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Evaluations of Health in Sympatric Free-Ranging Saguinus imperator and Leontocebus weddelli Using Neopterin, Urinalysis, and Blood Chemistry Analyses

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EVALUATIONS OF HEALTH IN SYMPATRIC FREE-RANGING *SAGUINUS IMPERATOR* AND *LEONTOCEBUS WEDDELLI* USING NEOPTERIN, URINALYSIS, AND BLOOD CHEMISTRY ANALYSES

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by

Alexandra Sacco

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Graduate Studies

We hereby approve the thesis of

Alexandra Sacco

Candidate for the degree of Master of Science

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ABSTRACT

EVALUATIONS OF HEALTH IN SYMPATRIC FREE-RANGING

SAGUINUS IMPERATOR AND *LEONTOCEBUS WEDDELLI*

USING NEOPTERIN, URINALYSIS,

AND BLOOD CHEMISTRY

ANALYSES

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This thesis is composed of one journal-ready article and one chapter that includes a preliminary interpretation of data for later publication. In summary, this thesis proposes the use of three different methods of evaluating the health status of New World Monkeys: urinary neopterin measurement, blood chemistry analyses, and urinalysis.

Chapter II addresses the question of whether or not the neopterin molecule, a biomarker for cell-mediated immune system activation, is conserved across primate taxa. To do so, we collected urine samples across three New World Monkey (NWM) families including captive *Leontopithecus rosalia* and *Pithecia pithecia*, and wild populations of *Leontocebus weddelli*, *Saguinus imperator*, *Alouatta seniculus*, and *Plecturocebus toppini*, to validate a commercial enzyme-linked immunosorbent assay (ELISA) for neopterin. To accommodate the use of this assay across a wide range of equipment and primate species, we assessed a range of variations in test conditions most likely encountered in field conditions. Urinary neopterin concentration (UNC) was successfully

quantified in all species across several wavelengths. However, from wild populations, we did not observe uniformity in UNC trends at each wavelength across the four species, and thus to evaluate species differences in immune function, we suggest that average levels be determined using a standardized wavelength. Our results indicate that neopterin, a valuable non-invasive biomarker for assessing immune status, appears valid in NWMs both in captivity and in the field.

Chapter III establishes the baseline values of blood chemistry and urinalysis parameters for wild *S. imperator* and *L. weddelli* using the Keto-Mojo device and multi-reagent urinary dipsticks. We propose that these affordable point-of-care methods of health assessment are effective ways of establishing baseline health data to facilitate future health monitoring initiatives in wild primate populations in the Neotropics. Notably, we found that the Keto-Mojo reliably produced accurate results for blood chemistry parameters when samples were run multiple times. We also found that urinalysis results were not influenced by variation in time elapsed between sample collection and urinalysis over a 12-hour period. These findings suggest that these point-of-care methods of evaluating non-human primate health are affordable, efficient, and ideal for researchers working in remote areas to conduct onsite health monitoring.

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source of inspiration and I hope my appreciation and respect for the incredible amount of work that has gone into creating and maintaining this longitudinal health-monitoring project has been apparent over the years.

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CHAPTER I

GENERAL INTRODUCTION AND LITERATURE REVIEW

Literature Review

Comprehensive health assessments of free-ranging populations are essential for establishing baseline parameters for a species, which can be used to improve captive care, evaluate population viability, and effectively monitor a population longitudinally. When carrying out health assessments, individual health status is typically evaluated by assessing their perceived physical condition through measuring a wide variety of biophysical parameters, and whether or not these parameters fall within the expected range of homeostasis for an individual. Homeostasis is the state of equilibrium in which an individual's physiological parameters are maintained within a given range that is considered "normal" for their age, gender, and species, and greatly influences an organism's survival (Maestripieri & Hoffman, 2011). Maintaining this state of equilibrium allows organisms to sustain a well-functioning immune system, which in turn promotes survival and resistance to infection (Behringer, Stevens, Leendertz, Hohmann & Deschner, 2017).

It is critical to establish baseline health parameters on a population level, as different populations and individuals have varying exposure to physical stressors. Physical stressors are external factors that cause a disruption of an organism's homeostasis, and typically they lead to feedback mechanisms that result in the adjustment of physiological parameters to compensate for this perturbation in homeostasis (Sapolsky, 2005; Maestripieri & Hoffman, 2011; Behringer et al., 2017). These stressors can either be acute or chronic, thus have varying effects on an individual's ability to

maintain homeostasis and promote individual survival and preserve their health status. Disparities in the normal parameters within and across populations can result from variation in physical stressors that influence their energetic demands and nutritional status due to factors such as habitat disturbance, disease, and food availability, that can influence an organism's ability to maintain homeostasis.

Fluctuating energetic demands are physical stressors that can play a significant role in the maintenance of health. Typically, if energetic demands fluctuate predictably within an organism's regulatory range, conditions in which an organism can cope with the range in variation, then the health status of "healthy" individuals should not be impacted significantly (Hämäläinen, Heistermann, & Kraus, 2015). In biology, energetic measurements include three major variables: current nutritional status, total energy expenditure, and energetic balance (Ellison et al., 1993). These variables can be influenced by a number of factors, such as environment, age, and breeding status, which in turn can influence health status. Environmental factors include abiotic factors, such as temperature and the nutritional value of food resources, as well as biotic factors, such as the genetic diversity of a host's pathogens (Lazzaro & Little, 2009). These can significantly impact an individual's ability to maintain homeostasis and resist infection, and with seasonal fluctuation of food availability and nutritional quality, it is possible that an individual's health status and susceptibility to infection can change seasonally.

Some individuals are better fit to maintain homeostasis and preserve a well-functioning immune system as a result of their genes. An organism's survival is greatly influenced by their own genetic structure, more specifically by genetic heterozygosity in the polymorphic genes of the major histocompatibility complex (MHC) (Penn,

Damjanovich & Potts, 2002; Kalbe et al., 2009). Genes in the MHC are responsible for an organism's immunological recognition of pathogens as they code for glycoproteins that destroy pathogens (Kalbe et al., 2009). Individuals with heterozygosity in MHC loci have enhanced resistance to diseases (Briles et al., 1983; Hill et al., 1991; Godot et al., 2000; Langefors et al., 2001; Grimholt et al., 2003; Bonneaud et al., 2005; Bonneaud et al., 2006; Harf & Sommer, 2005; Kalbe et al., 2009; Eizaguirre et al., 2012; Ryan & Cobb, 2012), and maximized lifetime reproductive success, allowing for them to maintain their health status more effectively than homozygotes (Kalbe et al., 2009).

An additional factor that influences individual health status is allostatic load, described as "the wear and tear that results from chronic over-activity or underactivity of allostatic systems" (McEwen & Wingfield, 2003). Different life history stages have different energetic costs, thus allostatic load can fluctuate throughout an individual's lifetime and is usually closely associated with an individual's energetic balance (McEwen & Wingfield, 2003). When an individual is experiencing an increased allostatic load and negative energetic balance, circulating glucocorticoid hormones, such as cortisol, increase to promote survival (for example, by regulating blood glucose levels) (Hämäläinen et al., 2015). This feedback mechanism is controlled by the hypothalamic-pituitary-adrenal (HPA) axis, which maintains homeostasis in response to stress and disease by controlling the release of glucocorticoids (Hoffman et al., 2011). Immune system activity is sensitive to high circulating levels of glucocorticoids, typically resulting in compromised immune function during times of stress (Hämäläinen, Heistermann, & Kraus, 2015).

Chronic stress is defined as long-term elevated glucocorticoid levels, which has been shown to increase susceptibility to disease, increase mortality risk (Hämäläinen et al., 2015; Hoffman et al., 2011), and accelerate cellular aging (Epel et al., 2006, Tomiyama et al., 2012). Women exposed to prolonged physiological stress exhibited shortened telomere lengths, highlighting the cellular aging consequences of long-term elevated glucocorticoid levels (Epel et al., 2006, Tomiyama et al., 2012). Age (Hämäläinen et al., 2015), reproductive state (Hoffman et al., 2011), reproductive output (Hoffman et al., 2011), and habitat fragmentation (Martínez-Mota et al., 2007) influence glucocorticoid levels in non-human primates. A study conducted on wild grey mouse lemurs (*Microcebus murinus*) revealed that individual stress levels spiked during the dry season when food and water availability were low, and this spike was more pronounced in older individuals, supporting the hypothesis that age impairs an individual's ability to cope under energetically demanding conditions (Hämäläinen et al., 2015). This is further evidence that glucocorticoid levels can fluctuate seasonally, contributing to seasonal differences in an individual's energetic balance and susceptibility to infections and disease.

The physiological demands of an individual's reproductive state and resulting changes in an individual's energetic balance can negatively influence their health status. A physiological correlation has been found between immune system performance and reproduction (Lazzaro & Little, 2009), which implies that individuals with higher energetic demands associated with their breeding status, such as a lactating female (Hoffman et al., 2011), are expected to be more susceptible to infection and potentially have worse health. Immune performance and reproduction limit each other as an

individual's resistance to infection can be compromised by the competing physiological demands of reproductive activity and vice versa (Lazzaro & Little, 2009). A study conducted on rhesus macaques (*Macaca mulatta*) found that pregnant and lactating females had higher glucocorticoid concentrations over time compared to non-pregnant and non-lactating females (Hoffman et al., 2011). This suggests that when an individual has a higher allostatic load and greater reproductive output (for example, animals that are primary breeders), they may be more susceptible to disease and cellular aging.

Assessing individual health status allows for researchers to analyze health trends within a population to evaluate the effects of age, sex, breeding status, group composition, seasonality, and the longitudinal effects of habitat disturbance on an individual's health. Comparative studies have revealed significant differences in the health trends observed in captive and wild primate populations, including significant variation in the intestinal flora (Uenishi et al., 2007; Villers et al., 2008; Clayton et al., 2016), genetic variation (Pastorini et al., 2015, Grogan et al., 2017), cortisol levels (Fourie et al., 2016), dental wear and tooth loss (Philips-Conroy & Jolly, 1988; Cuozzo et al., 2010), reproductive output (Sievert et al., 1991; Knott, 2001), and body size (Araújo et al., 2000; Terranova & Coffman, 2000; Turner et al., 2016). These differences are due to a variety of factors, including variation in diet and food availability, and most notably, the allostatic load of captive individuals is much lower without the presence of environmental stressors such as seasonality in food availability and limited energy expenditure in captive enclosures (Knott, 2001). Ovarian function is a reliable indicator of an individual's allostatic load, as it is sensitive to nutritional intake, energetic expenditure and net energy balance. The decreased allostatic load experienced by captive

individuals is evidenced by the shortened inter-birth intervals that have been observed in captive bonobos (Harvey, 1997), chimpanzees (Courtenay, 1987), and orangutans (Lippert, 1977) (reviewed in Knott, 2001). This provides further evidence that health trends and life history tradeoffs of wild populations cannot be modeled by captive populations due to the significant differences in allostatic load and energetic expenditure, and thus lifespan differences.

Health Evaluation in Wild Non-Human Primates

Health evaluations in wild non-human primates play an important role in modern conservation efforts. Identifying health trends in wild primate populations and establishing baseline health parameters not only allows for the potential prevention of disease transmission to threatened populations, but also enhances our understanding of naturally occurring pathogen tolerance and susceptibility in wild populations. Opportunities to conduct comprehensive health screenings in wild non-human primate populations are limited, and this is reflected in the dearth of published baseline health data. General health parameters, such as baseline hematology and serum biochemistry levels, morphometrics, and body weights, are published for less than 5% of primate species (reviewed in Crofoot et al., 2009). Baseline health parameters currently published for wild populations are summarized in Table 1.

Table 1

Comparisons of Baseline Health Data Collected From Health Assessment Studies in Wild Non-Human Primates

Species	**Authors**	***n***	**Body Mass**	**Morphometrics**	**Hematology**	**Serum Biochemistry**	**Parasitology**	**Dentition**
New World Monkeys (Platyrrhini)								
Saguinus fuscicollis	(Garber & Teaford, 1986)	77	✓	✓				✓
Saguinus mystax	(Garber & Teaford, 1986)	82	✓	✓				✓
Cebus apella	(Rosner et al., 1986)	40	✓	✓	✓	✓		
Alouatta palliata	(Glander et al., 1991)	42	✓	✓				
Ateles geoffroyi	(Glander et al., 1991)	14	✓	✓				
Cebus capucinus	(Glander et al., 1991)	8	✓	✓				
Brachyteles arachnoides	(Lemos de Sá & Glander, 1993)	12	✓	✓				
Ateles paniscus chamek	(Karesh et al., 1998)	8	✓	✓	✓	✓	✓	✓
Aloutta seniculus	(Vié, Moreau, de Thoisy, Fournier, & Genty, 1998)	122	✓		✓	✓		
Pithecia pithecia	(De Thoisy et al., 2001)	6	✓		✓	✓	✓	
Saguinus midas	(De Thoisy et al., 2001)	95	✓		✓	✓	✓	
Alouatta seniculus	(De Thoisy et al., 2001)	124	✓		✓	✓	✓	
Alouatta palliata mexicana	(Crissey et al., 2003)	6	✓		✓	✓		
Leontopithecus rosalia	(Monteiro et al., 2007)	206					✓	
Saguinus leucopus	(Fox et al., 2008)	29	✓		✓	✓		✓
Cebus capucinus	(Crofoot et al., 2009)	9	✓	✓	✓	✓	✓	✓
Alouatta pigra	(Rovirosa-Hernández et al., 2012)	34	✓		✓	✓		
Cebus flavius	(Teixeira et al., 2013)	20	✓		✓	✓		✓
Callithrix penicillata/ Callithrix goeldii hybrid	(Silva et al., 2014)	33	✓	✓	✓	✓		

Species	Authors	*n*	Body Mass	Morphometrics	Hematology	Serum Biochemistry	Parasitology	Dentition
Saguinus leucopus	(Soto-Calderón, Acevedo-Garcés, Álvarez-Cardona, Hernández-Castro, & García-Montoya, 2016)	36	✓		✓	✓	✓	
Alouatta pigra	(García-Feria, Chapman, Pastor-Nieto, & Serio-Silva, 2017)	12	✓		✓	✓		
Lemurs and Lorises (Strepsirrhini)								
Propithecus tattersalli	(Garell & Meyers, 1995)	34	✓		✓	✓		
Varecia variegata	(Junge & Louis, 2005a)	7	✓		✓	✓	✓	
Varecia rubra	(Junge & Louis, 2005a)	4	✓		✓	✓	✓	
Lemur catta	(Dutton, Junge, & Louis, 2011)	20	✓		✓	✓	✓	
Propithecus verreauxi deckeni	(Junge & Louis, 2011)	20	✓		✓	✓	✓	
Eulemur fulvus rufus	(Junge & Louis, 2011)	20	✓		✓	✓	✓	
E. macaco macaco	(Junge & Louis, 2007)	25	✓		✓	✓	✓	
Old World Monkeys and Apes (Catarrhini)								
Macaca tonkeana / M. hecki hybrid	(Gotoh et al., 2001)	43			✓	✓	✓	
Macaca hecki	(Gotoh et al., 2001)	22			✓	✓	✓	
Pongo pygmaeus pygmaeus	(Kilbourn et al., 2003)	84	✓		✓	✓	✓	✓
Macaca sinica	(Ekanayake et al., 2003)	72			✓	✓		
Macaca radiata radiata	(Mythili et al., 2005)	10	✓		✓	✓		

In these studies, there is a significant amount of variation in the health information collected and the number of individuals sampled (Table 1). Most studies published after 1995 minimally collected blood samples to establish baseline hematology and serum biochemistry profile parameters. Earlier studies were limited to morphometrics and body mass data collection (Garber & Teaford, 1986; Rosner et al., 1986; Glander, Fedigan, Fedigan, & Chapman, 1991; Lemos de Sá & Glander, 1993).

In clinics, veterinarians routinely collect blood to assess the health and condition of animals, and these evaluations are largely possible due to established reference values that enable animal health specialists to draw conclusions about the diagnosis and prognosis for patients (Stoot et al., 2014). With new tools in the field physiology toolbox, a move towards establishing hematology and serum biochemistry profile parameters is now possible for field biologists. Establishing the physiological range for these biological parameters in wild primate populations is valuable for assessing individual health status and health trends in a wild population, and also allows for the future application and interpretation of the health status of captive individuals. Immobilizing and handling wild primates poses a risk to the animals and staff, and the incidence of immobilization (either via darting or trapping) should be limited to prevent habituation and minimize the health risks to the animals (Brett et al., 1982; Sapolsky & Share, 1998; Karesh et al., 1998; Cunningham et al., 2015). For this reason, researchers should maximize the amount of health information collected during these immobilizations. The large disparity in the health information collected during previous health evaluation studies (Table 1) highlights the necessary shift towards incorporating

emerging technology and novel techniques to gain as much health information as possible when there is an opportunity to collect biological samples from wild primates.

Point-of-Care Devices

Novel applications of point-of-care (POC) technology provide primate field researchers with the means to overcome a major challenge in the field, which is evaluating internal organ function. Blood chemistry values are useful for monitoring the internal organ functions of free-ranging populations (Vié, Moreau, de Thoisy, Fournier, & Genty, 1998; Fox et al., 2008). POC devices allow researchers to measure multiple parameters with a single sample and obtain extensive analysis without requiring specialized lab equipment or staff (Stoot et al., 2014). POC devices were first developed and used to improve at-home patient care in humans and allow for the rapid measurement of biochemical markers onsite (Stoot et al., 2014). Their use has since expanded to veterinary emergency patient care, allowing for sick animals to be identified more easily without requiring extensive lab work (Stoot et al., 2014). Stoot et al. (2014) reviewed literature from 1995-2014, and found that 79 peer-reviewed studies used POC devices in on non-domesticated vertebrate studies.

Many POC devices are handheld and battery operated, which adds an additional convenience for field researchers, allowing for easy transport to remote locations. Additionally, using POC devices eliminates the need for sample storage, a factor that is especially helpful for researchers working at remote field sites. Using POC devices in a field research setting also reduces handling time and stress on the animals. These devices also generate valuable comparative data, and local veterinarians can be trained to use the

device to standardize sample collection and analysis from captive and wild populations. This can establish baseline hematology and serum biochemistry values for these species, which may be valuable in the future.

Neopterin and Other Biomarkers Used in Health Assessments

With emerging POC technology and novel non-invasive methods of assessing health status, there are growing opportunities to gain vital health information from wild primates. A wide variety of biomarkers associated with illness and disease are used when assessing the health status of humans. Common examples include cortisol measured in blood and feces (Beehner & McCann, 2008; Foerster, Cords, & Monfort, 2012; Murray, Heintz, Lonsdorf, Parr, & Santymire, 2013; Setchell, Smith, Wickings, & Knapp, 2010; Shutt, Setchell, & Heistermann, 2012), the gut microbiome evaluated in feces (Amato et al., 2016, 2013; Barelli et al., 2015; Gomez et al., 2015; Nagpal et al., 2018; Trosvik, de Muinck, et al., 2018; Trosvik, Rueness, De Muinck, Moges, & Mekonnen, 2018), and c-peptide measured in urine (Deschner, Kratzsch, & Hohmann, 2008; Emery Thompson & Knott, 2008; Girard-Buttoz et al., 2011; Higham, Girard-Buttoz, Engelhardt, & Heistermann, 2011).

A little-explored but particularly promising biomarker for field biologists is neopterin (d-erythro-trihydroxypropylpterin), a molecule that is synthesized during the cell-mediated immune response (Huber, 1984). Neopterin is a pteridine derivative, secreted by monocytes and macrophages upon the activation of cytokine interferon gamma (IFN-γ) (Murr et al., 2002). The release of neopterin stimulates T-helper 1 cell activation in immune responses to acute stress, viruses, or inflammation (Murr et al.,

2002). In humans, elevated neopterin levels have been correlated with severe viral (e.g., cytomegalovirus infections, influenza) and parasitic infections (e.g., acute malaria), but not bacterial infections, since bacteria tend to induce a humoral (antibody-mediated) rather than cell-mediated immune response (Murr, Widner, Wirleitner, & Fuchs, 2002). Neopterin levels have been used in diagnoses and prognoses for individuals with autoimmune diseases such as HIV (Abita, Cost, Milstien, Kaufman, & Saimot, 1985). In addition to disease response, neopterin is also associated with immunosenescence, or age-related decrease in cellular immune response (Frick, Schroecksnadel, Neurauter, Leblhuber, & Fuchs, 2004; Hawkley & Cacioppo, 2004; Müller, Heistermann, Strube, Schülke, & Ostner, 2017). Thus, neopterin is a reliable indicator of immune system activity in cases of compromised health status.

Researchers can use neopterin to evaluate risk factors for disease transmission in threatened non-human primate populations, as the maintenance of a well-functioning immune system is critical to survival and resistance to infection (Murr et al., 2002). Neopterin concentrations can be measured in serum, arterial blood, and urine using marker-specific immunoassays (Murr et al., 2002). Urinary neopterin in particular is promising for use by field biologists due to its high stability even under field conditions, without refrigeration (Heistermann & Higham, 2015). For example, urinary neopterin concentration (UNC) can remain stable in urine stored without preservative for up to 21 days. UNC is typically correlated to creatinine (Cr) concentrations. However, Cr can be sensitive to degradation across different storage conditions and has been shown to decrease over time when stored at room temperature (Heistermann & Higham, 2015). Therefore, urine samples are often treated with ProClin200, a biocide preservative that

stabilizes both UNC and Cr at room temperature (Heistermann & Higham, 2015), but ProClin200 is not easily obtainable with many vendors placing restrictions on its sale within the United States. In lieu of this method, the degradation of Cr over time can also be corrected for by using the specific gravity of the urine sample (Anestis, Breakey, Beuerlein, & Bribiescas, 2009). Additionally, short-term fecal and soil contamination do not significantly degrade UNC when samples are kept in a dark place, limiting sun exposure (Heistermann & Higham, 2015). These factors make UNC an ideal indicator for the immune status of wild populations.

Urinary neopterin, which can be sampled with relative ease, has successfully been tested as an indicator of immune system activation in four Old World monkeys and ape species in seven studies, 50% of which are on wild populations (Behringer, Stevens, Leendertz, Hohmann, & Deschner, 2017; Heistermann & Higham, 2015; Löhrich, Behringer, Wittig, Deschner, & Leendertz, 2018; Müller et al., 2017; Wu, Behringer, Wittig, Leendertz, & Deschner, 2018). Elevated UNC was recorded in captive bonobos (*Pan paniscus*) with respiratory infections (Behringer et al., 2017), captive SIV-infected rhesus macaques (*Macaca mulatta*) (Fendrich et al., 1989), and during a respiratory outbreak in a wild population of Taï chimpanzees (*Pan troglodytes*) (Wu et al., 2018). Remarkably, elevated UNC levels have also been observed in aging semi-free ranging Barbary macaques (*Macaca sylvanus*), suggesting that UNC may be an indicator of immunosenescence in non-human primates (Müller et al., 2017). While these studies have confirmed that UNC can be used to accurately represent and monitor changes in immune system activity in some primates, seasonal fluctuation and temporal delays between time of infection and immune response can impact UNC (Löhrich et al., 2018).

Lohrich et al. (2018) found seasonal fluctuation in UNC of wild chimpanzees, with increased urinary neopterin levels in correspondence with low ambient temperature. Thus, UNC is a suitable nonspecific measure of health monitoring that requires frequent sampling over time to adequately detect changes in immune system activation during acute outbreaks (Löhrich et al., 2018; Murr et al., 2002).

All current validations of UNC have been conducted on Old World anthropoids (Behringer et al., 2017; Heistermann & Higham, 2015; Hoffman et al., 2011; Löhrich et al., 2018; Müller et al., 2017; Wu et al., 2018), which diverged from New World Monkeys ~37 Ma (Lynch Alfaro, Cortés-Ortiz, Di Fiore, & Boubli, 2015). It is advisable that future studies do not operate under the assumption that the neopterin molecule is conserved across all primate taxa. With 37 endangered primate species in the Neotropics, 15 of which are critically endangered (Seminoff, 2004), a stable biomarker of immune system activity could allow for future health monitoring and accurate assessment of disease onset in the Neotropics at the population level.

Current Investigation

In the current investigation, we seek to validate non-invasive biomarkers for future utilization in health assessments of wild primates (see Chapter II), as well as establish baseline blood and urinary chemistry parameters for *S. imperator* and *L. weddelli* to allow researchers to carry out real-time wildlife health screening in the field, in sanctuaries, and reintroduction initiatives (see Chapter III).

CHAPTER TWO

A NON-INVASIVE MEASURE OF IMMUNE STATUS: VALIDATION OF AN ASSESSMENT OF URINARY NEOPTERIN IN NEW WORLD MONKEYS

JOURNAL-READY ARTICLE

FOR SUBMISSION TO:

SCIENTIFIC REPORTS

A non-invasive measure of immune status: Validation of an assessment of urinary neopterin in New World Monkeys

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ABSTRACT

Non-invasive biomarkers can facilitate health assessments in wild primate populations by reducing the need for direct access. Neopterin is a biomarker that is a product of the cell-mediated immune response, with high levels being indicative of poor survival expectations. Urinary neopterin has been validated as a method for monitoring immune system activation in Old World monkeys, but no such study has been performed in New World Monkeys (NWMs). In this study, we collected urine samples across three NWM families including captive *Leontopithecus rosalia* and *Pithecia pithecia*, and wild populations of *Leontocebus weddelli*, *Saguinus imperator*, *Alouatta seniculus*, and *Plecturocebus toppini*, to validate a commercial enzyme-linked immunosorbent assay (ELISA) for neopterin. UNC was successfully quantified in all species across several wavelengths. However, from wild populations, we did not observe uniformity in UNC trends at each wavelength across the four species, and thus to evaluate species differences in immune function, average levels should be determined using a standardized wavelength. Importantly, neopterin, a valuable non-invasive biomarker for assessing immune status, appears valid in NWMs both in captivity and in the field.

Introduction

Health and disease monitoring in wildlife plays an important role in modern conservation efforts[1,2]. Identifying health trends and evaluating disease prevalence and onset not only allows for the prevention of disease transmission to threatened populations, but also enhances our understanding of naturally occurring pathogen tolerance and susceptibility in wild populations. Evaluating the health status of wildlife allows researchers to anticipate the likelihood of disease outbreaks and infection as well as predict survival outcomes on an individual basis[3].

There are two general approaches to assessing wildlife health. The first focuses on evaluating physiological parameters associated with homeostasis and immune system activity, such as temperature, blood pressure, hematology or serum chemistry[4-13]. The second approach focuses on biomarkers indicative of illness and disease, which in some cases can be measured non-invasively. Common examples include cortisol measured in blood and feces[14-18], the gut microbiome evaluated in feces[19-25], and c-peptide measured in urine[26-29].

A little-explored but particularly promising biomarker for field biologists is neopterin or d-erythro-trihydroxypropylpterin, a molecule that is synthesized during the cell-mediated immune response[30]. Neopterin is a pteridine derivative, secreted by monocytes and macrophages upon the activation of cytokine interferon gamma (IFN-γ)[31]. The release of neopterin stimulates T-helper 1 cell activation in immune responses to acute stress, viruses, or inflammation[31,32]. In humans, elevated neopterin levels have been correlated with severe viral (e.g., cytomegalovirus infections, influenza) and parasitic infections (e.g., acute malaria), but not bacterial infections, since bacteria tend to induce a

humoral (antibody-mediated) rather than cell-mediated immune responses[31]. Neopterin levels have been used in diagnoses and prognoses for individuals with autoimmune diseases such as HIV[33]. In addition to disease response, neopterin is also associated with immunosenescence, or the age-related decrease in cellular immune response[34-36]. Thus, neopterin is a reliable indicator of immune system activity in cases of compromised health status.

Researchers can use neopterin to evaluate risk factors for disease transmission in threatened wildlife populations, as the maintenance of a well-functioning immune system is critical to survival and resistance to infection[31]. Neopterin concentrations can be measured in serum, arterial blood, and urine using marker-specific immunoassays[31]. UNC is typically expressed as a ratio of neopterin to urinary creatinine (Cr), to control for variation in urine density and concentration[31].

Urinary neopterin in particular is promising for use by field biologists due to its high stability even under field conditions, without refrigeration[37]. For example, urinary neopterin concentrations (UNC) can remain stable in urine stored without preservatives for up to 21 days. However, urinary Cr can be sensitive to degradation across different storage conditions and has been shown to decrease over time when stored at room temperature[37]. Therefore, urine samples are often treated with ProClin200, a biocide preservative that stabilizes both UNC and Cr at room temperature[37], however ProClin200 is not easily obtainable with many vendors placing restrictions on its sale. In lieu of this method, the degradation of Cr over time can also be corrected for by using the specific gravity of the urine sample[38]. Additionally, short-term fecal and soil contamination do not significantly degrade UNC when samples are kept in a dark place, limiting sun

exposure[37]. These factors make UNC an ideal indicator for the immune status of wild populations.

Urinary neopterin, which can be sampled with relative ease, has successfully been tested as an indicator of immune system activation in four Old World monkeys and apes over seven studies, 50% of which were in wild populations[3,36,37,39,40,41]. Elevated UNC was recorded in captive bonobos (*Pan paniscus*) with respiratory infections[39], captive SIV-infected rhesus macaques (*Macaca mulatta*)[41], and during a respiratory outbreak in a wild population of chimpanzees (*Pan troglodytes*)[3]. Remarkably, elevated UNC levels have also been observed in aging semi-free ranging Barbary macaques (*Macaca sylvanus*), suggesting that UNC may be an indicator of immunosenescence in non-human primates[36]. While these studies have confirmed that UNC can be used to accurately represent and monitor changes in immune system activity in some primates, seasonal fluctuation and temporal delays between time of infection and immune response can impact it[40]. Lohrich et al. (2018) found season fluctuation in UNC of wild chimpanzees, with increased levels in correspondence with low ambient temperature. Thus UNC is a nonspecific measure of monitoring immune function that requires frequent sampling over time to adequately detect changes in immune system activation during acute outbreaks[31,40].

Since all current validations of UNC have been conducted on OWMs[3,36,37,39,40,42], which diverged from the NWMs ~37Ma[43], it would be advisable to validate UNC for the remainder of the Primate Order. With 37 endangered primate species in the Neotropics, 15 of which are critically endangered[44], a stable biomarker of immune system activity could allow for future health monitoring and accurate assessment of disease onset in the

Neotropics at the population level. To address this need, we aimed to validate the use of commercially available urinary neopterin assays across three primate families, including six species representing six different genera of NWMs.

To facilitate future comparisons to previous studies that have employed commercial neopterin kits[3,36,39,40,42], we chose to validate the same commercial ELISA kit used in these studies. To accommodate the use of this assay across a wide range of equipment and primate species, we assessed a range of variations in test conditions most likely encountered in field conditions. First, since equipment variation and frequency of calibration can contribute to variation in sample analysis and quantification[45-47], we analyzed UNC across three wavelengths to increase the likelihood of compatibility of the assay with researchers with filter constraints within their field laboratories. Second, since the kit was originally designed for humans and not NWMs, we assessed two different dilution factors that could affect the efficacy of neopterin measurements by this kit. Lastly, we assessed the precision of UNC measurements at each wavelength by comparing the measured concentrations of each control to the target UNC of each high and low control.

Results

Captive study. We successfully measured neopterin using a commercially available ELISA kit from IBL International GmbH (Art. No. RE59321, Hamburg, Germany) in all urine samples collected from captive primates. There was no significant change during the two weeks elapsed from time of collection to time of analysis in the specific gravity (SG) of urine collected from *Leontopithecus rosalia* (t = -0.048, df = 1.227, P = 0.968) or

Pithecia pithecia (t = 0.447, d.f. = 7.961, P = 0.667) (Table 1). However, the samples collected from *P. pithecia* turned a distinct black color during this period.

	Sample size	Population SG at time of collection	Population SG at the time of analysis
Leontopithecus rosalia	2	1.050 ± 0.014	1.0485 ± 0.042
Pithecia pithecia	5	1.037 ± SD 0.007	1.039 ± SD 0.007
Saguinus imperator	47	1.0119 ± 0.0063	1.0118 ± 0.0048
Leontocebus weddelli	39	1.0111 ± 0.0046	1.0090 ± 0.0039
Alouatta seniculus	1	1.0520	1.0520
Plecturocebus toppini	1	1.0140	1.0140

Table 1. Summary of the specific gravity measures of urine samples collected from captive and field study. Urine samples from golden lion tamarin (*Leontopithecus rosalia)* and white faced saki (*Pithecia pithecia*) individuals were collected at the Woodland Park Zoo, Seattle, Washington, USA. Urine samples from emperor tamarin (*Saguinus imperator*), saddleback tamarin (*Leontocebus weddelli*), Colombian red howler monkey (*Alouatta seniculus*), and brown titi monkey (*Plecturocebus toppini*) individuals were collected in southeastern Peru**.** Sample size denotes number of samples analyzed.

While samples diluted 1:100 had higher measured UNC than samples diluted 1:10 on average (Table 2), there was no significant difference between these measures (t = 0.217, d.f. = 11.049, P = 0.832).

	Sample size	1:100 Dilution	1:10 Dilution
		Mean UNC (ng/mL) ± SEM	
Leontopithecus rosalia	2	2350.96 ± 1469.78	2062.24 ± 544.26
Pithecia pithecia	5	402.56 ± 277.18	230.45 ± 71.71

Table 2. Summary of UNC (ng/mL) results for captive *Leontopithecus rosalia* and *Pithecia pithecia* at the Woodland Park Zoo, Seattle, Washington, USA across two different dilution factors. Samples were measured in duplicate and read at 450 nm. Sample size denotes number of samples analyzed.

The mean UNC for both species did not vary significantly across the three wavelengths assessed ($H(2)$ = 0.05, P = 0.975). The highest UNC values for both species were measured at 450 nm (Table 3). Larger samples sizes could affect these numbers.

	Sample size	405 nm	450 nm	490 nm
		Mean UNC (ng/mL) ± SEM		
Leontopithecus rosalia	2	1364.82 ± 99.31	2350.09 ± 1469.78	2223.09 ±1453.53
Pithecia pithecia	5	392.55 ± 269.96	402.46 ± 277.18	385.10 ± 259.01

Table 3. Summary of UNC (ng/mL) for captive *Leontopithecus rosalia* and *Pithecia pithecia* at the Woodland Park Zoo, Seattle, Washington, USA across three different wavelengths. The UNC values represent the final specific-gravity adjusted concentrations. All samples represented were diluted 1:100 and measured in duplicate. Sample size denotes number of samples analyzed.

Field study. The commercially available ELISA kit measured neopterin in all urine samples collected from the six primate species sampled from the wild. The measured specific gravity at time of collection did not vary significantly from the final measured specific gravity in samples collected from *Saguinus imperator* ($H(1)= 0.139$, $P = 0.709$) and *Leontocebus weddelli* ($H(1) = 2.272$, $P = 0.099$) (Table 1). The measured specific gravities for both samples collected from the individual *Alouatta seniculus* and *Plecturocebus toppini* were the same at time of collection and time of analysis (Table 1). We observed that the SG of urine from wild species was significantly lower than that of captive species ($t = 4.484$, d.f. = 6.066, P=.004)

We determined that the commercial ELISA kit also allowed for the measurement of UNC at three different wavelengths. A significant difference was observed between UNC in *S. imperator* across the three different wavelengths ($H(2)= 33.42$, $P < 0.0001$) (Table 4), with UNC measured at 490 significantly higher than the other wavelengths. No significant difference was observed in measured UNC of samples collected from *L. weddelli* across the three different wavelengths ($H(2) = 0.037$, $P = 0.981$). Unlike samples collected from *S. imperator*, samples collected from *L. weddelli* were highest at UNC at 405 nm (Table 4). While only one sample was obtained from *A. seniculus*, the range in

variation of measured UNC across the three wavelengths was similar to the variation observed for samples collected from *L. weddelli* individuals, with no significant difference observed across the three different wavelengths ($H(2) = 2$, $P = 0.368$). There was more variation observed in measured UNC for the single sample obtained from *P. toppini*, thus, we are unable to discern a wavelength-specific trend for this species. Overall, the sample collected from *A. seniculus* had the highest measured UNC across all wavelengths, followed by *P. toppini* UNC, and samples collected from *S. imperator* and *L. weddelli* had substantially and consistently lower measured UNC across all wavelengths. (Table 4).

	Sample size	**405 nm**	**415 nm**	**490 nm**
		Mean UNC (ng/mL) ± SEM		
Saguinus imperator	47	186.98 ± 167.69	185.62 ± 157.49	422.14 ± 220.45*
Leontocebus weddelli	39	478.25 ± 375.98	468.27 ± 484.30	458.69 ± 395.70
Alouatta seniculus	1	2060.31	2048.98	2071.76
Plecturocebus toppini	1	1317.51	1293.47	1189.92

Table 4. Summary of UNC results measured at three different wavelengths. All samples were diluted 1:100 and measured in duplicate. (*) Denotes measured UNC across wavelengths is significantly different than the other wavelengths. Sample size denotes number of samples analyzed.

We evaluated the high and low controls at each wavelength to evaluate the precision of results obtained when measured at each wavelength by quantifying deviation from the target concentration. The control measurements were closest to the known target values for high and low controls at 415 nm (Table 5). While control measurements at 405 nm and 490 nm had greater deviation from both high and low controls, results from one-sample t-tests revealed that measured UNC across all three wavelengths did not differ significantly from the known UNC of low and high controls (Table 5).

	Low Control (Target: 6.3 nmol/L)			High Control (Target: 22.6 nmol/L).		
Wavelength (nm)	Mean Measured UNC (nmol/L) ± s.e.m.	Deviation from Target (nmol/L)	p	Mean Measured UNC (nmol/L) ± s.e.m.	Deviation from Target (nmol/L)	p
405	5.79 ± 1.42	0.51	0.599	21.68 ± 4.73	0.92	0.769
415	6.11 ± 1.66	0.19	0.864	21.74 ± 4.16	0.86	0.755
490	10.10 ± 8.86	3.8	0.534	26.15 ± 8.45	3.55	0.542

Table 5. Summary of high and low controls read at three different wavelengths. Mean measured UNC represents the average urinary neopterin concentration of each high and low control in three assays across three wavelengths. The p-value represents the one-sample t-test results of each mean measured UNC at three wavelengths compared to the control from the target low control value (6.3 nmol/L) and high control value (22.6 nmol/L). All controls were measured in duplicate.

Discussion

We validated a commercially available ELISA kit, showing that it successfully measures neopterin in the urine of six NWM species. Together with previous non-human primate studies, the neopterin molecule appears to be conserved across primate taxa and can likely be used as a non-invasive marker of immune system activation in New World Monkeys. We confirmed that the neopterin molecule is present and detectable via commercial ELISA in the urine of three different Platyrrhine (Atelidae: howler monkeys, spider monkeys, and woolly monkeys, Callitrichidae: tamarins and marmosets, and Pitheciidae: saki monkeys and titi monkeys) across five different genera. Although this study did not include the two remaining Platyrrhine families, Aotidae (night monkeys) and Cebidae (capuchins and squirrel monkeys), we predict neopterin can also be measured in the biological fluids of members of these families, but further validation is advised.

In field settings during behavioral primate follows, Salivette swabs allowed for the recovery of urine from leaves, but it was difficult to collect a volume greater than 0.2 mL. Diluting samples 1:100 not only maximizes analysis for small samples in the field where it can be difficult to recover large volumes of urine, but it can also control for discrepancies in colorimetric readings for highly concentrated or dark urine samples. The variation in SG between urine samples collected in the captive and field study could be attributed to species differences, although we hypothesize it is more likely due to diet. Diet influences variation in hydration across these species and consequently the concentration of solutes in urine, both of which are reflected in the SG of urine samples, although the small sample sizes make it difficult to confirm these are the only contributing factors to the variation in SG observed in this study. Additionally, urine samples collected from captive *P. pithecia* turned a black color. We believe this could be associated with a rare inherited disease called alkaptonuria, a condition typically diagnosed when urine turns a black color upon exposure to air. It is possible that the color of the urine collected from *P. pithecia* impacted colorimetric performance of the ELISA, but diluting samples 1:100 reduces the chance of interference of this nature in the assay.

We also determined that wavelengths outside of the recommended 450 nm can be used to recognize and measure UNC using this ELISA kit. The equation used to calculate UNC is dependent on maximum absorbance (450 nm), thus measurement outside of the maximum absorbance could explain the significant differences in measured UNC for samples collected from *S. imperator* at wavelengths different from the optimal wavelength. These results indicate that filters at multiple wavelengths are compatible with this ELISA kit, but the results may not express absolute UNC. For this reason, future

comparisons should only be drawn within studies using the same wavelength. While the three different wavelengths used were successful in measuring variation between samples, we determined that 415 nm measures high and low controls closest to that of 450 nm, but 405 and 490 nm can also be used to measure UNC as indicated by non-significant variation in UNC for samples collected from *L. rosalia, P. pithecia,* and *L. weddelli.* This potentially allows for researchers in laboratories with limited filter availability (e.g., field molecular laboratories) to use this kit across a range of wavelengths, even if the manufacturer's recommended filters are not available.

The variation in UNC across the six study species suggests that there may be species differences in immune function, which could be related to variation in size, diet, and health status. Sample size could be a contributing factor to the observed variation as there was considerably different sample sizes for each species. To address health status and species differences, larger sample sizes are necessary to determine average values for each species before conclusions can be drawn regarding trends in UNC related factors such as environment and metabolism.

In conclusion, we were able to validate a method of measuring urinary neopterin, a non-invasive biomarker for immune system activation, in Platyrrhines. Future studies are necessary to determine the reliability of using UNC in response to disease outbreak and health trends in these species. This validation allows for future utility of UNC as a measure to evaluate the health status and disease outbreaks in wild and captive populations to predict survival outcomes. It is likely that UNC in these species mirrors the same seasonal fluctuation of other non-human primates with similar time delays following infections, thus frequent sampling is recommended in future studies to

adequately reflect the dynamic immune system response observed in humans and other non-human primates.

Methods

Captive sample collection. Seven urine samples were opportunistically collected from two golden lion tamarins (*L. rosalia)* and two white-faced saki monkeys (*P. pithecia*) in the Tropical Rainforest exhibit at the Woodland Park Zoo, Seattle, Washington from April 8 to 20, 2018. Five samples were collected from *P. pithecia* and two samples were collected from *L. rosalia* using swabs (Salivette ® Cortisol, Sarstedt, Nürmbrecht, Germany) previously validated for non-invasive urine collection prior to UNC measurement [48].The Salivette collection device has an upper compartment that houses a swab nested within a larger tube. This upper compartment has a hole at the bottom that allows for urine to be drawn out of the swab and pool within the tube when centrifuged. Using sterile technique, zookeepers removed the swab from the tube, swiped over the pooled urine to collect and absorb the sample in its entirety, and then used a manual oscillatory centrifuge composed of cardboard disks and string (a modified whirligig toy colloquially referred to as a "paperfuge")[49]. to generate enough rotational force to recover the volume of urine absorbed in the swab. Urine samples were stored at 4-8°C in a refrigerator until analysis.

Field sample collection. The field study was performed at the Estacion Biologica Rio Los Amigos (EBLA) (12°34'07" S, 70°05'57" W) in the Madre de Dios Department of southeastern Peru in collaboration with a longitudinal capture-and-release program by

Field Projects International[50]. In brief, animals are habituated to a baited multi-compartment trap that they enter voluntarily. Entire groups are processed simultaneously and released upon recovery at the same trap site on the day of capture. Teams of five trained wildlife handlers process animals using a novel two-step anesthesia protocol(Watsa et al., 2015). Each individual is tagged permanently with a microchip (Home Again, Merck). During recovery from anesthesia, animals are placed in individual holding cages with urine collection trays beneath that are checked every three minutes. For this study, urine samples were opportunistically collected from 40 emperor tamarins (*S. imperator*) and 34 saddleback tamarins (*L. weddelli*) during mark-recapture. Additionally, during primate follows, urine samples were opportunistically collected from one Colombian red howler monkey (*A. seniculus*) and one brown titi monkey (*P. toppini*) using Salivette collection devices to recover urine from the surface of leaves. All urine samples were stored immediately upon collection in portable cool packs at a temperature of 8°C.

Sample storage and specific gravity: Upon return to the field station, field samples were labeled in more detail and 10 μL of urine were removed from each sample using a sterile pipette to determine the specific gravity (SG) using a handheld refractometer (Ade Optics Tri-Scale Clinical Refractometer, Oregon City, USA) on the day of collection. For samples from captive animals, SG was also measured similarly by zoo staff. The remaining volumes of the recovered urine samples were then stored at room temperature sheltered from direct sunlight until further analysis. Urine samples from field study were stored in a freezer at -5 to -10°C.

Laboratory methods. Samples from the captive individuals were analyzed at Central Washington University, whereas field sample analysis was performed at The Green Lab, a molecular field laboratory at Inkaterra Guides Field Station in Puerto Maldonado, Peru. To measure the concentration of neopterin in urine, we used a commercially available Neopterin ELISA kit (Art. No. RE59321, IBL International GmbH, Hamburg, Germany) designed for quantifying neopterin in human serum plasma and urine. For captive sample analysis, two different dilution factors (1:10 and 1:100) were used to confirm that the ELISA would identify neopterin in these species. Previous work had shown 1:100 was adequate for non-human primates[39] and is recommended by the manufacturer. Diluted samples were mixed by inverting the sample tube three to four times to fully homogenize the samples. All samples, standards, and controls were measured in duplicate when possible given plate size constraints (only two urine samples were measured once).

The assay was performed using the manufacturer's instructions. Following dilution, 20 µL of each sample, 100 µL of enzyme conjugate, and 50 µL antiserum were added to each well on the 96-well plate provided in the kit. The plate was then covered with black adhesive foil, wrapped in aluminum foil and incubated at room temperature for 90 minutes. During this time, every 90 seconds, the plate was gently shaken three times side-to-side and three times forward and backwards. Following the 90-minute incubation period, the plate was washed four times using wash buffer. Using a repeater pipette, 150 µL of tetramethylbenzidine substrate solution was added into each well and incubated at room temperature for 10 minutes. The reaction was then stopped using 150 µL stop solution. The optical density was read photometrically at 405, 450, and 490 nm during the captive study using a BioTek Synergy 2 (BioTek Instruments, Inc., Winooski,

Vermont, USA), and at 405, 415, and 490 nm during the field study using a BioRad Model 550 Microplate Reader (BioRad Laboratories Inc., Hercules, California, USA). Although the manufacturer suggested that the optical density be measured at 450 nm, due to the filter availability constraints during the field study, we decided to further assess the efficacy of measuring the optical density above and below the recommended wavelength. Inter-assay coefficients of variation, which were determined by repeated measurement of high and low value controls in each assay, were 4.39% and 3.19% respectively. A 4-Parameter Logistic standard curve was used to calculate the neopterin concentration of each sample.

To account for differences in urine volume and concentration, specific gravity-corrected neopterin concentrations were calculated using the following formula[51]:

$$SG\ corrected\ concentration = raw\ value \times \frac{(SG_{mean} - 1.0)}{(SG_{sample} - 1.0)}$$

The final SG value was used to control for variation in urine volume, density, and concentration. A decrease in specific gravity is expected, due to its correlation with the degradation of creatinine in the urine sample. The final neopterin concentrations were transformed from nmol/L to ng/mL following the manufacturer's protocol.

Ethical statement. All sampling protocols adhere to guidelines outlined by the American Society of Mammalogists.[52] and were approved by authorities at the Woodland Park Zoo (WPZ#2018-5) as well as the Institutional Animal Care and Use Committees at Central Washington University (A011802) and the University of Missouri-St. Louis (733363-5). The Peruvian Ministry of the Environment (SERFOR) granted annual research and collection permits.

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CHAPTER THREE

HEMATOLOGY AND URINALYSIS REFERENCE VALUES IN NEOTROPICAL PRIMATES USING NOVEL POINT-OF-CARE TECHNOLOGY

Literature Review

Health monitoring plays a critical role in primate conservation and research. Established baseline biological parameters are an important pre-requisite for detecting infection and disease. Technological advances have expanded the methods available for sample analysis, allowing wildlife researchers to make tremendous strides to collect health data and effectively carry out real-time wildlife health screening in the field.

Urine and blood samples are frequently collected during primate health evaluations, as there are a variety of measurable biological parameters present in these bodily fluids that undergo physiological adaptation in response to changes in health status and environmental stressors (García-Feria et al., 2017; Soto-Calderón et al., 2016; Vié et al., 1998). Urinary dipsticks are commonly used for rapid assessment of organ dysfunction through the measurement of parameters such as pH, leukocytes, nitrite, and glucose (Beaman, Hesemeyer, Dominy, Savini, & Reichard, 2009; Kaur & Huffman, 2004; Kelly, Sleeman, & Wrangham, 2004; Leendertz et al., 2010; Löhrich et al., 2018; MacIntosh, Huffman, Nishiwaki, & Miyabe-Nishiwaki, 2012; Mariani, Luangphinith, Loo, Scottolini, & Hodges, 1984; Simerville, Maxted, & Pahira, 2005; Sleeman & Mudakikwa, 1998). Blood samples can be used for nutritional status evaluation (Ange-Van Heugten, Timmer, Jansen, & Verstegen, 2008; Crissey et al., 2003; Girard-Buttoz et

al., 2011), disease detection (Junge & Louis, 2005; Kilbourn et al., 2003), and parasite infection (Crofoot et al., 2009; Gotoh et al., 2001) by assessing the degree to which measured parameters deviate from reference values. To accurately assess health status in these ways, baseline hematology reference values and urinary parameters must be established at the genus level at a minimum.

Opportunities to conduct comprehensive health screenings in wild non-human primate populations are limited, and this is reflected in the lack of published baseline health data. General health parameters are published for less than 5% of primate species (reviewed in Crofoot et al., 2009). For field primatologists, blood sample collection requires immobilizing and handling wild primates. This poses a risk to the animals and handlers, and the incidence of immobilization (either via darting or trapping) should be limited to prevent habituation and minimize the health risks to the animals (Brett, Turner, Jolly, & Cauble, 1982; Cunningham, Unwin, & Setchell, 2015; Karesh et al., 1998; Sapolsky & Share, 1998). For this reason, POC devices are ideal because they enable researchers to maximize the amount of health information collected during immobilizations.

POC technology is a promising method for wildlife veterinarians and researchers to measure physiological parameters and streamline the process to obtain instant results onsite. POC devices were first developed and used to improve at-home patient care in humans and allow for the rapid measurement of biochemical markers (Stoot et al., 2014). Their use has since expanded to veterinary emergency patient care and field research, allowing for sick animals to be identified more easily without requiring extensive lab work. In contrast to freezing and sending blood samples to specialized labs for later

analysis, utilizing POC technology for instantaneous results allows wildlife veterinarians and researchers to diagnose and treat an animal during the same capture instance, thus maximizing the effort and effectiveness of a single capture. Stoot et al. (2014) reviewed the use of POC devices in non-domesticated vertebrate research studies and found that 79 studies involved these devices. The i-STAT (Abbott Laboratories) was the most commonly used device (53.2% of studies) but can be costly for studies with large sample sizes. In this research, we sought out a new POC device for primate health evaluation on a community-wide scale.

Keto-Mojo. The Keto-Mojo is an affordable POC device ($59.99 USD) that is lightweight, runs on two AAA alkaline batteries, and enables users to run many samples inexpensively. While the i-STAT requires expensive single-use cartridges (~$25 USD each) that require refrigeration, test strips for the Keto-Mojo can be stored at ambient room temperature, which is ideal for researchers at remote field sites. There are two types of affordable test strips compatible with the device. The glucose strip ($0.36 USD each) contains the enzyme glucose hydrogenase and measures glucose (blood sugar), hematocrit (the ratio of the volume of blood cells to the total volume of blood), and hemoglobin (a blood protein that is responsible for transporting oxygen). The ketone strip ($1 USD each) contains the enzyme β-hydroxybutyrate dehydrogenase and measures β-ketone (β-hydroxybutyrate). Both strips receive AC and DC signals from the device. Once a sample is added to the absorbent channel of each strip, the strength of the current produced by the enzymatic reaction is used to determine the concentration of glucose and β-ketone in the blood sample. This blood glucose monitoring device is suggested over

other POC devices as it has been shown to measure glucose and ketone levels with accuracy within 5% of laboratory readings, well above the 20% accuracy range required by the FDA (Keto-Mojo, 2018).

The Keto-Mojo allows for rapid screening, as the glucose test strip measures glucose (from 10-700mg/dL), hematocrit (0-70%), and hemoglobin within five seconds (Keto-Mojo, 2018). The ketone strip measures ketones (from 0.1 - 8.0 mmol/L) within ten seconds (Keto-Mojo, 2018). Each test only requires 1.0 µL of blood to run. This allows researchers to measure multiple parameters rapidly from a very small sample, which minimizes the handling and stress on the animals.

Urinary dipsticks. Multi-reagent urinary dipsticks contain reagent pads that detect and quantify the presence of leukocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, and glucose. These dipsticks can also measure pH and specific gravity. Rapid screening of these urological parameters is a common practice in human and veterinary medicine as a diagnostic tool. Previous studies have evaluated the prevalence of infections and noninfectious urological diseases as well as nutritional condition with multi-reagent dipstick urinalysis.

Each dipstick contains reagent pads for each chemical parameter that change color when fresh urine is applied to the strip. The final color of each reagent pad is then compared to a calibrated colorimetric chart to determine the specific levels of each parameter. The detection of leukocytes, nitrite, protein, and blood are all possible indications of urinary tract infections and renal disease (Simerville et al., 2005). Nutritional condition can be determined by evaluating metabolic activity through ketone

measurement, an indication that the body is in a state of starvation and metabolizing fat as a source of energy in the absence of glucose (Coetzee, Jackson, & Berman, 1980; Knott, 1998). Dietary composition has been evaluated through the measurement of urinary pH (Kelly et al., 2004; Krief et al., 2005; Leendertz et al., 2010; Siener & Hesse, 2002; Sleeman & Mudakikwa, 1998), and an individual's hydration status can be inferred from the measurement of urinary specific gravity (Leendertz et al., 2010).

Urine can be collected non-invasively with relative ease from many primate species, making this rapid and cost-effective urine assessment method a promising and useful tool for health evaluations in primates under field conditions. The use of urological screening in wild primate health monitoring practices is increasing, although there is a lack of baseline data on urinary parameters in wild New World Monkeys (Beaman et al., 2009; Kaur & Huffman, 2004; Kelly et al., 2004; Knott, 1998; Krief et al., 2005; Löhrich et al., 2018; MacIntosh et al., 2012; Sleeman & Mudakikwa, 1998). To establish urinary reference values in wild primates, it is recommended to conduct evaluations on a population basis, as a wide range of urinary specific gravity and pH values have been recorded in wild chimpanzees living in different habitats (Leendertz et al., 2010), which highlights possible interpopulation variation in normal physiological parameters.

The aim of our study was to investigate the urinary and hematology parameters of emperor tamarins (*Saguinus imperator*) and saddleback tamarins (*Leontocebus weddelli*) to establish reference data for future health monitoring. We utilized convenient and affordable techniques to promote the incorporation of POC devices, such as the Keto-Mojo, and urinalysis dipsticks into wildlife health monitoring initiatives. The

incorporation of POC techniques facilitates the establishment of these baseline parameters and allows for the future evaluation of demographic effects on blood and urine parameters. We aimed to evaluate the reliability of the Keto-Mojo device and compare the measured hematology ranges to reference hematologic values of closely-related primates including wild-caught white-footed tamarins (*Saguinus leucopus*) (Fox et al., 2008). We also aimed to confirm that the use of POC devices and urinalysis dipsticks are effective methods of monitoring health in free-ranging primates under field conditions.

Methods

Study site and subjects. This study was conducted for 4 weeks in June and July 2018 on free-ranging primates at the Estación Biológica Los Amigos (EBLA) (12°34′S 70°05′W) in the Madre de Dios Department of southeast Perú as a part of a long-term disease screening effort. Blood and urine samples were obtained from two primate species as a part of a long-term mark-recapture program conducted between 2010-2018 (detailed protocol in Watsa et al., 2015). In brief, we captured entire groups at baited compartment traps to which they were habituated and processed and released them on the same day to minimize disruption and discomfort to the subjects. We used a two-step chemical restraint method that has improved recapture rates in comparison to established methods (Savage, Giraldo, Soto, & Snowdon, 1996), virtually eliminates capture-related injuries, and has no visible effect on habituation (Watsa et al., 2015). Radio collars placed on the groups are replaced annually on the largest breeding female in each group to facilitate tracking for long-term behavioral monitoring.

Blood collection. A total of 40 emperor tamarins (*Saguinus imperator*) (21 males and 19 females) and 35 saddleback tamarins (*Leontocebus weddelli*) (22 males and 13 females) were captured at EBLA. Seven groups of saddleback tamarins, and one lone saddleback were captured. Eight groups of emperor tamarins, and one lone individual were captured. During each capture event, a small blood sample was collected and analyzed via the Keto-Mojo device. Glucose, hematocrit, and hemoglobin readings were collected for 38 emperor tamarins and all 35 saddleback tamarins. Ketone readings were collected for 38 emperor tamarins and 33 saddleback tamarins. To evaluate the reliability of the Keto-Mojo device within a sample, duplicate blood chemistry readings were carried out for 30 blood samples total across both species (17 emperor tamarins and 13 saddleback tamarins). Each week a quality check test was conducted for the device to ensure effective operation. This was performed using a control solution with known concentrations of glucose and ketones purchased from the Keto-Mojo manufacturer.

Urine collection. As individuals recovered from anesthesia during each capture event, they were placed in individual holding cages with urine collection trays beneath. These trays were checked for urine every three minutes. For this study, urine samples were opportunistically collected from 31 emperor tamarins and 32 saddleback tamarins. All urine samples were stored immediately upon collection in portable cool packs at a temperature of 8°C. Upon return to the field station, field samples were labeled in more detail, and one drop of urine was placed on each reagent pad of a urinary dipstick using a sterile pipette. Each urinalysis dipstick was then photographed in RAW with a Canon

EOS Rebel T2i DSLR camera. To confirm that the time elapsed between sample collection and urinalysis did not influence the results, six samples were analyzed and photographed in two-hour intervals over the course of twelve hours to evaluate the consistency of results over time. Photographs of each urinary dipstick were then analyzed using ImageJ to measure the Red, Green, and Blue (RGB) color values to objectively quantify color change for each reagent strip. Using RStudio statistical software v.1.1.463, we then performed a principal component analysis to cluster the RGB values to then determine the concentration urinary parameters. This was carried out for each parameter as the reagent pads vary significantly in their color changes and associated concentrations.

Statistical analyses. The dataset was established in Excel before being transferred to Microsoft Access for Windows. We mean-centered and scaled all RGB measurements for each reagent pad in a principal component analysis (PCA: FactoMiner package in R). To assign a category to each reagent pad from the measured RGB values, the distance between each data point and the reference value for each category were determined. The minimum distance was then determined to be the appropriate category to assign for the data point. We repeated this for all ten reagent pads on each urinalysis dipstick. Parametric tests were used for data analysis following a Gaussian distribution. We used a mixed-effects modle (GLMM) to evaluate effects of age, sex, and breeding status on variation in urinary parameters of individuals within this population.

To estimate reference ranges for blood chemistry parameters, sample means, standard deviations, and minimum and maximum values were calculated for glucose,

hematocrit, hemoglobin, and ketone values. Hematology values from males and females were compared using Kruskal-Wallis tests. We used one-sample t-tests to compare blood glucose, hematocrit, and hemoglobin values to mean values published for wild-caught white-footed tamarins in Fox et al. (2008).

Ethical note. This study follows the American Society of Mammalogists' Guidelines on wild animals in research (Sikes & Gannon, 2011). This study is part of an ongoing, long-term annual capture-and-release program that began at this site in 2009. The Peruvian Ministry of the Environment (SERFOR) granted annual research and collection permits, and the Animal Studies Committees of Central Washington University (#A011802) and the University of Missouri—St. Louis (#733363-5) approved protocols.

Results

There were no significant differences in hematology values between sexes across different groups in *S. imperator* for glucose ($H(1) = 0.91$, $p = 0.34$), hematocrit and hemoglobin ($H(1) = 0.35$, $p = 0.55$) and ketone ($H(1) = 1.97$, $p = 0.16$). There were no significant differences in hematology values between sexes across different groups of *L. weddelli* for glucose ($H(1) = 0.09$, $p = 0.76$), hematocrit and hemoglobin ($H(1) = 0.79$, $p = 0.375$) and ketone (Kruskal-Wallis: $H(1) = 1.00$, $p = 0.32$). Thus, the values for both sexes across all groups were combined to calculate reference values for each species (Table 1).

The mean values for each parameter were compared to published values for male and female white-footed tamarins (*Saguinus leucopus*) (Fox et al., 2008) using Mann-

Whitney U tests (Table 1). Mean blood glucose, hematocrit, and hemoglobin values for both *S. imperator* and *L. weddelli* varied significantly from published blood chemistry values for wild-caught white-footed tamarins (*Saguinus leucopus*) housed in captivity (Fox et al. 2008) (Table 1). Mean blood ketone values for *S. imperator* and *L. weddelli* were low, and a large number of samples (n = 71) that were analyzed with the Keto-Mojo showed a "Low" (< 0.1 mmol/L) reading on the device.

Table 1
*Summary of Mean Blood Chemistry Values Collected from Emperor tamarin (*Saguinus imperator*) and Saddleback tamarin (*Leontocebus weddelli*) Individuals.*

Species	*Saguinus leucopus*[1]		*Saguinus imperator*					*Leontocebus weddelli*				
Parameter (unit)	N	Mean	N	Mean	SD (±)	Range	P	N	Mean	SD (±)	Range	P
Glucose (mg/dL)	26	170.1[1]	54	140.46	49.83	88.00-284.00	<0.001	48	159.66	60.40	83.00-458.00	0.118
Hematocrit (%)	26	49.0[1]	54	34.55	4.39	17.00-43.00	<0.001	48	36.82	6.41	16.00-48.00	<0.001
Hemoglobin (g/dL)	26	16.1[1]	54	11.70	1.49	5.70-14.60	<0.001	48	12.52	2.17	5.40-16.30	<0.001
Ketone (mmol/L)	-	-	53	0.070	0.11	0-0.40	-	47	0.056	0.13	0-0.40	-

Note. Sample size denotes the number of blood chemistry device readings carried out using the Keto-Mojo. P-values indicate the difference between the recorded and published hematology values for white-footed tamarins (*Saguinus leucopus*)[1].
[1]Fox et al. 2008

Table 2
Summary of Mean Body Mass

Species	*Saguinus leucopus*[1]	*Saguinus imperator*	*Leontocebus weddelli*
Body Mass (g)	384 ± 53	464.88 ± 95	341.14 ± 66

Note. Body mass reported is from free-ranging emperor tamarins (*Saguinus imperator),* saddleback tamarins (*Leontocebus weddelli*) in southeastern Peru, and wild-caught white-footed tamarins (*Saguinus leucopus*) housed in captivity in Colombia (Fox et al. 2008).

Body weight was determined for each emperor and saddleback tamarin individual during mark-recapture events (Table 2). We found slight negative relationships between body mass and measured blood glucose for emperor tamarins (R^2 = -0.2258) (Figure 1) and saddleback tamarins (R^2 = -0.3093) (Figure 2).

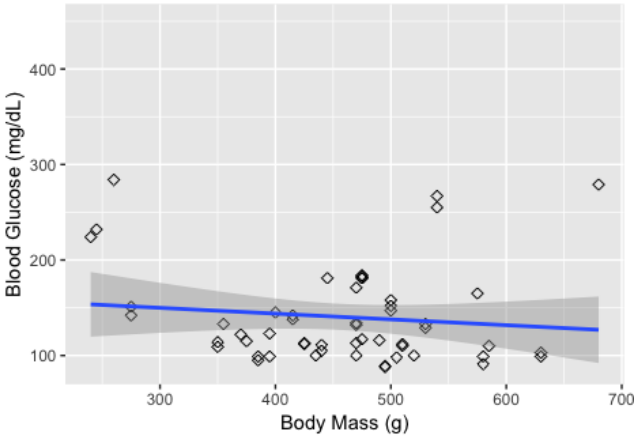

Figure 1. Relationship between measured blood glucose and individual body mass in emperor tamarins (*Saguinus imperator).* Plot includes regression line (R^2= -0.2258) and calculated 95% confidence interval of predicted blood glucose concentration for each body mass.

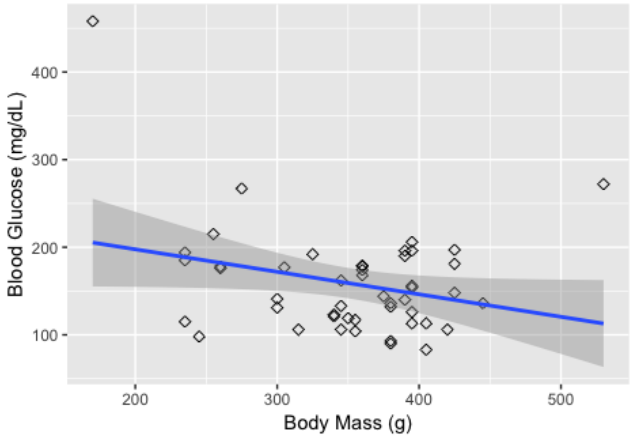

Figure 2. Relationship between measured blood glucose and individual body mass in saddleback tamarins (*Leontocebus weddelli).* Plot includes regression line (R^2= -0.3093) and calculated 95% confidence interval of predicted blood glucose concentration for each body mass.

To evaluate the reliability of the Keto-Mojo device, blood chemistry measurements were determined twice for 30 blood samples (13 samples collected from *L. weddelli* individuals, 17 samples collected from *S. imperator* individuals) during the mark recapture study. A two-tailed *t* test revealed blood glucose concentrations did not vary significantly across duplicate readings (t (56.49) = - 0.05, p = 0.96). Non-parametric Mann-Whitney U tests revealed no significant variation between duplicate results for hematocrit (U = 431, p = 0.74), hemoglobin (U = 431, p = 0.78), or ketone (U = 390, p = 0.98).

Urinalysis. All urine samples collected from emperor and saddleback tamarins tested positive for at least one of the parameters examined. Of the 63 samples collected from both species, the most common positive result was for protein (55% and 59% for *S. imperator* and *L. weddelli*, respectively). We present the results for each parameter below (Table 3 and Table 4).

Time series. Non-parametric Kruskal-Wallis tests revealed that time between sample collection and urinalysis did not significantly influence the measurement of leukocytes ($H(5) = 8.55$, $p = 0.13$), nitrite ($H(5) = 1.43$, $p = 0.92$), urobilinogen ($H(5) = 6.261$, $p = 0.28$), protein ($H(5) = 4.48$, $p = 0.48$), ketone ($H(5) = 3.71$, $p = 0.59$), bilirubin ($H(5) = 1.00$, $p = 0.96$), pH ($H(5) = 5.03$, $p = 0.41$), or specific gravity ($H(5) = 8.29$, $p = 0.14$) over the course of 12 hours. The glucose measurements for all urine samples in the time-series sample subset had the same recorded glucose concentration for all time points.

Table 3
Overview of Urinary Dipstick Parameters from 31 Urine Samples Collected From Saguinus imperator.

Parameter	Category 0		Category 1		Category 2		Category 3		Category 4		Category 5	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Leukocytes	9	**29**	16	**52**	6	**19**	-		1	**3**	-	
Nitrite	18	**58**	7	**23**	6	**19**	-		-		-	
Urobilinogen	8	**26**	18	**58**	2	**6**	3	**10**	-		-	
Protein	6	**19**	17	**55**	2	**6**	1	**3**	-		5	**16**
Blood	13	**42**	-		14	**45**	-		-		4	**13**
Ketone	21	**68**	3	**10**	1	**3**	6	**19**	-		-	
Bilirubin	14	**45**	9	**29**	6	**19**	2	**6**	-		-	
Glucose	20	**65**	1	**3**	3	**10**	2	**6**	-		5	**16**

Note. Percentage represents the percent of samples that are characterized at each level. All parameters, with the exception of urobilinogen, have positive results if characterized above Category 0. Urobilinogen levels are normal at Category 0 and Category 1. Highlighted cells represent categories in which more than 50% of individuals are assigned for that parameter.

Table 4

Overview of Urinary Dipstick Parameters from 32 Urine Samples Collected from Leontocebus weddelli.

Parameter	Category 0		Category 1		Category 2		Category 3		Category 4		Category 5	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Leukocytes	13	**41**	14	**44**	4	**13**	-		1	**3**	-	
Nitrite	19	**59**	7	**22**	6	**19**	-		-		-	
Urobilinogen	5	**16**	22	**69**	3	**9**	2	**6**	-		-	
Protein	4	**13**	19	**59**	3	**19**	-		1	**3**	5	**16**
Blood	12	**38**	-		18	**56**	1	**3**	-		**1**	**3**
Ketone	25	**78**	2	**6**	-		2	**6**	2	**6**	1	**3**
Bilirubin	14	**44**	10	**31**	7	**22**	1	**3**	-		-	
Glucose	19	**59**	2	**6**	5	**16**	-		-		6	**19**

Note. Percentage represents the percent of samples that are characterized at each level. All parameters, with the exception of urobilinogen, have positive results if characterized above Category 0. Urobilinogen levels are considered normal at Category 0 and Category 1. Highlighted cells represent categories in which more than 50% of individuals are assigned for that parameter.

Leukocytes. We detected leukocytes in 74% (*n* = 23) of the 31 samples collected from *S. imperator*. Of these positive results, 70% (*n* = 16) of samples only showed trace amounts of leukocytes (Category 1). We detected leukocytes in 59% (*n* = 19) of the 32 samples collected from *L. weddelli*. Of these positive results, 74% (*n* = 14) of samples only showed trace amounts of leukocytes (Category 1). No factors in our mixed-effects model affected variation in ketones across individuals (Table 5).

Table 5
Results of the Mixed-effects Model (GLMM) Examining Variation in Urinary Chemistry Parameters in Emperor and Saddleback tamarins.

Model[a]	Fixed Effects		Model Estimates			
	Factor	Level[b]	Estimate	Standard Error	t-value	*P* [c]
Leukocytes	(Intercept)	-	1.35	0.65	2.087	**0.040**
	Sex	Male	-0.17	0.19	-0.891	0.376
	Age Class	Juvenile	0.27	0.63	0.42	0.676
		Sub-adult	0.04	0.26	0.16	0.872
	Breeding Status	Primary	0.72	0.65	1.11	0.272
		Secondary	0.78	0.61	1.28	0.203

Table 5 (continued)

Model[a]	Fixed Effects		Model Estimates			
	Factor	Level[b]	Estimate	Standard Error	t-value	*P* [c]
Nitrite	(Intercept)	-	1.43	0.58	2.47	**0.016**
	Sex	Male	-0.38	0.18	-2.11	**0.041**
	Age Class	Juvenile	0.15	0.57	0.26	0.799
		Sub-adult	-0.24	0.26	-0.90	0.386
	Breeding Status	Primary	0.40	0.58	0.69	0.493
		Secondary	0.63	0.54	1.18	0.243
Urobilinogen	(Intercept)	-	2.28	0.55	4.16	**<0.001**
	Sex	Male	-0.13	0.19	-0.71	0.482
	Age Class	Juvenile	-0.31	0.54	-0.57	0.573
		Sub-adult	-0.39	0.29	-1.32	0.197
	Breeding Status	Primary	-0.19	0.55	-0.33	0.739
		Secondary	0.11	0.50	0.23	0.823
Protein	(Intercept)	-	5.40	0.79	6.88	**<0.001**
	Sex	Male	-0.91	0.37	-2.44	**0.018**
	Age Class	Juvenile	-2.56	0.83	-3.10	**0.003**
		Sub-adult	-1.30	0.60	-2.18	**0.034**
	Breeding Status	Primary	-2.52	0.80	-3.16	**0.002**
		Secondary	-0.98	0.64	-1.530	0.135
Blood	(Intercept)	-	1.52	0.86	1.77	0.081
	Sex	Male	-0.92	0.32	-2.89	**0.006**
	Age Class	Juvenile	0.51	0.86	0.59	0.557
		Sub-adult	0.48	0.50	0.95	0.347
	Breeding Status	Primary	1.69	0.87	1.94	0.057
		Secondary	1.48	0.77	1.91	0.062
Ketone	(Intercept)	-	2.78	0.45	6.23	**<0.001**
	Sex	Male	-0.38	0.33	-1.15	0.255
	Age Class	Juvenile	-1.04	0.54	-1.93	0.058
		Sub-adult	-1.06	0.54	-1.98	0.052
	Breeding Status	Primary	-1.00	0.46	-2.19	**0.032**
		Secondary	-0.01	0.18	0.03	0.974
Bilirubin	(Intercept)	-	2.24	0.54	4.19	**<0.001**
	Sex	Male	-0.29	0.23	-1.29	0.203
	Age Class	Juvenile	-0.57	0.55	-1.05	0.300
		Sub-adult	-0.45	0.36	-1.24	0.221
	Breeding Status	Primary	-0.22	0.54	-0.41	0.686
		Secondary	0.21	0.46	0.46	0.648

Note. [a] All variables were analyzed using generalized linear mixed-effects models using the lmeTest package in RStudio v 1.1.456. Because the same trends were observed in emperor and saddleback tamarins, the data was combined for both species to increase the sample size and power of the models.

[b] All analyses made against the intercept of first levels of each factor, e.g., sex = female, breeding status = non-breeding, age class = adult.

[c] Statistically significant results (P < 0.05) are in bold.

Nitrite and Urobilinogen. We detected nitrite in 42% (*n* = 13) of the 31 samples collected from *S. imperator*, and 41% (*n* =13) of the 32 samples collected from *L. weddelli*. Our mixed effects model indicated that breeding status and age class did not contribute to the variation in nitrite levels across individuals, but there were sex differences observed (Table 5). We detected urobilinogen in 16% (*n* =5) of the 31 samples collected from *S. imperator* and in 16% (*n* = 5) of the 32 samples collected from *L. weddelli*. No factors in our mixed-effects model affected variation in urobilinogen across individuals (Table 5).

Protein. We detected protein in 81% (*n* = 25) of the 31 samples collected from *S. imperator*. Of these positive results, 68% (*n* = 17) of samples only showed trace amounts of protein (Category 1). We detected protein in 88% (*n* = 28) of the 32 samples collected from *L. weddelli*. Of these positive results, 68% (*n* = 19) of samples only showed trace amounts of protein (Category 1). Our mixed effects model indicated that breeding status, age, and sex all contributed to variation in protein levels across individuals (Table 5). Female emperor tamarins were more likely to have higher urinary protein concentrations than males. For both species, secondary breeders and individuals in the adult age class were more likely to have higher urinary protein concentrations (Table 5).

Blood. We detected blood in 58% (*n* = 18) of the 31 samples collected from *S. imperator*. Of these positive results, 78% (*n* = 14) of samples only showed trace amounts of hemolyzed blood (Category 3). We detected blood in 63% (*n* = 20) of the 32 samples collected from *L. weddelli*. Of these positive results, 90% (*n* = 18) of samples only

showed trace amounts of hemolyzed blood (Category 3). Our mixed effects model indicated that breeding status and age class did not contribute to variation in test results for this parameter across individuals, but there were sex differences observed (Table 5).

Ketone. We detected ketones in 32% (*n* = 10) of the 31 samples collected from *S. imperator*. Of these positive results, 3% (*n* = 3) of samples only showed trace amounts of ketones (Category 1). We detected ketones in 22% (*n* = 7) of the 32 samples collected from *L. weddelli*. Of these positive results, 29% (*n* = 2) of samples only showed trace amounts of ketones (Category 1). No factors in our mixed-effects model affected variation in ketones across individuals (Table 5).

Bilirubin. We detected bilirubin in 55% (*n* = 17) of the 31 samples collected from *S. imperator*. Of these positive results, 59% (*n* = 9) of samples showed small amounts of bilirubin (Category 1). We detected bilirubin in 56% (*n* = 18) of the 32 samples collected from *L. weddelli*. Of these positive results, 56% (*n* = 10) of samples showed small amounts of bilirubin (Category 1). No factors in our mixed-effects model affected variation in bilirubin across individuals (Table 5).

Glucose. We detected glucose in 35% (*n* = 11) of the 31 samples collected from *S. imperator*. Of these positive results, 1% (*n* = 1) of samples showed small amounts of glucose (Category 1). We detected glucose in 41% (*n* = 13) of the 32 samples collected from *L. weddelli*. Of these positive results, 15% (*n* = 2) of samples showed small

amounts of glucose (Category 1). A generalized linear mixed model for glucose could not be constructed because there were not enough positive test results for this parameter.

Table 6
*Summary of Measured pH in Urine Samples Collected from Emperor tamarins (*Saguinus imperator*) and Saddleback tamarins (*Leontocebus weddelli*)*

pH	5.0		6.0		6.5		7.0		7.5		8.0		8.5	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Saguinus imperator	2	**6**	3	**10**	4	**13**	2	**6**	11	**35**	5	**16**	4	**13**
Leontocebus weddelli	-		1	**2**	2	**4**	-		11	**34**	7	**22**	11	**34**

Note. Percentage represents the percent of samples characterized at each pH.

pH and Specific Gravity. Forty-nine (78%) of 63 samples total collected from emperor and saddleback tamarins had an alkaline pH reading (>7.0) (Table 6). Forty-two (67%) of 63 samples total collected from emperor and saddleback tamarins had a measured specific gravity of 1.005 (Table 7).

Table 7
*Summary of Measured Specific Gravity in Urine Samples Collected from Emperor tamarins (*Saguinus imperator*) and Saddleback tamarins (*Leontocebus weddelli*)*

Specific Gravity	1.000		1.005		1.010		1.015		1.020	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Saguinus imperator	3	**7**	21	**74**	5	**14**	1	**2**	1	**2**
Leontocebus weddelli	7	**22**	21	**66**	4	**13**	-		-	

Note. Percentage represents the percent of samples characterized at each specific gravity.

Discussion

In this study, we examined blood chemistry parameters via the Keto-Mojo device, and urine chemistry parameters via multireagent dipstick analysis in wild emperor and saddleback tamarins to establish baseline health data and investigate preliminary trends in health status. All mean blood chemistry parameters varied significantly from

published values. While our intent for comparing the measured blood chemistry ranges to published values was to evaluate the accuracy of the Keto-Mojo device, we believe that the significant variation between our recorded values and the published values does not necessarily indicate that the device is not accurately measuring these parameters. One explanation is that the baseline data for *Saguinus imperator* and *Leontocebus weddelli* are not comparable to the wild-caught *Saguinus leucopus* population investigated by Fox et al. (2008). White-footed tamarins have an average body mass that is less than emperor tamarins but greater than saddleback tamarins (Table 2), and as noted in Figures 1 and 2, a negative relationship between blood glucose concentration and body mass is observed in these species. The blood chemistry ranges of saddleback tamarins were more closely related to the white-footed tamarins than emperor tamarins, and this could be due to their smaller body mass relative to emperor tamarins, and closer proximity to the average body mass of white-footed tamarins recorded by Fox et al. (2008) (Table 2). This variation in body size can explain the significant differences in blood chemistry parameters between emperor and saddleback tamarins from the published values of white-footed tamarins. Additionally, the white-footed tamarins that were sampled in Fox et al. (2008) were wild-caught but had been housed in captivity for periods ranging from 1 week to 2 years. This variation in diet, allostatic load, and energetic expenditure could contribute to variation in their blood chemistry values.

The average ketone levels in both *S. imperator* and *L. weddelli* individuals were very low (0.070 ± 0.11 and 0.056 ± 0.13 respectively). Of the 100 samples in which ketone readings were taken with the Keto-Mojo, 71 produced "Low" (<0.1 mmol/L) readings. This suggests that the ketone ranges of these species do not fall within the

detectable range of the Keto-Mojo device. It is possible that these populations had low circulating blood ketone levels due to overall positive energetic balance and low allostatic load, but a more likely explanation would be that these species experience much lower spikes in blood ketone values during times of "starvation" that are not in the detectable range of the Keto-Mojo device. While the device may not be recommended for measuring blood ketone concentrations in these species, we found no significant variation in blood chemistry values measured in duplicate with the Keto-Mojo. The replicability of results with little variation provides promising evidence that this POC device produces results with high reliability.

The high percentage of urine samples that fell within the normal range for all urinalysis parameters for emperor and saddleback tamarins suggests that the normal range in these species is similar to that of humans. The pH of their urine was slightly more basic than the normal range for humans (most of the samples fell within the 7.5-8.5 range) (Table 6), which suggests that the normal pH range for these species may be more alkaline than the urine of humans. One explanation for this could be their diet. The diet of emperor and saddleback tamarins varies with respect to the seasonality of their food resources. To explore the influence of diet on their urinary pH, a future study should analyze samples across the wet and dry seasons. Measured specific gravity for all emperor and saddleback tamarin individuals (aside from two emperor tamarins) indicated normal hydration levels in these populations (Table 7). These findings suggest that multireagent urinary dipsticks are suitable for evaluating clinical signs of kidney and liver function in emperor and saddleback tamarins as the normal ranges of these species appear to be similar to the clinically normal ranges of humans.

Of all parameters measured, the most common positive result for both emperor and saddleback tamarins was protein (Tables 3 & 4). These positive results most frequently were attributed to "trace amounts" of protein for emperor tamarins (55% of individuals) and saddleback tamarins (59% of individuals) (Tables 3 & 4). A possible explanation for this is that the normal concentration of protein in the urine of these species is higher than that of humans. A large percentage of individuals also tested positive for trace amounts of hemolyzed blood in their urine (45% of emperor tamarins and 56% of saddleback tamarins) (Tables 3 & 4). While this may seem indicative of infection or urinary tract disorder, the Renal Association and British Association of Urological Surgeons released a joint statement in 2008 prompting caution when interpreting results, as urinary reagent dipsticks are highlight sensitive to hematuria detection (Anderson et al., 2008). They suggest that "trace" results of blood in urine samples should be considered negative.

Three of the six saddleback tamarins that had the highest reported of glucose in their urine (Category 6), also had blood glucose values significantly higher than the mean blood glucose for the population. This could be indicative of an underlying metabolic disorder similar to diabetes.

Most factors included in our mixed-effects models showed minor effects on urinary parameters in these populations of emperor and saddleback tamarins. A possible explanation for this is the small sample size analyzed with this method. In the future, we recommend incorporating other clinical measures of health (i.e., injury status, biomarkers of health and immunity, parasite load, etc.) in these models to gain a holistic picture of individual health and further evaluate trends in primate populations.

We present promising evidence for the use of two affordable and effective POC methods in evaluating the health of free-ranging non-human primates onsite in remote, field settings. The Keto-Mojo device reliably recorded blood glucose, hematocrit, hemoglobin, and ketones in emperor and saddleback tamarins. The urinalysis results produced over a 12-hour time period using multireagent urinary dipsticks suggest that this method is suitable for field primatologists that are unable to analyze urine samples immediately at the time of collection. While both of these POC technologies are designed for use in humans, our results suggest that they can be easily applied to primate health monitoring initiatives as the normal ranges for both species in this study fell well-within the ranges of human blood chemistry and urinary parameters.

CHAPTER IV

GENERAL CONCLUSIONS

Chapter II Conclusions

Our study confirms that the neopterin molecule is conserved across primate taxa and is present in five genera of Platyrrhines (New World monkeys) across three different families. The commercially-available enzyme-linked immunosorbent assay (ELISA) kit that was previously validated to recognize the neopterin molecule in the urine of Catarrhines (Old World monkeys and apes) successfully measured neopterin in urine samples collected from captive *Leontopithecus rosalia,* and *Pithecia pithecia,* and free-ranging *Saguinus imperator, Leontocebus weddelli, Plecturocebus toppini,* and *Alouatta seniculus*. In addition to confirming that researchers can use this ELISA kit to evaluate immune function in New World monkeys, we also confirmed that the kit is compatible with a range of spectrophotometer filters at different wavelengths. This allows field primatologists to analyze urine samples without being constrained to a single filter wavelength that may not be available in remote field laboratories. While a range of wavelengths can be used to measure neopterin, we suggest that researchers only compare urinary neopterin measurements within similar studies that have used the same wavelength, as different filters may produce results that do not reflect absolute neopterin concentrations, and there has been significant variation in UNC recorded in different laboratories.

Our findings present urinary neopterin as a promising biomarker for use by field primatologists to evaluate immune system activation in New World monkeys. Further investigation is needed to evaluate baseline concentrations of neopterin to determine how

significantly and reliably urinary neopterin increases in these species during the onset of infection. This requires repeated urinary neopterin measurement, which will also facilitate studies examining how seasonality, diet, breeding status, demographics, and environmental factors influence urinary neopterin concentrations. Our study is an initial validation of the presence of urinary neopterin in New World monkeys indicating it as a valuable non-invasive biomarker of health for field primatologists to utilize in health-monitoring initiatives.

Chapter III Conclusions

With the lack of published baseline health data for free-ranging non-human primates, the aim of our study was to present affordable and efficient point-of-care technologies to evaluate health in the field in real-time. Devices designed for use in animals are typically expensive and require specialized lab equipment and trained personnel, which is why we chose to evaluate the reliability and accuracy of health evaluation techniques designed for human use. We measured blood and urine chemistry parameters in free-ranging *Saguinus imperator* and *Leontocebus weddelli* using the Keto-Mojo device and multireagent urinary dipsticks. Our results indicate that the Keto-Mojo measures glucose, hematocrit, and hemoglobin within close range of published hematology values for non-human primates. For many individuals, blood ketone concentrations were lower than the range detectable by the device. In addition to producing comparable results, this device also showed high reliability when duplicate readings were performed. The results from the urinary dipsticks indicated that the normal ranges of urinary parameters for tamarins fell within the range of humans, as their

"normal" was considered clinically healthy for human urine. We also found that time elapsed between urine collection and urinalysis did not significantly influence the measurement of any urinary parameter. These findings provide primatologists with affordable and efficient methods of evaluating the health of non-human primates at remote field sites, which can allow for real-time health monitoring, and facilitate the collection of baseline health parameters to be used as reference ranges for wild populations during reintroduction protocols and conservation initiatives.

Future Directions

This study allowed us to collect health data in populations with known demographics, which will allow for investigation of the influences of a number of factors on health status in these species. During mark and recapture events, we collected a significant amount of data, including information regarding injuries, blood parasites, morphometrics, and DNA. With this information, we plan to use the urinary neopterin concentrations, blood chemistry values, and urinary parameters to gain a holistic view of individual health status and evaluate what factors co-vary and respond to different ecological and social variables.

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