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Hematologic Evaluation of Reptiles: A Diagnostic Mainstay

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With their increasing popularity as pets, reptiles are becoming routine patients in more veterinary practices. Just as with cats and dogs, basic hematologic evaluation is a mainstay of diagnosis for reptiles, and technicians who can conduct these tests are becoming a vital part of daily operations in many clinics.

As with any pet, reptiles should undergo regular checkups to safeguard their health and well-being. One of the basic parameters used in these evaluations is the complete blood count (CBC). Veterinary technicians should develop proficiency in interpreting reptilian hematologic findings so that this information can be used to assist the veterinarian in making a diagnosis.

Blood Collection

Reptiles vary in size from the tiny green anole to the formidable Nile crocodile and can weigh from 3 g to 700 kg. Approximately 0.3 mL of blood is required to perform a manual reptilian CBC. The blood volume in reptiles is approximately 5% to 8% of their body weight, and approximately 10% of the total blood volume of a healthy patient can be collected.[1] For example, a snake weighing 100 g has a total blood volume of 5 to 8 mL, 10% of which (0.5 to 0.8 mL) can be safely drawn from the patient.[2] For patients that weigh <50 g, one drop of blood can be used for a blood smear; then the Natt-pette Test Kit (Exotic Animal Solutions, LLC, [www.exoticanimalsolutions.com] Melbourne, FL) can be used to perform a manual CBC because it requires only 5 µL of blood (**BOX 1**).

The collection site should be disinfected with either alcohol or diluted chlorhexidine solution (3 oz of chlorhexidine in 1 gal of water). The choice of needle size and syringe depends on the size of both the reptile and the blood vessel. For smaller patients or if a slow draw is anticipated, the syringe can be heparinized with lithium heparin to help reduce the likelihood of hemolysis and prevent clotting. This can be accomplished by drawing just enough heparin through the needle to "rinse" the needle hub. This reduces the chance of sample dilution while allowing enough anticoagulant to prevent clotting. However, allowing cells to remain in sodium heparin for too long can cause thrombocyte and leukocyte aggregation.[3] Ethylenediaminetetraacetic acid anticoagulant should be chosen as a last resort because it causes hemolysis in certain reptile species, such as sea turtles and iguanas.[2,4]

Box 1. Manufacturers of Manual Counting Solutions and Kits

ENG Scientific, Inc.
PO Box 1589, 82 Industrial East, Clifton, NJ 07012
800-922-0223, www.engscientific.com
Product: Natt-Herrick solution (4-oz bottle)

Exotic Animal Solutions, LLC
PO Box 410104, Melbourne, FL 32941
800-268-8189 or 205-461-4163, www.exoticanimalsolutions.com
Products: Natt-pette (Natt-Herrick solution) Test Kit; Eopette (eosinophil) Test Kit

Vetlab Supply, Inc.
18131 SW 98th Ct., Palmetto Bay, FL 33157
800-330-1522, www.vetlab.com
Product: Leukopet kit

Glossary

Azurophils—cells found only in reptiles; resemble reptilian monocytes but often are smaller with an irregular shape

Hemacytometer—a device used in manual blood cell counts, consisting of a microscopic slide with a depression where the base is marked in grids and into which a measured volume of a sample of blood is placed; the number of cells and formed blood elements in the squares is counted under a microscope and used as a representative sample for calculating the unit volume

Hemipenes—paired, vascular, eversible sacs that open into the posterior cloaca in snakes and lizards

Phlebotomy—needle puncture of a vein for the drawing of blood

Scutes—any squama or scale-like structure, especially one of the thick epidermal plates on the head of snakes or the shell of a tortoise

Sinus—anatomic nomenclature for a cavity, channel, or space, such as a venous sinus

Wright-Giemsa stain—solution containing azure II–eosin, azure II, and glycerin for differential staining of blood smears and viral inclusion bodies; stained elements appear pink to purple to blue

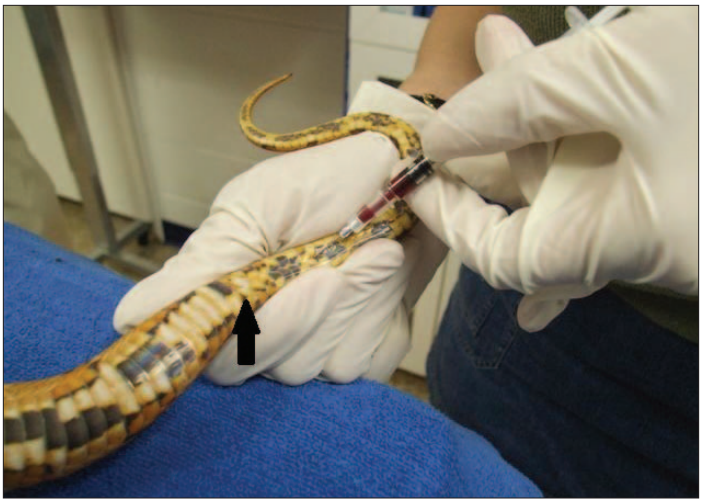

Figure 1. Blood collection from the ventral coccygeal vein of a snake. The *arrow* points to the cloacal opening.

Figure 2. Blood collection from the occipital sinus of an aquatic turtle.

Snakes

In snakes, the collection site depends on the type and size of the snake and the availability of sedatives or anesthetics. For large, unsedated snakes, the preferred collection site is the tail (ventral coccygeal) vein, with the needle inserted between the scutes (the scales). Because this vessel narrows as it descends from the cloaca, blood should be collected as close to the cloaca as possible. In male snakes, however, the insertion site must be far enough away from the cloaca to avoid puncturing the hemipenes or musk glands[2] (**FIGURE 1**). Small snakes can be supported in a vertical position with the head up to facilitate collection by promoting "pooling" of blood in the tail area. For large snakes, only the tail portion can be comfortably held in a vertical position.

Alternatively, blood can be collected from snakes through cardiac puncture. This works well for small snakes or if large samples are required. Brief sedation with sevoflurane is generally recommended because of the mobility of the heart.[2] Some practices might choose to use isoflurane, but it takes effect more slowly than sevoflurane in reptiles. The heart is located about one-third of the way down the snake's body. It is best to restrain snakes in dorsal recumbency to visualize heartbeats on the ventral side. Snakes with dark pigmentation may require the use of Doppler ultrasonography to locate the heart. Once it has been located, the heart can be marked with a piece of tape or a surgical marker. The heart should then be held firmly in position with one hand or held by an assistant and the site swabbed with alcohol or diluted chlorhexidine. With the other hand, the needle should be inserted into the heart ventrally between the scutes. If the needle is not placed into the heart on the first attempt, it should be removed completely before redirecting it in order to prevent dire consequences.

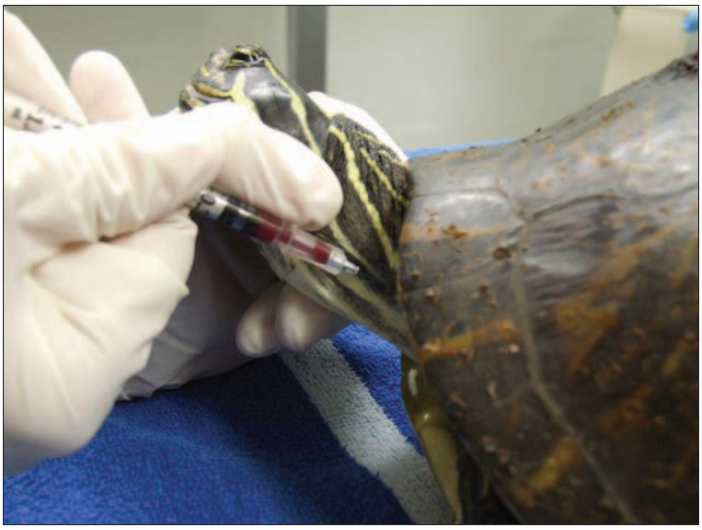

Figure 3. Blood collection from the jugular vein of an aquatic turtle.

Turtles and Tortoises

For turtles and tortoises, there are four common locations for phlebotomy: the occipital sinus, jugular vein, dorsal coccygeal venous sinus, and subvertebral venous sinus. The occipital sinus, which is commonly used in aquatic turtles, is located dorsolateral to the cervical vertebrae on the right side of the neck and caudal to the base of the skull (**FIGURE 2**). The head is held firmly, and the needle is inserted at a 30° angle. Pulling the head outward and slightly downward helps to visualize the base of the skull and the spinous process.

For larger sample requirements, the jugular vein can be used in aquatic turtles. If access is hindered by the strength of the neck, sedation with a gas anesthetic may be required. The needle and syringe should be placed parallel to the vein, with the needle inserted in the direction of venous blood flow, which is away from the head (**FIGURE 3**).

Although the dorsal coccygeal sinus is an alternative collection site, this small vessel is not ideal for large-volume sampling (i.e., 1 mL or more). The vessel is easily accessible with a 25-gauge or

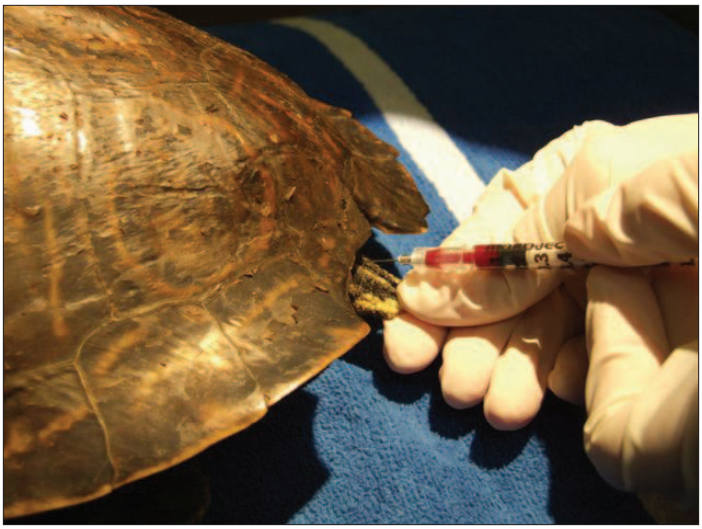

Figure 4. Blood collection from the dorsal coccygeal sinus of an aquatic turtle.

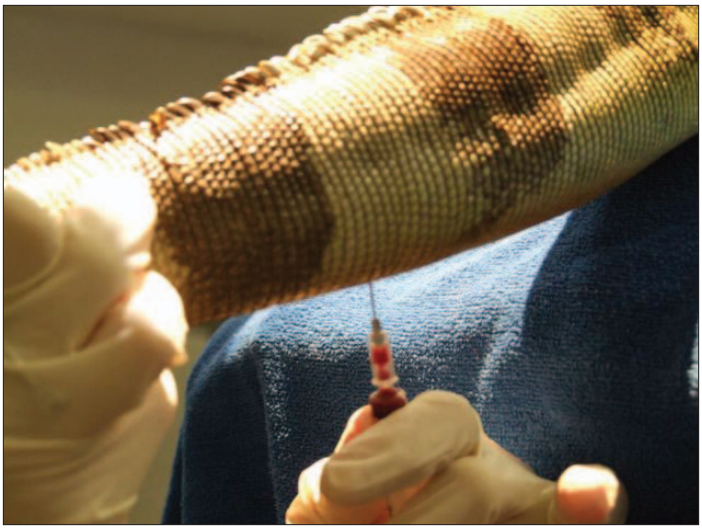

Figure 6. Blood collection from the coccygeal vein (ventral approach) of an iguana.

Figure 5. Blood collection from the subvertebral sinus in an aquatic turtle. The needle, which is being withdrawn, is pointing to the collection site. For collection, the needle is inserted more parallel to the carapace.

Figure 7. Blood collection from the coccygeal vein (lateral approach) of an iguana.

tuberculin needle, and sedation is not required (**FIGURE 4**). The turtle can be placed on a surface with a diameter smaller than the ventral shell; then the tail is pulled out and held firmly with one hand while blood is collected using the other hand.

The subvertebral sinus (also known as the *subcarapacial sinus*) is also commonly used for collection. It is considered a sinus because several blood vessels are grouped in this location at the level of the eighth cervical vertebra, just caudal to the nuchal scute.[2] This site should be used for collection only as a last resort because of the risk for sample contamination from the lymphatic vessels.[5] The advantage of using this site is that blood can be drawn whether the head is extended or retracted, meaning that no sedation is required (**FIGURE 5**).

The brachial vein, which is located in the forelimb, is not commonly used for phlebotomy because it requires a "blind stick" and the sample can frequently be contaminated with lymph.[2] The advantage of collecting blood from the brachial vein is that the leg can be extended and a sample obtained while the patient's head is retracted into the shell. However, extending the limb in certain chelonians can be challenging.

Lizards

In large lizard and crocodilian species, blood can be collected from the ventral coccygeal vein with minimal restraint. (The occipital sinus is commonly used for crocodilian species.) If necessary, cotton balls can be placed over the closed eyelids and secured with an elastic bandage around the head. This restraint technique calms the patient without the use of chemical restraint. This technique can also be used for other procedures, such as radiography and physical examination. The coccygeal vein, which lies just beneath the vertebrae, can be accessed laterally or ventrally. For the ventral approach, the tail can be held horizontally and draped off the end of the examination table (**FIGURE 6**). The needle is then inserted

perpendicular to the vein between the scales and distally enough from the cloaca in males to avoid the hemipenes. Some clinicians prefer the lateral approach, in which the needle is inserted midway between the dorsal and ventral sides of the tail, where a natural groove or midline often serves as a landmark (**FIGURE 7**). Caution must be used when collecting blood from tails of large lizards, as they often use their tails for defense and can injure the phlebotomist. Caution must also be used in lizard species that can "drop" their tail as a defense mechanism (i.e., caudal autotomy), such as geckos. Blood should be collected from an alternate location to prevent these species from self-amputating.

The central abdominal vein can be used for blood collection in small lizard species and can be visualized as a dark line along the midline of the ventral abdomen in lizards with light pigmentation (e.g., leopard geckos, bearded dragons). The disadvantages of this site are that the vein can be easily lacerated and there is a risk for hematoma formation because pressure cannot be easily applied after venipuncture. Using cotton-tipped applicators may help to focus pressure directly on the puncture point.

Smear Preparation and Staining

Blood smears should be made immediately after blood collection (before hemoparasites can leave the blood cells).[6] Regardless of the technique used, a thin monolayer is needed for proper evaluation.

The common 30° angle or "push" smear technique, which works well for mammals, may cause reptilian blood cells to rupture.[7]

Box 2. Methods for Estimating the White Blood Cell Count

Method 1[12,13]

Using the 40× objective

Total WBC estimate per microliter =
Average number of WBCs counted in 10 fields × 2000

Method 2[2,15]

Using the oil immersion (1000×) objective

Total WBC estimate per mm^3 =
Average number of WBCs per five fields ÷ 1000 × 3,500,000

This method is used if the PCV is within normal limits. If the reptile is anemic, a corrected count must be conducted using the following formula:

Corrected total WBC estimate per mm^3 =
Estimated WBCs × Observed PCV ÷ Normal PCV (45%)

PCV = packed cell volume; *WBC* = white blood cell

The coverslip method avoids this problem but uses a smaller drop of blood, representing a smaller portion of the circulating blood than the 30°-angle method. In addition, the coverslip may be difficult to stain if a Coplin jar is not used for dipping.

The parallel (slide-to-slide) smearing technique uses a larger drop of blood and works well for all nonmammalian species. This technique requires practice, but once it has been perfected, there is minimal distortion or rupturing of the cells. The blood is placed

Table 1. Normal Hematologic Values for Reptiles Commonly Seen in Practice[a–e]

	Box Turtle (*Terrapene carolina*)	Red-Eared Slider (*Trachemys scripta elegans*)	Green Iguana (*Iguana iguana*)	Red-Tailed Boa (*Boa constrictor*)	Ball Python (*Python regius*)	Bearded Dragon (*Pogona vitticeps*)
WBCs (× 10^3/µL)	5.1 (+2.339)	3.2–25.5	3–14	4–10	7.9–16.4	6–15
RBCs (× 10^6/µL)	0.49 (+0.57)	0.3–0.8	1.5–3.5	1.0–2.5	0.3–1.3	0.8–1.2
Hct (%)	25.6 (±3.2)	25–33	30–45	20–40	16–21	24–36
Hb (g/dL)	5.0 (±0.2)	8.0	6–12.2	3.3–15.3	5.5–7.9	8.4–11.4
Heterophils (%)	41–44	36	40–70	20–65	56–67	—
Lymphocytes (%)	26–28	24	20–45	10–60	7–21	54–76
Monocytes (%)	0–4	0–1	0–2	0–3	0–1	0–8
Azurophils (%)	0–6	3–4	—	0–6	12–22	—
Eosinophils (%)	25–30	11	0–1	0–3	—	—
Basophils (%)	0–3	25–27	0.2	0–20	0.2	—

Hb = hemoglobin; *Hct* = hematocrit; *RBCs* = red blood cells; *WBCs* = white blood cells.

[a]Mean values or ranges are listed; standard deviations are in parentheses. Reptile hematology results vary depending on the season of the year, sex, breeding condition, whether the species hibernates, and seasonal diet changes.

[b]International Species Inventory System (ISIS) physiologic normals from the Los Angeles Zoo.

[c]Campbell T, Ellis C. Appendix B. In: *Avian and Exotic Animal Hematology and Cytology.* 3rd ed. Ames, Iowa: Blackwell Publishing; 2007.

[d]Johnson-Delaney C, Harrison L. *Exotic Companion Medicine Handbook for Veterinarians.* Reptile section. Lake Worth, FL: Wingers Publishing; 1996.

[e]Ellman M. Hematology and plasma chemistry of the inland bearded dragon, *Pogona vitticeps. Assoc Reptilian Amphib Vet* 1997;7(4):10-12.

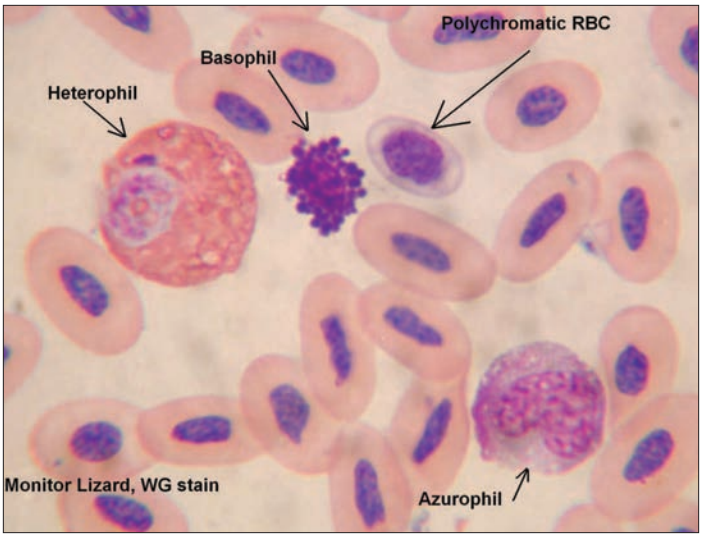

Figure 8. Blood smear from a monitor lizard (*Varanus* sp). Note the size, shape, and color of the polychromatic erythrocyte. (Wright-Giemsa stain, original magnification ×1000)

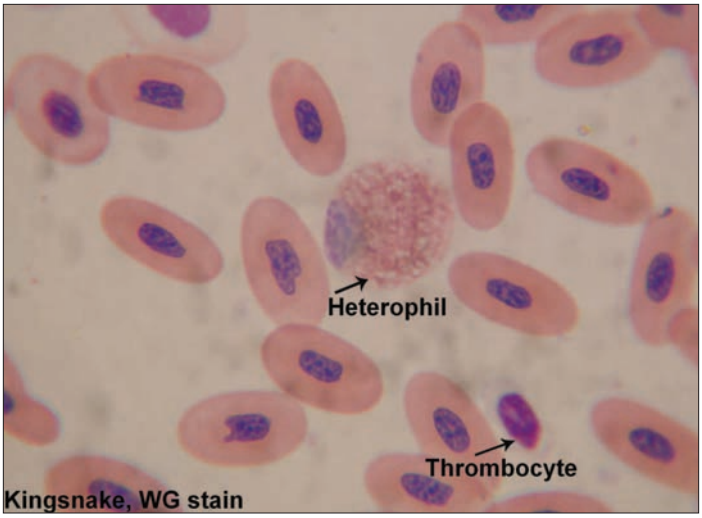

Figure 9. Blood smear from a kingsnake (*Lampropeltis* sp). Thrombocytes are often in clusters. The heterophil in the center is densely packed with granules, making the granular shapes almost indistinguishable. (Wright-Giemsa stain, original magnification ×1000)

in the center of a slide with another slide on top, and the slides are gently pulled apart. The cells are evaluated from the center of the smear rather than from the feathered edge as with the 30°-angle technique.[7]

Rapid (quick) stains or Wright-Giemsa stain can be used with little time or labor. Rapid (quick) stains work well, but some brands do not adequately stain erythrocyte nuclei. They also do not stain the granules of basophils; the basophils are visible, but the granules may appear ghost-like, or the cells may take on a spiderweb appearance. These cells are often mistaken for smudged cells.[8] By contrast, Wright-Giemsa stain, which is preferred, takes longer than quick stain methods but stains all

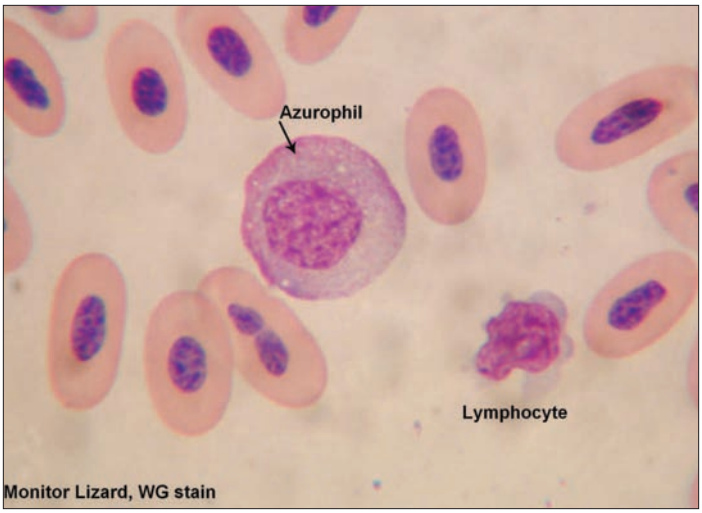

Figure 10. Blood smear from a monitor lizard (*Varanus* sp). Azurophils (*center*) have a round to oval nucleus and blue cytoplasm and contain very fine azurophilic granules. Lymphocytes are round to irregularly shaped, have an eccentric nucleus with dense chromatin, and have a large nuclear:cytoplasmic ratio. (Wright-Giemsa stain, original magnification ×1000)

cells well. Wright-Giemsa must be filtered regularly because of the formation of precipitate.

Methods of Analysis

Because reptiles have nucleated erythrocytes, accurate results cannot be obtained from commercial automated cell counters. Therefore, CBCs must be done manually.

The Phloxine B Method

The phloxine B method is an indirect counting method, so a differential analysis must be performed to obtain a white blood cell (WBC) count. However, this method is not recommended for conducting a WBC count in certain species of reptiles because studies have shown discrepancies between results of the phloxine B method and the direct WBC count in sea turtles.[9] A red blood cell count cannot be obtained using the phloxine B method. The historically used Eosinophil Unopette #5877 test (Becton Dickinson, Rutherford, NJ) is no longer available. However, the Eopette Test Kit (Exotic Animal Solutions, LLC) and the Leukopet kit (Vetlab, Inc., Palmetto Bay, FL) are now commercially available.

The Natt-Herrick Method

The Natt-Herrick method (Natt-pette Test Kit, Exotic Animal Solutions, LLC) requires only 5 µL of blood (versus 25 µL of blood for the Eosinophil Unopette #5877 test). The Natt-Herrick method is a direct counting method[3] that can be used to obtain WBC and red blood cell counts. One disadvantage of this method is that blood cells tend to aggregate in the hemacytometer chamber. This can be avoided by charging the hemacytometer immediately after mixing the blood and stain.[3] It may be difficult to distinguish lymphocytes from thrombocytes when counting. Thrombocytes tend to stain light purple and may have a "fluffy" appearance,

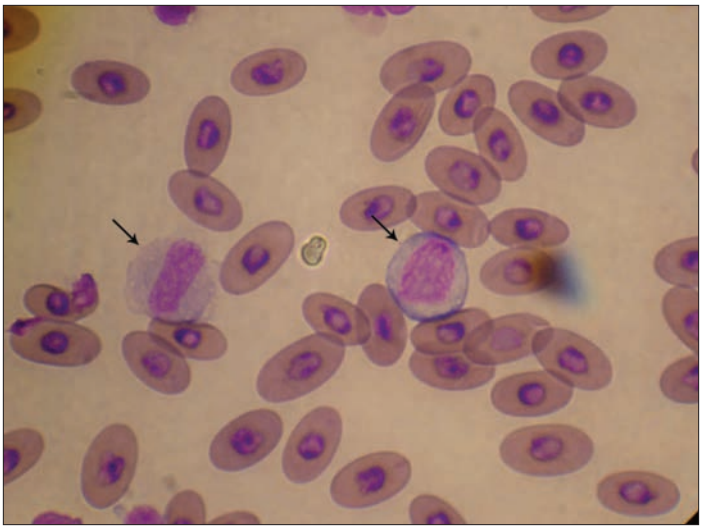

Figure 11. Blood smear showing monocytes (*arrows*) from a green iguana (*Iguana iguana*).

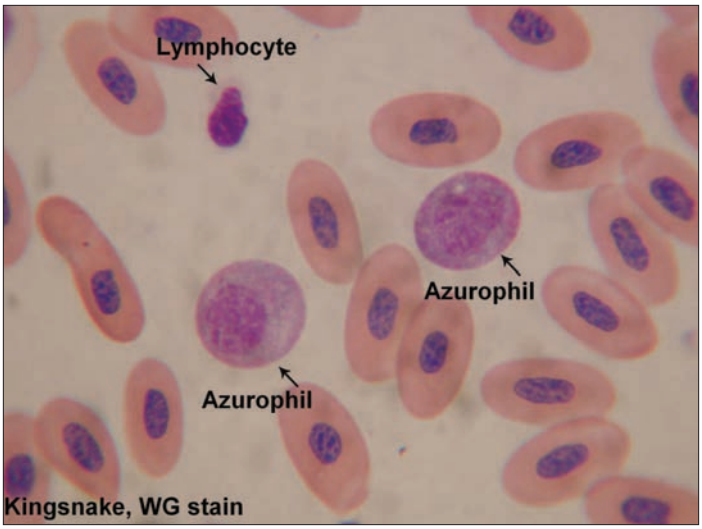

Figure 12. Blood smear from a kingsnake (*Lampropeltis* sp) showing two azurophils and a lymphocyte. (Wright-Giemsa stain, original magnification ×1000)

whereas lymphocytes tend to be round and stain dark purple. The ability to differentiate these cells comes with experience.

The Estimated WBC Count

An estimated WBC count (**BOX 2**) is recommended only if the sample size is insufficient for testing by any other means.[2,10–13] Many factors can render the estimated WBC count inaccurate. The blood smear must be of the highest quality (i.e., a good monolayer, no clots, and few smudged cells). Accurate counts can be obtained only if the sample is not diluted by anticoagulant. This method works primarily for establishing a "ballpark" high or low WBC count. It is not recommended for establishing normal values for a species.[2]

Differential Evaluation

After the blood smears have been stained and calculations completed, the differential evaluation can begin. If a clinician is evaluating a species for the first time, it is best to scan the slide and try to identify the cell types before obtaining the differential count. The morphology of reptilian blood cells can be quite challenging, with great variation among species. In addition, different reference texts classify the cell types differently. **TABLE 1** lists normal hematologic values for common reptile patients.[2,14]

The erythrocytes of reptiles are round to oval, with a nucleus that stains dark purple. The nuclear chromatin pattern becomes denser and the cytoplasm less basophilic as the cell ages.[2] Reptiles occasionally have immature polychromatic erythrocytes in the peripheral blood that may be mistaken for azurophils or monocytes (**FIGURE 8**). These cells are rounder and more basophilic than more mature erythrocytes.

Thrombocytes typically appear as small cells that are oval to spindle-shaped, with a central, dark purple nucleus (**FIGURE 9**). In some snake species, these cells may appear similar to airplane propellers. Thrombocytes are often confused with lymphocytes because they are generally the same size. However, thrombocytes may be identified in small clusters on the slide, and their cytoplasm tends to be colorless to pale blue. Activated thrombocytes tend to be clustered, with decreased cytoplasm, vacuoles, and irregular cytoplasmic borders.[2]

Nongranulocytic White Blood Cells

As with mammalian cell types, nonmammalian WBCs can be divided morphologically into granulocytes and nongranulocytes (i.e., mononuclear cells).[2] Reptile lymphocytes are nongranulocytes and may be the predominant WBC in some species, such as green iguanas. Mature lymphocytes are round and have an eccentric nucleus with a chromatin pattern that is dark, clumped, and dense. The cytoplasm of lymphocytes is pale blue but darker than that of thrombocytes. Lymphocytes have a larger nuclear-to-cytoplasmic ratio than that of thrombocytes; therefore, the nucleus of lymphocytes takes up most of the cell.[7] Lymphocytes and thrombocytes may have an occasional azurophilic granule (**FIGURE 10**).

Monocytes are nongranulocytes and tend to be the largest WBCs in the peripheral blood smear (**FIGURE 11**). The nuclear shape is usually lobed but may appear round. The nuclear chromatin pattern is lighter and lacier than that of lymphocytes. The cytoplasm is typically battleship gray and may have vacuoles or a foamy appearance.[2,7]

Azurophils are found only in reptiles, and there is some debate as to the classification of these cells; they are counted as separate cells in the differential evaluation. They resemble reptilian monocytes in many ways but differ in that they are irregularly shaped and often smaller. The nucleus is round to oval with a coarse chromatin pattern and may occasionally have two lobes (bilobate).[15] The cytoplasm is darker blue than that of monocytes and contains very fine azurophilic granules. Azurophils may also have vacuoles (**FIGURE 12**).

Reptiles may occasionally have plasma cells in their blood. The plasma cell nucleus is round and eccentric and has a very

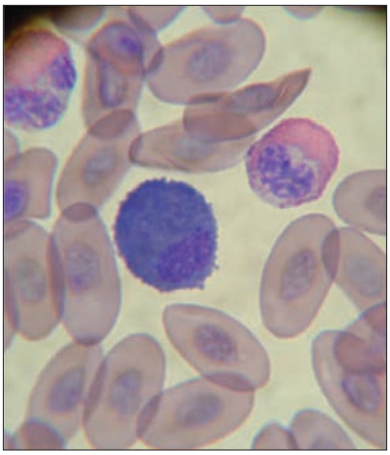

Figure 13. Blood smear from a green iguana (*Iguana iguana*). In some species of reptiles, eosinophils contain blue granules (*center*). Two heterophils are also present. (Dip Quick Stain [Jorgensen Laboratories, Inc., Loveland, CO], original magnification ×1000)

clumped and coarse chromatin pattern. Plasma cells are similar in size to azurophils and may be mistaken for them. Unlike azurophils, however, plasma cells have dark blue staining in the cytoplasm and do not contain granules. Plasma cells also have a "halo," called the *Golgi apparatus*, surrounding the nucleus.[2]

Granulocytic White Blood Cells

Reptilian granulocytes are predominantly heterophils. Heterophil granules are usually refractile, appearing bright orange to brick red (**FIGURE 8**; **FIGURE 9**). The granules can be round, rod-shaped, or spindle-shaped, depending on the species.[2] Heterophil nuclei are eccentric and vary from round to lobed, with a dense chromatin pattern. Heterophil cytoplasm is usually colorless and contains granules.

Eosinophils are granulocytes with a round to oval nucleus that is slightly eccentric. Some lizard species have eosinophils with lobed nuclei. The chromatin pattern is usually dense. Unlike heterophils, eosinophils have light blue cytoplasm in which most of the granules tend to be round. The granule color varies among species[15]; for example, in green iguanas, the granules are blue (**FIGURE 13**), whereas in some tortoises (e.g., the gopher tortoise), the granules are bright orange (similar to heterophils). In sea turtles (e.g., the loggerhead), the granules are brick red and sparse, and the cells may appear to be partially degranulated heterophils. (Mammalian eosinophils stain red.)

Basophils are generally the easiest granulocyte to identify. They have the typical round shape and are smaller than heterophils and eosinophils. The nucleus is generally round, with a dense chromatin pattern. It is often difficult to see the nucleus because of the abundance of granules in the cytoplasm. Some basophils are easily distorted on a smear, clearly revealing the nucleus (**FIGURE 14**). The granules stain very dark purple and may appear as smudged cells. Basophil granules do not stain with some of the Quik-Dip methods; if this is the only stain available, fixing the slide with methyl alcohol for 1 minute before staining and allowing the slide to dry may help prevent the granules from dissolving.[2] By contrast, Wright-Giemsa stain delineates the granules clearly.

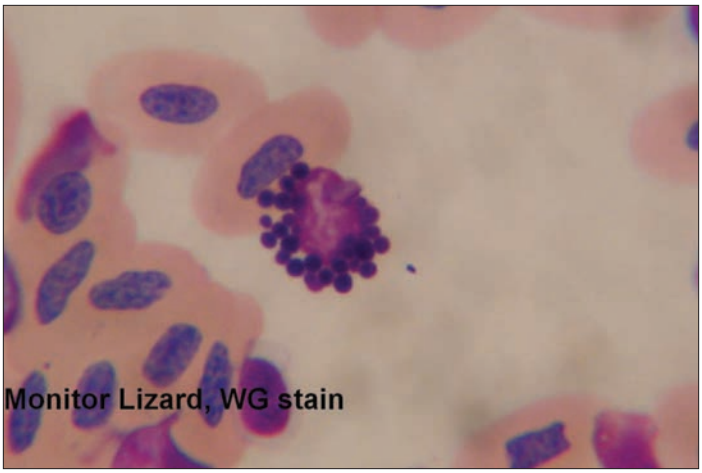

Figure 14. Blood smear from a monitor lizard (*Varanus* sp). Slight distortion of the basophil (*center*) reveals the nucleus and granules. (Wright-Giemsa stain, original magnification ×1000)

Conclusion

The veterinary technician's ability to conduct in-house hematologic diagnostic procedures can be vital to the practice. Most veterinary practices can treat reptile patients but must send samples to outside laboratories and then wait for the results. Veterinary technicians who can conduct in-house blood tests for critically ill reptile patients obtain results in minutes, benefiting the patient and the practice.

Acknowledgments

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1. **What percentage of a reptile's body weight is its blood volume?**
   - a. 2% to 8%
   - b. 3% to 5%
   - c. 5% to 8%
   - d. 8% to 10%

2. **Which anticoagulant causes leukocyte aggregation if the sample is allowed to sit too long?**
   - a. sodium heparin
   - b. ethylenediaminetetraacetic acid
   - c. sodium citrate
   - d. lithium heparin

3. **When reptilian blood smears are processed,**
   - a. the smear should be prepared immediately after collection.
   - b. the blood should first be placed in the anticoagulant to prevent cell rupture.
   - c. any standard smearing technique can be used because reptilian blood cells are not easily damaged.
   - d. the blood can be refrigerated for 4 to 6 hours without deterioration.

4. **One limitation of Eopette analysis is that it**
   - a. only stains thrombocytes.
   - b. is an indirect counting method.
   - c. precludes differential evaluation.
   - d. distorts the granulocyte count.

5. **When the Natt-Herrick method is used,**
   - a. thrombocytes stain a darker purple than lymphocytes do.
   - b. the cells must remain in the stain for at least 10 minutes before the hemacytometer is charged.
   - c. the cells have a tendency to aggregate in the hemacytometer chamber.
   - d. the sample must contain at least 25 µL of blood.

6. **Which of the following blood cell counting methods should be reserved as a last resort?**
   - a. the Natt-pette Test Kit
   - b. toluidine blue staining
   - c. an estimated WBC count
   - d. the Eopette method

7. **Which of the following blood cells is often confused with thrombocytes when the Natt-pette Test Kit is used?**
   - a. monocytes
   - b. basophils
   - c. erythrocytes
   - d. lymphocytes

8. **Which blood cell is unique to reptiles?**
   - a. the heterophil
   - b. the azurophil
   - c. the basophil
   - d. the lymphocyte

9. **Reptilian heterophils**
   - a. are classified as nongranulocytic.
   - b. always have pale blue cytoplasm.
   - c. are classified as granulocytes.
   - d. none of the above

10. **Which of the following is true regarding reptilian lymphocytes?**
    - a. They are often confused with reptilian thrombocytes.
    - b. They may contain azurophilic granules.
    - c. The nucleus takes up most of the cell.
    - d. all of the above