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Accuracy of LeadCare® Kit in Veterinary Wildlife Applications

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Accuracy of LeadCare® Kit in Veterinary Wildlife Applications

A Major Qualifying Project Report

Submitted to the Faculty of the

Worcester Polytechnic Institute

In partial fulfillment of the requirements for a

Bachelor of Science Degree

By:

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and

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April 2012

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Professor Jill Rulfs

Abstract

Wildlife veterinarians often need fast blood lead level results for sick or injured animals. The LeadCare® kit, which quickly reads human blood lead levels, was tested for accuracy in veterinary applications at Tufts Wildlife Clinic using avian blood and post-mortem fluid samples. Accuracy was determined by comparison to results from a reference lab. Data suggest that the LeadCare® kit is accurate for clinical diagnosis of lead poisoning in avian species; however, further studies are warranted to increase sample size.

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Dr. Jill Rulfs

Dr. Robert Gegear

Tufts Cummings School of Veterinary Medicine Wildlife Clinic Staff

Worcester Polytechnic Institute

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JAVMA Manuscript

The following is the manuscript, based on this project, submitted to the Journal of the American Veterinary Medical Association (JAVMA) for publication.

Accuracy of LeadCare® Kit in Veterinary Wildlife Applications

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This manuscript represents the senior Major Qualifying Project submitted by Ms. Pearce and Ms. Boschetto to the Worcester Polytechnic Institute Department of Biology & Biotechnology as fulfillment of the requirement for a Bachelor of Science degree.

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The authors thank Dr. Jill Rulfs for her guidance and Dr. Robert Gegear for his assistance with the statistical analyses.

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Abstract

Objective – To determine the accuracy of the LeadCare® kit in testing avian blood lead levels.

Design – Prospective evaluation study.

Sample – Blood samples from 12 wild birds representing the Muscovy duck (*Cairina moschata*), mute swan (*Cygnus olor*), turkey (*Melegris gallopavo*), herring gull (*Larus argentatus*), crow (*Corvus brachyrhynchos*), common loon (*Gavia immer*), red tailed hawk (*Buteo jamaicensis*), bard owl (*Strix varia*), and Canadian goose (*Branta canadensis*) species.

Procedures – Whole blood samples were obtained and stored either refrigerated or frozen in sterile tubes containing the anticoagulant heparin. Blood lead concentration was measured at room temperature using the LeadCare® kit (Magellan Diagnostics, Chelmsford, MA). The remainder of the sample was frozen and sent to the Pennsylvania Animal Diagnostic Laboratory (Harrisburg, PA) for testing. A Wilcoxon matched-pair signed rank test and paired t-test were used to assess agreement between reference lab and LeadCare® values.

Results – Agreement between the LeadCare® kit and the reference lab results was good. The blood lead levels were statistically not different ($p > 0.05$) with a Wilcoxon matched-pairs signed rank test including an outlier yielding $p = 0.0674$ and a paired t-test excluding an outlier yielding $p = 0.0900$.

Conclusions and Clinical Relevance – Results suggest that the LeadCare® kit is appropriate for measuring blood lead levels in avian species for rapid clinical diagnosis.

Background

Lead poisoning is a serious medical issue that has become an expanding topic in the veterinary world over the past few decades. Wild birds are especially at risk for ingesting lead fishing weights or being shot with lead pellets.[1] There are also many other environmental hazards such as lead-based paint or contaminated water that can put animals and people at risk .[2] Despite some existing legislation restricting the use of lead, this issue continues to be a topic of much debate as more legislation either advocating for further restriction or protecting hunters' rights has come up for review[3].

There are various methods used to evaluate blood lead concentrations. The LeadCare® kit utilizes electrochemistry with unique, disposable sensors. Whole blood is mixed with the treatment reagent which lyses the red blood cells and releases the lead. The analyzer applies an electrical potential to the sensor that collects the lead which is then measured.[4] The Pennsylvania Animal Diagnostic Laboratory System typically uses atomic absorption spectroscopy (AA) to analyze blood lead content.[5] AA is simple absorbance spectrophotometry where the sample is volatilized and the absorption of light (at a wavelength specific to lead) is measured. The lead concentration is then determined by comparison to a standard curve.[6] Another test less commonly used by the lab is an inductively coupled plasma/mass spectrometry (ICP/MS).[5] In this test, the high temperature ICP source converts the elemental atoms to ions which are separated and detected by the mass spectrometer.[7] The results from this test can then be quantified for lead to give the overall sample content.

For humans, the LeadCare® blood test is much quicker and more affordable than these lab tests, making it easier for health care professionals to test a wide range of people. However, this type of test has not yet been validated for veterinary use despite the existing need to

efficiently test animals. Therefore, the purpose of this study is to determine the accuracy of the LeadCare® kit in avian species; our hypothesis is that the LeadCare® kit is clinically accurate. In a clinical setting, a quick primary blood test will help veterinarians diagnose lead poisoning earlier and consequently begin life-saving treatment sooner.

Methods

Animals - Birds brought to the Wildlife Clinic at the Tufts Cummings School of Veterinary Medicine were used in this study. There were twelve different birds from eight species including one Muscovy duck (*Cairina moschata*), three mute swan (*Cygnus olor*), one turkey (*Melegris gallopavo*), two herring gulls (*Larus argentatus*), one crow (*Corvus brachyrhynchos*), one common loon (*Gavia immer*), one red tailed hawk (*Buteo jamaicensis*), one bard owl (*Strix varia*), and one Canadian goose (*Branta canadensis*).

Sample Collection - Blood samples were obtained from the birds while under anesthesia for radiographs by the wildlife clinic staff. The amount of blood varied by bird based on their size and the amount of blood that could be safely drawn. The blood was stored in heparin tubes in either the refrigerator or freezer for a period of time ranging from 1-30 days until testing.

Measurement of Blood Lead Concentration - The samples were brought to room temperature and tested following the LeadCare® instructions.[3] The LeadCare® machine was calibrated for each lot of test strips according to the manufacturer's instructions. 50µl of blood was added to a supplied tube containing a set amount of the lysis reagent. This tube was then gently mixed for approximately one minute, allowing the reagent to lyse the red blood cells and causing the solution to turn a darker brown color. 50µl of the lysed sample was then applied to the test strip, just coving the exposed metallic circle. The strip was inserted into the LeadCare® machine and the analysis was run. After three minutes, the result appeared on the LeadCare® display and was manually recorded on a laptop in an Excel® file. The remainder of the whole blood sample was then frozen and packaged with icepacks to be shipped to the reference lab for testing. These results were received 4-8 days later, recorded, and compared to the results from the LeadCare® Kit.

Statistical Analysis – All statistical analyses were performed using Graph Pad Prism® software. The data from the 12 blood samples were tested for normality and a Wilcoxon matched-pairs signed rank test was used to compare the reference lab results with those from the LeadCare® kit. Next, a single outlier was removed from the sample set and the normality test was rerun. It was concluded that a paired t-test could then be used to compare the LeadCare® and reference lab data.

Results

Blood samples collected from twelve different birds from eight species were tested for lead in this study. Any value outside the measurement range of the machine was given the highest (65µg/dL) or lowest (2.5µg/dL) possible measurable value. The mean lead level of the LeadCare® results for all twelve samples was 16.15µg/dL and of the reference lab results was 18.33µg/dL. A normality test revealed that the sample values were not normally distributed; therefore, a Wilcoxon matched-pairs signed rank test was performed with a resulting p = 0.0674. Table 1 shows the comparison of each sample's values. For eleven of the twelve samples, the difference ranged from -9.7 to 2.8 with the average being -2.2. Sample 7 had a difference of -53 and therefore was determined to be an outlier.

Table 1: The LeadCare® results versus the reference lab results.

Sample #	Lead Level (µg/dL)	Lab Result (µg/dL)	Signed Difference
1	5.1	6.5	-1.4
2	15.2	13.6	1.6
3	12.2	9.4	2.8
4	4.1	5.3	-1.2
5	26.3	34.7	-8.4
6	5.4	7.7	-2.3
7	**12.0**	**65**	**-53.0**
8	13.8	13.6	0.2
9	13.2	22.9	-9.7
10	9.2	11.5	-2.3
11	8.2	11.4	-3.2
12	65	65	0.0

The outlier was removed and a normality test was run again, this time showing normal distribution. A paired t-test was then performed yielding p = 0.0900. Visual comparison of each

sample (Figure 1) showed a good agreement between sample results within clinical ranges for lead poisoning.

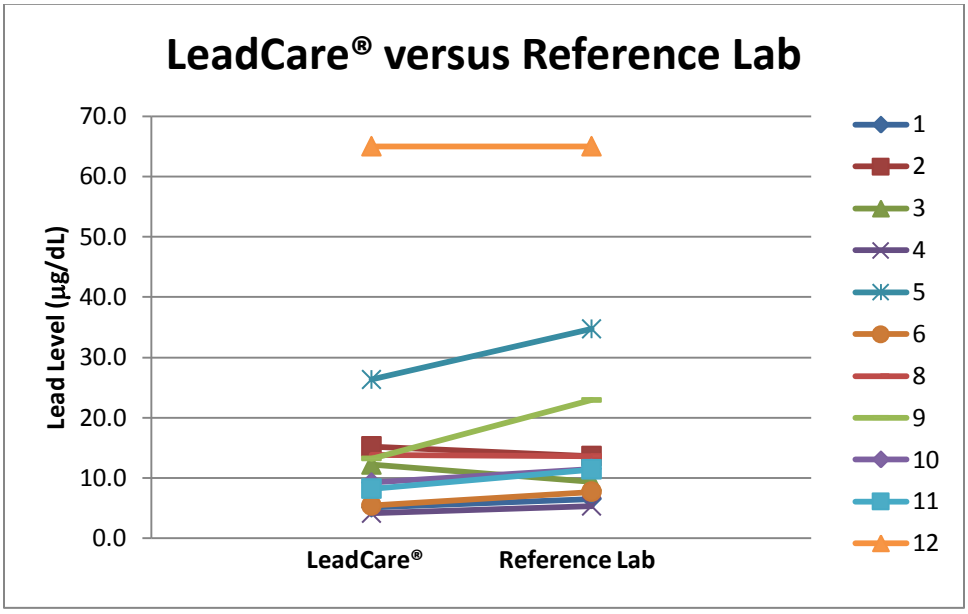

Figure 1: Plot of the LeadCare® results versus the reference lab results without the outlier. The slope of the lines is the difference between the values.

Discussion

In this study, the LeadCare® and reference lab results were found to be not statistically different as $p > 0.05$, with a value of 0.0900 from the paired t-test. Even including the outlier in the Wilcoxon matched pair singed rank test, $p > 0.05$ with a value of 0.0674. This result suggests that the LeadCare® kit does provide an accurate means for measurement of blood lead levels in birds.

Even though the p value is high enough to be considered not statistically different, it is still very close to the statistical cutoff of significance. Further study is warranted with a larger and more diverse sampling. As seen, many of the lead levels tested were in a lower range with only a few in the medium to high range. More samples that are better distributed within the different ranges could yield more promising statistical results.

It is also important to mention that the overall data support LeadCare® use in the clinic to initially diagnosis lead poisoning and start treatment when needed. With the exception of the outlier, all of the LeadCare® values were within range of the reference lab. If a more accurate value is needed, the sample could be sent to a reference lab while treatment is initiated. This would save critical time that could determine the life or death of a patient.

The LeadCare® kit would also be very cost effective in the field of wildlife veterinary medicine where there is typically no owner to pay for the testing. After the cost of the initial machine, each test averages around $7.50[8] which is substantially less than a reference lab test which costs around $50[9]. This kit makes lead testing much more affordable for clinics, allowing them to test many more patients at a much reduced price.

Overall, these results do suggest that the LeadCare® kit is accurate in measuring lead levels in avian blood and could be used in the diagnosis of lead poisoning in birds supporting the original hypothesis. However, additional study is recommended to further support this claim.

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Tissue Fluid Study

In addition to the preceding manuscript, a study on post-mortem fluid samples was completed as part of this project. Unfortunately, due to a low sample size and inconclusive results, it could not be published as part of the JAVMA article. However, this study is relevant because many wildlife researchers, rehabilitators, and veterinarians often find themselves with deceased animals, and it is important to be able to identify the cause of death. By using this cheap and efficient test, they would be able to test the deceased animals for potential lead poisoning as the reason for fatality. If the animal did indeed have lead poisoning, they could then locate the cause and possibly prevent further individuals from being poisoned.

Methods

Tissue fluid samples were collected from five various birds from two species, four common loons (*Gavia immer*) and one bald eagle (*Haliaeetus leucocephalus*), post-mortem during necropsy by wildlife clinic staff at the Tufts Cummings School of Veterinary Medicine. The samples were stored refrigerated in sterile tubes containing no anticoagulants or other reagents for a period of time ranging from 0-30 days until testing. The same protocol was used to test the lead levels of the tissue fluid samples as described previously for the blood samples and as seen in the LeadCare® manual in Appendix A. The results were also recorded and the samples were prepared accordingly to be sent to the reference lab with the blood samples. Once these results were received from the reference lab, they were recorded and compared to those obtained from the LeadCare® kit. The raw data for both the tissue fluid and blood sample studies can be seen in Appendix B.

Graph Pad Prism® software was used to perform statistical analysis on the fluid sample data. Due to the low sample size, the normality test could not be run. However, a paired t-test was done to compare the LeadCare® and reference lab fluid data.

Results

Post-mortem fluid samples collected from the five different birds from two species were tested for lead in this study. Any value outside the range of the machines was given the highest (65µg/dL) or lowest (2.5µg/dL) possible measurable value. The mean lead level of the LeadCare® results was 50.96µg/dL and of the reference lab results was 52.50µg/dL. A normality test on all the blood sample data could not be run due to the small sample size. Nonetheless, a paired t-test was performed yielding p = 0.8481. Figure 2 gives a visual comparison of each sample's values.

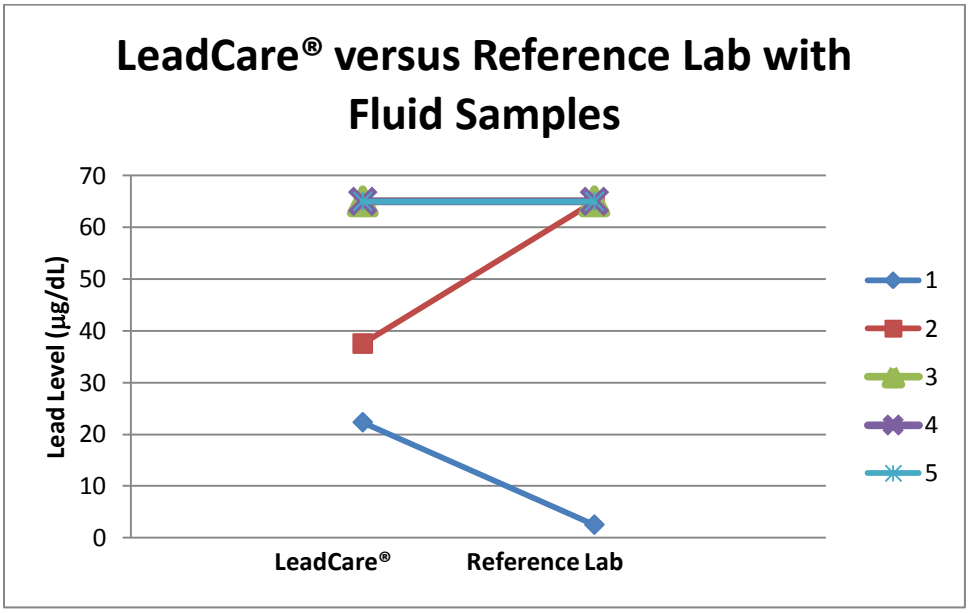

Figure 2: Plot of LeadCare® results versus reference lab results for fluid samples. The slope of the line shows the difference between the values.

As can be seen from the data for these few samples, three out of five had high readings for both tests. The average difference was only -1.54 but samples 1 and 2 varied greatly in opposite directions which effectively cancelled each other out.

Discussion

The LeadCare® and reference lab results were found to be not statistically different as $p > 0.05$, with a value of 0.8481. Although this high p value indicates that the results are similar, the paired t-test was not a proven valid measure of the data set. That fact combined with the low sample size and the extreme difference in two of the LeadCare® versus reference lab samples deemed the results inconclusive. As seen in Figure 2, the large positive difference and large negative difference in samples 1 and 2 act to offset each other which, along with the difference of zero of the other samples, make it appear statistically not different. These differences, however, would have a major effect on the clinic diagnosis of the lead poisoning in the animals, as they would test in the positive range for lead poisoning by one test but not the other.

The discrepancies in results could have been caused by various factors. Lab error could have occurred either by the reference lab or with the LeadCare® kit. Unfortunately, due to the small volume of the samples, they were only able to be run once on each of the tests. In future studies, a larger volume of fluid should be taken when available so that the sample can be tested multiple times with both tests to help minimize lab error, both human and machine. Furthermore, the LeadCare® kit was developed solely for use with blood samples, not fluid samples, which could also explain the large differences between the LeadCare® values and the reference lab values. Blood contains many components such as hemoglobin, whose interference may be anticipated and automatically be accounted for during the test. If these components are factored into the algorithms for determining the lead level with the LeadCare® kit, it is likely that the test would give an incorrect result for the fluid samples which do not contain these same components. Further study with fluid samples is needed to see if this is true.

Overall, the fluid sample study was inconclusive and further study is needed. A larger sample size with a wider range of values is recommended. Although the data analysis showed no statistical difference, the sample size was too small to conclude that the LeadCare® kit can be used in the clinic diagnosis of lead poisoning in post-mortem avian fluid samples.

Further study on the affects of sample storage before testing with the LeadCare® kit is also recommended. Some initial tests were run as part of this project but the results were inconclusive and incomplete due to several limiting factors such as time and money, and therefore are not included here. However, both refrigerating and freezing samples were storage methods used for variable amounts of times in this study. The LeadCare® kit was designed for use with fresh blood, so refrigerating or freezing the blood could potentially have effects on the results. Also, the time for which the samples were stored varied and therefore should also be investigated as changes in the blood over time could potentially result in differences with the results.

Additional studies to validate the LeadCare® kit for use in other veterinary species would be beneficial as well. In this study only birds were used; however, the LeadCare® kit could potentially be used to test blood lead levels in other species. It was designed solely for human use so there is currently no known data on its accuracy with different types of animals. However, the quickness and cost effectiveness of the LeadCare® kit would make it advantageous to veterinarians of all practice types and therefore warrants further study.

As discussed in the JAVMA manuscript, the LeadCare® kit can be considered a viable option for the clinic diagnosis of lead poisoning in birds using blood samples. However, further study is required to determine its accuracy with post mortem fluid samples. The LeadCare® kit

will allow wildlife veterinarians a quick and low cost way to diagnose lead poisoning in living birds. At only $7.50 per test, it is very cost effective for clinics that receive minimal funding where there is often no owner to cover the cost of the patient's tests and treatments. Using the LeadCare® kit, veterinarians will be able to quickly diagnosis lead poisoning and initiate treatment much faster, therefore potentially saving the lives of birds that would have otherwise died waiting 4-8 days for lab results. Although further studies are recommended to help support these conclusions, the LeadCare® kit is a great option for wildlife veterinarians in the efficient and economic diagnosing of lead poisoning in birds.

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Appendices

Appendix A: LeadCare® Instructions

Instructions retrieved from: http://www.woongbee.com/POCT/leadcare.htm

LeadCare® Childhood Blood Lead Testing

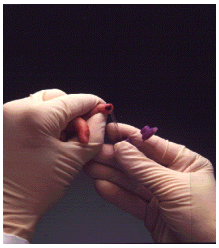
The LeadCare System is for the determination of lead in whole blood. When you test young patients for lead levels, you want fast, accurate, inexpensive results. You want the LeadCare system, a simple, foolproof way to perform blood lead measurements using a finger stick or venous sample. No more waiting days for expensive lab analyses. You get quantitative blood lead results equivalent to those reported by outside laboratories in just three minutes.

A LeadCare system analysis costs far less than you'd pay an outside laboratory, and it qualifies for reimbursement as a quantitative blood lead. You'll also cut your staff's result-tracking and administrative time. You'll save your patients days of possibly needless worry plus the time-consuming inconvenience and cost of a return visit. Blood lead measurement couldn't be easier.

LeadCare is easy and safe to use. The hand-held analyzer is portable and requires neither manual calibration nor refrigeration. Its unique gold electrode sensor contains no mercury or other toxic materials. The point-of-care LeadCare system was developed by ESA and Andcare with a grant from the CDC. It's the diagnostic tool which makes sense medically and economically.

Fast! *Easy as 1- 2- 3*

STEP ONE



Draw a capillary or venous blood sample using EDTA or heparin as anticoagulants.

STEP TWO

Using the pipette provided with the kit, dispense 50 µl, about two drops of blood, into the reagent and mix.

STEP THREE

Transfer it to the sensor strip. Press the button. Just three minutes later, you have your result.

Accuracy

LeadCare System vs. Atomic Absorption Spectroscopy performed at a major lead outreach and referral clinic/hospital
Number of Samples: 112
Slope: 1.07
Intercept: -0.57 µg/dl
Correlation coefficient: 0.97

Portable

Power source: 9V battery or AC adapter
Dimensions: 7.7 in x 4.2 in x 2.5 in. (19.6cm x 10.7cm x 6.4cm)
Weight: 14 oz

Specification

Test method: Electrochemical with disposable sensors
Blood lead level range: 1.4 - 65 µg/dl
Blood sample volume: 50 µl
Test time: 3 minutes
Calibration: Electronic calibration button
Classification: Moderately complex under CLIA guidelines. Suitable for use in physician's office laboratory.

Theory of Anodic Stripping Voltammetry (ASV)

ASV Method

Anodic Stripping Voltammetry is a highly precise, virtually interference-free method.

1. Whole blood is added to the reagent solution (Fig. 1),
2. Any lead present is released from the blood components (Fig. 2).
3. Now any lead in the reagent solution is concentrated (plated) onto a thin-film electrode during the plating step of the analysis cycle (Fig. 3).
4. The plated lead is removed from the electrode by applying a stripping current (Fig. 4) and the amount of lead is measured by integration of the electrical current released during this rapid electrochemical step.

Anodic Stripping Voltammetry

Figure 1

Figure 2

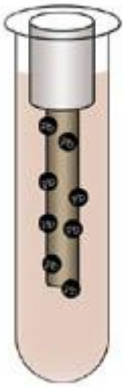


Figure 3

Figure 4

The current released during the stripping step, is a directly proportional to the amount of lead present in the blood sample.

Accurate Results

LeadCare® System vs. Atomic Absorption Spectroscopy performed at a major lead outreach and referral clinic/hospital

Number of Samples	**112**
Slope	**1.07**
Intercept	**-0.57 µg/dl**
Correlation Coefficient	**0.97**

Method Correlation

Results from the Model 3010B Lead Analyzer have shown close correlation with the widely accepted graphite furnace methodologies. This is further supported by results from numerous proficiency surveys.

The Model 3010B provides the sensitivity you need for the detection of blood lead in childhood lead screening, industrial hygiene and occupational health monitoring programs.

The LeadCare system operates by fundamentally the same principal but uses a single-use electrode contained on a disposable slide.

Appendix B: Raw Data for JAVMA Manuscript and Fluid Studies

Patient ID	Species	Sample Type	Lab Result Converted to (µg/dL)	Lead Level (µg/dL)	Signed Difference
W111636	Crow	Blood	6.5	5.1	1.4
W111791	Mute Swan	Blood	13.6	15.2	-1.6
W111853	Turkey	Blood	9.4	12.2	-2.8
W111854	Herring Gull	Blood	5.3	4.1	1.2
W111886	Muscovy Duck	Blood	34.7	26.3	8.4
W111907	Herring Gull	Blood	7.7	5.4	2.3
W111913	Mute Swan	Blood	103	12.0	91.0
W112002	Mute Swan	Blood	13.6	13.8	-0.2
W111980	Barred Owl	Blood	22.9	13.2	9.7
W112015	Common Loon	Blood	1230	HI	N/A
W111936	Red Tailed Hawk	Blood	11.5	9.2	2.3
W120037	Canadian Goose	Blood	11.4	8.2	3.2
TV11102	Common Loon	Tissue Fluid	<5	22.3	N/A
TV11121	Bald Eagle	Tissue Fluid	84.7	37.5	47.2
TV11124	Common Loon	Tissue Fluid	169	HI	N/A
TV11098	Common Loon	Tissue Fluid	80.3	HI	N/A
TV11135	Common Loon	Tissue Fluid	95	HI	N/A