Exotics

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AVIAN BLOOD COLLECTION AND HEMATOLOGY

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Presenter Qualification

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Paper Text

Introduction

Anatomic differences necessitate the modification of venipuncture techniques between avian species and selection of appropriate blood collection sites. Moreover, a venipuncture site in a individual patient is chosen after consideration of a number of important factors. The volume of blood required dictates whether a relatively small or large blood vessel is sampled. Patient status, from healthy to critical, influences how the patient is restrained and thereby affects the vein that is selected. History of recent venipuncture in a particular vessel may preclude the clinician from sampling the same vessel again. Risk of hematoma formation varies between blood collection sites and should be considered. Coagulopathies may be associated with certain diseases, including liver failure and aflatoxicosis, and may influence the choice of blood collection site. Furthermore, for veterinarians who perform phlebotomy in an examination room with the bird's owner present, the client's perception and acceptance of the venipuncture site must be considered. Finally, the clinician's experience also plays a role in the site selected. Several common and uncommon blood collection sites will be described.

In-clinic blood testing is steadily gaining popularity as veterinary practices continue to recognize its tremendous value. For avian patients, the turnaround time from outside laboratories is often longer than these patients can afford. An immediate diagnosis aided by the in-clinic laboratory, during the initial 30-minute exam, allows the veterinarian to determine, within minutes, whether the patient requires hospitalization or outpatient treatment. The doctor can easily prescribe medication based on scientific conclusions, not speculation. As a result, the hospital is able to practice better medicine, provide a more effective emergency service, and overall provide superior, faster service.

Additionally, the in-house lab results offer an instant prognostic indicator. Most clients consider their pets valued family members. The sooner the animal can be diagnosed and the owner given a prognosis, the less anxiety the client feels waiting for test results. Whether the prognosis is good or bad, the in-house lab helps decrease the client's stress level by reducing wait time and ambiguity about their pet's fate, and replaces "educated guesswork" with solid diagnostic capability. Especially with exotics that often present in critical condition, any means to expedite results is of paramount importance to a successful outcome.

Two extremely important components of blood testing in birds and exotics include the complete blood count (CBC) and biochemistry analysis. Together, these two pieces of data yield a comprehensive glimpse into the patient's state of health or disease. However, many of the commonly kept companion exotics are quite small compared to traditional species such as dogs and cats. Small body sizes restrict the veterinarian to withdrawal of small blood sample sizes, which can be a limiting factor for performing both the CBC and biochemistry analysis. With the advent of some modern chemistry analyzers, which require only 0.1 cc of whole blood, testing even the smallest exotic patient, through hematology and biochemical analysis, has become a reality whereas this was not possible before.

Blood Collection

A general rule of thumb for phlebotomy in avian and exotic patients is that 1 mL of blood can be safely removed per 100 grams of body weight. This equates to removal of 1% of a patient's body weight, or 10% of its total blood volume. For example, a parakeet weighing 30 grams can have 0.3 mL of blood taken; a parrot weighing 300 grams can have 3 mL of blood taken. Patient status must be taken into account; severely debilitated or anemic patients may not withstand phlebotomy (or even restraint) and should be handled with care. With the advent of modern blood analyzers, the avian practitioner now has the luxury of considering ancillary blood tests, including both a complete blood count (CBC), serum biochemistry panel, and so forth, in patients as small as budgerigars and canaries.

Venipuncture Techniques

In most birds, jugular venipuncture is the most accessible and reliable technique for obtaining adequate blood samples. A useful tip for this procedure involves entry of the needle with the bevel facing down. This approach has the following two advantages: 1) it allows one to "tent" up

the blood vessel, allowing expansion of the jugular vein and engorgement of the vessel; and 2) there is less risk of puncturing the medial wall of the jugular, as the distance the needle must be inserted is comparatively less when the bevel is oriented down.

Alternate venipuncture sites include the basilic or ulnar vein (hematoma formation is likely, especially in awake patients, so prolonged pressure is needed in this location) and the medial metatarsal vein (prominent in pigeons and waterfowl). Toenail clips are generally discouraged as sample contamination can lead to altered biochemistry or DNA probe results and micro-clotting or hemolysis may occur.

Blood Handling

Following collection of the sample, the blood must be properly stored or preserved to prevent artifactual changes and enable diagnostic accuracy. Multiple sizes of blood containers are available; for small blood samples under 1 mL, 0.5 mL-capacity containers may be used. The proper anti-coagulant to be used will be discussed in lecture; it is usually dependent on the diagnostic tests that will be performed.

Blood Smear Preparation

A stained blood smear is an essential part of the hematologic examination. Blood films are made using blood with or without anticoagulant and using a variety of techniques. Standard push-slide methods are often used; alternatively, blood films can be made by using a slide and coverslip, or two coverslips. Blood-cell stains include Wright, Wright-Giemsa, Wright-Leishman, and May Grünwald-Giemsa staining methods. Quick stains or modified Wright stains (i.e. Diff-Quik, Dade Diagnostics or Hemacolor, Miles Laboratories) are the most commonly used stains in veterinary practice settings.

Evaluating Blood Smears

The major differences between mammalian and avian hematology include the presence of nucleated erythrocytes, thrombocytes, and heterophil granulocytes in the blood of birds. Reading the slide provides the opportunity to determine the differential leukocyte count which is a key component in calculating the total white blood cell count. Additionally, it allows assessment of pathologic abnormalities of the various blood cells.

Erythrocyte: Flattened, elliptical cell with an elliptical, centrally positioned nucleus. Nuclear chromatin is uniformly clumped and becomes increasingly condensed as the cell ages.

Lymphocyte: Typically round cells that often show cytoplasmic irregularity. Round, occasionally indented, centrally or slightly eccentrically positioned nucleus. Nuclear chromatin is heavily clumped. Cytoplasm is typically scant, giving a high nucleus to cytoplasmic ratio, and typically appears homogenous and weakly basophilic (pale blue) and lacks vacuoles or granules.

Monocyte: Largest leukocyte in avian blood. Vary in shape from round to amoeboid. Nucleus may vary in shape from round to lobed, and is relatively pale with less chromatin clumping when compared to lymphocytes. Cytoplasm is abundant, blue-gray in color, and may appear slightly opaque. Vacuoles may be present.

Heterophil: Functionally equivalent to the mammalian neutrophil, and the most abundant granulocyte. Nucleus of the mature heterophil is lobed (2-3 lobes) with coarse clumped purple-staining chromatin. Cytoplasm appears colorless and contains granules that stain an eosinophilic color (dark orange to brown-red). The shape, size, and general morphology of the granules vary among the different avian species. Typically these granules appear rod-shaped but may appear oval to round as well. Immature, toxic, and band heterophils may be present.

Eosinophil: Similiar in size to heterophil. Nucleus is lobed and usually stains darker than the nucleus of a heterophil. The cytoplasm stains clear blue in contrast to the colorless cytoplasm of normal mature heterophils. Cytoplasmic granules are present and are strongly eosinophilic in appearance.

Basophil: Round cells with a round, non-lobed nucleus. The nucleus is centrally located and light blue. The cytoplasmic granules stain deeply basophilic and often hide the nucleus.

Performing the Complete Blood Count (CBC)

Avian leukocyte counts should include both a differential leukocyte count and a total leukocyte count. The differential is performed by evaluating 100 leukocytes in a monolayer portion of the slide and determining the percentage of each type. The total leukocyte count is best made using a method that involves the staining of avian heterophils and eosinophils using phloxine B as the diluent. Commercial kits for this purpose are available (Avian Leukopet, Vetlab Supply, USA). A measured amount of blood (typically 25 uL) is diluted with the phloxine B test kit. A Neubauer-ruled hemacytometer is then charged with the blood-phloxine mixture and allowed to stand for at least 5 minutes. Then, the stained heterophils and eosinophils are counted on one side of the chamber (9 large squares).

The total leukocyte count is then obtained using the following calculation:

TWBC/uL =eosin-stained cells (counted in 9 squares) x 2 x 1.1 x 16

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% of heterophils + eosinophils

An alternative method to the above is a crude estimation of cell numbers from a blood smear. Obtain the average number of leukocytes observed in 10 monolayer fields using the 40x lens objective. This number is multiplied by a factor of 2000 (though this number seems variable depending on the laboratory) to yield a TWBC/uL.