Health Assessment Procedures for the Mojave Desert Tortoise (*Gopherus agassizii*): A Handbook Pertinent to Translocation

U.S. Fish and Wildlife Service
Desert Tortoise Recovery Office

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This document was developed to accompany a hands-on training course in conducting health assessments on Mojave desert tortoises for translocation screening and monitoring purposes. Wendland et al. (2009), Handbook on Gopher Tortoise (*Gopherus polyphemus*): Health Evaluation Procedures for Use by Land Managers and Researchers, served as a template and with permission of the authors, several sections were adapted or modified only slightly.

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1. Introduction

Background

The Mojave desert tortoise (*Gopherus agassizii*) (i.e., all tortoises living north and west of the Colorado River in Arizona, Utah, Nevada, and California) is federally protected as Threatened under the Endangered Species Act (USFWS 1990). The Sonoran desert tortoise (*Gopherus morafkai*), south and east of the Colorado River, is listed as a Candidate under the Endangered Species Act, having been determined to be warranted for listing, but precluded by higher priority actions (USFWS 2010). State laws also protect the two desert tortoises. The protocols herein were developed for the Mojave desert tortoise, but could be appropriate for the Sonoran desert tortoise should that become needed.

Some projects that occur in desert tortoise habitat are not compatible with the continued existence of tortoises in and around the project sites. Recently, numerous energy-development projects (primarily solar and wind) have gained approval to construct such facilities in areas inhabited by desert tortoises. In an attempt to minimize impacts on desert tortoises, many projects propose to displace tortoises from the project sites and relocate them to other areas. Many steps must be taken to minimize the risks to the tortoises being moved, as well as to tortoises that they might encounter after translocation from the project site. Among the many precautionary steps is assessing the health of tortoises at both the project and recipient sites, as well as at any reference sites as may be required for specific projects.

While upper respiratory tract disease (URTD) is commonly assumed to be a threat to populations and was implicated in declines of the desert tortoise when it was federally listed, other diseases and conditions do exist. These conditions could have significant impacts on populations should pathogen spread be influenced by human-mediated movements of tortoises. Health assessments and subsequent decisions on whether or not translocation should be allowed must be done in concert with knowledge of the distance between proposed project and recipient sites, the prevalence of disease at the sites, the behaviors of tortoises after translocation, population density, food availability, and other factors that pertain to assessing the risk of the translocation. Lack of knowledge with respect to the precise impacts of potential infectious diseases present within desert tortoise populations, the impact of disease status relative to translocation of the species, and disease impacts to long-term population viability create a major dilemma for wildlife biologists, veterinarians, conservationists, and public policy makers. However, we do have enough information now to develop an approach to minimize known risks.

Objective

This handbook is designed to serve as a reference for biologists conducting translocation-related health assessments, as well as for individuals making decisions based on the results of the assessments. While health is only one of the many factors that need to be considered when planning the translocation of desert tortoises, it is a complex and important one. This handbook includes detailed protocols and photographic presentation of various conditions that may be encountered, and it is expected that formal, specialized hands-on training will be acquired by those individuals wishing to conduct health assessments.

Scope

While the information in this handbook was developed in response to projects affecting primarily the Mojave desert tortoise, generally it is applicable to all desert tortoises across their range. The health assessments are designed to minimize the risk of spreading disease within and among populations and to promote survival of individuals when relocating tortoises from project sites. Health assessments conducted with additional or different goals (e.g., health and disease-related research) may differ from the assessments described here. We do encourage, however, a standardized approach to health assessment data collection in order to monitor populations across projects and over temporal and spatial scales.

Approach

We present an overview of the importance of monitoring health and disease, followed by more specific information about the health assessments and decision points that are applicable specifically to relocating desert tortoises from project sites. Additional information is provided in the appendices. Some of the technical terms specific to tortoise biology and anatomy, as well as veterinary medical terminology, are defined in the Glossary.

2. Preparing to Work with Desert Tortoises

Permits

Field workers should be aware that several permits may be needed before they begin to handle and assess the health of desert tortoises. When working with the federally listed Mojave desert tortoise, a permit or incidental take statement from the U.S. Fish and Wildlife Service is required. In addition, each state wildlife agency requires permits authorizing handling activities. Permits may also be required by other federal or state agencies, so workers should thoroughly inquire about necessary permits prior to beginning work. Because conducting health assessments requires specialized skills, workers should also expect to provide documentation of training received and experience in conducting health assessments on desert tortoises to both state and federal agencies. Any research being conducted on vertebrate animals generally requires an approval from the Institutional Animal Care and Use Committee (IACUC) for academic institutions and industry or from other similar review panels in order to meet the Animal Welfare Act regulations.

Human-risk minimization protocols

Field workers must ensure that protocols are in place for disposal of medical waste, disposal of sharps, and response to accidental exposure to biological samples through needle sticks or other means. The protocols for biological sampling techniques in the appendices of this handbook include some details to address these issues.

3. Health and Disease Overview

Importance

Health is vital to the success and well-being of any population. Certain management activities may negatively impact the health of a population by imposing additional stressors to which the animals may have difficulty adapting. Implications of health and disease in wildlife management, especially in translocations, are a topic of increasing concern (Wolff and Seal 1993; Cunningham 1996; Kock et al. 2010, IUCN/SSC 2013). In the past, infectious diseases were not considered in wildlife management because it was generally believed that wild animal populations were large enough to adapt to, or deal with, any potential impacts from disease (Spalding and Forrester 1993). However, substantial increases in human-induced impacts to the natural environment have occurred over the past 50 years. The primary issues facing all species of wildlife, including desert tortoises, are habitat loss and habitat degradation due to human development activities (Mitchell and Klemens 2000).

In the case of desert tortoises, translocation is a management tool that has become increasingly popular. While translocation can be a useful tool to help restore viable tortoise populations, its misuse has the potential to cause great damage (IUCN 1987), and numerous safeguards must be taken to protect the translocated tortoises, as well as tortoises in the resident recipient population. Because desert tortoises are long-lived and do not reach reproductive maturity for 13-20 years, a disease outbreak that causes the death of a large number of tortoises may result in devastating population losses, making it difficult for the population to recover. A key step in minimizing disease risks is screening animals that will be translocated, as well as those in the existing population at the recipient site, for pathogens (Beck et al. 1993; Cunningham 1996). Because tortoise health can substantially influence the success of a translocation, baseline information should be collected before implementing the action and then again afterwards to measure the results.

How health is measured

Health is measured in all species using parameters such as food/water intake and fecal/urine output (physiological balance), body weight or condition in relation to a known standard, reproductive performance, blood biochemical parameters, social and environmental factors, amount of physical activity, availability of suitable habitat, absence of clinical signs of disease, and several other variables. Many of these variables are easy to measure in captive animals; however, most are extremely difficult to measure in wild species. The most common components of a comprehensive health assessment of animals include examinations and tests for:

- Physical condition, including evidence of trauma and body condition scoring
- Complete blood count and biochemical profile
- Vitamin and mineral assays
- Internal and external parasites
- Infectious diseases
- Reproductive capacity
- Exposure to environmental toxins

Unfortunately, normal reference ranges for some of these variables are simply not available for free-ranging wild desert tortoises (but see Christopher et al. 1999 and Dickinson et al. 2002). Increasing concern about tortoise health has led to several recent studies conducted at multiple institutions that ultimately will provide much health-related data in the near future.

4. Health and Diseases of Desert Tortoises

Disease is a natural phenomenon within wild animal populations, and epidemic outbreaks can have catastrophic effects on small or declining populations. To date, the available evidence indicates that upper respiratory tract disease is probably the most important infectious disease for desert tortoises. Less is known about other diseases that have been identified in the desert tortoise (*e.g.*, herpesvirus, shell diseases, bacterial and fungal infections, and urolithiasis or bladder stones) (Jacobson *et al.* 1994, 1995; Homer *et al.* 1998; Berry *et al.* 2002; Origgi *et al.* 2002). Additional research is needed to clarify the role of disease in desert tortoise population dynamics relative to other threats and the level of effort we should expend in disease control as compared to other threats.

At least two pathogenic species of *Mycoplasma* known to cause upper respiratory tract disease in desert and gopher tortoises have been identified (*M. agassizii* and *M. testudineum*) (Brown *et al.* 1994, 1999, 2001; 2002, Jacobson and Berry 2012). The pathogens are likely transmitted by contact with an infected individual or aerosols (airborne liquid droplets or solid particles). Once infected, tortoises may develop lesions in the nasal cavity, excessive nasal discharge, swollen eyelids, sunken eyes, lethargy, and possible death (Jacobson *et al.* 1991; Schumacher *et al.* 1997; Homer *et al.* 1998; Berry and Christopher 2001). These clinical signs, which may not always be evident in an infected individual, may also be symptomatic of other conditions, such as dehydration or infection with other pathogens (Brown *et al*. 2002). Various tests have been developed to detect the presence of antibodies to *Mycoplasma* or to help determine active infection (Schumacher *et al.* 1993; Brown *et al.* 1995; Wendland *et al.* 2007; Hunter *et al*. 2008). Johnson *et al.* (2006) uncovered a positive link between tortoises with anti-*Mycoplasma* antibodies and the severity of clinical signs of upper respiratory tract disease, as well as with age class, with adults being more likely to test positive for presence of antibodies. However, in-depth study of the desert tortoise's immune system and epidemiological study of disease dynamics across space and time are necessary to more thoroughly understand the factors involved in the spread and virulence of the disease in the wild (Braun *et al*. 2011b; Boarman 2002; Sandmeier *et al*. 2009). See Sandmeier et al. (2009) and Jacobson et al. (2014) for reviews of information on mycoplasmosis.

The most commonly described shell disease in desert tortoises is cutaneous dyskeratosis, which manifests itself as lesions along scute sutures of the plastron and sometimes on the carapace, which then spread to the scutes themselves (Jacobson *et al*. 1994; Homer *et al*. 1998). The shell can appear discolored, dry, rough and flakey, and there is also peeling, pitting and chipping through multiple cornified layers. In advanced cases, exposed areas become infected with bacteria, fungus, and exposed tissue and bone may become necrotic (Homer *et al*. 1998, 2001). Shell diseases have been seen in tortoise populations in the eastern Mojave and Colorado deserts of California but less so in the western Mojave Desert (Jacobson *et al*. 1994; Christopher *et al*. 2003). Shell diseases occur in all sizes and ages of desert tortoises but are usually more common in adults (Jacobson *et al*. 1994; Homer *et al*. 1998). It appears that shell diseases reflect metabolic and physiological changes that involve more than the shell itself (Homer *et al*. 1998, 2001). Little is known about the causes of shell disease; no evidence indicates a bacterial or viral origin, despite directed research efforts by pathologists to find one (Jacobson *et al*. 1994; Homer *et al*. 1998). Pathologists also report that the location and histologic appearance of cutaneous dyskeratosis lesions seen in tortoises are suggestive of a nutritional deficiency, a toxicosis, or both (Jacobson *et al*. 1994, Homer *et al*. 1998). The extent to which shell diseases contribute to population declines in desert tortoises remains unclear (Jacobson *et al*. 1994).

In tortoises with herpesvirus infection, clinical signs include lesions and plaques on the tongue and hard palate (Johnson *et al*. 2006). The contribution of herpesvirus to population declines of the desert tortoise is unknown; however, herpesvirus infection has been reported in wild desert tortoises (Jacobson *et al.* 2012; Jacobson *et al.* 2011). In captive desert tortoises herpesvirus infections have been associated with illness and mortality (Johnson *et al*. 2006; Braun et al. 2011a). Clinical herpesvirus infections can be rapid and progressive, resulting in large die-offs in other species of vertebrate animals as well (Johnson *et al*. 2006). Therefore, at least in theory, herpesvirus could become a serious threat to desert tortoise populations.

There is evidence that harboring one disease may predispose desert tortoises to other diseases (Christopher *et al*. 2003); however, it is not known whether this is a cause or effect. That is, it is not known whether disease in an individual increases susceptibility to other diseases in that individual or whether an individual's baseline susceptibility to disease causes that individual to get more diseases. Nevertheless, positive nasal cultures for *Mycoplasma agassizii* had relatively high positive predictive values for tortoises with moderate to severe shell disease (Christopher *et al*. 2003).

In some cases, a disease may become apparent and get very severe soon after an animal has been exposed to the pathogen. Such diseases are generally called 'acute diseases' because the onset and progression of the disease is rapid and severe, sometimes resulting in death. The direct impacts of an acute disease to a population often become apparent quickly because these diseases usually spread through a population of animals very rapidly. In other cases, the disease may be more insidious, and exposure to the pathogen results in a chronic illness that progresses slowly over time. Animals with a chronic disease may have reduced reproductive capacity, may not grow normally, may be more susceptible to secondary infections or conditions such as predator attack or vehicular trauma, and in some cases, may have a reduced life span. Mycoplasmosis in tortoises is increasingly considered to be such a chronic disease (cf. Berish *et al*. 2010). Identifying the impacts of such diseases can be a difficult task. There is an abundance of literature describing the potentially severe, long-term consequences of chronic disease on animal populations (Spalding and Forrester 1993; Hess 1996; Heffernan *et al*. 2005). However, the full effect of chronic disease on a long-lived species such as the desert tortoise may take decades to be seen. Therefore, it is important that populations are monitored using standardized techniques so that any changes associated with health problems may be detected over time.

Table 1 provides a list of clinical signs that may be encountered when examining tortoises and some of the potential causes of these signs. Although it is not an exhaustive list of every potential problem that may be encountered when conducting physical examinations of tortoises, it covers the most common signs of illness.

Table 1. Clinical signs that may be observed when conducting physical examinations on desert tortoises in the field.

Clinical Signs	**Possible Conditions/Causes**
Behavioral	
Extending neck, increased respiratory effort, mouth gaped open	Respiratory disease (e.g., tracheal obstruction, reduced lung capacity secondary to coelomic cavity effusion, pneumonia - many potential causes such as bacterial and/or viral)
Weakness, non-responsive, outside burrow during cold weather	Severe debilitation (many potential causes)
Head	
Eye discharge (serous or mucous), red and/or swollen conjunctiva and	Eye infection, URTD*, abrasion/irritation, foreign bodies, nutritional imbalance

periocular tissue	
Nasal discharge (serous or mucous)	URTD*, clear nasal discharge sometimes seen after a tortoise has been drinking, do not mistake frothy oral discharge from stressed or overheated tortoise for nasal discharge
Erosion and/or depigmentation of skin around nares	Trauma, chronic nasal discharge associated with URTD*, skin infection
Asymmetrical or abnormally shaped nares	Normal, impacted with sand/soil, scarring or exudate secondary to chronic infection such as URTD*
Abnormal breath sounds (wet/crackling sounds)	URTD*, pneumonia (many potential causes)
Swollen tympanum (membrane over ear)	Abscess (bacterial infection), vitamin deficiency, trauma
Oral cavity	
Pale mucous membranes	Anemia, severe debilitation, shock
Ulcers, crusts, or plaques on tongue or inside mouth	Viral (herpesvirus, iridovirus), bacterial or fungal infection, embedded foreign body
Limbs/body	
Skin swelling	Abscess/infection, parasite, tumor, trauma, over hydration
Skin discoloration	Incomplete shed, infection, scar from prior injury
Emaciation/reduced muscle mass	Starvation, severe debilitation (many potential causes), chronic disease
Lameness, swollen joints	Trauma/injury, nutritional disease, metabolic disease (such as gout), neurological disease
Firm mass/object in coelomic cavity	Substrate ingestion, feces, eggs, urolith (bladder stone), tumor
Shell	
White or yellow discoloration, flaking	Healing traumatic injury, bacterial or fungal infection, dyskeratosis (nutritional deficiency, toxicity, autoimmune disease, infectious disease, metabolic disorder, or other)
Soft spots, especially at mid-carapace	Nutritional disorder, toxicity, trauma
Red blotches	Trauma, infection
Malformed shell	Trauma, nutritional disorder

* URTD: Upper respiratory tract disease caused by *Mycoplasma spp* or other pathogen.

5. Conducting Health Assessments

Disinfection/sanitation protocols

Caution must be taken whenever handling or sampling desert tortoises to ensure that field personnel do not aid in the spread of infectious microorganisms or contaminate samples. Cleaning refers to the physical removal of organic debris (dirt, feces, urine, blood, etc.) from objects or living tissue. Disinfection refers to the elimination or inhibition of the growth of microorganisms (except bacterial spores) on non-living objects, whereas antisepsis involves the same process for living tissue. In contrast, sterilization is the complete

elimination or destruction of all forms of microorganisms (including bacterial spores) on non-living objects and is generally not possible in the field.

Field personnel must follow a standard protocol that includes the disinfection of all equipment. Field personnel should wear disposable latex or nitrile gloves whenever handling tortoises, and change gloves between individual tortoises. If gloves are torn when handling a tortoise, a new glove should be placed over the torn glove and an antiseptic should be used on the hands after handling of that individual is complete. A disinfection protocol is provided in Appendix A.

Tortoise capture/handling

Desert tortoises will be captured and transported according to existing protocols (USFWS 2009). Health assessments may occur at the capture site or within a project's quarantine pens.

Handling of tortoises should occur in a manner that minimizes stress. Effort should be made to keep the tortoise as close to ground level as possible and in its normal spatial orientation. Tortoises can be slightly rotated out of normal spatial orientation for necessary data and sample collection. Due to increased risks of voiding and stress, tortoises should not be turned upside down. Caution should also be taken to hold tortoises away from the handler's body (pants, shoes) to minimize risk of contamination from contact or voiding.

Handling a tortoise for health assessment and sample collection must be completed within 30 minutes or less. This time period does not include rehydrating tortoises that void.

Physical examinations

Health studies in wildlife typically include physical examinations and the collection of biological samples for a number of diagnostic tests (Christopher et al. 1999; Karesh *et al*. 1999; Berry and Christopher 2001; Hanni *et al*. 2003; Kilbourn et al. 2003; Deem *et al*. 2005; Deem *et al*. 2006; Uhart *et al*. 2006). Excellent guidelines have been published describing techniques for the evaluation of health in chelonians (Jacobson *et al*. 1999; Berry and Christopher 2001). Berry and Christopher (2001) provide sample data sheets and have helpful line drawings that show, in detail, how to examine the eyes and periocular area of desert tortoises. The manuscript also describes abnormal behaviors observed in desert tortoises that may be indicative of a health problem. Anyone planning to conduct a health study in wild tortoises should read that journal article (available online at http://www.jwildlifedis.org/cgi/reprint/37/3/427). The health assessments that are required in conjunction with translocations of desert tortoises include a physical exam, during which very specific clinical signs of interest are evaluated, and collection of several biological samples. These health assessments are specifically designed to minimize risks when translocating tortoises and are not intended to be in-depth studies into the diseases that may affect an individual.

Performing a physical examination requires, at minimum, knowledge about normal tortoise behavior (attitude and activity) and physical appearance. For this reason, we consider conducting health assessments to be an advanced skill that novice tortoise biologists are not yet prepared to learn. Additional training is required if biological samples are going to be collected. The basic components of the physical exam include an overall assessment of the attitude and activity of the tortoise, an examination of the eyes, nares, beak, oral cavity, skin, muscle mass, and shell. Body condition scoring (BCS) is a system for evaluating the condition of domestic

animals, and BCS has been modified for desert tortoises (Appendix D). The BCS is based on an evaluation of muscle mass and fat deposits in relation to skeletal features. A tortoise's body mass can vary greatly with hydration state or other physiological processes, so mass is of limited value when used alone. Other scoring systems exist such as condition index based on body mass and size (Nagy et al. 2002). The BCS is a rapid method of evaluating muscle mass and other characteristics and is currently being used by the U.S. Fish and Wildlife Service for these health assessments. Morphometric (i.e., body) measurements are used to determine the maturity of individual tortoises and to track growth over time. A brief explanation of the physical examination and procedures for completing the data collection forms are described in Appendix B. Appendix C provides photographic examples of numerous clinical signs that may be encountered when conducting these exams.

Diagnostic tests

Diagnostic assays (i.e., tests) greatly complement the physical examination because many diseases have an incubation or subclinical period when tortoises may not exhibit outward signs of illness. These diagnostic tests provide extremely valuable information regarding the immunological, nutritional, and physiological status of the animal and can indicate specific organ dysfunction or disease. Therefore, specific assays may detect a disease process before clinical signs develop. In some cases, the presence of a specific set of clinical signs observed in tortoises may prompt one to run diagnostic tests to try to determine the underlying cause of the signs.

Biological sample collection

Hands-on training is necessary to learn appropriate sample collection and handling techniques, and even more expertise is often required to interpret test results. Proper sample collection, processing, labeling, and handling is essential for meaningful, reliable, and repeatable test results. Samples that have not been collected, processed, labeled, or stored correctly may provide inaccurate results and can lead to inappropriate conclusions or decisions. For example, lymphatic vessels lie directly adjacent to the most commonly used blood vessels for sample collection in tortoises. Often blood samples are contaminated with clear lymphatic fluid, which may alter diagnostic test results dramatically, and therefore, when it occurs, it should always be noted on the sample tube and on data collection sheets, and results should be interpreted cautiously.

In this handbook and accompanying training, we instruct students on the particular techniques that should be used when collecting blood and oral cavity samples specifically for translocation-related health assessments. Information regarding collecting nasal fluids is included in the handbook for future reference. This technique may be necessary for specific research projects. The laboratories that will be analyzing these samples have specialized experience working with reptilian samples. This is important because reptilian blood cells and body temperatures differ from those of mammals, so standard laboratories may not have the appropriate laboratory setups or diagnostics. Sample handling and storage protocols, supply lists, and laboratory shipping information are included in Appendices E and F.

Biological sample storage

Even when diagnostic tests are not being performed at the onset of the project, blood samples, oral swabs, cloacal swabs, nasal lavages, and ectoparasites (e.g., ticks) may be collected, processed, and stored for future use in the event that a problem develops in the population. These stored samples can be used for retrospective studies in the event that a disease outbreak occurs, can be analyzed with new diagnostic tests that become available, or can simply be saved for analysis until sufficient resources become available in the future. The red blood cell (RBC) pellet that results from centrifugation of a blood sample to separate the serum should be saved because it may be used for DNA-based studies and, therefore, may be of value in the future. It is absolutely critical that blood products be stored appropriately to prevent degradation of the samples. Instructions for banking blood, nasal fluid, oral cavity, and cloacal samples are included in Appendix F.

Hydration after voiding

Voiding occasionally occurs while tortoises are handled for health assessment, data collection, and biological sampling. Probability of voiding generally increases with increased handling time and winter precipitation (Agha et al. 2015). Tortoises store urine for long periods of time and use this for maintaining hydration and proper physiological balance. Loss of urine due to handling-induced voiding may have an adverse affect on long-term survivability based on a previous retrospective study (cf. Averill-Murray 2002).

Given the potential for adverse effects on health occurring from voiding urine, it is recommended that all tortoises that void urine be offered or administered fluids to replace voiding losses. Options for fluid replacement include injectable epicoelomic fluids, nasal-oral offering of fluids, and soaking in a shallow water bath. Each option has positive and negative aspects that will be discussed below. Injectable epicoelomic fluids and nasal-oral administrations are the preferred two of the three USFWS authorized methods of fluid replacement. For these two methods, a dose of 20ml/kg should be administered. A maximum volume of 60ml will be injected for all weights exceeding 3kg. Tortoises with an MCL < 150 mm should not be offered injectable epicoelomic fluids due to increased potential for harm if fluids are administered improperly. Instead, these small tortoises should be offered nasal-oral fluids or soaked as their primary route of fluid administration.

Epicoelomic fluid administration in desert tortoises has been commonly and safely used by veterinarians for treatment of dehydration as well as for other reasons. The technique of epicoelomic fluid administration is not technically difficult and with proper training can be performed by most biologists in the field. Epicoelomic describes a location between the coelomic cavity/membrane and the shell. This is a potential space similar to subcutaneous areas. The benefit of using this location versus other subcutaneous locations is that fluids in this area do not appear to affect mobility or the ability of the tortoise to withdraw its limbs into the shell prior to complete absorption of the fluids. This procedure has the benefit of providing a known amount of fluid replacement and does not require significant amounts of equipment. It does require advanced training and permitting by USFWS prior to use on federally protected desert tortoises.

Nasal-oral administration has been used successfully to administer fluids to tortoises that void. A dose of 20 ml/kg of drinking water should be offered to the tortoise. This is done by slowly emptying a syringe of water into the nares or mouth. Water should not be forced into the tortoise. If the tortoise does not accept water this way, epicoelomic fluids should be administered or the tortoise should be soaked. This process has the

benefit of requiring less water to be carried by the biologist, and minimal equipment is needed; however, this method may not work for all tortoises.

Soaking is the last alternative for fluid replacement for tortoises that void. This requires a plastic container to hold water and it requires significantly more water than the other methods. The tortoise is placed in the plastic tote and water is added to the level between the skin-plastron junction and the chin. The tortoise remains in the tub soaking for 30 minutes. Tortoises may drink water through their nose or mouth or absorb it through their cloaca. Tortoises may void more urine while being soaked in an effort to eliminate urine with high solute levels and replace it with the fresh water being offered. If a tortoise voids in its soaking tub and additional fresh water is available, the water should be discarded and replaced with fresh water until the completion of the 30 minutes. Otherwise the tortoise can be soaked for 30 minutes in the water that contains the voided urine and urates. The tortoise should also be rinsed with water to remove urine odor on the limbs and shell, which might attract predators.

Complete protocols for all three of these methods are provided in Appendix F.6.

6. Interpreting Results and Making Decisions

Interpreting results

Data interpretation must be placed within the context of the overall goals, the questions asked, the limitations of the data collected, and the experience level of the individuals conducting the study. Although the collection of health related data from tortoises requires some level of basic training, interpretation and decision-making based on the results require a thorough understanding of normal tortoise biology and the health assessment tools used. For example, if clinical signs were observed in tortoises when conducting physical examinations, to interpret the findings one must be aware of the severity of the clinical signs (relative to normal), potential causes for the signs, and their biological significance. Alternatively, if clinical signs are not observed, managers must recognize that many diseases have subclinical periods (e.g., times when clinical signs are not exhibited) or have clinical signs that are intermittently expressed. Thus, although physical examinations are extremely useful, they do not provide conclusive evidence about the overall health of the individual tortoise.

There are a number of important considerations when interpreting results of diagnostic tests. For serological tests that detect antibodies, a positive result indicates that the tortoise has been exposed to a given pathogen, but it does not tell you if the tortoise is currently infected. The use of assays that have been validated properly and have had positive results correlated with pathological lesions is important to help make decisions regarding the significance of a positive test. Additionally, false positive and false negative results occur, which obviously complicates interpretation. Test performance varies with prevalence, stage of infection, concurrent diseases, immunological competence, experience of personnel, and cut-off values for positive tests. Tests should be applied and interpreted at the population level where they are effective at diagnosing disease within sick populations and confirming that healthy populations are uninfected. Conversely, tests are not as effective at identifying infected individuals among a mostly healthy population or uninfected individuals among a mostly sick population. We will be using the results of diagnostic tests for long-term monitoring (USFWS 2012, currently under revision), but not in determining an individual's eligibility for translocation. If hematology or biochemistry data are being used, one must be able to discern normal values from abnormal and additionally must understand the influence of season, reproductive status, and age on the test results. In particular, awareness of the limitations of the established sampling schemes, sample sizes, and methods selected for the project, as well as limits of the diagnostic tests mentioned above, are all very important considerations.

While field biologists will be preparing proposed disposition plans for the tortoises at their project sites after completing health assessments and receiving results of diagnostic tests, these plans MUST be submitted to the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office PRIOR TO relocating any tortoises. Final interpretation of results will be made by the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office in coordination with the state wildlife agencies, and partnering veterinarians and pathologists. Once interpretation is complete, USFWS will provide field biologists with an approved or modified disposition plan.

Management decisions

Most management decisions, including those based on the results of diagnostic assays and presence or absence of clinical signs, will have some degree of uncertainty associated with them. Management decisions must be made with a clear understanding of these limitations. The establishment of clear objectives greatly assists in weighing the importance of the limitations and making better management decisions based on

findings of the study. It is important to note that definitive or clear-cut answers may not be available, and therefore judgments must be made based on the best available scientific information at the time. A strong commitment to follow-up monitoring, within a well-designed research framework, will be critical to document results of management decisions and to adapt conservation strategies as deemed necessary.

Our objectives with regards to translocation-related health assessments are to minimize the risk of spreading disease within and among populations and to promote survival of individuals when relocating tortoises from project sites. Perturbation of populations will occur through translocation, and previous studies of desert tortoise translocations have given us information about likely movement patterns and behaviors. Using these data along with information about disease, we can devise a strategy that should meet our objective. Appendices G and H show the algorithm and disposition plan for making recommendations on whether or not individual tortoises should be translocated. The USFWS's guidance on translocation (USFWS 2012, currently under revision) describes how to choose appropriate recipient sites when considering the results of diagnostic tests and evaluation of clinical signs. As described above, the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office, in coordination with the state wildlife agencies and partnering veterinarians and pathologists, will make final decisions about translocating tortoises after reviewing data from the health assessments and from the proposed disposition plans that will be submitted along with those data.

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Glossary

Active (pertaining to lesions)
Status of a wound or lesion that is very recent or unhealed.

Aliquot
A portion of a total amount of solution or sample.

Anemia
A reduction in the number or volume of red blood cells in the blood.

Antisepsis
The elimination or inhibition of the growth of microorganisms (except bacterial spores) on living tissue (i.e., skin).

Atrophy
Decrease in size or wasting away of body part or tissue.

Axillary
The cavity or area around the forelimbs.

Beak
An external anatomical structure with a keratinized, horny covering, which serves as the mouth in some animals.

Bevel
The angled surface of the tip of the needle containing the opening.

Biochemistry panels
A number of tests relating to the chemistry of a living organism and vital processes that occur in living cells. Tests provide information regarding the function of major organ systems and metabolic processes.

Biological samples
Samples collected from living organisms or their products (i.e., blood, feces, urine, etc.).

Body condition score (BCS)
Body condition scoring is a system developed for domestic animals to estimate the average body condition of animals in a herd. This system provides managers a relative score based on an evaluation of muscle mass and fat deposits in relation to skeletal tissue. This scoring system has been adapted for the desert tortoise. A score of 1-3 is under condition, 4-6 is acceptable to good condition, and 7-9 is over-condition.

Catastrophic event
A sudden, short-lived, violent event that has a profound impact on a population (i.e., hurricane or other natural disaster, substantial population mortality associated with a disease, etc.).

Chelonians
Belonging to the order Chelonia, which includes the turtles and tortoises.

Choana
Opening of the upper respiratory tract in the upper oral cavity.

Coelomic Cavity
Internal cavity containing organs.

Complete Blood Count (CBC)
A series of tests that assess the quantity of each type of blood cell in a sample of blood, often including the amount of hemoglobin and the proportions of red blood cells and various white blood cells. Tests provide information regarding the immunological status of the animal and function of specific organ systems.

Chronic disease
A disease with slow progression and a long duration.

Cleaning
The physical removal of organic debris (dirt, feces, urine, blood, etc.) from an inanimate object or living tissue.

Clinical sign
An objective indication of some medical fact or characteristic that may be detected by a physician/veterinarian during a physical examination of a patient. A sign is not the same a symptom, which is subjective and observed by a patient.

Compressible
Capable of being flattened by pressure or pressed into a smaller space.

Conjunctiva
Mucous membranes that line the inner surface of the eyelids including the third eyelid.

Corneal opacity
A local or generalized loss of transparency of the surface of the eye.

Crust
An outer layer of solid material formed by drying of a bodily exudate or secretion

Debilitation
Being in a state of severe weakness; lack or loss of strength.

Disease
An abnormality in structure or function of a living organism. May be identifiable with clinical signs or may be subclinical (i.e., with no outward visible signs).

Disinfection
The elimination or inhibition of the growth of microorganisms (except bacterial spores) on inanimate objects.

Dyskeratosis
Abnormal development of keratin cells in the shell and skin of tortoises. Causes are many and may include toxins, auto-immune disease, infection, trauma, metabolic problems, or due to a nutritional deficiency.

ELISA (Enzyme-Linked Immunosorbent Assay)
A biochemical technique used to detect the presence of specific antibody or antigen in a plasma or serum sample.

Epicoelomic
A potential space between the coelomic membrane and the shell in reference to the location injectable fluids are administered.

Epidemiology
The frequency and distribution of disease in populations, and the detection of the source and cause of disease.

Eroded nares
Loss or wearing away of scales and/or skin around nares.

Erosion
A localized to extensive, superficial defect of the epithelial surface of a tissue (e.g. tongue, skin)

Etiologic agent
The cause or origin of a disease.

Evidence of foraging
Presence of food or coloration (green, pink) on beak.

False positive
A test result that is read as positive but actually is negative; a test that shows evidence of a disease or immune response when it is not present.

Feces
Bodily waste discharged through the digestive tract; excrement.

Generalized
Spread throughout most of an area

Hematology
The study of the nature, function, and diseases of the blood and of blood-forming organs.

Hydration
The process of providing an adequate amount of liquid to bodily tissues.

Hypersalivation
Increased saliva in or around the oral cavity.

Immunological
Pertaining to immunology, or the study of all aspects of the immune system, immunity from disease, the immune response, and immunologic techniques of analysis.

Impaction
Lodgment or instance of lodgment of something, such as food or debris, in a body passage or cavity.

Inactive
The condition of a wound or lesion that is no longer changing significantly or is healed.

Incubation
The maintenance of control over temperature, humidity, and oxygen concentration in order to provide optimal conditions for growth and development of an organism.

Incubation period
The time period between invasion of the body by a pathogen and development of the initial signs of disease. This period may range from days to years, depending on the type of disease.

Inflammation
A local response to cellular injury that is marked by redness, heat, pain, swelling, and often loss of function.

Lavage
The process of washing out a body cavity, such as the nasal sinuses, for diagnostic or therapeutic purposes.

LB
Luria-Bretani media used as a nutrient preservative for storing biological samples.
Lesion
An abnormal change in structure of an organ or part due to injury or disease

Lethargic
Sluggish or lacking energy.

Localized
Restricted to a particular place or area.

Lymph
A yellowish, transparent fluid that is composed of water, plasma proteins, chemical substances and lymphocytes (a type of white blood cell).

Lymphatic vessels
The vessels that collect lymph and transport it between tissues and the bloodstream.

Mental glands
Paired symmetrical glands on the chin.

Metabolic disease
A disease in which normal body metabolism is altered resulting in an excess, absence, or shortage of specific enzymes or substances needed for normalcy.

Microorganisms
An organism of microscopic or submicroscopic size, including bacteria, viruses, some fungi and protozoa.

Morphometric measurement
The measurement of forms and shapes (i.e., for these purposes, the measurement of tortoise body shapes, including plastron/carapace length, body thickness, body width, plastron concavity, and other body measurements).

Mucous
Thick viscous discharge that may be clear, cloudy, or discolored.

Multifocal
Having to do with two or more places or areas.

Naïve population
A population that has not been exposed to a pathogen of interest.

Naris (s), Nares (pl)
The external opening(s) of the nasal cavity in reptiles and birds; nostrils.

Necropsy
The examination of an animal's body after death; similar to an autopsy, which is an examination of a human's body after death.

Occluded naris
Narrowing or reduced opening of the naris due to debris, exudates, or scarring.

Oral mucosa
Membranes lining the oral cavity.

Packed cell volume (PCV)
The percentage of red blood cells relative to the total volume of blood in the sample.

Palpation
Physical examination by pressure of the hand or fingers to the surface of the body to determine the condition of an underlying organ or body part.

Pathogen
Any disease-causing agent; includes viruses, bacteria and other microorganisms.

Pathology
The study and diagnosis of disease through examination of organs, tissues, cells and bodily fluids.

PCR
The **polymerase chain reaction** (**PCR**) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Periocular
The tissues surrounding the eye including the upper and lower eyelids.

Physiological
Pertaining to physiology, or the study of the physical and chemical factors and processes associated with the normal functioning of a living organism.

Plasma
Non-cellular portion of the blood that includes clotting factors.

Plaque (oral)
A localized patch or flat area in the oral cavity. Plaques tend to have a white or yellow appearance.

Potential space
A space that can exist within the body because adjacent organs, membranes, etc. are not tightly adjoined. The space does not exist during normal functioning, but can be created during activities such as epicoelomic fluid administration.

Prefemoral
The cavity or area in front of the hind leg.

Prevalence
A ratio of the number of cases of a disease to the number of individuals at risk in the population for a given time period.

Recipient population
The population receiving individuals.

Sagittal crest
The ridge of bone running lengthwise along the midline of the top of the skull.

Scute
A hard, keratinized, external plate or scale overlying the partially bony dermis of the tortoise shell.

Serology
Dealing with the immunological properties and actions of serum (i.e. blood); evaluation of antigen-antibody reactions in a laboratory setting to determine if an animal has been exposed to a specific pathogen.

Serous
Clear, colorless, watery discharge

Shock
A life-threatening medical condition where blood flow to the body tissues is inadequate, often resulting in reduced oxygen and nutrient delivery to the tissues, and sometimes cardiac arrest (the heart stops beating).

Sterilization
The complete elimination or destruction of all forms of microorganisms (including spores).

Subclinical
Not showing characteristic clinical signs or symptoms of a condition.

Temporal muscle
A large muscle that serves to raise the lower jaw and attaches to the sagittal crest.

Total Protein (TP)
A rough measure of all the proteins found in the plasma portion of the blood.

Ulcer
A localized defect or excavation of the epithelial surface of a tissue (e.g., tongue, skin), usually produced by sloughing of necrotic inflammatory tissue.

Upper Respiratory Tract Disease (URTD)
An illness caused by an infection of the upper respiratory tract (nasal passages, nasopharynx, pharynx, larynx, and extrathoracic trachea). Can be caused by several bacteria, viruses, or fungi.

Urate
A salt of uric acid.

Urine
Waste material that is secreted by the kidney, is rich in end products of protein metabolism (such as urea, uric acid, and creatinine) together with salts and pigments, and forms a clear fluid.

Urolith
A calculus (i.e., stone or concretion) in the urine or the urinary tract.

Variable strain virulence
Differences in disease severity caused by different strains of a pathogenic microorganism.

Vent
External opening of the cloaca.

Void
To eliminate solid or liquid waste from the body.

Appendix A: Disinfection and Sanitation

Caution must be used whenever handling or sampling desert tortoises to ensure that pathogens (i.e., disease causing microorganisms) are not introduced to a field site through contaminated equipment or clothing items. Equally important, field personnel must take necessary precautions to ensure that they do not aid in the unintended transmission of pathogens among the individual tortoises sampled or contaminate the samples that have been collected. Therefore, development and implementation of a step-by-step disinfection protocol is essential for field studies. An effective disinfection protocol must address the microorganisms being targeted, the characteristics of the disinfectant, and the impact a disinfectant may have on the environment. The health and safety of field personnel and desert tortoises are of vital importance.

A number of products are available for disinfection/sanitation purposes. The most common antimicrobial products fall within one of the following classes: alcohols (i.e., hand gels), chlorine (i.e., bleach), iodine/iodophors (i.e., povidone, iodine), chlorhexidine (i.e., Nolvasan), oxidizers (i.e., VirkonS), phenols (i.e., Lysol), quaternary ammonia (i.e., Roccal), and aldehydes (i.e., Wavicide). Each has varying effectiveness for different classes of microorganisms, and the reader is referred to the University of Nebraska - Lincoln Extension website entitled, 'Selection and Use of Disinfectants' for more information about disinfectants.[1] The effectiveness of any disinfectant or antiseptic is determined by the amount of time that the cleaning agent is allowed to contact the surface, concentration of the product used, the organic load (amount of dirt/debris), the level of microorganism contamination, the condition of the object being cleaned (cracks, crevices, wood vs. plastic surfaces), ambient temperature, and sometimes the environmental pH. In field conditions where high organic loads are almost always present, effective antisepsis and disinfection are not possible without first cleaning to remove excessive debris. Therefore, regardless of the agent used, an initial cleaning is required.

The protocol listed below describes methods to be used when working with desert tortoises. Certainly, other protocols may be developed to meet the needs of specific projects or management goals, upon approval from the U.S. Fish and Wildlife Service. Users should follow local and state regulations for transport and disposal of disinfectant solutions.

Disinfectant solutions

Solutions should be stored in dark bins or in opaque bottles and should be made fresh regularly (i.e., daily or weekly depending on storage conditions). Take care to keep all solutions off of skin and out of eyes. Gloves should be worn when using disinfectants. Appropriate choices as disinfectants include those listed below.

Trifectant®

This product is sold as a powder or tablet and is effective against viruses, bacteria (including mycoplasma), and fungi. It is also fairly resistant to inactivation by hard water and organic matter. Once mixed, the solution is stable for seven days. Contact time for disinfection is 5-10 minutes and metal instruments should not be soaked for more than ten minutes. A 1-2% solution should be mixed according to instructions on the packaging.

[1] Available through URL: http://www.ianrpubs.unl.edu/epublic/live/g1410/build/g1410.pdf

Chlorhexidine diacetate (e.g. Nolvasan®)
This disinfectant is sold as liquid and is effective against viruses, bacteria, and fungi. It should be prepared according to the instructions on the packaging.

Bleach
While no longer the preferred option, a standard bleach solution may be used (1:20 dilution of 5 percent household bleach in water). Bleach should be purchased in small bottles or dispensed into small bottles to minimize deterioration from opening/closing the lid. In the event of a high organic load, thoroughly remove or wash the organic material off before using bleach to disinfect to prevent the bleach from being inactivated. Bleach solutions are also readily inactivated by sunlight.

Protocol for cleaning and disinfecting equipment and sanitizing personnel

All equipment must be thoroughly cleaned and disinfected prior to arriving at a field site. Additionally, all equipment and work surfaces (if not on natural ground) must be cleaned before and after handling each tortoise. Efforts should be made to first remove organic debris (i.e., dirt, feces, etc.) by rinsing the area with water, brushing debris off with paper towels, or cleaning the equipment/work surface with the chosen disinfectant solution and wiping with paper towels. The equipment should then be thoroughly moistened with disinfectant at appropriate dilution and appropriate contact time and dried or allowed to air dry. Heat and direct UV light exposure will improve the disinfection process.

Gloves are to be worn and changed between handling individual tortoises to prevent the transfer of pathogens from one tortoise to another. Additionally, wearing gloves will protect field personnel from the effects of potentially irritating disinfectants and pathogens. Contaminated gloves should be removed after handling and cleanup and prior to the disinfection process. New gloves should be used while disinfecting equipment. All items touched with contaminated gloves should be considered contaminated (e.g. pens, clipboards, clothing). Skin must also be sanitized between tortoises, if contact with a tortoise and/or its excretions occurs. Acceptable sanitizing solutions include soap and water (if available) or a hospital grade ethyl alcohol hand wash (minimum alcohol concentration of 60%). Example product: Alcare Foamed Alcohol Scrub (Steris Co.). Follow manufacturers' recommended contact times for all sanitizing products. Removal of organic debris is essential for proper sanitation; therefore, a water rinse before using the product will be more effective when hands are extremely dirty. Alcohol hand washes should be allowed to air dry while rubbing hands vigorously in order to appropriately distribute the product.

Any individual that has broken skin as a result of a needle stick should notify their supervisor. Supervisors should have appropriate protocols in place and review them with all field personnel prior to the initiation of any fieldwork.

Appendix B: Completing the Health Assessments and Data Collection Form

This appendix provides an overview for conducting a physical examination of a desert tortoise and completing the data collection form, but it is not a substitute for appropriate training and hands-on experience. A thorough and accurate physical examination can only be performed if the individual performing the exam has knowledge of normal tortoise physical appearance and behavior. Prior training in assessing desert tortoise health is required. Physical examinations provide valuable information about the health of individual tortoises and insight into population level issues. Because the desert tortoise is a long-lived species, it is extremely important to use standardized techniques for data collection so that information can be compared over time.

This appendix provides a description of how to conduct a basic physical examination of a desert tortoise and follows the order of the provided data collection form. A minimum of two people will be needed in order to conduct a full health assessment that includes the collection of biological samples. One person will serve as the examiner and will also complete the data collection form. This person should be able to stay clean (i.e. not touch the tortoise or contaminated items) until he/she begins to collect the biological samples. The other person (handler) will handle the tortoise such that the examiner can complete the examination and collect samples. If a third person is available, this person (rather than the examiner) may record the data to further reduce contamination risks. It is important to complete certain observational aspects of the physical exam first, so that potential clinical signs are not affected by handling of the animal. For example, head restraint may cause eye bulging or serous discharge so it should be done after initial evaluation. Most of the health assessment can be conducted with minimal, if any, handling of the tortoise. We recommend using the order described below for a systematic health evaluation of every animal. Generally, the tortoise will be evaluated from a distance before direct contact (far to near), beginning at the head and working towards the tail (head to tail), and least invasive procedures will be conducted prior to more invasive procedures (least to most). This approach also helps field staff remember to collect all of the data consistently because they fill out the form as they proceed with the exam. Be sure to carefully describe all anomalies such that comparisons can be made with subsequent examinations. Handling a tortoise for health assessment and sample collection must be completed within 30 minutes or less. This time period does not include rehydrating tortoises that void.

Unless tortoises are being moved into a quarantine holding facility for their health assessment, they should not be moved far (<20 m, if possible) from the location at which they are found in the field. Before the handler touches the tortoise, find a flat shaded area with ample work space where you will not disturb a tortoise's shelter sites. If shade is not available, it can be created by placing a lightweight cloth over vegetation. Be sure to record the temperature where you plan to work to ensure that it falls within the requirements established by USFWS for handling tortoises. If the temperature is appropriate, lay out equipment in the order that it will be used and place it out of the reach of where you will place the tortoise. Depending on the substrate, the top few centimeters of soil can be cleared away, such that the tortoise is in contact with cooler soil while you work. Prepare all the biological sampling equipment and supplies and set them up in an orderly manner for easy access during the health assessment. An area on one side of the examiner should be designated for clean supplies and equipment and an area on the other side for contaminated items. After taking pertinent initial data (date, start time, project name, site description, tortoise ID, transmitter frequency if applicable, GPS location, temperature, and full names of the biologists), record the time at which you approach the tortoise and are within 2 meters of it. Take care not to startle the tortoise as you approach it. While wearing gloves,

the handler should gently lift the tortoise a short distance off the ground by grasping the sides of the shell and slowly carry it to the shaded area while maintaining it close to the ground in its normal spatial orientation.

While placing the tortoise on the ground in the work area, be sure that the tortoise does not touch any of the supplies or equipment. The examiner should perform as much of the visual observation portion of the health assessment as possible before the handler holds the tortoise again. Note that when completing the data collection form, right and left refer to the tortoise's right and left sides (not right and left from the examiner's perspective if looking at the tortoise head-on). Descriptions of how to complete each data field on the data collection form follow the presentation of the actual form below.

http://www.fws.gov/nevada/desert_tortoise/dtro/dtro_trans.html

Desert Tortoise Health Assessment (HA) Data Collection Form—Translocation Projects U.S. Fish and Wildlife Service May 2016

Date (ddmmmyy)	Project name	Site description / current pen #	Tortoise ID#	Transmitter frequency
				N/A or _____

GPS datum	UTM zone	UTM easting	UTM northing	Temp ◦C	Full name of biologist(s)	HA start time (24h)

Attitude/activity:	Initial Weight	MCL mm	Width V3 mm	Height V3 mm	Respiration: Abnormal sounds (describe in notes)	Beak: Physical defect Staining
Appropriate Lethargic-weak	g				Increased effort None of above	Evidence of foraging None of above

Left naris: Eroded Occluded	Left naris discharge and severity:	Right naris: Eroded Occluded	Right naris discharge and severity:
None of above	None Serous: 1 2 3 Mucous: 1 2 3	None of above	None Serous: 1 2 3 Mucous: 1 2 3

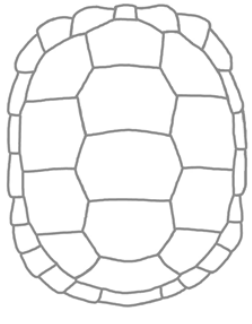
Left eye:	Right eye:
Sunken Corneal opacity Partially closed Fully closed	Sunken Corneal opacity Partially closed Fully closed
Serous discharge Mucous discharge Periocular swelling Periocular redness	Serous discharge Mucous discharge Periocular swelling Periocular redness
Conjunctival swelling Conjunctival redness None of above	Conjunctival swelling Conjunctival redness None of the above

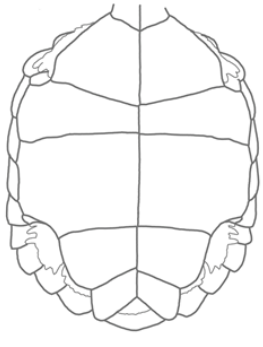
Skin lesion location:	Condition of skin lesion(s):	Ticks: 0 1-10 >10 Collected? N/A Yes No
None Head Neck L / R forelimb	N/A Active Inactive	All Removed? N/A Yes No
L / R axillary region L / R hindlimb L / R pre-femoral region Vent-tail		

Shell characteristics:	Shell characteristics location:	Condition of shell trauma:	Circumstances of skin/shell trauma:
Sunken scutes Whitish flaking Trauma	Carapace Plastron N/A	N/A Active Inactive	N/A Unknown Suspect predator Vehicle
None of above Other _____(describe below)	(Describe or draw below)		Other_____

Sex:	Body condition score:	Coelomic cavity palpation:	Photos (take all*):
M F Unknown	1 2 3 4 5 6 7 8 9	No mass L / R mass Not done	____Label or data sheet / ____Front face ____Left side face / ____Right side face / ____Carapace ____Plastron (*only if abnormal) / ____Abnormalities / ____Other

Draw, label, and describe abnormalities, trauma, anomalies, lesions, missing body parts, and identifying features (including gulars and head/limbs/tail *if noteworthy*).





Oral cavity characteristics and tongue color:	Choana:	# oral swabs collected
Not examined Hypersalivation Impaction	Not examined	
Crust-Plaque-Ulcer White Pink Red other _____	White Pink Red	_____

Total volume blood + lymph collected	Total # hep tubes	Indicate ml lymph (L) / ml total in each hep tube (number each tube)
_____ml	0 1 2 3 4	1)_____ml L/_____ml total 2)_____ml L/_____ml total 3)_____ ml/L/_____ml total 4)_____ml L/_____ml total

Void during processing:	Hydration method: N/A Soak Nasal-oral Epicoelomic
None Urine-urates Feces	Fluid type _____ Vol _____ml

Disposition:	End handling time (24h)	Total # of tubes/vials collected
Wild capture location Same pen _____ New pen _____ Translocated _____ Other _____		

Blood processing time (24h)	Plasma color:	UFL plasma aliquots:	USFWS plasma aliquots:	Total tubes with RBCs saved:	Total # tubes saved (for submission/banking):
	Colorless Red Pink Yellow Green	0 1	0 1 2 3	0 1 2 3 4	

Notes:

<u>Description of data fields on the May 2016 data collection form</u> Keep a copy with you in the field as reference.

Date (ddmmmyy) – 2 digit day, 3 letter month, and 2 digit year (e.g. 04Mar11).

Project name – Name and phase of the project.

Site description/current pen # – If the tortoise is newly caught from the wild, provide a description of the capture location, or if the tortoise was already in captivity, provide the quarantine pen number.

Tortoise ID # - Each tortoise will be assigned a unique identification number from a series of numbers assigned by USFWS for that project. The tortoise may have been numbered prior to the health assessment or you may need to number it (refer to numbering protocols provided by the USFWS).

Transmitter frequency – Numbers of the radio transmitter frequency, if applicable. Usually includes 3 digits, a decimal, and 3 more digits (e.g. 164.020). Mark N/A, if there is no transmitter.

GPS datum – **Make sure your GPS is set to UTM, WGS 1984.** Datum is the model used to match the location of a feature on the ground to the coordinates of the feature on a map.

UTM zone – In the UTM system, the earth is divided into 60 zones of 6 degrees of longitude wide. Your GPS displays zone as 2 digit number (e.g. 11, 12 that may be followed by N denoting north of the equator).

UTM easting – 6 digit number displayed on your GPS. Easting is measured from the central meridian of the zone, which is given the value of 500,000 m, and increases as you travel east.

UTM northing - 7 digit number displayed on your GPS. Northing is measured relative to the equator, which is assigned a value of 0 in the northern hemisphere, and increases as you travel north.

Temp °C – Temperature in degrees Celsius measured 5 cm above the ground in the shade and protected from wind where you set up your equipment to process the tortoise, or as specified in the most recent USFWS Desert Tortoise Field Manual.

Full name of biologist(s) - First and last names of the biologists handling the tortoise and recording the data. List the biologist primarily responsible for conducting the health assessment and biological sampling as the first name, followed by the name of the person assisting by handling the tortoise, then the name of person recording the data, if applicable.

HA start time – Health assessment start time. This is the time at which you approach a tortoise and get within 2 meters of it to begin assessing its condition, even without touching it or moving it. If a tortoise must be extracted from its shelter, that activity must be included in the start time. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45). This time goes on all sample tubes.

Attitude and activity – Circle one.

Appropriate – Tortoise paddles its forelimbs when held, attempts to escape, and repeatedly retracts into its shell when handled; or is shy and tends to remain retracted into its shell when being handled,

but has normal strength. During cold temperatures, activity may be minimal, but is considered appropriate for the temperature or time of year.

Lethargic-weak – Forelimbs may hang limp when tortoise is lifted, tortoise appears weak, is slow to respond to stimuli, and/or does not resist gentle tugging on the limbs.

Initial weight g - Weigh the tortoise prior to extensive handling to avoid the risk of the tortoise voiding. Spring scales or electronic balances may be used. When using a spring scale, weigh the tortoise with the smallest scale appropriate for the individual to get an accurate weight. Record in grams.

MCL (mm) – Straight midline carapace length measured in millimeters by holding the calipers directly over the center line of the tortoise and measuring from the center of the outer edge of the nuchal scute to the most caudal aspect of the carapace. Note that the most caudal end of the carapace may not be the edge of the supracaudal scute.

Width V3 (mm)- Width of the carapace measured in millimeters by holding calipers directly over the center line of the tortoise crosswise in the middle of the 3rd vertebral scute. Keep the calipers level with the ground.

Height V3 (mm)- Height of the carapace measured in millimeters by holding the tortoise so that one arm of the calipers is held across the center of the 3rd vertebral scute on the carapace and the other arm of the calipers is held in the same position across the plastron so the tortoise is between the arms of the calipers. The arms of the calipers should extend all the way from one side of the tortoise to the other while being held level against the tortoise's carapace and plastron.

Plastron – This measure was discontinued in 2015. If your project still requires it, see precautions below. Length of the plastron measured in millimeters measured by having a handler tip the tortoise to one side (the tortoise's left or right side) so the examiner can see the plastron. The examiner then uses calipers to measure from the notch between the gular scutes to the notch between the anal scutes. If the calipers do not fit into the notch, then hold them outside the notch on the plastron where the notch comes to a V to get the most accurate measurement. Do not tip the tortoise too far to the left or the right and do not put it on its carapace to take this measurement.

Respiration – Circle all that apply.

Abnormal sounds - Includes wet, crackling, or gurgling sounds associated with congestion. Describe in notes section at bottom of data form.

Increased effort – Tortoise pumps forelimbs up and down symmetrically when breathing, may indicate lower respiratory disease or compromised lung volume. Open-mouthed breathing or neck extended while breathing may indicate increased respiratory effort, but this must be distinguished from occasional normal gaping.

None of above – If neither of the above apply, circle this choice. This choice includes no audible sounds and appropriate respiration sounds (such as faint, high-pitched whistle when expelling air out of nares).

Beak – Circle all that apply.

Physical defect – Unusual shape, size, or structure. Describe in notes.

Staining – Discoloration not associated with food

Evidence of foraging – Presence of food or coloration associated with food, usually green or black, but may be more colorful (e.g. pink) in spring depending on food sources.
None of above – This choice includes beaks of appropriate shape, size, color, and texture. May include beaks with dirt stuck on them.

Left/right naris – Circle all that apply.
Eroded - Loss of scales and skin around naris opening.
Occluded – Plugged or reduced size of naris opening.
None of the above – Includes usual shape and/or size, as well as conditions not listed.

Left/right naris discharge and severity – Circle one description of discharge and one number of severity.
None – No discharge present.
Serous - Clear, watery discharge present.
Mucous – Cloudy, thick discharge present.

1. Mild - Moisture present around nares.
2. Moderate - Discharge coming out of the nares, but not running far from the nares themselves.
3. Severe - Discharge coming from nares that is running down the beak.

Left/right eye – Circle all that apply.
Sunken – Eye recessed within the orbit.
Corneal opacity- Eye is cloudy, hazy, or there is a loss of transparency of the cornea.
Partially closed – Eye is not fully open.
Fully closed – Eye is not open at all.
Serous discharge - Clear, watery discharge present.
Mucous discharge - Cloudy, thick discharge present.
Periocular swelling – Area around the eye is swollen.
Periocular redness – Area around the eye is abnormally pink or red.
Conjunctival swelling - Membranes around the eye are swollen.
Conjunctival redness – Membranes around the eye are abnormally pink or red.
None of above – Includes eyes without any clinical signs or those with signs not listed.

Skin lesion location – Circle all that apply, describe in notes, and draw on diagram.
None – No skin lesions.
Head
Neck
L/R forelimb – Circle left, right, or both.
L/R axillary region – Circle left, right, or both.
L/R hindlimb – Circle left, right, or both.
L/R prefemoral region – Circle left, right, or both.
Vent-tail – Circle if found anywhere on the vent or other areas of the tail.

Condition of skin lesion(s) – Circle one, but for multiple lesions circle all that apply and describe in areas provided.
N/A – Not applicable.

Active – Very recent or unhealed lesion.
Inactive - Old or healed lesion.

Ticks (*Ornithodoros spp*) – Circle one. See Appendix F.2 for details regarding tick collection.
0 – No ticks were observed.
1-10 – 1 to 10 ticks were observed.
>10 – More than 10 ticks were observed.

Collected? - Circle one.
N/A – No ticks were observed so no ticks were collected.
Yes – Ticks were collected following the tick collection protocols.
No – Ticks were present but not collected.
All Removed? – Circle one.
N/A – No ticks were observed so there were no ticks to remove.
Yes – All detected ticks were removed (collected and/or disposed of).
No – All detected ticks were not removed (some or all were left on the tortoise).

Shell characteristics - Circle all that apply and describe in areas provided. Note that these characteristics may not always be considered abnormalities, as in the case of sunken scutes on an older tortoise.
Sunken scutes – Scutes are depressed lower than the seams of the shell.
Whitish flaking – Thin pieces of whitish material present on scutes and/or seams.
Trauma – Injury or damage to the shell caused by physical harm by an external source.
None of above – Includes shells with no abnormal characteristics or those with notable characteristics not listed above.
Other – If none of above was chosen due to unlisted characteristics, other may also be chosen to list the characteristic observed.

Shell characteristics location - Circle all that apply, describe in notes, and draw on diagram. If you record something in the section "Shell characteristics" above, then you must circle a location in this section.
Carapace
Plastron- Have a handler tip the tortoise to one side (the tortoise's left or right side) so the examiner can see the plastron. Do not tip the tortoise too far to the left or the right and <u>do not put it on its carapace to observe the plastron</u>.
N/A – There are no noteworthy shell characteristics.

Condition of shell trauma – Circle all that apply (may be both active and inactive, if multiple traumas are present) and describe in areas provided.
N/A – Not applicable.
Active – Very recent or unhealed condition.
Inactive - Old or healed condition

If present, circumstances of skin/shell trauma – Circle one.
N/A – Not applicable
Unknown – There is no way to determine the cause of the trauma.

Suspect predator - Suspected or known predation by coyote, dog, felid, badger, raven, etc. Provide details, if known, in the notes area at bottom of datasheet.

Vehicle – Trauma is suspected or known to be caused by a vehicle, including but not limited to a car, truck, military tank, ATV, dirt bike, dune buggy, etc. Provide details in the notes area.

Other _____ - Provide details if the other options do not provide an adequate description of the circumstances of the trauma.

Sex – Circle one.

M – Male

F – Female

Unknown – Unknown sex

Body condition score – Circle one. Scores range from 1 (emaciated) to 9 (morbidly obese). Descriptions of each body score are included in Appendix D. If the tortoise seems to score between two numbers, choose the lower number.

Coelomic cavity palpation – Circle one.

No mass – The tortoise was palpated but no masses were detected.

L/R mass – Circle left, right, or both.

Not done – The tortoise was not palpated

Photos (take all*) – Photographs should be taken with a good quality digital camera. Whenever possible, take photos using a macro lens to show detail, especially for photos taken to show trauma and signs of disease. Please review photos for clarity and usefulness prior to leaving the tortoise.

Label or datasheet - Prior to taking photos of the tortoise, take a photograph of the datasheet clearly showing the Tortoise ID number, date, site. Alternatively, photograph a label card that contains the pertinent information. This photograph is to help distinguish between sets of photographs of different individuals.

Front face and body - Show nares, forelegs, gular, and anterior shell.

Left side face – Full frame close up.

Right side face – Full frame close up.

Carapace – Full frame close up.

Plastron (*only if abnormal) – Take this photo only if the plastron is abnormal. Have a handler tip the tortoise to one side (the tortoise's left or right side) so the examiner can see the plastron. Do not tip the tortoise too far to the left or the right and <u>do not put it on its carapace to photograph the plastron</u>.

Abnormalities – Full frame close up.

Other – Any of other photos of the animal.

Draw, label, and describe abnormalities, trauma, anomalies, lesions, missing body parts, and identifying features (including gulars and head/limbs/tail, *if* noteworthy) – On the line drawings of a tortoise carapace and plastron, draw in any abnormalities, trauma, anomalies, lesions, missing body parts, and identifying features of the tortoise, and circle or point an arrow to the location of missing body parts. Draw in the shape of the gular on the picture of the plastron. Draw head/limbs/tail only if noteworthy. Provide a written

description in the space provided for details about the tortoise that may require clarification. Detailed notes should be provided in the notes section and clarifications and labels for your drawing in the present section.

Oral cavity characteristics and tongue color - – Circle all that apply. See Appendix F.3 for details regarding the oral cavity examination. Note that color of the choana also is observed during the oral exam, but is recorded in a separate field.

Not examined – The oral cavity examination was not conducted
Hypersalivation - Increased saliva in or around the oral cavity.
Impaction – Lodgment of something, such as food or debris, in the oral cavity
Crust-Plaque-Ulcer – Circle this entire option if any of the three are present. We are no longer asking for differentiation of crusts, plaques, and ulcers. A crust is an outer layer of solid material formed by the drying of a bodily exudate or secretion. An ulcer is a localized defect or excavation of the surface of a tissue, usually produced by sloughing of necrotic inflammatory tissue. A plaque is a localized patch or flat area in the oral cavity. Plaques tend to have a white or yellow appearance.
White – Tongue is white or very pale (sometimes yellowish) in coloration.
Pink – Tongue is pink in coloration
Red – Tongue is red in coloration.
Other - Provide details in notes if provided options do not provide an adequate description of the oral cavity and tongue color.

Choana – Circle one. See Appendix F.3 for details regarding the oral cavity examination.

Not examined – An oral cavity examination was not conducted or it was conducted but the choana was not observed.
White – White or very pale (sometimes yellowish) in coloration.
Pink – Pink coloration.
Red –Red coloration.

 # oral swabs collected – Write the number of oral swabs collected. You can collect 0, 1, or 2 oral swabs. See Appendix F.3 for details regarding oral swab sample collection.

Total volume blood + lymph collected – Total volume in milliliters of all blood collection attempts. Up to three attempts to collect total maximum volumes are allowed. See Appendix F.5 for details regarding blood sample collection**.**

Total # hep tubes – Circle one. You can have 0, 1, 2, 3, or 4 tubes containing whole blood and/or lymph samples.

Indicate ml lymph (L)/ml total in each hep tube – Number each tube and estimate the amount of lymph per total sample volume of each tube.

Void during processing – Circle all that apply.

None – The tortoise did not discharge bladder or digestive tract contents.
Urine-urates – Waste material that is secreted by the kidney as a clear fluid or as a semi-solid salt of uric acid that may be white, yellow, or brown

Feces – Bodily waste discharged through the digestive tract; firm excrement

Hydration Method – If the tortoise voided urine/urates, circle one, or if multiple rehydration methods were employed, circle all that apply and describe in the area provided. See Appendix F.6 for details regarding rehydration techniques.

N/A – The tortoise did not void urine/urates, so it was not provided with fluids. This should also be chosen in the unusual case that fluids were not provided to a tortoise that voided

Soak – The tortoise was placed in a plastic tote with shallow fresh water for 30 minutes.

Nasal-oral – The tortoise was offered fresh water ad lib with a syringe through the nares and mouth.

Epicoelomic – The tortoise was given an epicoelomic injection of sterile saline.

Fluid type – Fill in blank with fluid(s) used. If multiple rehydration methods were employed, describe in the notes area provided. See Appendix F.6 for details regarding rehydration techniques**.** If the tortoise did not void, skip this field.

Water – Fresh drinking water.

Saline – 0.9% sodium chloride.

Vol - Amount of fluids in milliliters injected into the tortoise or offered ad lib. Do not provide a volume if you used the soaking method. If the tortoise did not void, skip this field.

Disposition - Circle one.

Wild capture location - For tortoises that were caught in the wild and are being placed back at their site of capture immediately following processing (i.e, the tortoise is not being brought into captivity after processing).

Same pen – For tortoises that were already in captivity at the time of processing and are being placed back in the same pen. Provide the pen number or name for clarity.

New pen – Provide the number or name of the new pen where the tortoise is being placed after processing, regardless if it was newly captured from the wild, or if it was already in captivity but moving to a new pen.

Translocated – For tortoises that are immediately translocated following this health assessment, provide the name of the translocation site, if a descriptive name exists.

Other- Describe the new location (e.g., veterinary hospital, etc.)

End handling time (24h) – The time at which you complete the processing of the tortoise and have returned it to its original location or new disposition (point of capture, pen, etc). Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Total # tubes/vials collected: – When you are done with the assessment, count the total number of tubes/vials containing samples from the individual. This is the number that you have as you are walking away from the tortoise.

Blood processing time (24h) – The time at which samples were removed from the cooler and placed in the centrifuge. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Plasma Color – Circle one. This is the plasma color of the sample you will use for the "Hep plasma UFL" cryovial. This should be the best sample you collected for each tortoise, meaning the one with the least lymph contamination. Choose from colorless, red, pink, yellow, or green.

UFL plasma aliquots – Circle one. You can have 0 or 1 aliquots of plasma for UFL. See Appendix F.7 for details regarding sample processing**.**

USFWS plasma aliquots - Circle one. You can have 0, 1, 2, or 3 aliquots of plasma for USFWS. See Appendix F.7 for details regarding sample processing**.**

Total tubes with RBCs saved – Circle one. You can have 0, 1, 2, 3, or 4 heparinized microtainers with RBCs in them to save. All of the plasma should have been pipetted out of these microtainers into the UFL or USFWS plasma and plasma/lymph tubes prior to storage, leaving only RBCs in the microtainers. See Appendix F.7 for details regarding sample processing**.**

Total # tubes saved (for submission/banking) – Count the total number of tubes/vials after blood processing that are being saved for submission and banking (including oral swabs and ticks). Tubes that had known contamination, that over-heated since time of collection, etc. should have been discarded by this stage.

Notes – Other pertinent observations that do not fit into other data fields.

Appendix C: Clinical Signs of Disease in Desert Tortoises

Desert Tortoise Health Assessment Cheat Sheet

Eroded nares

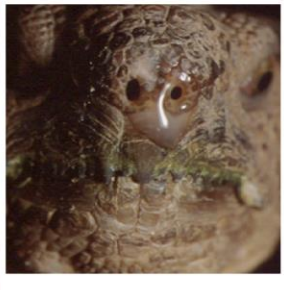
Occluded nares

Serous discharge
1 = mild

Serous discharge
2= moderate

mucoid discharge
1 = mild

mucoid discharge
2 = moderate



Pink tongue

Red choana

Red tongue

White tongue

Sunken eye

Serous discharge

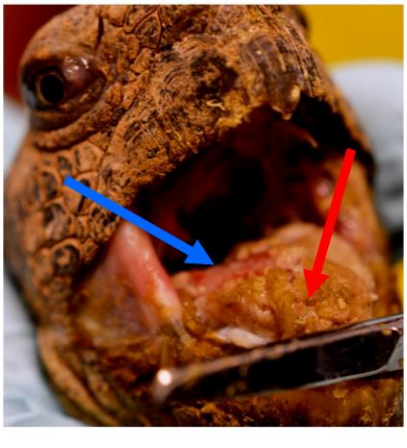
Partially closed, Periocular Swelling, redness & eroded nares

Periocular swelling

Conjunctival swelling & redness, periocular swelling & serous discharge

Oral plaque

Oral ulcer and plaque



Appendix D: Body Condition Scores for Desert Tortoises

Download at http://www.fws.gov/nevada/desert_tortoise/dtro/dtro_trans.html

Body Condition Scoring in Desert Tortoises

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Introduction

Body condition scoring is a visual appraisal system that estimates body energy reserves without using scales, calipers, or calculators (Bewley and Schutz, 2008; Stevenson and Woods, 2006). Since individuals can vary in size and shape, weight alone is not a good indicator of body condition. The body condition score (BCS) is based on an evaluation of muscle and fat deposits in relation to skeletal features (Bewley and Schutz, 2008). We adapted the BCS to the desert tortoise (*Gopherus agassizii*) by evaluating temporal muscle mass in relation to the sagittal crest and estimating the amount of subcutaneous fat in the prefemoral, axillary, and/or cervical spaces (Lamberski et al. 2012; Lamberski 2012).

Assessing BCS in tortoises

BCS range from one to nine, where one is emaciated and nine is extremely obese. Assigning a BCS is a two-step process. The numbers are divided into three groups.

STEP 1: Choose the grouping that best describes the tortoise at the current point in time.
a) Under condition (1-3): best assessed be degree of temporalis muscle atrophy and prominence of the sagittal crest;
b) Good condition (4-6): best assessed by degree of temporalis muscle development;
c) Over-condition (7-9): best assessed by degree of subcutaneous fat deposition.

STEP 2: Fine tune the score by selecting one of the three numbers in the given range. Choose the best fit for that individual at the current point in time.

Conclusion and Relevance

- The BCS is dynamic and should improve if the animal is eating and body energy reserves increase.
- Conversely, the score will decrease if inanition persists or body energy reserves are depleted.
- A tortoise's body condition will change with life stage, stage of reproduction, season of the year, drought, food availability, and disease.
- This management tool can be used to monitor and compare populations over time or to assess tortoise condition following reintroduction or translocation.
- Since energy reserves can have a strong effect on response to pathogens and susceptibility to disease, reintroduction or translocation of tortoises with low BCS (1-3) is not recommended.

Comparison to RLW and CI

The subjective BCS was compared to the objective measures of relative liver weight (RLW) and condition index (CI) (Nagy et al., 2002). RLW and CI were calculated from animals submitted for post mortem examination. Associations were evaluated with correlation coefficients and linear or logistic regression models. A strong positive relationship was identified between BCS and RLW (Fig. 1, Table 1). This supports the premise that BCS reflects energy reserves and desert tortoises store fat in the liver consistent with other reptiles. The correlation was stronger in adults than in juveniles.

BCS and CI (Fig. 2, Table 1) also had a significant positive correlation but the relationship was not strong. This is not surprising since BCS is a visual assessment of muscle mass and fat deposits while CI is calculated as the ratio of body mass to shell volume. They reflect similar trends but CI may be more affected by hydration status, recent food ingestion, reproductive status, and presence of large bladder stones.

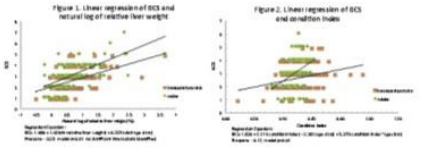


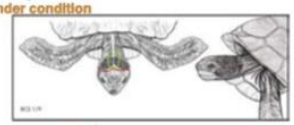
Table 1. Spearman correlation coefficients (R) and p-values for BCS and + regarded variables*

Correlate	No correlation ([illegible])	Mild correlation ([illegible])	Moderate correlation ([illegible])
Relative liver weight (%)			
Adults (SVL > 200 mm)			0.62 p<0.01)**
Hatchlings/juveniles (SVL < 200 mm)			0.49 p<0.01)**
Condition index (g/cm)			
Adults (SVL > 200 mm)			0.43 p<0.01)**
Hatchlings/juveniles (SVL < 200 mm)		0.24 p<0.05)**	

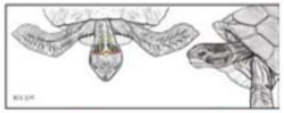
*Included by co-linear variables of: Relative liver weight (adults n=120, hatchlings/juveniles n=170); condition index (adults n=170, hatchlings/juveniles n=168).
**Linear regression of relative liver weight shown in Figure 1.

Body condition scores for tortoises

Under condition



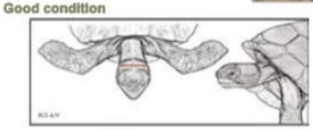
BCS 1: Severe atrophy of the temporalis muscle, visible sagittal crest



BCS 2: Moderate atrophy of the temporalis muscle, visible sagittal crest

BCS 3: Slight atrophy or waviness of the temporalis muscle, visible sagittal crest

Good condition

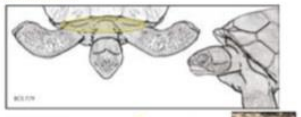


BCS 4: Flat appearance to temporalis muscle, sagittal crest not visible

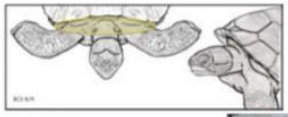
BCS 5: Developed temporalis muscle with a bi-lobed appearance

BCS 6: Well-developed temporalis muscle with rounded appearance

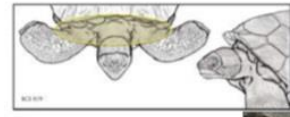
Over-condition



BCS 7: Subcutaneous fat present in prefemoral, axillary, and cervical regions



BCS 8: Moderate amount of subcutaneous fat present



BCS 9: Large amount of subcutaneous fat present

Acknowledgments

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Appendix E: Biological Sampling Supplies

Supplies for individual tortoise sampling

The following supplies will be needed to process a single tortoise. You can prepare zipper sealed plastic bags containing these supplies for individual tortoises to decrease preparation time in the field. It is also important to carry extras of all of these supplies in case of contamination or other unforeseen circumstances. We recommend laying out all the supplies from this bag before touching the tortoise to prevent potential contamination while processing the tortoise.

- (4) Alcohol or diluted povidone iodine wipes
- (2) Sterile cotton tipped plastic stick swabs for oral swabs
- (1) Small trash bag
- (1) Zipper sealed plastic bag to hold completed sample tubes
- (1) 20cc or 60cc luer lock or slip tip syringe with (1) 22G 1.5", 22G 1", or 25G 1" needle for injectable epicoelomic fluid administration – syringe and needle sizes depend on size of tortoise
- (3) 1cc or 5 cc slip tip (luer) syringe with (3) 23G 1", 23G 1.5", or 25G 1" needle for blood sampling - syringe and needle sizes depend on weight of tortoise
- (1) 1-2.0ml O-ring cryovial labeled "Ticks"
- (2) 2.0ml O-ring cryovial labeled "Oral swab"
- (4) 1.3ml heparinized snap top microtainers* labeled "RBC"

Supplies to keep in the cooler (must be with you while processing a tortoise)

The following supplies should be kept in a cooler for use with multiple tortoises.

- Frozen gel packs or dry ice
- (1) bottle liquid sodium heparin
- (2) 250ml sterile saline bags encased to protect them from damage

General supplies to keep in the field kit

The following supplies should be kept in your field kit for use with all the tortoises that you process. Remember to keep your field kit free from contamination by laying out your supplies before touching a tortoise.

- Gloves
- Hand sanitizer
- Disinfectant in a spray bottle
- Sharps container
- Ultra fine permanent markers
- Tweezers, hemostats, and/or multi-tool
- Bottle of water (to soak tortoise for rehydration or to clean tortoise face and nares)
- Paper towels
- Clipboard
- Data collection forms
- Extra packaged syringes – 1cc, 3cc, and 5cc slip tip (luer), 20cc and 60cc luer lock or slip tip
- Extra packaged needles - 22G 1.5", 22G 1", 23G 1.5", 23G 1", 25G 1"
- Extra 1.3ml heparinized snap top microtainers labeled "RBC"
- Extra plastic disposable pipets

- Camera
- Calipers

Supplies to keep in your vehicle and/or lab
The following supplies and equipment should be kept in your vehicle and/or lab, in addition to extras of all the supplies listed above.

- Disinfectant
- Big cooler with dry ice
- Inverter
- Portable centrifuge
- Plastic disposable pipets
- 0.5ml O-ring cryovial labeled “Hep plasma UFL” – 1 vial per tortoise
- 1-2.0 ml O-ring cryovials labeled “Hep plasma” – up to 2 vials per tortoise
- 1-2.0 ml O-ring cryovials labeled “Hep plasma/lymph” – up to 2 vials per tortoise

Appendix F: Protocols for Biological Sampling, and Sample Processing, Storage, and Shipment

These protocols describe the collection of biological samples from the tortoise; handling, aliquoting, and storage of the samples; and shipment of samples to the appropriate laboratories. Teams of two biologists (an examiner and a handler) will work together to collect samples, with at least one (the examiner) being certified and permitted to conduct all of the activities involved with sample collection. The handler must be trained in handling tortoises. Ideally, a third person will record data on the data form, but a third person is not required.

Conduct a physical examination of a tortoise prior to taking any samples. Follow the order of data collection as presented on the data form and as described in Appendix B. Prior to handling the tortoise, your sampling equipment should be removed from your field pack and/or cooler and placed in the order that you will need it. Avoid re-entering your field pack and supply bags while you work on an individual tortoise, but if you need to do so, remove your gloves to enter the pack and put on clean gloves before continuing with processing. If you forget to remove your gloves before entering your pack, disinfect everything you touched and put on clean gloves to continue. During the processing of a tortoise, you can avoid contaminating supplies and equipment by using the one-handed glove technique in which you wear gloves on both hands, but the glove on your dominant hand is one size too big so you can easily slide your hand into and out of it as needed to avoid contamination while handling samples, recording data, etc. In addition to preparing and laying out all of the necessary supplies and equipment prior to handling the tortoise, label all the sampling tubes you will be using for that animal with the tortoise ID and date. Appendix F.1 describes all of the preparations that should be made for sampling tortoises prior to handling the animal.

In general, samples collected with the intention of detecting an immune response (e.g., plasma for ELISA tests) require that the immune system be functioning and active. Because a desert tortoise's immune response is temperature dependent, thus depressed during periods of winter inactivity, it is important to collect such samples after the immune system resumes more typical active-season functioning. While individual activity does vary across the range of the desert tortoise, we consider blood samples to be used for ELISA tests valid when collected between 15 May and 31 October. Upon specific approval from USFWS, exceptions to the start date may be made. For example, a sample may be considered valid when collected four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground (not in association with a shelter site).

Appendix F.1: Preparing for Biological Sampling (Before Handling the Tortoise)

Label cryovials and microtainers

Goag ID_____ Date_____	Goag TC281 Date 20May16
Time _____ Site _____	Time 13:25 Trout Canyon
Contents _____ PI _____	Hep Plasma 1 Jones

- Prepare cryolabels in advance using a laserjet printer. Some information can be printed on the labels, while other information will need to be recorded in the field using a black ultra-fine permanent marker. For information that will be recorded in the field, it is helpful to print the word, such as time, to remind yourself to record the actual time while in the field. Labels always display Goag to indicate that the sample came from a desert tortoise (*Gopherus agassizii*).

- Print the words "Date" and "Time" using the printer, but leave space after it to record while in the field. The date format is DDMMMYY (e.g., 30MAR16) and time is using the 24-h clock (e.g., 13:25).

- Print site name and last name of your PI on the labels using a printer to avoid having to write these in the field.

- In place of the word "Contents", you will print one of the following contents and tube numbers on each label with your laserjet printer. Remember that you will be using multiple tubes for some of these samples so you will need to print labels for all potential tubes of those samples. Example: You could need 4 RBC tubes for an individual tortoise so you will need to print 4 labels for RBCs labeled RBC 1-4 and place one on each 1.3ml heparinized microtainer. Also be sure to print extra labels in case of unforeseen circumstances in the field.

Contents & tube #	Number of tubes per tortoise	Type of tube
RBC 1, RBC 2, RBC 3, RBC 4	4	1.3ml heparinized microtainer
Hep plasma UFL	1	0.5ml O-ring cryovial
Hep plasma 1, Hep plasma 2, Hep plasma 3	3	1-2 ml O-ring cryovial
Hep plasma+lymph 1, Hep plasma+lymph 2, Hep plasma+lymph 3	3	1-2.0 ml O-ring cryovial
Oral swab 1, Oral swab 2	2	2.0 ml O-ring cryovial
Ticks	1	1-2.0 ml O-ring cryovial

- While working in the field, you will record the tortoise ID next to GoAg ID using an ultra fine black permanent marker. Samples with unreadable or questionable ID numbers may be discarded when they arrive at the labs so be sure to record the ID clearly.

- Record the date and health assessment start time. This time needs to match the health assessment start time as recorded on your data form.

- Complete the fields for "GoAg ID" and "Date" in the field with an ultra fine black permanent marker when you find a tortoise, but BEFORE approaching it. Complete these fields when you prepare and set out all of your supplies and equipment for processing.

Heparinize needles for blood sampling

In order to keep blood from coagulating when it is drawn, the needles and syringes need to be treated with the anticoagulant liquid sodium heparin. Prepare needles and syringes on the day that they will be used and if prepared before going into the field, store them in a zipper sealed plastic bag in a cooler at all times.

Supplies for heparinizing needles

- Bottle of liquid sodium heparin (to be kept in cooler)

- (3) 1cc or 3cc slip tip (luer) or luer lock syringe with (3) 23G 1", 23G 1.5", or 25G 1" - syringe and needle sizes depend on size of tortoise

Tortoise weight	Maximum blood volume to collect	Syringe size	Needle size
100-199g	0.25 ml	1cc	25G 0.5" or 1"
200-249g	0.5 ml	1cc	25G 0.5" or 1"
250-499g	0.75 ml	1cc	25G 1"
500-999g	1.5 ml	3cc	23G 1" or 1.5"
1000-1999g	2.0 ml	3cc	23G 1.5"
2000g+	3.0 ml	3cc	23G 1.5"

Procedure for heparinizing needles

- Take a bottle of liquid sodium heparin out of the cooler and use an alcohol swab to wipe the top of it.
- Attach the appropriate size needle and syringe for the tortoise you are sampling according to the table, and push and pull the plunger to loosen it.
- Remove the cap of the needle and insert the needle into the bottle of heparin.
- Tip the bottle upside down and draw in the heparin, ensuring that the bevel of the needle is in the liquid.
- When the syringe is full, keeping the bottle upside down, inject the heparin back into the bottle.
- Do not push the heparin out of the needle completely because you want a little heparin to remain in the hub.

- Remove the needle from the heparin bottle and recap it using the one-handed recapping technique (protocol below).

Safely discard heparinized needles and syringes that are not used on the day they were prepared. Note that heparinizing needles and syringes in advance instead of heparinizing them in the field as you need them can save you time in processing a tortoise. However, this could cause you to heparinize a large number of syringes that you may not need, and you will ultimately have to dispose of unused heparinized syringes and needles at the end of the day.

<u>One-handed needle recapping technique</u>
Only use this technique to recap needles that have NOT been used on an animal (i.e., after heparinizing syringes and needles or after drawing up saline for nasal lavages).

- While holding the open needle on a syringe in your dominant hand, use your other hand to lay the cap of the needle on a solid surface with the open end of the cap facing you.

- Remove your hand and guide the needle into the cap without your hands touching it. You can use a solid object to hold the cap in place to make it easier to guide the needle in.

- Once the needle is inside the cap, continue moving your hand forward against the capped needle and syringe until the cap is pushed against a solid surface.

- Then angle the end of the syringe closest to you upwards with the needle inside the cap facing downward and push the needle to secure it inside the cap. Make sure it clicks closed.

- If you are heparinizing a syringe prior to going into the field, you may then place it in a zipper sealed plastic bag in a cooler for use that day.

- If you are drawing up saline for nasal lavage, remove the capped needle and place the saline-filled syringe with your clean supplies to be used while processing the tortoise.

Note that you must NEVER recap a needle that has been used on an animal, but you may use the one-handed recap technique to recap needles that are being ***prepared*** for use on an animal.

Appendix F.2: Ticks

Equipment and supplies

- 1-2.0ml O-ring cryovial labeled “Ticks”
- Tweezers or hemostats

Ticks procedure

For all tortoises (translocatees, residents, and controls)

- Examine the entire body of the tortoise including the soft tissue and shell.
- If ticks are seen, record the number and locations on the data collection form.
- Collect up to 20 ticks in an O-ring cryovial labeled “Ticks”.
- Recheck that the ID and date are correctly labeled on the tube.
- Put the cryovial in a zipper sealed plastic bag on ice in the cooler. They must remain cold until they are placed directly in the freezer.

For tortoises that are being translocated

- Carefully remove the remaining ticks with a solid object, such as a stick or rock and let them fall to the ground.
- Be sure that all visible ticks are removed to prevent translocating them to another field site along with the tortoise.
- If there are some ticks that are difficult to remove, use tweezers, hemostats, or even a toothbrush to scrub them off.

For tortoises that are NOT being translocated (residents, controls, and post-translocation)

- Do not remove the remaining ticks.

Check your skin, clothes, and equipment for ticks that may have landed there or walked there after being removed from the tortoise, and remove any that you find. WEAR GLOVES. DO NOT HANDLE TICKS WITH YOUR BARE HANDS.

Appendix F.3: Oral Cavity Exam and Oral Swab

Equipment and supplies

- Sterile cotton tipped plastic stick swabs
- 2.0ml O-ring cyrovials labeled “Oral swab 1” and “Oral swab 2”.

Oral swab procedure

- The handler is the only person that will touch the tortoise during this procedure. The examiner will wear gloves, but will not touch the tortoise.
- The handler holds the tortoise with one hand and places the other hand behind its head so that its neck is extended.
- The handler uses their thumb and middle finger to stabilize the head, leaving the index finger free.
- When stabilizing the tortoise’s head, the handler holds the head behind the jaw at the neck, and avoids putting pressure on the jugular vein to minimize the chance of trauma.
- The handler uses the free index finger to gently push down on the mandible to make the mouth accessible.
- If you can’t get the tortoise’s head out or you can’t open the mouth after a few attempts, and you believe the tortoise is experiencing stress, do not proceed. You can attempt to collect the sample during subsequent interactions with the tortoise. Another attempt may be made as soon as 24 hours from the failed attempt, if venipuncture was not conducted on the day of the failed attempt. If venipuncture was done on the day of the failed attempt, the next attempt should occur no sooner than 7 days later to allow for soreness associated with the venipuncture to resolve.
- Once the mouth is open, the examiner looks inside the oral cavity paying particular attention to the choana, tongue, and mucosa, and records the observations on the data form.
- The examiner gently inserts two cotton tipped swabs inside the oral cavity. The swabs should be held far enough back from the tips such that the part touched by gloves will not be inserted in the sample cryovial.
- Move them around the entire oral cavity to sample the choana, the tongue, the inside of the beak, and all mucosal surfaces; however, do not attempt to swab beneath the tongue. In addition to moving around the oral cavity with the two swabs, gently rotate the swabs as well to cover all surfaces of the swabs, and spend extra time swabbing abnormalities.
- The handler should now release the tortoise’s head as the nasal lavage procedure is no longer implemented in the standard basic assessment.

- After collecting the samples, the examiner quickly places each swab in its own cryovial labeled “Oral swab 1 and 2”. Put the cotton tipped end of the swab inside the cryovial so the plastic stick is pointed outward. Be sure not to contaminate the swabs by touching other surfaces or by touching them with your hands.

- Hold the cotton tipped end of the swab inside the cryovial lifted just slightly off the bottom.

- Bend and break off the plastic stick so it will fit inside the vial when it is closed. You can also use a disinfected multi-tool to clip the stick. The stick should be broken below the place it was touched, in case your gloves were contaminated (i.e., everything inside of the tube is considered an oral cavity sample).

- Close the tops tightly, make sure the vials are labeled properly, and place them in an area that you have designated for collected samples so you can quickly move on to the blood sample.

- Record on the data form the number of tubes containing oral swab samples.

Appendix F.4: Blood Sampling

Equipment and supplies

- Alcohol or povidone iodine swabs, or 10% povidone iodine solution
- Gauze or cotton
- 1.3ml snap top heparinized microtainers with labeled "RBC"
- Appropriate size heparinized syringes and needles

Blood sampling volumes

The following table shows the maximum whole blood volume to collect based on tortoise weight and collection device. Note that blood is only drawn on tortoises over 100g and always with heparinized syringes and needles. After the initial collection from an individual tortoise, no more than 1 ml is needed in subsequent health assessments of tortoises greater than 500 g.

Tortoise weight	Maximum blood volume to collect	Syringe size	Needle size
100-199g	0.25 ml	1cc	25G 1"
200-249g	0.5 ml	1cc	25G 1"
250-499g	0.75 ml	1cc	25G 1"
500-999g	1.5 ml	3cc	23G 1" or 1.5"
1000-1999g	2.0 ml	3cc	23G 1.5"
2000g+	3.0 ml	3cc	23G 1.5"

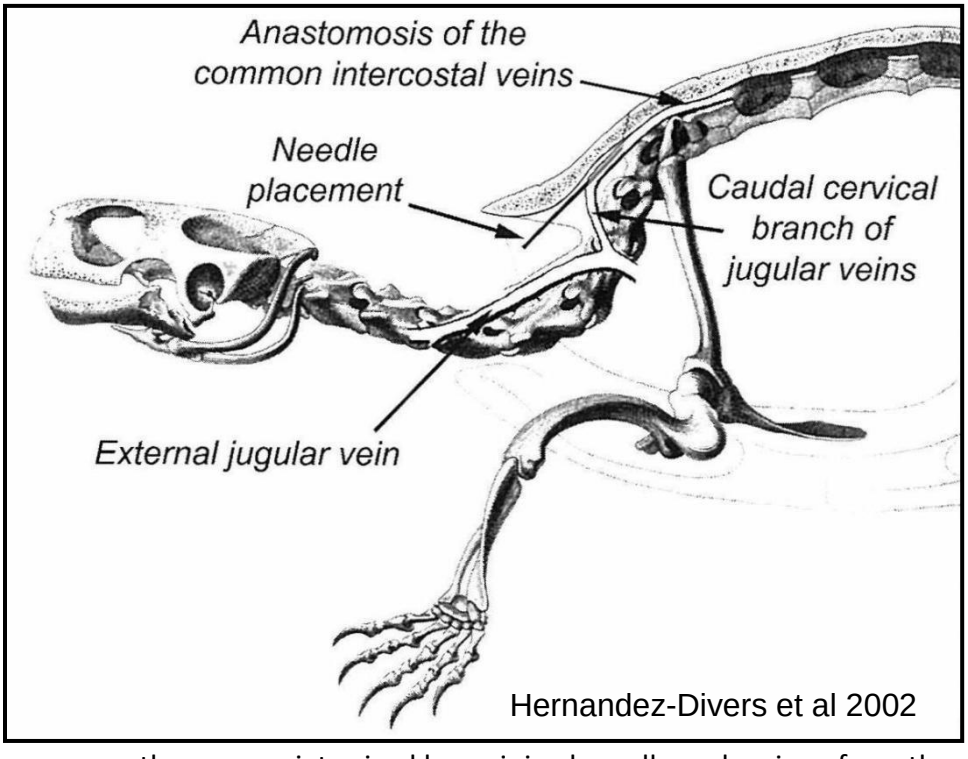
Blood sampling procedure

- Blood will be drawn from the subcarapacial vein as described in Hernandez-Divers et al. (2002), and it is recommended that all individuals using this technique should read the publication.

- The subcarapacial venipuncture site is located underneath the carapace, posterior to the nuchal scute and beneath the anterior portion of the 1st vertebral scute, cranial or anterior to the 8th cervical vertebra.

- The site is accessed by inserting the needle a few millimeters posterior to the junction of the skin-carapace junction and then angling the needle dorsally toward the 8th cervical vertebra.

- While the handler restrains the tortoise's forelimbs, the examiner palpates the subcarapacial area by inserting a finger along the midline posterior to the skin-carapace junction and feels for the depression anterior to the 8th cervical vertebra. This allows you to estimate the proper location of the injection site, as well as determine the angle at which to bend the needle (see next step).



- The examiner removes the appropriate sized heparinized needle and syringe from the cooler. Prepare the needle by bending it upwards with the bevel facing up (using the cap, not your fingers!) to a position that will allow it to easily puncture the subcarapacial vein. It should be bent at a slight angle to correspond with the angle of the dome between the nuchal and 1st vertebral scute.

- The examiner cleans the venipuncture site with alcohol or 10% povidone iodine solution using swabs, gauze, or cotton prior to insertion of the needle. Be careful not to get alcohol or povidone iodine in the tortoise's eyes.

- Before inserting the needle, the handler should position the tortoise at a level and angle that allows best access to the subcarapacial venipuncture site. The appropriate angle is likely to vary by size and doming of the carapace, but an upright position with the head approximately 75 degrees to the horizontal surface provides a good access point for the needle is often easier to see than when tortoises are held in other positions, especially on very large adults.

- Do not allow the needle to touch the head of the tortoise, which may be moving. With one finger on the beak, gently press the head in and downward toward the plastron and hold it in place, being careful not to touch or place pressure on the eyes.

- Insert the needle through the skin before pulling up on the plunger.

- Pull up on the plunger just slightly to locate the vein, pushing the needle in gently and slowly. You can move the needle very slowly and gently inward and outward with tiny motions along a single path of entry and exit. The needle should not be moved side to side or up and down.

- During the process of drawing blood, be careful not to push the plunger down in the syringe.

- When you see a flash of blood in the syringe, draw the maximum amount of blood indicated in the table for the size tortoise you are working with.

- If you cannot get the full amount of blood on the first or second try, you can try again, but you can only make 3 attempts to draw blood on a single tortoise in one day. If after 3 attempts, you cannot draw the minimum amount of blood required for ELISA testing (0.5ml), you can try again in seven days.

- Lymph
    - In order to be useful, blood should have as little lymph contamination (clear or yellow fluid) as possible and not be coagulated.

    - If the sample you collect is contaminated with lymph, the needle has likely been placed posterior to the subcarapacial venipuncture site.

    - If the sample you are drawing appears to be pure lymph, stop collection at 0.3 ml for adults and immediately for small tortoises. If you start to get blood at 0.3 ml continue to draw until you obtain 1 ml of blood.

    - Put the lymph-contaminated blood sample in a heparinized microtainer and be sure to note it on the label with an "L" so the sample can be transferred to the hep plasma/lymph cryovial after centrifuging.

    - If you have not used the maximum number of attempts and have not collected the maximum blood volume, insert the needle anterior to the location of the previous stick and try to get a clean sample.

    - When placing a subsequent clean sample in a heparinized microtainer, do not combine the clean sample with the first lymph-contaminated sample.

    - Samples that are estimated to contain greater than 10% lymph can be discarded, unless it is the first and only sample that exists for the tortoise. Prior to discarding samples, be sure to record the volumes as required on the data form. Discarded samples should be removed from the desert with your trash, rather than dumped from tubes or syringes onto the ground.

- After removing the needle from the venipuncture site, if bleeding occurs, apply pressure with a cotton tipped swab and 10% povidone iodine solution for at least 30 seconds or until bleeding has stopped.

- **DO NOT recap the needle**. Remove the needle using a disinfected multi-tool or hemostats (not your hand). Immediately dispose of the needle directly into a sharps container.

- Pick up a heparinized microtainer labeled "RBC". You may use a "dirty" gloved hand to do this, if ungloving is not feasible; however, the outside of microtainer should then be considered contaminated. Slowly transfer the whole blood from the syringe into the heparinized microtainer. If

you collected too much blood to fit in a single heparinized microtainer, use as many heparinized microtainers as you need

- Close the snap top lid(s) with and gently invert the microtainer(s) two or three times to mix in the heparin to prevent coagulation and place the tube(s) down temporarily in a location that has not been contaminated.

- Dispose of the syringe in the trash bag.

- Recheck that the ID and date are correctly labeled on the tube.

- Place the microtainers of blood in a zipper sealed plastic bag in a cooler for later processing. It is best if you can place a barrier, such as newspaper, between the zipper sealed plastic bag and the ice to prevent the cells in the sample from lysing. Remember to disinfect your cooler, if you opened it with dirty hands.

- Remove gloves.

- Record all the necessary information on the data collection form.

<u>Multiple attempts to collect blood</u>

- An attempt is considered one full motion inward and outward with the needle, even if the needle does not leave the injection site. Any repositioning of the needle is considered another attempt and should not be done with the needle inside the skin.

- After each attempt to collect blood (with a maximum of 3 attempts in one session), if there is fluid in the syringe, follow the protocol above to transfer the fluid into a heparinized microtainer labeled "RBC", and label the microtainers in the order in which their contents were drawn (1, 2, or 3). Also include on the label the letter "L" if the sample in that tube is contaminated with lymph.

- When completing the data collection form, record the total sample volume of all of the attempts combined, and circle the total number of tubes (microtainers) containing blood-related samples. There can be up to 4 tubes in total because in the case that you collect a clean sample of blood in your last attempt, you may have collected too much blood during that attempt to fit in a single microtainer so that sample will be divided into 2 tubes.

- Also record on the data collection form the estimated volume of lymph contained in each microtainer.

If the tortoise voided during processing, continue to the rehydration protocol in Appendix F.5. Record the end handling time when you have finished processing the tortoise and have returned it to its point of capture or placed it in a pen.

Appendix F.5: Rehydration

Options for fluid replacement include injectable epicoelomic fluids, nasal-oral offering of fluids, and soaking in a shallow water bath. Injectable epicoelomic fluids and nasal-oral administrations are the preferred two of the three methods of fluid replacement that the USFWS may authorize.

Fluids for rehydration are not prepared during pre-handling preparations for processing tortoises because only tortoises that void will be rehydrated. You can wait until you are ready to rehydrate the tortoise (i.e., after you have conducted all of the biological sampling) to prepare these fluids.

<u>Injectable epicoelomic fluid administration</u>

Supplies for epicoelomic fluid administration

- 250ml bag of sterile saline (0.9% sodium chloride - to be kept in cooler)
- 20cc or 60cc luer lock or slip tip syringe with 22G 1.5“, 22G 1”, or 25G 1” needle – syringe and needle sizes depends on size of tortoise

Tortoise MCL	Syringe size	Needle size	Total volume of sterile saline
150-180mm	20cc or 60cc	25G 1”	20ml/kg Up to 60 ml
180-200mm	60cc	22G 1.5” or 22G 1”	
200mm +	60cc	22G 1.5”	

Procedure for epicoelomic fluid administration

Epicoelomic describes a location between the coelomic cavity/membrane and the shell. This is a potential space similar to subcutaneous areas. The benefits of using this location versus other subcutaneous locations is that fluids in this area do not appear to affect mobility or the ability of the tortoise to withdraw its limbs into its shell prior to complete absorption of the fluids. This procedure has the benefit of providing a known amount of fluid replacement to the tortoise and does not require a lot of specialized equipment. It does, however, require advanced training and permitting by USFWS, if you would like this method to be among your options.

- Put on a fresh pair of gloves and select a syringe and needle based on tortoise size (see table for proper size). The needle size selected should go no further than the caudal aspect of the pectoral scute upon insertion.

- Use an alcohol swab to wipe the port on a 250ml bag of sterile saline, and insert the needle into the port. Be sure to insert the needle directly into the port and be careful not to puncture any part of the bag. If the bag becomes punctured, dispose of it, as well as the needle and syringe, and start fresh with sterile supplies.

- Draw up the total volume of sterile saline required for the tortoise’s size (see table for volume).
- Carefully withdraw the needle from the port and use a tool (not your hand) to remove the needle from the syringe. Dispose of the needle in the sharps container.

- The location of fluid administration is on the lateral aspect of the plastron anywhere from the caudal area of the pectoral scute to the cranial area of the abdominal scute, just inside the bridge.

- The handler should position the tortoise presenting the axillary fossa to the examiner. The handler can either restrain the forelimb into the fossa where the fluids are to be given or pull the forelimb outward from the fossa, forward and towards the head.

- Insert the needle bevel down in the lower outer aspect of the axillary fossa (above the ridge of bone at the skin junction) at approximately a 45˚ angle, directed caudally. When contact with the plastron bone is felt, move the needle caudally and parallel to the plastron and bridge.

- Pass the needle directly along the bones of the plastron to its appropriate depth (*i.e*. caudal aspect of pectoral scute).

- Pull the plunger out just slightly to ensure negative pressure occurs, and that no blood enters the hub. If there is no negative pressure or blood is seen in the hub, remove the needle from the tortoise, remove it from the syringe, and replace it with a new needle. Attempt to insert the needle into the tortoise again, aiming the needle more closely toward and in contact with the plastron.

- Once you have negative pressure in the syringe when pulling the plunger, steady the needle and inject the saline.

- Heavy pressure on the plunger may be needed to inject the fluids if a small needle size is used.

- If flow stops, slightly redirect the needle and continue administering the fluids.

<u>Nasal-oral fluid administration</u>
This procedure is easy to carry out with minimal equipment, but may not work for all tortoises. In such a case, use one of the other rehydration methods.

Supplies for nasal-oral fluid administration
- Syringe (any size, no needle)
- Fresh drinking water (20ml/kg)

Procedure for nasal-oral fluid administration
- The tortoise can be placed on the ground if it is not very active or the handler can hold the tortoise facing the examiner, near to and parallel with the ground, without restraining its legs.
- The examiner holds the water-filled syringe (no needle) facing downward just in front of the tortoise's face, very close to the nares and slowly pushes the plunger allowing the water to enter the nares and/or mouth.

- The tortoise will sometimes actively accept and drink the water, but if the tortoise refuses, do not force the tortoise to take in the water; use one of the alternate rehydration methods.

Water bath

Supplies for water bath rehydration

- Plastic tote slightly longer and wider than the tortoise
- Fresh drinking water

Procedure for water bath rehydration

- Place the tortoise in the tote and add water until it reaches the level between the skin-plastron junction and the chin.
- Allow the tortoise to soak for 30 minutes. It is acceptable that the total handling time will exceed the standard 30 minute limit, due to soaking a tortoise.
- Tortoises may drink water through their nose or mouth or absorb it through their cloaca.
- Tortoises may void more urine while being soaked in an effort to eliminate urine with high solute levels to replace it with the fresh water it is taking in. If a tortoise voids in its soaking tub and additional fresh water is available, the water should be discarded and replaced with fresh water until the completion of the 30 minutes. Otherwise the tortoise can be soaked for 30 minutes in the water that contains the voided urine and urates. At the end of the soak time, rinse the tortoise with fresh water to remove urine odor on the limbs and shell, which might attract predators.

After rehydrating the tortoise

- Complete the fields on the data collection form for hydration method, fluid type, and volume.
- Record the end handling time once you have finished processing the tortoise and have returned it to its point of capture or placed it in a pen.

Appendix F.6: Processing Biological Samples (After Handling the Tortoise)

Note: Change your gloves before processing the following samples to be sure they do not become contaminated.

Processing oral swab samples

- Make sure the vials are labeled properly, and place them in a zipper sealed plastic bag in the cooler.

Processing whole blood samples (at your vehicle or lab)

Supplies for processing whole blood samples

- Centrifuge (with inverter, if at vehicle)
- 0.5ml O-ring cryovial with label for "Hep plasma UFL"
- 1-2.0 ml O-ring cryovials with label for "Hep plasma"
- 1-2.0 ml O-ring cryovials with label for "Hep plasma/lymph"
- Pipets

Procedure for processing whole blood samples

If you will be centrifuging blood samples in the field, test run the centrifuge from the vehicle's cigarette lighter using the inverter before your first day in the field. The centrifuge/inverter setup might not work on some older or smaller vehicles. Always try to keep the centrifuge cool and shaded.

- Centrifuge the blood as soon as possible after collecting the sample, and always within 4 hours, especially if the temperature is higher than 90F (32C). The longer the blood sits before centrifuging, the more likely it is to clot, which will render the sample useless.

- Before starting the centrifuge, ensure that the centrifuge is properly balanced by placing samples with the same amounts across from each other. If you do not have matching sample amounts, use tubes with water in the same amounts of the samples to balance the centrifuge.

- Record on the data form the time that you placed the samples in the centrifuge to spin (blood processing time).

- Spin the blood for the amount of time required by your centrifuge. This could be 3-10 minutes.

- While the blood is spinning, record the ID and date on the labels of the cryovials that you intend to use for each sample (one for "Hep plasma UFL", one to three for "Hep plasma" and/or "Hep plasma/lymph").

- When the centrifuge stops, record on the data form the plasma color of the sample you will use for the "Hep plasma UFL" cryovial. This should be the best sample you collected for each tortoise, meaning the one with the least lymph contamination.

- Use a pipet or syringe to draw out the plasma (clear layer on top of the red blood cells), being careful not to touch the red blood cells with the pipet. If you drop the sample with the lid closed or you touch the red blood cells with the pipet, re-centrifuge the sample before pipetting to ensure that no red

blood cells are removed during the process. It is important to draw up only plasma with no red blood cells in it at all.

- Transfer 0.5ml of the plasma into a cryovial labeled “Hep plasma UFL”.

- Transfer the remaining plasma using the same pipet to cryovials labeled “Hep plasma” (or “Hep plasma/lymph” if applicable) with the tube number, and save the red blood cells in the original microtainer labeled “RBC” plus tube number.

- Recheck that the ID, date, and time are correctly labeled on all of the tubes (microtainers and cryovials).

- Record on the data form for each tortoise how may cryovials of plasma there are for UFL (UFL plasma aliquots), the total number of other cryovials containing plasma and plasma/lymph (USFWS plasma aliquots), and the number of heparinized microtainers you are saving that contain RBCs (all plasma should have been transferred out of these so only RBCs are left behind).

- Check the lids on the microtainers and cryovials several times to make sure they are closed tightly before placing them in a zipper sealed plastic bag.

- Freeze the samples immediately. If you do not have access to a freezer on site, place the samples in a cooler with dry ice and either transfer them to a freezer while they are still frozen or keep them on dry ice.
    - Note: If the freezer you will be using is a self-defrost freezer (like most residential freezers), be sure that it does not self-defrost at any time when there are samples inside it. Also remember not to leave samples in any location where they can become warm at any time and do not hold samples for more than a few seconds with warm fingers.

Appendix F.7: Shipping Samples

The samples that you collect will be shipped to three labs. If diagnostic tests will be performed as part of your study and they are not related to the required samples, please contact laboratories well in advance to determine if they have expertise performing the specific diagnostic tests you need, if they can accommodate your samples, and how they would like the samples to be collected, stored and shipped, as their protocols may differ from the ones presented here.

Shipping plasma samples to University of Florida for ELISA testing

- This protocol is only for the heparinized plasma samples in the 0.5ml cyrovials labeled "Hep plasma UFL".
- Samples should only be shipped to UFL Monday through Thursday.
- Call (352) 294-4086 or (352) 294-4071 before shipping to make sure someone will be in the lab to receive the samples the next day.
- Complete the UFL Mycoplasma Lab submission form.
    - All samples will be analyzed for antibodies to *Mycoplasma agassizii* and *Mycoplasma testudineum*. Mark the check boxes for ELISA *Mycoplasma agassizii and Mycoplasma testudineum*. Culture/PCR will not be done at this time.
    - If you complete the form by hand, you will need to scan it into a computer so you can email it to the lab after shipping the samples.
    - If you complete the form in Word or Excel, or on UFL's electronic form, you will also need to print it to include it in the shipment.
- If there is a time sensitive issue, such as relocation or temporary holding of animals, note it on the submission form so the lab can expedite your request whenever possible.
- Put the completed submission form inside a zipper sealed plastic bag.
- Put the samples in a separate zipper sealed plastic bag, making sure all caps are secure and cryovials are clearly labeled.
- Place the zipper sealed plastic bag with the samples in a small cooler with frozen gel packs. DO NOT use ice or any other substance that can melt into liquid. Note that you may ship the samples on dry ice.
- Place the zipper sealed plastic bag with the submission form in the cooler on top of the frozen gel packs and samples before sealing the package.
- Tape up the cooler or box. Note that hard sided coolers can usually be shipped without an additional cardboard box.

- Take the package to a FedEx office. Choose Priority Overnight for the delivery service. Sample shipments should always be shipped overnight to arrive at the lab no later than 11am unless otherwise instructed.

- Send to:

  Dr. Mary Brown
  University of Florida Mycoplasma Laboratory
  Room V2-234
  2015 SW Archer Rd
  Gainesville, FL 32608
  (352) 294-4086

- After you ship the package, email the tracking number and a copy of the submission form as soon as possible to ALL of the following email address: mbbrown@ufl.edu, amburne87@gmail.com

Sample Submission Form for Tortoise Mycoplasma Testing
Mycoplasma Research Laboratory, University of Florida

Species of animal(s): _____

County and state of origin (free-ranging only): _____

Date of sample(s): _____

Identification #(s): _____

_____

Is this animal awaiting relocation? ☐ **Yes** ☐ **No** ☐

NOTE: Price discounts (20%) are available for larger numbers of samples (>20) that are submitted for testing or for large volume contracts. Large volume costs are reduced; discounted costs are $28/assay; $11 for dilution curve.

Test requested (please check appropriate box):

↑ ☐ **ELISA serology for *Mycoplasma agassizii*- $35 each.** This is the most commonly used test to detect exposure to *M. agassizii*, a cause of URTD in tortoises; submit serum or plasma.

↑↑☐ **ELISA serology for *Mycoplasma testudineum*- $35 each.** This test detects exposure to a second species of mycoplasma that can be associated with URTD and eye infections; submit serum or plasma. Please note that this test has not been extensively validated at this time.

↑↑☐ **ELISA serum dilution curves - $14 each**

↑ ☐ **Culture/PCR- $35 each.** To avoid false negative results, this test is recommended primarily for clinically ill animals with active nasal discharge; submit nasal lavage sample.

↑ ☐ **PCR sequence if amplicon obtained and RFLP suggests new species- $35.**

Person submitting sample(s): _____

Report results to:_____

Phone: _____ **Email** _____**Fax:** _____

Preferred method of getting results (check one)↑ ☐ **Email** ↑ ☐**Fax** ↑ ☐**Mail**

Preferred method of billing (check one)↑ ☐ **Email** ↑ ☐**Fax** ↑ ☐**Mail**

Name & address of person to be billed:

_____

_____

_____

Email contacts: mbbrown@ufl.edu; amburne87@gmail.com

Please ship samples from Monday to Thursday only:

Dr. Mary Brown
University of Florida
Dept. of Infectious Disease and Pathology
2015 SW 16th Ave
Room V2-234/232
Gainesville, FL 32608
Telephone: (352) 294-4071 or 294-4086
Fax: (352) 392-9704

*****Please label tubes with investigator's name, tortoise species, tortoise identification name/number, and date sample collected.**

*****NOTE: Federal Express is the only carrier that delivers directly to our laboratory. Other carriers deliver to the VMTH, and samples may take 1-2 days to be delivered to our lab. In such cases, the quality of the samples may be compromised.**

For laboratory use only

Date Received:_____ Samples cold: ☐Yes ☐ No

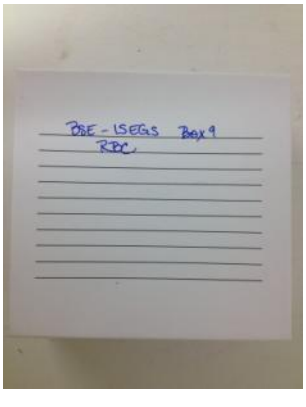
No. ELISA: Comments:

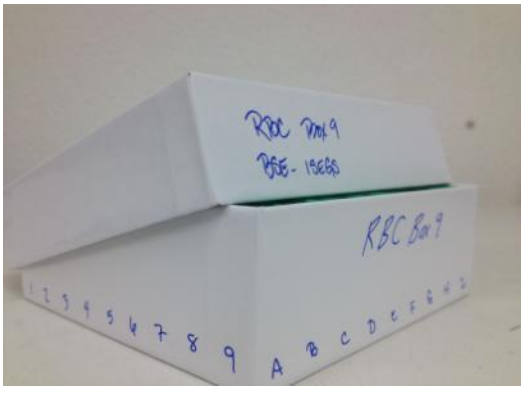
Shipping plasma, oral swab, tick, and RBC samples to the USFWS's sample bank at UCLA

- This protocol is for ALL samples EXCEPT the heparinized plasma samples in the 0.5ml cyrovials labeled "Hep plasma UFL" and one oral swabs that goes to San Diego Zoo (please consult with USFWS to determine if you need to submit one oral swab to San Diego Zoo.
- A laboratory at University of California, Los Angeles (UCLA) will be serving as the USFWS sample bank.
- Samples need to be frozen when shipped.
- Samples should only be shipped to UCLA Monday through Thursday. Alternatively, arrangements can be made to drop off samples by calling 310-825-5063 and speaking with Sarah Wenner to coordinate.
- Prepare a sample log spreadsheet using the column headings below to be submitted in hard copy with samples and electronically.

Box #	Grid Code	Date of sample collection	Tortoise ID #	Sample Type	Easting	Northing	UTM Zone	Datum	Project/Site Name

- Make sure all caps are secure and tubes are clearly labeled.
- Prepare a freezer box with 81 place dividers by labeling the top of the lid, one side of the lid, and one side of the box with the species (*Gopherus* agassizii), project or site name, type of sample, and number of box out of total number of boxes sent in with this submission. Also, label the bottom front of the box A-I and the bottom left side 1-9 to create a grid ID for each slot used (see below). Labeling the box with the date range of the samples within the box is also helpful, but not required.
- Place samples of the same type in boxes together, organized by date then by tortoise number.





- Record samples on the sample log spreadsheet.

- Be sure that samples are in the appropriate slots in the appropriate boxes.

- Prepare a sample submission letter that at minimum includes the project or site name, PI or contact person name and contact information, name of person submitting the samples, and number of boxes being submitted. The rest of the critical information should be contained in the sample log spreadsheet that you will also submit.

- Place the hardcopy of the sample log spreadsheet and submission letter into a zippered freezer bag.

- Place the banking boxes into an insulated box or cooler with dry ice or frozen gel packs. Pack the boxes such that they are protected from damage by moisture. Place the zippered freezer bag with the log and letter on top of the boxes.

- Tape up the cooler or box. Note that hard sided coolers can usually be shipped without an additional cardboard box.

- Take the package to a FedEx office. If using dry ice, be sure to affix the appropriate notification label to the package. Choose Priority Overnight for the delivery service. Sample shipments should always be shipped overnight to arrive at the office no later than 11am the next morning unless otherwise instructed.

- Send to:
  Brad Shaffer
  Department of Ecology and Evolutionary Biology
  610 Charles E. Young Drive East
  Terasaki Loading Dock
  Box 957239
  University of California, Los Angeles
  Los Angeles, CA 90095-7239 USA

- After you ship the package, call the lab (310-825-5063) to inform Sarah Wenner that a package of samples will be arriving the next morning. Also, email an alert and the package tracking number to Robert Cooper (rdcooper408@gmail.com) and cc Brad Shaffer (brad.shaffer@ucla.edu), Sarah Wenner (sarahwenner2@gmail.com), and Evan McCartney-Melstad (evanmelstad@ucla.edu). Include the sample log spreadsheet and submission letter as attachments to the email.

Protocol for submitting oral swabs to the Amphibian/Molecular Diagnostics Lab for qPCR

Ship by overnight courier (Fed Ex, UPS, DHL) on dry ice with submission form.

Sample: oral swab (send 1 swab per tortoise, if instructed by USFWS to do so)

Required tests*: qPCR for *Mycoplasma agassizii* and *M. testudineum*.
Optional tests: qPCR for testudinid herpesvirus 2, other.

Submission form: Fill out the attached submission form. Include a hard copy in the shipment (seal in a plastic bag if necessary) and submit an electronic copy to amphibianlab@sandiegozoo.org.

Sample packaging: Samples are to remain frozen during packaging and shipment. Put samples into a plastic envelope or cryobox and place into a Styrofoam box with dry ice. The samples should be surrounded with sufficient dry ice to prevent thawing for at least 48 hours. The Styrofoam box should be in a cardboard casing.

Shipping address:
Amphibian Disease Lab
15600 San Pasqual Valley Road
Escondido, CA 92027

Shipping specifications: Overnight to arrive on a weekday (Monday through Friday).

Questions?
Call the Amphibian/Molecular Diagnostics Lab at 760 291 5471 or
email amphibianlab@sandiegozoo.org.
Contacts: Josephine Braun, Jennifer Burchell, Asa Preston

* Update as per current USFWS instructions, if necessary.

SAN DIEGO ZOO INSTITUTE FOR CONSERVATION RESEARCH

Amphibian/Molecular Diagnostics Laboratory Submission Form

qPCR for *Mycoplasma agassizii* and *M. testudineum*

Institution Name: _____ Contact Name: _____
Billing Address: _____ Contact Phone: _____
City: _____ Email (required for reporting): _____
State: _____ Total Number of Samples Submitted: _____
Zip code: _____ Date Submitted: _____

FOR LAB USE ONLY: Arrival Date_____Plate_____Set ID_____

***** Please make sure the information on this list matches your sample tubes*

	Animal ID #	Sample type (e.g. oral swab)	Species / Common Name (e.g. desert tortoise)	Collection Date	Purpose of Testing (e.g. routine surveillance, pre-relocation, sick animal)	Mycoplasma PCR (Mag=M. agassizii Mtes=M. testudineum)	Comments/ Other PCR test (optional)
1						Mag and Mtes ☐	
2						Mag and Mtes ☐	
3						Mag and Mtes ☐	
4						Mag and Mtes ☐	
5						Mag and Mtes ☐	
6						Mag and Mtes ☐	
7						Mag and Mtes ☐	
8						Mag and Mtes ☐	
9						Mag and Mtes ☐	
10						Mag and Mtes ☐	
11						Mag and Mtes ☐	
12						Mag and Mtes ☐	
13						Mag and Mtes ☐	
14						Mag and Mtes ☐	
15						Mag and Mtes ☐	

Ship overnight to: Amphibian Disease Lab, 15600 San Pasqual Valley Road, Escondido, CA 92027 USA
Email amphibianlab@sandiegozoo.org.
Questions? Call us at (760) 291-5471

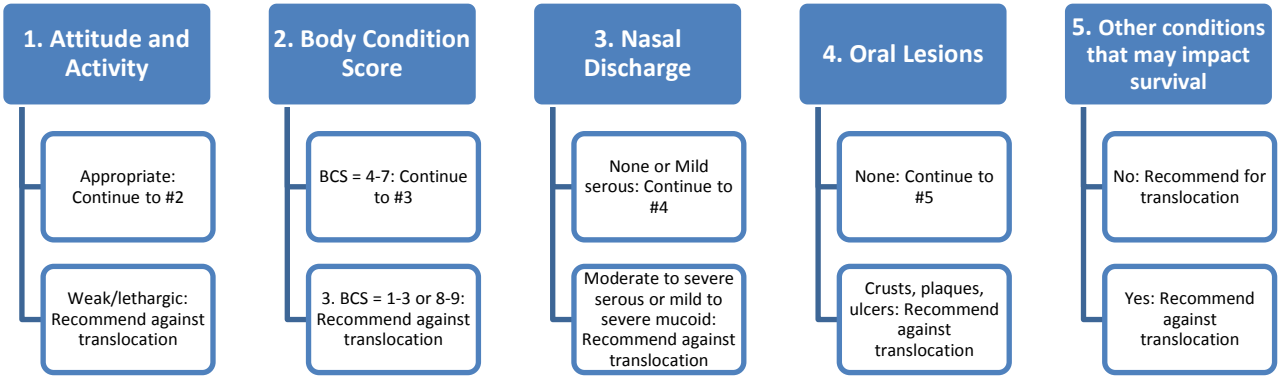
$35/swab for Mycoplasma PCR (includes both *M. agassizii* and *M. testudineum*); please contact lab for pricing updates

Appendix G. Algorithm for Evaluating if Desert Tortoises are Suitable for Translocation

Goals:

Relocate individuals that have high chances of survival.

Minimize the risk of spreading disease



All recommendations regarding translocation are to be recorded in the proposed disposition plan and submitted to the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office prior to relocating any tortoises.

Appendix H: Disposition Plan Template

Please fill out this summary table using the data field choices from your data sheet, except where yes/no or other information is requested.

ID	Sex	MCL	Mass	Attitude /activity	BCS	Nasal discharge	Severity	Oral lesion[1] (yes or no)	Other defect (yes[2] or no)	MYAG ELISA Titer	MYTE ELISA Titer	Photos (file names)	Comments	Recommended disposition (see examples below)	Proposed release location (descriptive + UTMs)
														translocate	
														on-site quarantine until further consultation with FWS	
														Transport to veterinary clinic for evaluation and treatment	

[1]Oral lesion = crust-plaque-ulcer

[2]Describe in Comments