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Eight species of sea turtle are currently found in the world, with six inhabiting the Atlantic Ocean. Some species, such as the Loggerhead sea turtle (*Caretta caretta*) and the Green sea turtle (*Chelonia mydas*) are commonly seen in coastal waters off the United States, while others like the Leatherback sea turtle (*Dermochelys coriacea*) are rarely seen due to their pelagic lifestyle. Sea turtles are listed as threatened or endangered under the U.S. Endangered Species Act and they are also listed in Appendix I of C.I.T.E.S. (Convention on the International Trade in Endangered Species of Flora and Fauna). Numerous factors have been implicated in the decline of sea turtle populations in North America and around the world from fishing nets to construction on beach front property used as nesting sites for turtles.

The database for information on sea turtles is miniscule in comparison with many other marine and terrestrial animals. The lack of information on natural history, reproduction, and disease processes makes diagnosis and treatment of disease entities a challenge for the most seasoned veterinarian. Despite these obstacles, a significant amount of information can be obtained from collection and analysis of biological samples. The following will review the techniques for sample collection and analysis.

**Sample Collection**

**Blood collection (Venipuncture)**

The anatomy of a sea turtle limits the availability of many of the common sites for venipuncture used in other species. Vessels commonly utilized for sample collection in turtles cannot be visualized without aid of an ultrasound, or other, more sophisticated technology. Therefore; knowledge of the peripheral cardiovascular anatomy and landmarks used to locate vessels and sinuses make sample collection a more successful endeavor.

Common sites for venipuncture include:

1. Dorsal occipital sinus
2. Jugular vein
3. Digital (metacarpal) vessels

The dorsal occipital sinus is the most frequently utilized site for sample collection because it is easier to locate and large amounts of blood can be collected for diagnostic testing. The location of the occipital sinus and other vessels may change depending on how debilitated, dehydrated, and cachectic a turtle is on presentation. Blood is collected into lithium heparin tubes, microhematocrit tubes, and multiple slides are made immediately upon collection. Sample preparation and interpretation will be covered below.

**Fecal collection**

Reptiles, like avian species, have a cloaca that is the termination point for the gastrointestinal tract, reproductive tract, and the urinary system. The unique anatomical configuration of the cloaca must be taken into account when evaluating a supposed “fecal sample” which may in fact include urinary and reproductive components.

Collection of samples is frequently volunteered by patients during manual restraint and transport. If fecal samples are not easily obtained, a cloacal flush can be preformed. A cloacal flush is accomplished by placing the turtle in dorsal or ventral recumbency and passing a red rubber catheter of appropriate diameter into the cloaca or colon. Many times the catheter is passed, infuse 0.9% saline (approximately ½-1% of the body weight) and withdraw the sample. Occasionally, fecal material will freely flow without instilling saline due to stimulation, a full cloaca, or capillary action of the catheter. Sample preparation and interpretation will be reviewed below.

**Urine collection**

Urine collection and interpretation has come under much debate within the last few years as a diagnostic tool in many turtle species. The same confounding factors that affect analysis of fecal samples also plague urine sample interpretation. Urine collection is similar to fecal collection with a few minor differences. The patient is placed in dorsal recumbancy to increase the likelihood of entering the urinary bladder. A red rubber catheter is directed cranioventrally and advanced into the cloaca followed by the urinary bladder. Capillary action and a distended urinary bladder typically result in an adequate urine sample. If urine does not freely flow out of the catheter, a syringe can be attached to the catheter and gentle negative pressure can be applied. If urine is still not evident, the catheter may not be positioned in the bladder, or the bladder may be empty. Sample preparation and interpretation will be reviewed below.
Interpretation of Results

Similar to many other exotic animals, interpretation of results is dependent on numerous factors. Knowledge of anatomy, physiology, natural history, reproductive behavior, season, and age may all affect how results are interpreted. In addition, many of these variables are poorly documented or understood, increasing the level of difficulty during evaluation. Taking this into account, there is a small body of information that has been obtained and can be referenced to better aid the clinician in interpretation of results.

The close association of the lymphatic system with the vascular system may result in lymph dilution which is commonly seen in chelonian samples. Samples suspected of lymph dilution have extremely low total protein levels (<1mg/dl), low hematocrit (<10%), and lower than expected white blood cell counts (<200 WBC/ul). Despite these criteria, anemic, hypoproteinemic, and leukopenic animals have been seen by the author that fall within these ranges. These individuals are severely debilitated and support the low values obtained. If there are any questions to the relevance of the results, a second sample should be collected and resubmitted for evaluation.

Complete blood count

The hemogram is calculated similar to that for other species with minor differences in values due to their aquatic lifestyle. Differentiating white blood cells into the proper classification has brought about much controversy. Despite twenty years of variance, there appears to be some clarity that has surfaced in the last 6-7 years. A combination of light microscopy, electron microscopy, and cytochemical staining has aided in this clarification. The most current and complete description of erythrocytes and leukocytes that occur in green sea turtles are listed below. Many of these descriptions may also apply to the other species of marine turtles, but those of the green sea turtle are the most complete to date.

Erythrocyte
- Oval with pale orange/green cytoplasm and purple-blue nucleus
- 17-20µm long
- Many have small amorphous intracytoplasmic inclusions

Heterophil
- Cytoplasm filled numerous round, dull-red granules
- 10-18µm in diameter
- Nuclei are dense, round to oval, purple-blue, and eccentric

Eosinophil (two types)
- Small
  • 12-16µm long
  • Round dense purple blue nucleus
  • Blue cytoplasm with dull orange or clear round granules
- Large
  • 14-22µm long
  • Amorphous purple-blue eccentric nucleus
  • Clear to lightly granular cytoplasm with large round granules (occasionally with a refractile “halo”)

Lymphocyte
- Cytoplasm is basophillic with a round purple-blue nucleus
- Nucleus to cytoplasm ratio is approximately 2:1
- 6-14µm in diameter

Monocyte
- Rounded to amoeboid
- 11-26µm - larger than lymphocytes
- Cytoplasm is moderately basophilic with intracytoplasmic vacuoles
- Nucleus is round, oval, or indented and purple-blue

Basophil
- Rarely seen and poorly documented

Thrombocyte
- Oval with clear cytoplasm
- Densely blue-purple, oval to angular nucleus
- 9-12µm long

Red blood cell count

In terrestrial chelonians, variations in red blood cell parameters vary significantly depending on season and hibernation status. Marine turtles do not appear to follow this seasonal trend, yet there is still variation based on age of the individual and captive versus wild status. Anemia may be seen with lymph dilution, malnutrition, blood loss, intravascular hemolysis, and anemia of chronic disease. Many of the turtles that enter into a rehabilitation program are anemic and dehydrated. Rehydration of these individuals’ is necessary, so clinicians should be aware that it will further lower the packed cell volume.

Lymph dilution is the most common cause for an inaccurate packed cell volume.

White blood cell count

Automated methods have not been developed to accurately calculate the total white blood cell count. Two methods are currently employed for white blood cell counts: the direct and indirect methods. The direct method utilizes Natt and Herrick’s solution and the indirect method utilizes the eosinophil Unopette method which requires a manual differential count. Of these two, the eosinophil Unopette method is used more frequently due to its availability and longer shelf life. Questions concerning accuracy have arisen after a
study on loggerhead turtles showed a 38% discrepancy in the white blood cell counts between the two methods (the Natt and Herrick’s solution resulting in a higher WBC count). More research in this area is needed to substantiate this finding in other species of marine turtles, but should be considered when reporting results. Currently, the Natt and Herrick’s method appears to be the most reliable indicator of white blood cell counts in turtles.

Evaluating the differential count can be challenging, as it relates to immune function and response, due to the lack of information available for marine turtles. As an alternative, information from other reptile and chelonian species can be extrapolated to assist in the interpretation process, keeping in mind the wide variety of factors that may affect those results (i.e., age, season, etc.). A few points to add to the confusion include the fact that in many differential counts eosinophils and heterophils are lumped together as granulocytes by many laboratories and lymphocytes appear similar to immature red blood cells and thrombocytes.

Leukocytosis has been documented with oil exposure by Lutz and Lutcavage which also resulted in a decreased hematocrit and hemoglobin. Leukocytosis was also suspected and documented with cold stunned Loggerhead turtles in Cape Cod Bay, Massachusetts. Various other articles list elevations in the white blood cell counts, but fail to mention the values or the methods employed to achieve those results. In the authors’ experience, leukocytosis is infrequently observed in marine turtles. Turtles in rehabilitation with severe pneumonia, osteomyelitis, and other critical, life threatening disease may have white blood cell counts within acceptable ranges. Despite the lack of elevation in the leukocytes commonly found in circulation, recognition of an elevation should immediately raise concern for life threatening illness and appropriate diagnostics should be executed.

As a general rule, the primary white blood cell type found in marine turtles is the heterophil, but this may be dependent on age and species of turtle. Heterophils are consistently the primary cell type in Loggerheads, Green sea turtles, and Kemps’ Ridley sea turtles, while lymphocytes appear to be the predominant cell type in immature Green sea turtles. Much of the discrepancies over predominant cell type has been due to difficulty in identifying characteristics of the cells and their “official” classification over the last two decades.

Heterophilia may result with change of season, infection, inflammation, trauma, and neoplasia. Lack of heterophilia does not rule out infectious or inflammatory diseases. Frequently there will be no change in the heterophil count with severe disease processes. Toxic heterophils may occasionally be seen. Lymphopenia has not been routinely recognized in marine turtles.

Eosinophilia may result with change in season and inflammation. In this author’s experience eosinophilia is not a common finding with mild or severe parasitism.

Lymphocytosis can be seen based on season, inflammation, and in younger animals. In the authors’ experience, this is more frequently seen in captive marine turtles with no apparent disease processes. In addition, in other reptile species, lymphocytosis may indicate leukemia, viral disease, and neoplasia. These disease entities should be considered when evaluating marine turtles with elevated lymphocyte counts.

Leukopenia may be seen with anorexia and malnutrition. In terrestrial chelonians lymphopenia is seen with stomatitis, during hibernation, and with lymphoproliferative disorders.

Monocytosis has been documented in Green sea turtles with fibropapillomas by researchers in Hawaii. Other causes include acute or chronic infection, inflammation, and chronic renal failure in terrestrial species.

Basophilia has not been recognized in the authors’ practice.

Biochemical Parameters

Fortunately, for the clinician and laboratory technicians, biochemical values are viewed with fewer discrepancies than the complete blood count. Many of the tests used for terrestrial chelonians are also used for marine species with similar results correlating to disease processes. Knowledge of chelonian biochemical parameters in disease for terrestrial chelonians will assist the clinician in evaluation for marine species. The commonly performed parameters are listed and interpreted below.

Alkaline phosphatase (ALP) is present in the kidney and intestines, but may also be found in bone due to the elevations seen in terrestrial species with metabolic bone disease. Other elevations seen in chelonians are secondary to preovulatory stasis and in immature animals. Alkaline phosphatase is not routinely included on many of the standard laboratory profiles and should be requested.

Alanine transferase (ALT) is present in the kidneys with a small amount found in the liver. ALT is rarely, if ever, elevated, and serves as a poor prognostic indicator for renal or hepatic disease.

Aspartate transferase (AST) is present in the liver, kidney, and heart. Ramsey and Dotson (1995) oddly did not note AST in skeletal muscle during their evaluation. In this authors’ experience, elevations in AST frequently correlate with elevations in CPK, indicating there may be skeletal muscle or cardiac muscle involvement. If the elevations are not skeletal in origin, there may be an increased incidence of cardiac disease, but this has not
been recognized by the author. The elevations in AST in association with an elevated CPK are interpreted as skeletal muscle in origin.

Creatinine phosphokinase (CPK) is present in skeletal and heart muscle with lesser amounts in the kidney. Elevations may be seen with muscle trauma, administration of intramuscular injections, and cardiocentesis. Frequently elevations of >1000U/L may be seen with physical restraint or repeat attempts for blood collection.

Lactate dehydrogenase (LDH) is present in the liver, kidney, skeletal muscle, cardiac muscle, with small amounts in the intestines. Though elevations are infrequently seen in marine turtles, gastrointestinal obstruction, septic arthritis, and renal failure have all resulted in elevations in terrestrial chelonians.

Gamma-glutamyl transferase (GGT) is found in small amounts in the kidney. GGT does not appear to elevate significantly with any disease processes documented in the terrestrial chelonians.

Ammonia and urea appear to be the primary excretion byproducts utilized by marine turtles. Water, urea, and ammonia retained in the urinary bladder can be reabsorbed into the body. Evaluation of urea and ammonia is of questionable value due to this unique physiological adaptation.

Creatinine is not actively secreted or absorbed by the kidney and is considered a poor indicator of renal disease.

Amylase and lipase have not been evaluated in marine turtles to date.

Cholesterol and triglycerides, though not documented in marine turtles, elevate in preovulatory and reproductively active female terrestrial chelonians.

Glucose levels can be invaluable in formulating a treatment plan for marine turtles presented for rehabilitation. Hypoglycemia is frequently seen with severely debilitated, dehydrated, cachectic turtles. Starvation, septicemia, anorexia, and depletion of body storage reserves can all result in hypoglycemic patients.

Calcium may be significantly elevated in reproductively active females, termed vitellogenic hypercalcemia. Osteolytic changes have also been documented in terrestrial chelonians to cause elevations in calcium levels. Hypocalcemia has been documented with nutritional secondary hyperparathyroidism in captive Loggerhead turtles. This has also been seen by the author in a young, growing Loggerhead turtle not supplemented with calcium. The calcium to phosphorus ratio, as it relates to renal disease, has not been documented to date.

Phosphorus elevations should make the clinician first consider the possibility of hemolysis. If hemolysis is not present, nutritional secondary hyperparathyroidism should be included on the differential diagnosis. Similar to calcium, phosphorus does not appear to be a good indicator of renal function in chelonians. Variation in laboratory reference ranges exist and should be considered when interpreting results.

Hypoalbuminemia is seen frequently in marine turtles presented for rehabilitation. Etiologies include starvation, emaciation, anorexia, intestinal obstruction, severe infectious and inflammatory processes, and lymph dilution.

Globulin levels are best interpreted (as are albumin levels) by utilization of an electrophoresis. Recent research with electrophoresis in Loggerhead turtles has been performed to aid in interpretation of the results and should be reviewed if this methodology is used.

Iron levels have been documented in loggerhead sea turtles and green sea turtles but their significance is not yet understood.

Blood gases and lactates have been documented in Loggerhead sea turtles following two capture techniques in South Carolina and Georgia. Results revealed that turtles submerged for longer periods exhibited a marked acidemia and lactic acidosis. The results were interpreted as a mixed metabolic and respiratory acidosis with correction by respiratory alkalosis and titration of bicarbonate by lactic acid.

**Fecal Interpretation**

Fecal examination is infrequently performed in marine turtles, but may provide valuable information in diagnosis and treatment of parasitism and other diseases entities.

The primary reason that fecal analysis is rarely performed is due to its poor diagnostic yield. Knowledge of dietary habits of the species of sea turtle in question may aid the clinician in fecal evaluation. Some animals are primarily herbivorous while others are omnivorous. Rehabilitation facilities frequently document dietary intake of hospitalized turtles further aiding the clinician in evaluation of submitted samples.

Direct examination of fresh fecal samples with a few drops of saline is a reliable method for identifying protozoa. A fecal flotation aids in concentration of parasite eggs for examination. Sucrose and zinc sulfate are the most widely used media, but zinc sulfate appears more diagnostic when attempting to identify spirurid, cestode, and trematode eggs. Finally, sedimentation may be implemented for identification of protozoans, their cysts, and trematode eggs.
Few parasites have been consistently documented in marine turtles except for trematodes. Coccidia (Caryospora and Eimeria) have been documented in captive green sea turtles, as have leeches and barnacles. Trematodes appear to be the most consistently identified parasite in marine turtles and have been implicated in vascular disease, pneumonia, and neuromuscular diseases. Sea turtles entering rehabilitation are frequently administered praziquantel due to the increase prevalence of trematodes that may complicate recovery.

**Urine Interpretation**

As stated previously, the primary nitrogenous excretory products of the renal system are urea and ammonia. Measurement of these values in serum or urine has not proven to be a good indicator of renal function. In addition, urine specific gravity is of questionable diagnostic value due to the physiological adaptation of marine turtles to reabsorb water and fluid contents of the urinary bladder. In terrestrial herbivorous chelonians, an acidic pH (<7) is indicative of fat or protein catabolism and is a feature of prolonged anorexia. This has not been evaluated in marine turtles. Cytology of urine frequently reveals casts, bacteria, erythrocytes, leukocytes, and other inflammatory cells. Many of these findings are considered normal due to the non-sterile environment of the urinary bladder and its association with the cloaca.

Urine samples may show some promise in measurement and evaluation of hormone levels in males and females as an indicator of reproductive status, but this remains to be seen.

**References**