Health Assessment of Captive and Wild-Caught West Indian Manatees (*Trichechus manatus*)

M. ANDREW STAMPER AND ROBERT K. BONDE

As human populations increase along coastlines and waterways, their various activities impact the health of sirenians in these fragile environments. Medically examining these animals in their natural habitats and gauging how both individual and overall population health alter as the environment changes serves as a tool for the conservation and management of the animals and their habitats¹. To construct useful databases, it is critical that observations are recorded and samples taken in consistent ways. Most important is the appropriate collection and careful management of biological samples. Collection and handling of samples takes experience even when conducted in controlled situations, such as at a captive facility. Even greater skill and quality control

needs to be attempted in field situations, where biologists and veterinarians often work in difficult conditions, with fluctuations in temperature, direct sunlight, rain, wind, and tides. Despite the unpredictability of fieldwork, tissues and the associated documentation must be handled carefully; otherwise biases caused by artifact interference could influence results, especially when interpreting blood analyses.

Guided by experience obtained from handling manatees in Florida, Puerto Rico, Belize, Mexico, and Brazil, and dugongs in Australia, this chapter is designed to give scientists information on the appropriate protocol to examine a sirenian for a detailed health assessment (figure 16.1).



Figure 16.1. Health assessment of wild-caught West Indian manatee in Puerto Rico. (Courtesy of U.S. Geological Survey.)

Date:	Set/Cap Time:	Deck Time:	Release Time:
Location:		Latte	ng:
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Urine			Skin		
Feces Milk			Parasites Microbiology	-	
Tear film			Biopsy		
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entral (umb, u	ro, anal)				
duncle (tail) -					
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Most health assessment projects develop their own data sheets. These usually include one form each for the overall examination (figures 16.2 and 16.3), specimen collection, documentation of temperature, pulse and respiration (TPR) monitoring, ultrasound recording, radio tagging, etc.

External Examination

Prior to capture, researchers and clinicians should note the general disposition of the animal. Once beached, the animal should be made as comfortable as possible and placed on foam pads. Special consideration must be made for pregnant animals (enlarged abdomen and, during late stages, distended vulva), which should not be left without appropriate support while out of water and should be processed as quickly as possible or released right away. The overall condition of the animal should be thoroughly evaluated for any evidence of lesions, nutritional status, and deformities². This must be performed in a consistent and systematic method (i.e., head to tail) for every animal.

First, overall appearance should be noted, including movement characteristics (i.e., freedom of movement of the head and neck, pectoral flippers, and tail) and body condition (i.e., emaciated, thin, or overweight). Eyes and nares should be examined for abnormalities. The examiner should document any skin scarring with drawings and photographs. As part of an evaluation of body condition, determine the thickness of the adipose (fat/blubber) layer of manatees using ultrasound or a sonogram³. Morphometric measurements should include weight; total length (straight line and curvilinear); girths at axilla, umbilicus, anus, and peduncle; and measurements of any scars or mutilations (figure 16.1). This information, coupled with results of lab analyses, is a valuable tool for gauging health status.

In most countries where sirenian research is ongoing, all captured animals should be scanned for passive integrated transponder (PIT) tags⁴. Tag types should be standardized between projects and countries, since animals often travel across borders. The standard protocol for West Indian manatees is to place one tag in each shoulder. However, be aware that it is possible, though rare, for the implanted transponders to migrate

Figure 16.2. (*top left*) Health assessment sheet used in Florida for wild-caught manatees. (Courtesy of U.S. Geological Survey.) Figure 16.3. (*bottom left*) Example of a detailed physical examination form used for captive manatees. (Courtesy of U.S. Geological Survey.)

through tissue, and examiners should be careful to scan the whole animal. If a PIT tag is not present, then the animal should be fitted with two tags, using the protocol described in Wright et al5.

Vital Signs

Respiration

During restraint and observation note the number of respirations per five-minute interval. Average rested animal respirations should be around one breath every minute with a range of 3-15 per five minutes⁶. Attempts can be introduced to increase breathing by pouring water over the closed nasal passages. This procedure often results in a voluntary inhalation. Describe if the inspiration and expiration are shallow or deep, and document the odor of the expiration (no odor, sweet/sour, or foul). Though this may be difficult in large animals, a stethoscope can be used to listen to each lung along the dorsum while the animal is taking a breath; note if the lung field is clear.

Heart rate and capillary refill

To obtain heart rate, either use an electrocardiography (ECG) monitor or place a stethoscope against the body

at the sternum. Check the heart for palpitations, trills, or arrhythmias and evident murmur sounds. Siegal-Willott and colleagues⁷ demonstrated a heart rate of 51-66 bpm for adults and 61-75 bpm for calves using an ECG monitor (figure 16.4). Capillary refill time will give an indication of blood circulation and can be determined by pressing a finger against the upper inner lip pad, then removing and noting capillary refill time when the blanched tissue returns to normal color. Notice if the gum color is pink or pale and whether the tissue appears cyanotic (bluish) or icteric (yellowish). If the animal is showing signs of distress, try supporting it in the water (if possible and safe) to see if parameters normalize. On very rare occasions, some animals may be lethargic, and these individuals should be introduced back into the water with care. If the animal does not stabilize satisfactorily, transport it to the nearest rehabilitation center if possible.

Temperature

Oral temperature in West Indian manatees is generally between 34 and 36°C. Temperature can be monitored with a flexible oral probe placed as far as possible into the posterior of the mouth between the outer cheek teeth and gum. Care must be taken since the animal's

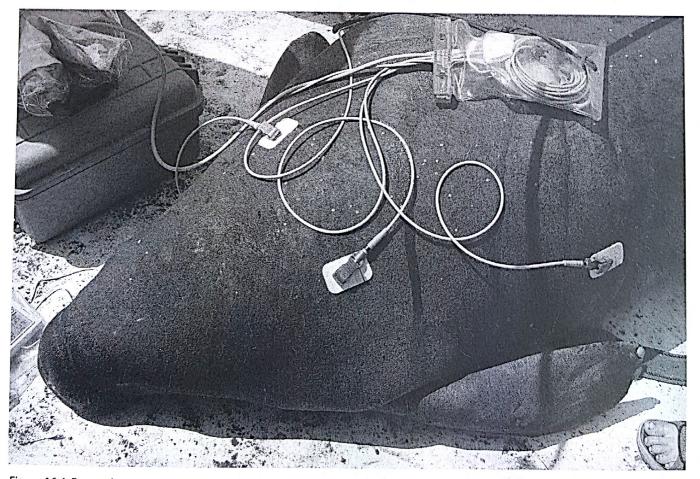


Figure 16.4. Researchers monitor a West Indian manatee in Belize using an electrocardiogram (ECG) monitor. (Courtesy of U.S. Geological Survey.)

molars can crush fingers and probes. Rectal temperature is unreliable since the fermentation characteristics of the manatee gastrointestinal system create dynamic temperature fluctuations, with rectal temperature ranging between 27 and 32°C8. Increases in temperature should be mitigated by shading the animal as well as applying copious amounts of water over the body, especially over the flippers and tail. In addition to cooling the animal, this also helps to keep skin from drying. Be aware that a dramatic decrease in body temperature can lead to shock. Lower oral temperature can indicate hypothermia during periods of exposure to cold ambient temperatures. In this event, attempt to raise the body core temperature by placing a layer of survival blankets over the animal.

Mentation/reflexes (reactiveness)

During handling, care should be taken to note alertness and reaction to eye reflex. Eye reflex can be examined by placing the finger adjacent to the eye, which should elicit a response.

Skin and Epibionts

Skin

The skin should be examined for abnormalities. Make descriptive notes detailing the characteristics of any abnormal tissue or lesions. Photographs of lesions are helpful to the pathologist examining tissues submitted for analysis (they can also be used for future identification). Tissues may be collected cleanly with a scalpel and forceps after a local anesthetic has been applied. Counter to intuition, skin biopsies should not be surgically prepped since this will remove portions of the tissue that need to be examined. The use of forceps must be performed carefully to prevent crushing tissue. For correct preservation, tissues should be collected in a fashion that allows efficient perfusion of formalin. Since formalin cannot penetrate more than 1 cm into tissues, this should be the maximum depth to the center of any tissue collected. Collected tissues should be representative of the surrounding normal tissue as well as any suspected pathology9. After removal of tissues the biopsy site should be thoroughly cleaned.

Special care needs to be taken when handling tissues for histological examination. Use a 1:10 tissue to formalin ratio to preserve tissues in 10% neutral buffered formalin. Label all samples with a field number unique to the animal; record date, organ sampled, and sampler's initials; and detail any lesion using descriptors for size, shape, density (soft, firm, hard), and color.

Parasites and Epibionts

Note the presence or absence of external parasites, which should be properly collected. Commensal associates are commonly found on the skin of sirenians. Signs of copious amounts of algae and invertebrate growth do not necessarily mean the animal is in poor condition since there are large variations between individuals and habitat types. However, there may be an underlying issue that warrants further investigation. Parasites, algae, commensal associates (e.g., barnacles), when in fresh condition, should be preserved in equal volume of 70% ethanol.

Urinary and Reproductive Systems

Urine

Urine should be clear, but it will have a strong odor if the animal is captured from a marine environment and the urine is concentrated. Semen is sometimes present in the urogenital canal of adult male breeders. A complete urinalysis includes chemistries, hormones, bacterial cultures, parasite screening and identification, and examination for blood and other cells. Generally the collection and processing methods of Pratt¹⁰ are used, although they must be adapted to working in the field with a large mammal. Urine can be collected from the manatee by placing a sterile circular container (a Frisbee or soft-edged flying disc, for example) beneath the manatee covering the urogenital aperture. Make sure the area is thoroughly cleaned prior to placement of the collection container. Positive bacterial cultures should still be viewed with skepticism since it is very difficult to collect a sterile urine sample in this fashion. Manatees that are dehydrated and/or in marine environments do not urinate frequently. Some success has been achieved by external manual massage of the skin by applying pressure above the urinary bladder. If urine is acquired it should be placed in a sterile centrifuge tube, protected from sunlight and excessive heat, and refrigerated until analysis. To reduce the effects of chemical and cellular changes, urine should be examined as soon as possible, within two hours if fresh or six hours if refrigerated. Water contamination can also be a factor with dilution in freshwater environments, or high pH/NaCl content indicating saltwater contamination if animals are in marine systems. To examine for solid elements in the urine, one drop of 40% formalin should be added to 30 ml of urine. This test should be conducted after all chemical tests are performed, as formalin interferes with some chemical analysis, especially for urobilinogen and pH11.

Reproductive system

An overall examination of the external genitals should be conducted. For adult females, total body length, abdominal distention in lean animals, enlarged/swollen vulva, and enlarged axillary mammary glands (teats) should be documented, as these are often indicators of near term pregnancy. Currently serum immunoassay to measure progesterone is the only reliable test for determining pregnancy in sirenians¹². Collection of milk from lactating females is accomplished by manual massage of the mammary region on the main body, caudal to the insertion of each flipper, while gently pulling on the teat. The area should be thoroughly washed with alcohol prior to milk withdrawal. Expressed milk should placed into a sterile container and frozen as soon as possible after collection.

Digestive System

Manatees have a single stomach and some 40 m of small and large intestine. They are hindgut fermenters. Flatulence is a common function of the manatee's digestive system and should be expected.

Mouth

The mouth should be examined for vegetation, ulcers, or dental anomalies. Naturally, use caution as manatees do not tolerate oral examination well and can bite down or struggle.

Abdomen

Palpate the abdomen for tenderness and listen with a stethoscope to the lower abdomen for intestinal movement (peristalsis) and gas production.

Feces

Collect a fecal sample if the animal defecates. Describe the frequency, color, and consistency of the stool. Feces should be semi-solid, well formed, and soft and may or may not float in water. Fecal samples are used for identification of gastrointestinal tract contents, internal parasites, and bacteria and fecal hormones. Fresh fecal samples are valuable, as rapid changes occur in bacterial population and parasite eggs once they are passed from the animal's body; the death of some protozoa that may be present and identified by their movement can occur quickly¹³. Unfixed fecal samples should be refrigerated as soon as possible. For hormonal analysis, samples should be frozen. When used for vegetative content analysis or parasite identification, fecal samples should be placed in equal volume of fecal matter and 70% ethanol.

Hematology and Blood Chemistries

Value of Blood for Health Assessment

Blood provides researchers with another tool for assessing the health status of the animal. Generally, the complete blood count (CBC) is a profile of tests including the cell types, sizes, and numbers used to describe the quantity and quality of the cellular elements in blood and a few substances in serum. Common abnormalities may alert one to events such as infections, immunosuppression, homeostasis abnormalities, anemias, and/or clotting abnormalities. Serum chemistries in conjunction with other ancillary tests can indicate issues involving the liver, kidney, brain, intestines, muscle, endocrine system, and pancreas and give information on overall nutritional and electrolyte status.

Collection of Blood Samples

Blood must be handled delicately and quickly to ensure accurate results for comparisons to previous and subsequent analyses. Blood tubes, containing a variety of anticoagulants or supportive media, are used to assure accuracy and validate quality control (table 16.1). Blood should be collected as soon as possible after capture since stress-released hormones can influence body metabolism (shifting enzymes and blood cell populations). The brachial arteriovenous plexus on the medial or lateral aspect of the pectoral flipper is the preferred draw site (figure 16.5). It should be noted that an unknown mixture of arterial and venous samples will be collected at this site. This can dramatically impact some values, especially blood gases, electrolytes, tissue-derived enzymes, and pH values. Thorough cleaning of the sampling site (medial or lateral aspect of the flipper) is extremely important as manatee skin has high loads of bacteria; use alternating scrubs, three each of betadine or Nolvasan and isopropyl alcohol, employing a circular pattern, working from the center outward. Blood can be obtained using the Vacutainer method (recommended) or by syringe. Use of a Vacutainer collection method (figure 16.6) will fill multiple tubes rapidly and result in the least sampling artifact. Generally, 3.8 cm long, 18-21 gauge needles are used. An extension set (approximately 20 cm) with a Luer adapter and collar are handy and give flexibility to change tubes away from the needle insertion site. To reduce trauma to the arteriovenous plexus, use larger needles (18 gauge) for larger manatees and smaller needles (21 gauge) for calves. If a lateral approach is used, a 5 cm needle may be needed in larger manatees. Tubes with anticlotting

agents should be filled first (table 16.1). To prevent damage to blood cells, the needle should be removed from the syringe (if used) prior to filling the blood tubes. The tubes should be filled slowly to the labeled line or approximately 80% full. All anticlotting tubes (i.e., green or purple/lavender tubes) should be capped and rocked gently and slowly for 15–30 seconds immediately after collection. Caps should be tightly secured.

Minimal blood collection for basic health measurements includes two 5 ml EDTA purple/lavender top tubes for complete blood count, one to three 10 ml lithium heparin green top tubes for plasma archival banking, and two to five 10 ml red top tubes for serum biochemistries and archival banking, totaling some 40–90 ml of blood (table 16.1).

To ensure proper interpretation of each processed

sample, history must be carefully documented and should include times and conditions. Times to be recorded include when, how, and for how long the animal was followed, time of actual capture, and exact time blood was collected. All tubes should be labeled with the date, time, animal ID, and any other pertinent information. Use a pencil or indelible pen that will not smear.

Protecting the sample from environmental conditions once it is collected is paramount to ensuring its integrity. Blood exposed to a rise of 10°C can double the rate of chemical or enzymatic reaction. Blood should be stored as close to refrigerator temperature (4°C) as possible. Wrap the tubes in paper towels so that they do not directly touch the ice or ice packs, since this may freeze the blood cells and rupture them.

Table 16.1. Different types of tubes used in various studies for collecting blood during sirenian health assessments.

Anticoagulants and preservatives	Description	Diagnostic use	Minimum to collect	
EDTA (Ethylene- diaminetetraacetic acid)	Used for whole blood	Hematology, complete blood cell count	2 5-ml tubes	
None	Used for extracting serum	Biochemistry standard panel, electrophoresis, protein evaluation, triglycerides, hormone, vitamin, mineral and contaminant analysis	2–5 10-ml tubes	
Sodium heparin	Used for extracting heparinized plasma	Emergency biochemistry standard panel, electrophoresis, triglycerides, hormones, lymphocyte proliferation, cytogenetics	1 10-ml tube	
Lithium heparin	Used for extracting heparinized plasma	Emergency biochemistry standard panel, electrophoresis, triglycerides, hormones, blood gas analysis	2–4 10-ml tubes	
None	Acid washed, zinc-free stopper. Designed for mineral and heavy metal analysis	Biochemistry standard panel, protein evaluation, electrophoresis, triglycerides, hormones, minerals, heavy metals	Optional*	
Sodium citrate	Used for extracting citrated plasma	Coagulation testing, fibrinogen quantization	1 5-ml tube	
Thrombin and soybean		Determining fibrin degradation products	Optional	
Thrombin	Used for extracting serum, faster clotting	Biochemistry standard panel, protein evaluation, electrophoresis, triglycerides, hormones	Optional	
Acid citrate, dextrose		DNA or red cell survival	Optional	
Diatomaceous earth		Measure activated clotting time	Optional	
Sodium fluoride, and/or potassium oxalate		Measure glucose and lactate	Optional	
	preservatives EDTA (Ethylene-diaminetetraacetic acid) None Sodium heparin Lithium heparin None Sodium citrate Thrombin and soybean Thrombin Acid citrate, dextrose Diatomaceous earth Sodium fluoride, and/or	EDTA (Ethylene-diaminetetraacetic acid) None Used for whole blood None Used for extracting serum Used for extracting heparinized plasma Lithium heparin Used for extracting heparinized plasma None Acid washed, zinc-free stopper. Designed for mineral and heavy metal analysis Sodium citrate Used for extracting citrated plasma Used for extracting citrated plasma Used for extracting citrated plasma Thrombin and soybean Thrombin Used for extracting serum, faster clotting	EDTA (Ethylene- diaminetetraacetic acid) None Used for extracting serum None Used for extracting None Used for extracting None Used for extracting None Lithium heparin Used for extracting None Acid washed, zinc-free stopper. Designed for mineral and heavy metal analysis Sodium citrate Used for extracting citrated plasma Thrombin and soybean Thrombin Used for extracting serum, faster clotting Acid citrate, dextrose Diatomaceous earth None Measure activated clotting time Measure glucose and lactate	

Note: Tube top color may vary according to maker/laboratory. Please contact your local lab to assure correct tube for your diagnostic use.

^{*}Use of optional tubes should be at the discretion of the assessment team; generally only collected for special clinical purposes.



Figure 16.5. Blood draw sampling site on a West Indian manatee. (Courtesy of Mike Walsh.)

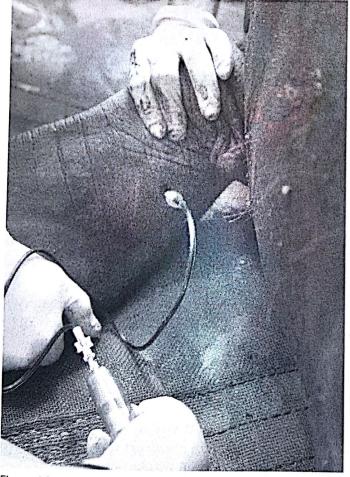


Figure 16.6. Manatee blood draw using an extension set. (Courtesy of U.S. Geological Survey.)

Processing Blood Samples

Timely preparation is vital for accurate analysis. Blood for biochemical analyses should be separated within two to three hours of collection but may still give useful information if processed within 12 hours. Separated, chilled serum and plasma are still useful for biochemical analyses four days after collection. Always check the collection protocol and test procedures with the laboratory you are utilizing.

An on-site centrifuge is necessary if blood is to be processed in the field for diagnostic analysis. An electrical outlet is essential for many units; however, some will work off a 12-volt battery. A voltage converter also can run a machine from a battery, or a small portable generator can be used. The centrifuge should be capable of operating at 2,000–3,000 RPM in a stationary position. Hematocrit centrifuges can be used to spin blood that can be used to analyze hematocrit. Total protein can be obtained using a refractometer. On the water, to avoid breaking tubes, a boat must be stationary with little movement when running these machines.

Cells should be separated from the plasma fluid phase. Cells are separated in whole blood containing an anticlotting agent (such as EDTA, citrate, or heparin) by centrifugation for approximately ten minutes at 2,000–3,000 RPM. Plasma fluid is obtained by decanting the supernatant (fluid above the blood cell pellet) from a sample tube. Excessive RPM may cause hemolysis or cell breakage. Use a sterile pipette to remove the plasma and place it into appropriately labeled containers.

Serum is plasma with fibrinogen removed during the clotting process and is obtained when a blood sample is drawn in either a thrombin or red-top tube and allowed to clot for roughly 20–30 minutes. It is then processed similarly to plasma. Serum can be used to measure hormones, vitamins, minerals, and contaminant concentrations as well as serum biochemistries and analysis of immunologic compounds. Prior to use, check with the laboratory running the desired tests to ensure that the type of blood tube used will not impact the results.

Obtaining blood smears on glass slides for hematological analysis takes experience, and personnel should be well trained before attempting this in the field. Please refer to clinical pathology textbooks to master these techniques. Make sure to label all slides with pencil as some staining techniques will remove ink. Slides made in the field should be protected at all times from moisture, flies (which will eat the blood film), and other contaminants. Blood slide holders can be obtained to assure that the slides are protected. Deterioration due to excessive humidity is common in the tropics, and samples should be kept cool and dry if possible.

Interpreting Results

Caution should be used when comparing values generated by different methods or analytical machines, as these factors can result in marked differences¹⁴. Manatee CBC and serum chemistries do not always follow conventional domestic or wild animal trends. Always use caution and consult experienced clinical veterinarians during all components of blood data interpretation and treatment. All interpretation should be performed in conjunction with other physical examination results. Basic clinical diagnostic manuals such as Willard et al. 15 give fundamental information on diagnostic analysis on domestic animals, and Harvey et al.16 detail biochemistry analyses of blood from Florida manatees. Also, reported reference ranges for serum chemistry from manatees from Colombia and Puerto Rico are presented by Montoya-Ospina¹⁷. Walsh and Bossart¹⁸ and Bossart¹⁹ can be referenced to give insights into marine mammal medicine, but proper interpretation is always based on experience.

Microbiology

Bacteriology

At least 48 species of bacteria have been reported from West Indian manatees²⁰. In characterizing bacteria from a wild or captive manatee, the first rule is to choose samples carefully, always mindful of potential contaminates found in the water or air or on the human collecting the sample. Samples for isolation of microbes should be collected under sterile conditions. Special culture media are available for anaerobic and aerobic pathogens, and researchers and clinicians must consult with a microbiologist for the most appropriate media given the diagnostic interest.

Bacterial samples are only worthwhile if fresh. Tissues (blocks) or fluids (several milliliters of purulent discharge, exudate, or feces) are better than culture swabs because they preserve the bacteria's environment for longer periods of time. If using tissues, pack individual tissues in separate sterile containers to avoid cross-contamination. If specific transport media are not available, use plastic bags for collection and then transfer the specimens to screw-capped, water-tight containers before sending them to a laboratory. If a swab must be

used, transport medium should be used to increase the chance of isolation and identification of organisms and to prevent desiccation. If possible, refrigerate samples as soon after collection as possible. If this is not feasible. assure that the samples are not exposed to direct sunlight or extreme temperatures. Culturette brand swabs are supplied with transport media for aerobes. Because most anaerobes cannot survive exposure to air for more than 20 minutes (at most), collecting samples for anaerobic culture on swabs is not recommended. Acceptable anaerobic specimen collection techniques include: (1) blocks of tissues in a closed sterile container with the air evacuated (sealed thioglycollate broth tube is likely best), (2) material placed in Becton-Dickinson's "anaerobic specimen collector," and (3) purulent discharge and other exudate specimens drawn into a sterile syringe with the air expelled and the needle plugged with a rubber stopper or bent backward on itself. The specimens should be cultured as soon as possible after collection and refrigerated. Do not deep freeze any bacterial samples unless there will be extensive delay before the samples can be examined. Freezing often desiccates bacteria or disrupts the bacterial cell wall.

Mycology

Often fungal samples can be collected similarly to aerobic bacterial cultures. Some skin-related fungi need to be collected on special media. Care must be taken to avoid environmental contamination.

Virology

Viruses have not been extensively studied in manatees other than current work with papilloma and herpes viruses in Florida manatees²¹. If a virus is suspected, contact a veterinary specialist and individual laboratories to determine the method of collection, as each may have different requirements. A biopsy should be collected and frozen for molecular analysis. Additional samples from the lesion should be preserved in 10% formalin for supportive histological and immunohistochemical examination. Formalin fixation may not be the only preservative of choice, and researchers should contact a virologist for specific instructions.

Cytology

Health and disease diagnosis may also be accomplished through cytology or cellular diagnostics. With minimal equipment, samples can be obtained to be sent to diagnostic laboratories for analysis. The major goal is to obtain a significant number of well-stained cells reflecting

the composition of the sample. Samples of deeper tissue are more likely to be diagnostic but are more difficult to obtain. Fluid or secretions should be collected in a specimen cup as well as spread on a microscope slide with a sterile applicator. Fine needle aspirates and impression smear collecting techniques are used to obtain cytology samples. Please refer to a cytology textbook to determine appropriate technique.

Cytology does have its limitations. Histopathology may be more diagnostic and definitive because more information (i.e., tissue architecture) is available from a histopathology sample than from a cytologic smear. Histopathology has limitations for assessing organisms in infected tissue or evaluating cellular detail useful in diagnosing some tumors.

Conclusion

To garner insight into human health issues as well as to identify potential impacts of anthropogenic threats such as hunting, pollution, climate change, and habitat destruction, it is important to determine proactively the health status of populations of animals in the wild. In the proactive study of wild populations, the first step is obtaining baseline information to give a reference point for future efforts. The key to this endeavor is to obtain good quality centralized data so that efforts can translate among groups in an effort to maximize our understanding of health and risk maladies in fragile sirenian populations.

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