing immune-mediated hemolytic anemia (Figure 5). Other potential causes of spherocyte formation include erythrocyte parasites, oxidant damage and transfusion of stored blood. Microspherocytes may result from erythrocyte fragmentation.<sup>1</sup>

### Elliptocytes

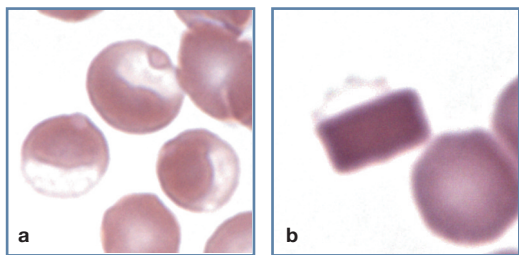
Erythrocytes that are elliptical or oval in shape are classified as elliptocytes (or ovalocytes). They have been recognized in cats with myeloid neoplasms, hepatic lipidosis, portosystemic shunts (Figure 11b) and doxorubicin toxicity.<sup>12,13,16,17</sup>

### Dacryocytes

Tear-drop shaped erythrocytes are called dacryocytes (Figure 11c). They are commonly seen in people with myelofibrosis, but not in animals. They have been observed in cats with portosystemic shunts and myeloid neoplasia.<sup>1</sup>



**Figure 11** Abnormal erythrocyte shapes. (a) A polychromatophilic erythrocyte (top left), discocyte (top right) and spherocyte (bottom) in blood from a cat with immune-mediated hemolytic anemia. (b) Three elliptocytes (right) in blood from a cat with a portosystemic shunt. A large activated platelet (center) with aggregated granules that may be confused with a nucleus is also present. (c) A tear drop-shaped dacryocyte appears below two discocytes, each of which contains a pale, barely visible Heinz body in blood from a cat with a portosystemic shunt. (d) Stomatocytes in the thick portion of a feline blood film. This shape was not present in thinner areas of the film. Wright-Giemsa stain



**Figure 12** Abnormal erythrocyte shapes. (a) Three eccentrocytes in blood from a cat with acetaminophen toxicity. Pale Heinz bodies are also visible in two of the eccentrocytes (left and top). (b) A hemoglobin crystal is present within an erythrocyte. The faintly stained cell membrane (top) is visible. Wright-Giemsa stain

### Stomatocytes

Cup-shaped erythrocytes that have oval or elongated areas of central pallor when viewed in stained blood films are called stomatocytes (Figure 11d). They generally occur as artefacts in thick blood film preparations in cats.<sup>1</sup>

### Eccentrocytes

Eccentrocytes form when oxidant damage to erythrocyte membranes results in the adhesion of opposing areas of the cytoplasmic faces of the membrane. As a result, hemoglobin is eccentrically localized to part of the cell, leaving a hemoglobin-poor area visible in the remainder of the cell (Figure 12a). Eccentrocytes have often been recognized in dogs secondarily to exogenous (eg, onions) and endogenous (eg, ketoacidotic diabetes) oxidants. Eccentrocytes may be present in cats with oxidant damage resulting from acetaminophen administration.<sup>1</sup>

### Hemoglobin crystals

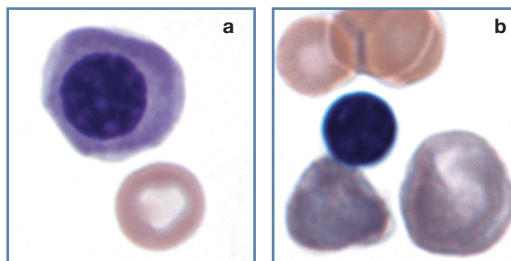
Crystallized hemoglobin is occasionally observed within erythrocytes in blood films from cats (Figure 12b). Crystals may be eccentrically located in the cells and resemble eccentrocytes. No hemoglobin abnormalities have been recognized by hemoglobin electrophoresis in cats with hemoglobin crystals.<sup>1</sup>

### Nucleated erythrocytes

Low numbers of rubricytes and metarubricytes (see box) occasionally occur in the blood of normal cats. When present in blood these cells are usually described simply as NRBCs. Increased numbers of NRBCs are

#### Stages in the development of erythrocytes

Erythrocytes are produced from nucleated precursors in bone marrow. The earliest recognizable precursor is the *rubriblast*, and is followed by the *prorubricyte*, *basophilic rubricyte*, *polychromatophilic rubricyte* and *metarubricyte*. Nuclear extrusion from the metarubricyte produces a *reticulocyte*.



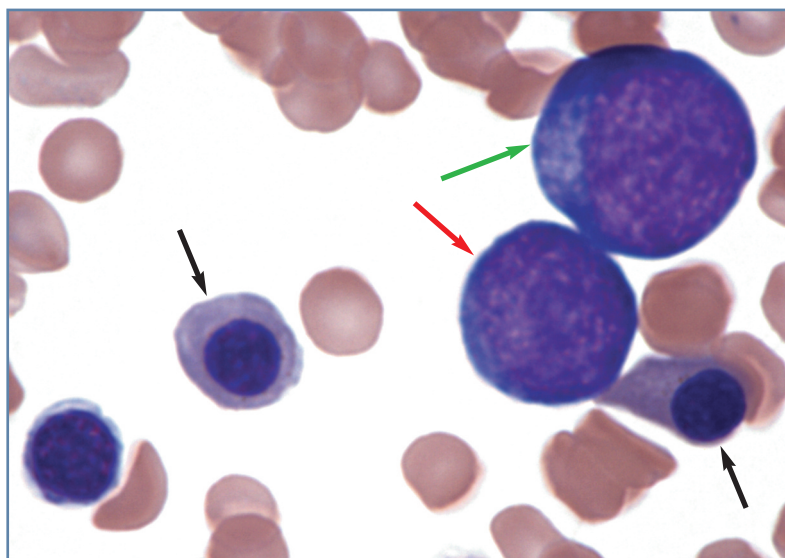
**Figure 13** Nucleated erythrocytes. (a) Polychromatophilic rubricyte and discocyte in blood from a cat. (b) Metarubricyte with nuclear extrusion (left), a polychromatophilic erythrocyte (right) and discocytes (top) in blood from a 3-week-old kitten with a regenerative response to anemia. Wright-Giemsa stain

often seen in blood in association with regenerative anemia (Figure 13); however, their presence does not necessarily indicate a regenerative response. Nucleated erythrocytes may be seen in cats with lead poisoning, with or without anemia,<sup>19</sup> and in non-anemic conditions in which bone marrow is damaged, such as septicemia, endotoxic shock and hypoxia, as well as associated with certain drug administrations.<sup>20</sup>

When nucleated erythrocyte precursors are frequent in the blood of an animal with non-regenerative anemia, conditions including myelodysplastic syndrome, erythroleukemia, infiltrative marrow disease and impaired splenic function should be considered. The presence of rubriblasts and prorubricytes in blood from an animal with non-regenerative anemia strongly suggests that a myeloid neoplasm is present (Figure 14). Nucleated erythrocytes may also be excessively large and erythrocyte nuclei may be lobulated or fragmented in cats with myeloid neoplasms.<sup>1</sup>



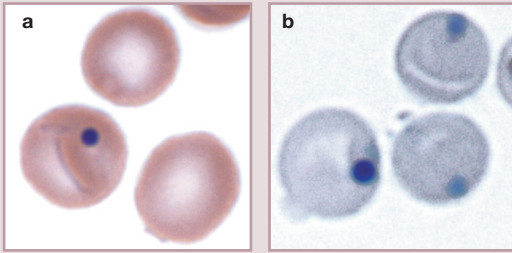
**Rubriblasts and prorubricytes in blood from a cat with non-regenerative anemia are strongly suggestive of a myeloid neoplasm.**



**Figure 14** Two metarubricytes (black arrows), a prorubricyte (red arrow) and a rubriblast (green arrow) in blood from a cat with erythroleukemia (AML-M6). Wright-Giemsa stain



## Howell-Jolly or Heinz body?



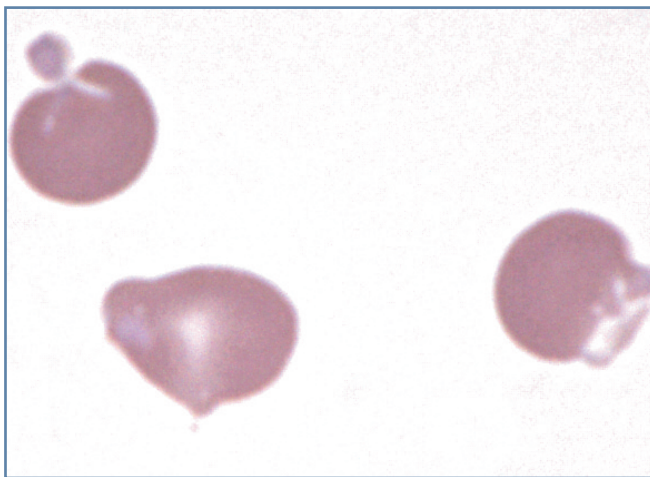
**Figure 15** Staining characteristics and appearance of Heinz bodies versus Howell-Jolly bodies in a cat with acetaminophen toxicity. (a) The erythrocyte on the top contains a barely visible, pale-pink staining Heinz body at the margin at the 7 o'clock position; in contrast, the erythrocyte below it contains a Howell-Jolly body that stains dark blue. Wright-Giemsa stain. (b) For comparison, erythrocytes from the same cat were stained with a new methylene blue reticulocyte stain. The erythrocyte on the left contains a dark-blue staining Howell-Jolly body and the other two cells each contain a lighter blue staining Heinz body. Reproduced from Harvey (2012),<sup>1</sup> with permission

**Howell-Jolly bodies and Heinz bodies are often present in low numbers in normal cat blood.**

## Erythrocyte inclusions

## Howell-Jolly bodies

Howell-Jolly bodies (micronuclei) are small, spherical nuclear remnants that appear dark blue when stained with routine Romanowsky-type blood stains (Figure 15). They form in the bone marrow, following nuclear fragmentation or rupture of the nuclear membrane, with some nuclear material left behind when the metarubricyte nucleus is expelled. In contrast to dogs, Howell-Jolly bodies are often present in low numbers in normal cat blood. These inclusions are removed or 'pitted' by the spleen as erythrocytes squeeze through interendothelial slits of the splenic sinus. Their presence in cat blood results from a poor pitting function of the feline spleen. Howell-Jolly bodies are often increased in regenerative anemias.<sup>1</sup>



**Figure 16** Heinz body hemolytic anemia in a cat with acetaminophen toxicity. The two erythrocytes on the left contain light-pink staining Heinz bodies, with prominent protrusion of the Heinz body in the top cell. The erythrocyte on the right is an eccentric erythrocyte. Wright-Giemsa stain

## Heinz bodies

Heinz bodies are inclusions composed of oxidized, precipitated hemoglobin. They attach to the internal surfaces of erythrocyte membranes. Heinz bodies stain red to pale pink with Romanowsky-type stains, in contrast to Howell-Jolly bodies, which stain dark blue (Figure 15). Heinz bodies appear lighter blue than Howell-Jolly bodies when stained with reticulocyte stains. If they bind extensively to the inner surface of erythrocyte membranes, they may be recognized as small surface projections when the membrane binds around much of an inclusion (Figure 16). When intravascular hemolysis occurs, they may be visible as red to pale pink inclusions within erythrocyte 'ghosts' (Figure 17).

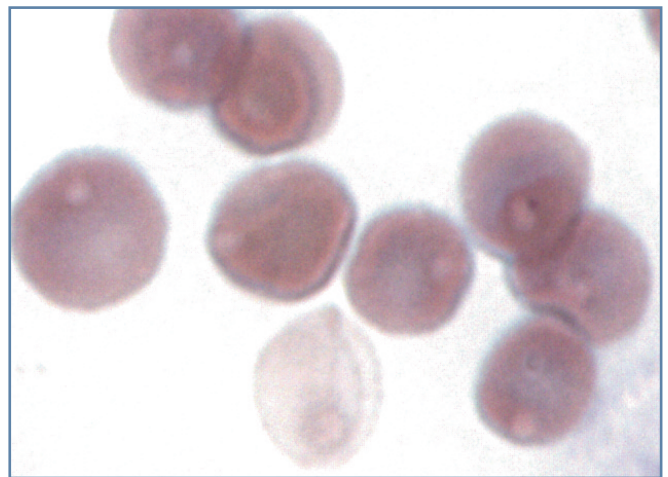
In contrast to other domestic animal species, normal cats may have up to 5% Heinz bodies within their erythrocytes. Not only is the cat spleen less efficient in the removal (pitting) of Heinz bodies from erythrocytes than are spleens of other species, but cat hemoglobin is more susceptible to denaturation by endogenous oxidants.<sup>1</sup>

Increased numbers of Heinz bodies may occur:

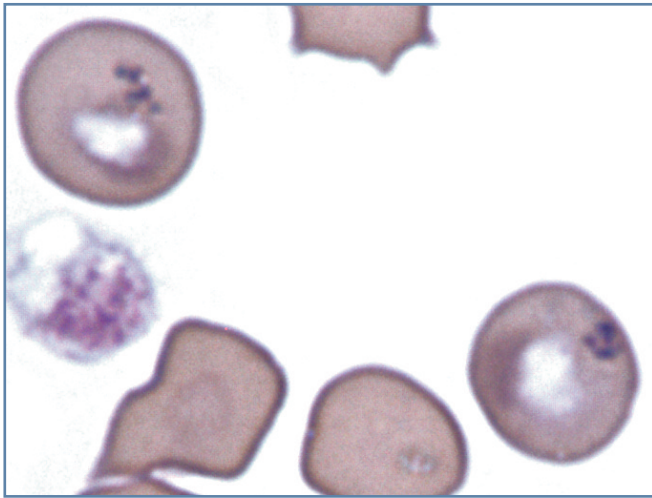
- ✦ In cats with spontaneous diseases such as diabetes mellitus (especially when ketoacidosis is present), hyperthyroidism and lymphoma;<sup>21</sup>
- ✦ In cats fed either commercial soft-moist diets containing propylene glycol or baby food containing onion powder;<sup>22,23</sup>
- ✦ In cats undergoing repeated propofol anesthesia.<sup>24</sup>

Although erythrocyte survival tends to be shortened, anemia is either mild or absent in the above conditions.

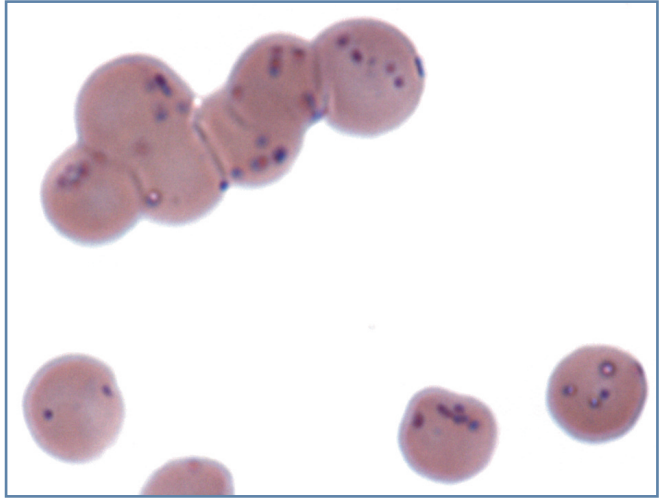
✦ Acetaminophen toxicity is the most common cause of severe Heinz body hemolytic anemia in cats. Other reported causes of Heinz



**Figure 17** Heinz body hemolytic anemia in a cat with acetaminophen toxicity. The cell at the bottom has lysed and a pale-pink Heinz body is visible in the erythrocyte 'ghost'. The remaining intact erythrocytes all contain pale-pink staining Heinz bodies. Wright-Giemsa stain



**Figure 18** Focal basophilic stippling in two erythrocytes from a cat with dyserythropoiesis and persistent mild anemia. These are called siderotic inclusions because they stained positive for iron using the Perl's iron stain. In the human literature, siderotic inclusions have been referred to as Pappenheimer bodies. A platelet (left) is also present. Modified Wright's stain. Photograph of a stained blood film from a 2012 ASVCP slide review submitted by P Cazzini, M Camus, J Messick, B Garner and K Latimer

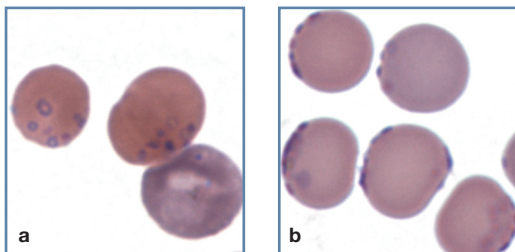


**Figure 19** Large numbers of small blue/purple-staining *Mycoplasma haemofelis* bacterial organism on the surface of erythrocytes from a cat with a natural infection. Wright-Giemsa stain

body hemolytic anemia in cats include methylene blue, benzocaine, phenazopyridine and methionine administration.<sup>1</sup>

### Basophilic stippling

Diffuse blue punctate inclusions in erythrocytes stained with Romanowsky-type blood stains are composed of aggregates of ribosomes and polyribosomes. These inclusions are similar in composition to precipitated ribosomes and polyribosomes produced using reticulocyte stains, but they form during the process of cell drying prior to staining, rather than during the staining process itself. Like other domestic mammals, diffuse basophilic stippling is often present, along with nucleated erythrocytes, in cats with lead poisoning.<sup>19</sup> Diffuse basophilic stippling commonly occurs in ruminants with regenerative anemia but it seldom occurs in cats with regenerative anemia.<sup>1</sup>



**Figure 20** Large numbers of small blue/purple-staining *Mycoplasma haemofelis* organisms on the surface of erythrocytes from two cats with a natural infection. (a) Ring forms of the organism can be seen on the surface of an erythrocyte. A polychromatophilic erythrocyte (aggregate reticulocyte) without organisms is also present (bottom right). (b) Organisms appear on the periphery of erythrocytes in a thin area of the blood film. Wright-Giemsa stain

Focal basophilic stippling is typically positive for iron, as demonstrated with the Prussian blue or Perl's iron stain. Iron-positive focal stippling has been reported in cats with dyserythropoiesis and myeloid neoplasms (Figure 18).<sup>1</sup>

### Infectious agents of erythrocytes

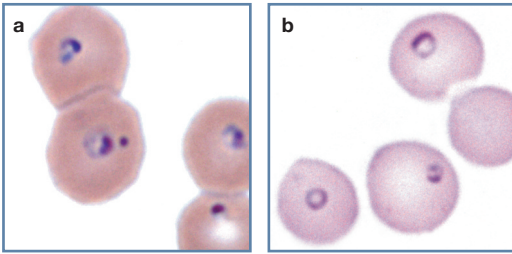
A number of infectious agents are recognized to occur on or in the erythrocytes of cats. These include epicellular hemotrophic mycoplasmas (formerly *Haemobartonella* species) and intracellular protozoal parasites (*Babesia felis* [not in the United States] and *Cytauxzoon* species). Protozoa have a nucleus within their cytoplasm. Hemotrophic mycoplasmas are bacteria and, therefore, lack nuclei. These infectious agents of erythrocytes generally cause hemolytic anemia, which may be mild to severe depending on the pathogenicity of the organism and the susceptibility of the host.

Infectious agents must be differentiated from staining artefacts, as well as from non-infectious inclusions.

### Hemotrophic mycoplasmas

Three distinctly different hemoplasmas have been identified in cats based on 16S rRNA gene sequences:

✦ *Mycoplasma haemofelis* (formerly *Haemobartonella felis*) appears as small, blue staining cocci, rings or rods on the surface of erythrocytes (Figures 19 and 20a).<sup>25</sup> Typically these organisms are observed on the periphery of erythrocytes in thin areas of the blood film (Figure 20b). These bacteria are smaller than Howell-Jolly bodies and *Cytauxzoon felis* organisms. *M haemofelis* occurs in cyclic parasitemias; consequently, it is not always



**Figure 21** Protozoal parasites in feline erythrocytes. (a) Erythrocytes containing *Cytauxzoon felis* merozoites in blood from a domestic cat with a natural infection. Organisms have a magenta staining nucleus and pale-blue staining cytoplasm. Wright-Giemsa stain. (b) Erythrocytes containing *Babesia felis* in blood from a cat from South Africa. Wright stain. Reproduced from Harvey (2012),<sup>1</sup> with permission

identifiable in blood even during acute infections. In contrast to dogs infected with *Mycoplasma haemocanis*, splenectomy or splenic pathology is not required for *M haemofelis* to be visualized in stained blood films and cause anemia in cats.<sup>25</sup>

✦ *Mycoplasma haemominutum* is rarely observed in stained blood films from infected cats. When seen, *M haemominutum* organisms appear to stain less densely and are about half the size of *M haemofelis* organisms. *M haemofelis* is an important pathogen in cats, but *M haemominutum* appears to be minimally pathogenic.

✦ *Candidatus Mycoplasma turicensis* has been documented in blood from many cats using PCR-based assays, but it has not yet been identified in stained blood films from infected cats, presumably because of low numbers of organisms present.

### *Cytauxzoon felis*

*C felis* is a protozoal organism that infects feline erythrocytes and results in high mortality in the United States (Figure 21a).<sup>26</sup> It is morphologically similar to small *Babesia* organisms. The nuclei of the merozoites in erythrocytes stain magenta or blue and their cytoplasm appears light blue. *C felis* has both a tissue phase and an erythrocyte phase. The schizonts of *Cytauxzoon* develop in macrophages that are adherent to many vessels within the body. These schizonts are generally not seen in blood films (see Part 2, Figure 18), but can easily be identified by exfoliative cytology or histopathology of tissues from infected cats.

Different *Cytauxzoon* species have been reported in domestic and wild cats outside of the Americas. These infections appear to be subclinical with persistent erythroparasitemia.<sup>27</sup>

### *Babesia* species

In contrast to *Cytauxzoon*, *Babesia* protozoa proliferate only in erythrocytes. Both large and small *Babesia* species have been reported in wild and domestic cats. Diseases associated

## Infectious agents that occur on or in erythrocytes of cats must be differentiated from staining artefacts and non-infectious inclusions.



with these organisms have not been well characterized, with the exception of *Babesia felis* (Figure 21b). This small *Babesia* species can cause a life-threatening hemolytic anemia in cats in South Africa.<sup>28</sup>

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## KEY POINTS

- ✦ There are important hematology findings that can only be determined by microscopic examination of stained blood films.
- ✦ Blood films should be prepared as soon as possible after blood sample collection to minimize artefactual changes that can distort blood cell morphology.
- ✦ When using Romanowsky-type stains, the appearance of stained blood films depends on the quality of the fixative and stains, the staining and rinse times, and the pH of the stains and rinse water.
- ✦ Stained blood films should first be scanned using a low-power objective to look for erythrocyte autoagglutination, leukocyte aggregates, platelet aggregates, microfilaria and abnormal cells that might be missed during the differential leukocyte count.
- ✦ As a quality control measure, the number of leukocytes and platelets present should be estimated to assure that the number present on the slide is consistent with the total leukocyte and platelet counts measured by the analyzer.
- ✦ Erythrocytes may exhibit rouleaux, agglutination and aggregate reticulocytes, and include Howell-Jolly bodies, Heinz bodies and basophilic stippling; nucleated red blood cells may also be present.
- ✦ Feline erythrocytes are anucleated bioconcave discs, but there may also be abnormally shaped erythrocytes present (poikilocytosis). These include echinocytes, acanthocytes, keratocytes, schistocytes, spherocytes, elliptocytes, dacryocytes, stomatocytes, eccentrocytes and erythrocytes with hemoglobin crystals.
- ✦ A number of infectious agents occur on or in the erythrocytes of cats. These include epicellular hemotrophic mycoplasmas and intracellular *Cytauxzoon* and *Babesia* protozoal parasites.



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