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*Ornithological Methods*

# Comparability of hemoglobin concentration measurements from the HemoCue Hb201+ and Hb801 in birds and a mammal with and without nucleated red-blood cells

## Comparación de las mediciones de concentración de hemoglobina del HemoCue Hb201+ y Hb801 en aves y un mamífero con y sin glóbulos rojos nucleados

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ABSTRACT. HemoCue analyzers are standard point-of-care devices for measuring blood hemoglobin concentration ([Hb]). The utility of these photometers has been demonstrated for all vertebrate lineages including birds, yet because most vertebrates except mammals possess nucleated red-blood cells, their [Hb] readings are inflated upward in these taxa and require a correction factor relative to the reference calibration. We field tested the newest of these analyzers, Hb801, against its predecessor, the Hb201+. Because of differences in the photometry methods, these two analyzers were not found to be equivalent for taxa with nucleated red-blood cells, such as birds. Whereas a correction factor of 1.0–1.4 g/dL is required for the Hb201+, it may be that no correction factor is required for the Hb801. Further study of the Hb801 relative to a gold-standard reference in non-mammalian lineages is warranted.

RESUMEN. Los analizadores HemoCue son dispositivos estándar de puntos de atención para medir la concentración de hemoglobina en sangre ([Hb]). La utilidad de estos fotómetros se ha demostrado para todos los linajes de vertebrados, incluidas las aves, pero como la mayoría de los vertebrados, excepto los mamíferos, poseen glóbulos rojos nucleados, sus lecturas de [Hb] aumentan en estos taxones y requieren un factor de corrección relativo a la calibración de referencia. Hemos probado a campo el más reciente de estos analizadores, el Hb801, comparándolo con su predecesor, el Hb201+. Debido a las diferencias en los métodos de fotometría, estos dos analizadores no resultaron equivalentes para taxones con glóbulos rojos nucleados, como las aves. Mientras que para el Hb201+ se requiere un factor de corrección de 1,0-1,4 g/dL, es posible que para el Hb801 no se requiera ningún factor de corrección. Está justificado seguir estudiando la Hb801 en relación con una referencia estándar de oro en linajes no mamíferos.

Key Words: *avian hematology, HemoCue, hemoglobin concentration*

## INTRODUCTION

Opportunities to go to the field and obtain lab-quality diagnostic measurements have blossomed in recent decades with the proliferation of point-of-care devices (e.g., i-STAT Abbot Laboratories). While principally designed for health care professionals and veterinarians, these devices also have broad applications to animal health and physiological studies of many different taxa, including studies of wild birds.

In hematology, HemoCue analyzers have become a standard point-of-care tool for measuring blood hemoglobin concentration ([Hb]) with results that can be obtained within minutes from a small quantity of blood. Such studies relate to ailments such as the hemoglobinopathies, certain cancers, and kidney disease that result in low [Hb], or cardiovascular and pulmonary disease that elevate [Hb] (Luks et al. 2021), such as chronic mountain sickness (Monge 1943). Hemoglobin has also been a focal point for studies of exercise performance and acclimatization and adaptation to the soaring heights of the Andes and Himalayas (Storz and Scott 2019), as well as for studies of breath-hold divers foraging underwater (Ponganis 2015). Indeed, HemoCue analyzers can provide reliable measurements across all major groups of vertebrates, so long as systematic correction factors are applied to account for differences in erythrocyte size, structure, and nucleation (Neufeld et al. 2002, Posner et al. 2005, Clark et el. 2008, Kutter et al. 2012, Harter et al. 2015, Andrewartha et al. 2016). Recently, Linck et al. (2023) and Williamson et al. (2023) published taxonomically extensive surveys of [Hb] from Andean birds. Schell et al. (2024) reported [Hb] across diving waterfowl. All of these recent cross-taxonomic surveys utilized the HemoCue Hb201+ analyzer. Finally, HemoCue analyzers have also been found to be more accurate than other less invasive methods relative to reference [Hb] values obtained via the complete blood count (CBC), which is performed on an expensive hematology analyzer at a diagnostic laboratory and is considered to be the gold standard (Young et al. 2021).

The Hb201+ analyzer measures azidemethemoglobin absorbance at 570 nm and 880 nm. In this photometer, sodium deoxycholate in the microcuvette hemolyzes the erythrocytes to release hemoglobin, which is then converted to azidemethemoglobin by sodium nitrite and sodium azide, with methemoglobin as an intermediate. The dry reagents contained in the microcuvette have a recommended shelf life of three months once opened. However,

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because the HemoCue analyzers were developed and calibrated for human blood, in which erythrocytes are non-nucleated (like other mammals but not vertebrates in general), [Hb] is overestimated for non-mammalian lineages, and correction factors must be applied because of differences in erythrocyte size and structure (Andrewartha et al. 2016). For birds, a correction factor of –1 g/dL was recommended for blood samples from birds using this particular analyzer (Simmons and Lill 2006).

## METHODS

Here we report field testing of the newest HemoCue analyzer, the Hb801. The Hb801 analyzer is distinct from its predecessors the Hb201+ (and Hb301 which shares the same chemistry) because the Hb801 measures Hb absorbance directly at 506 nm and 880 nm without any chemical conversion step or hence any reagents in the microcuvettes. This allows the microcuvettes to be stored indefinitely, a major advantage for long-duration studies such as those by Linck et al. (2023) or Williamson et al. (2023) in which data from many different field seasons were combined over a 14-year period. In our study, this field testing was necessary to compare Hb201+ and Hb801 results and determine the accurate correction factors for species with nucleated blood cells or if a correction factor may not be needed at all given the novel characteristics of the Hb801. While the Hb801 was previously field tested on humans (Young et al. 2021), this is the first comparison we are aware of for other vertebrate species.

For each paired sample, up to 1 ml of avian blood was drawn from the brachial vein of various duck species (family *Anatidae*) using a 23-gauge syringe pre-rinsed with 0.5 M ethylenediaminetetraacetic acid (EDTA; pH 8.0) as an anticoagulant but with a fresh, unfilled needle so as not to bias the measurement by residual EDTA present in the needle itself or the hub. Blood samples were mixed and then dispensed into the Hb201+ and Hb801 cuvettes in random order with the needle removed to prevent hemolysis of the red blood cells. All measurements were obtained immediately following the blood draw.

This research was authorized by the Servicio Nacional de Áreas Naturales Protegidas (SERNANP #002-2022-SERNANP-RNT/J) in Perú and the University of Miami Animal Care and Use Committee (IACUC #22-049) in Coral Gables, Florida, USA. Use of pre-existing human blood sample data was authorized by the University of Miami's Institutional Review Board (#20231181). All birds were released back to the wild.

## RESULTS AND DISCUSSION

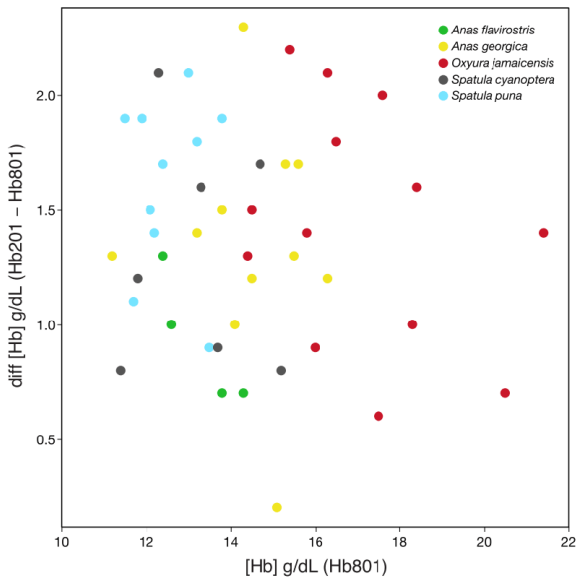
Using a total of $n = 48$ South American ducks that were banded and released for ongoing hematology projects involving measurement of total Hb mass, we made paired Hb201+ and Hb801 measurements of mixed veinous blood from five species: *Oxyura jamaicensis* ($n = 13$), *Spatula puna* ($n = 10$), *Spatula cyanoptera* ($n = 8$), *Anas georgica* ($n = 11$), and *Anas flavirostris* ($n = 6$) on Lake Titicaca, in the vicinity of Puno, Perú, during June 2023. When comparing the Hb201+ and Hb801 across all species and samples combined, we found that [Hb] was significantly different between the two analyzers, on average ~1.4 g/dL higher for the Hb201+ ($\bar{x} = 15.8 \pm 0.3$) than the Hb801 ($\bar{x} = 14.4 \pm 0.3$; paired two-tailed t-test $P < 0.00001$; df = 45; all means reported with standard error of the mean; Table 1). Pearson's correlation coefficient for the readings from the two analyzers was not significant ($r = –0.077$; $P > 0.60$). Thus, we observed a mean difference corresponding to 0.4 g/dL greater than the 1 g/dL correction factor cited by Simmons and Lill (2006). We next considered variation among species and found that, despite some variation in [Hb], these ratios varied from about 1.2–1.6, excluding the least well-sampled species (Table 1, Fig. 1). Although we did not see a significant trend between [Hb] and the observed difference between the Hb201+ and Hb801 readings, the data for *Oxyura jamaicensis*, which had the highest average [Hb], suggested that blood samples with the very highest [Hb] might yield smaller differences between the Hb201+ and Hb801 analyzers (Fig. 1), which would be expected because blood turbidity is highest in such species.

**Table 1.** Summary of paired hemoglobin concentration ([Hb]) measurements obtained side by side from the Hb201+ and Hb801 analyzers across all five species.

| | Hb201+ [Hb] | | Hb801 [Hb] | | Difference (Hb201 - Hb801) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Min, Max | *P*-value[†] |
| *Oxyura jamaicensis* ($n$ = 13) | 18.5 | 2.0 | 17.1 | 2.1 | 1.4 | 0.5 | 0.6, 2.2 | 0.00001 |
| *Spatula puna* ($n$ = 10) | 14.2 | 0.9 | 12.5 | 0.8 | 1.6 | 0.4 | 0.9, 2.1 | 0.00001 |
| *Spatula cyanoptera* ($n$ = 8) | 14.2 | 1.6 | 13.0 | 1.5 | 1.2 | 0.5 | 0.8, 2.1 | 0.00022 |
| *Anas georgica* ($n$ = 11) | 15.8 | 1.5 | 14.4 | 1.4 | 1.3 | 0.5 | 0.2, 2.3 | 0.00001 |
| *Anas flavirostris* ($n$ = 4) | 14.2 | 0.7 | 13.3 | 0.9 | 0.9 | 0.3 | 0.7, 1.3 | 0.00759 |
| Overall ($n$ = 46) | 15.8 | 2.4 | 14.4 | 2.4 | 1.4 | 0.5 | 0.2, 2.3 | 0.00001 |

[†] Two-tailed paired t-test. Note that two *Anas flavirostris* with lower [Hb] for the Hb201+ than the Hb801 were excluded from the analysis.

**Fig. 1.** Difference in hemoglobin concentration ([Hb]) measured with the HemoCue Hb201+ vs. Hb801. A total of $n = 46$ avian blood samples are depicted because two individual *Anas flavirostris* had Hb801 measurements that were greater than the Hb201+; otherwise this outcome was not observed, and these samples were excluded from analysis. *Oxyura jamaicensis*, a species with relatively high [Hb], seemed to showed a trend toward smaller differences between the two photometers at higher [Hb]; however, this was not statistically significant ($R^2 = 0.10$; $P > 0.28$).



For comparison with a mammal lacking nucleated red-blood cells and to verify that the analyzers utilized were functioning properly in the field, the first author performed a repeated measures analysis ($n$ = 25) using his own capillary blood sampled using a lancet. In contrast to the birds, there was no difference between the Hb201+ ($\bar{x}$ = 14.3 ± 0.1) and the Hb801 ($\bar{x}$ = 14.5 ± 0.1; paired two-tailed t-test; $P > 0.26$; df = 24), and Pearson's correlation coefficient for the two analyzers was significant ($r$ = 0.535; $P$ = 0.0058) for the mammal's non-nucleated red-blood cells, therefore suggesting that the Hb801 could plausibly be reading the correct [Hb] in birds as well as mammals. It should be noted that despite the repeated measures analysis, this constituted a sample size of one, which is not the most robust comparison. More mammalian comparisons would be useful.

In conclusion, the Hb201+ and the Hb801 analyzers are outstanding tools for researchers working in the field of hematology, with applications to human and animal health, but also other areas of physiology and ecology. Both analyzers are factory calibrated for human blood using the hemiglobincyanide (HiCN) method, which is the international reference (i.e., gold standard) for the determination of [Hb] in human blood, results of which also can be obtained from a CBC test performed on a hematology analyzer in a diagnostic laboratory (Young et al. 2021). Although we cannot yet confirm that the Hb801 is reading the correct value in birds, this could be examined further by other researchers in a setting where captive birds can be sampled near a diagnostic laboratory. Nonetheless, our results do show that these two photometers are not equivalent for taxa with nucleated red-blood cells, like the birds studied here, which may also be the case for reptiles, amphibians, and fishes, in which the [Hb] is known to be overestimated with the Hb201+ (Andrewartha et al. 2016). On the other hand, mammals, because they do not have nucleated red blood cells, are the only exception where the two analyzers should be expected to give equivalent results. Our study, although limited in scope, illustrates the need to establish taxon-specific correction factors across lineages and/or to verify that such correction factors are not needed relative to reference [Hb]. The requirement for a correction factor is likely not only driven by erythrocyte size and structure, but also that the chemistry that converts hemoglobin to azidemethemoglobin, which, in the presence of cell nuclei and mitochondria, contributes to a denser read and therefore artificially higher [Hb] value (Simmons and Lill 2006). Hence, these calibrations are both platform and lineage specific. In sum, this new information should be taken into account, especially for researchers wishing to combine data from different generations of HemoCue analyzers or working across vertebrate taxa with both nucleated and enucleated red-blood cells. Such studies will have to pay particular attention to these details. Finally, the utility of these small portable analyzers has great potential to contribute to both basic sciences as well as the health and monitoring of wild animal populations. Their portability is a major feature contributing to their easy use and utility for diverse taxa, but this portability and ease-of-use also raises issues. Further study would benefit from larger sample sizes across more diverse nucleated vertebrate lineages as well as calibration of the new Hb801 across lineages. Until then, researchers combining data from the Hb201+ and Hb801 should proceed with caution and seek to obtain a reference if mammalian blood is not the focus of the study.

**Author Contributions:**

*KGM and LA designed and executed the field study and analyzed the data and wrote the manuscript. HBJ, KAM, RMO, JAOS, and RHTA executed the field study and provided comments on the manuscript.*

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**Data Availability:**

*Data sharing is accessible through Dryad at https://duckdna.org/data/*

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