

Feasibility of Reagent Test Strips to Estimate Blood Urea Nitrogen Concentrations in Egyptian Fruit Bats (*Rousettus aegyptiacus*)

Eshar D.,^{1*} Lapid R.,² Weinberg, M.³ and King R.⁴

¹ Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, Manhattan, Kansas 66506, USA.

² The Hai Park Zoo, 79th Ha-Hashmonaim Street, Qiryat Motzkin, 2633761, Israel.

³ Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel.

⁴ The Science and Conservation Division, Israel Nature and Parks Authority, 3rd Am Ve'Olam Street, Jerusalem, 9546303, Israel.

* **Correspondence:** Dr. David Eshar, College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, Manhattan, Kansas 66506, USA. Tel: 785-532- 5690. Email: deshar@vet.k-state.edu

ABSTRACT

The Egyptian fruit-bat (*Rousettus aegyptiacus*) has been studied in both field and laboratory settings. As bats are expected to encounter diseases potentially affecting their blood urea nitrogen (BUN) concentrations, it may be beneficial to use a rapid and accurate test for determining BUN, which can indicate azotemia and guide appropriate therapy. This study has evaluated the feasibility of commercial reagent tests strips to estimate whole-blood BUN concentration in Egyptian fruit bats. For each blood sample, the BUN strip result was compared to a predetermined color scale provided by the manufacturer, and compared to the BUN as measured by a point-of-care (POC) chemistry analyzer. There was a 100% agreement between the reagent test strip and the biochemistry analyzer's BUN results. However, the naturally occurring low BUN of this species precluded an indication for azotemia as all tested bats were assigned to a single predetermined category. Continued investigation into alternative point of care testing in bats is warranted. Standard biochemistry analyzer should still be considered the most accurate method of BUN testing and identifying azotemia in Egyptian fruit bats.

Keywords: Egyptian fruit-bat; *Rousettus aegyptiacus*; Blood Urea Nitrogen; Reagent Tests Strips; Point of Care Testing

INTRODUCTION

Although not as common as small rodents, bats are also studied in laboratory settings in research centers, as well as in the field (1-6). Bats used in studies are either wild-captured or originate from captive breeding colonies (2). Second to rodents, bats are the most abundant and diverse mammalian group in nature. The order *Chiroptera* is divided into suborders *Microchiroptera* and *Megachiroptera*, with further subdivision into 18 families and 1,116 recognized species (7).

The Egyptian fruit-bat (*Rousettus aegyptiacus*) (EFB) belongs to the *Megachiroptera* suborder, within the *Pteropodidae* family (8). It has a wide geographical distribution, from

Northern Africa, through the Middle East to South-western Asia, where it inhabits wild and urban areas (8). Its diet consists of wild and cultivated fruits with high water content (up to 90%) and low sodium (Na) content (3, 5, 8).

Researchers and veterinarians encounter diseases in bats, which might affect blood urea nitrogen (BUN) concentration. The EFB, like other species, might be affected by renal dysfunction of various causes, including neoplasia (9). Iron storage disease is another major cause of morbidity and mortality in the adult EFB, potentially resulting in hepatic failure and abscesses and hepatocellular carcinoma (10). Blood testing in bats is carried out for clinical evaluation or

research. Monitoring BUN concentration, especially in the light of azotemia, via low-volume blood sample is warranted, and using tests which provide immediate results is beneficial.

With the advantages of their relative simplicity of operation and prompt results, point of care (POC) testing is commonly used in human medicine, and is increasingly more common in veterinary medicine (11, 12). A variety of commercial reagent test strips have been evaluated for use as POC methods for BUN concentration measurement in both human and veterinary medicine (13-19). The evaluation of these reagent strips in animals has focused in companion animals, and these have been shown to be highly sensitive and specific (17-19). However, their performance in bats has yet to be investigated. Commercial reagent test strips provide a rapid estimation of BUN concentration, using only a single drop of blood, making them an attractive and useful method in small-sized species, such as bats, especially when repeated sampling is required. Diagnostic testing in bats is often limited by the relatively challenging venipuncture, the small blood sample volume obtained, and financial and technical constraints.

The purpose of this study was to evaluate the performance of a commercial, cost-efficient BUN reagent test strip, requiring a small whole-blood sample volume, in wild-captured EFB. We hypothesized that this reagent test strip will provide an accurate estimate of BUN when compared to a chemistry analyzer, and may therefore be clinically useful in this species.

MATERIALS AND METHODS

Animals

The experimental procedures carried out in this study were approved by the Institutional Animal Care and Use Committee, College of Veterinary Medicine, Kansas State University (IACUC#3667).

Free-living captured EFBs were tested, as part of the Israel Wildlife Diseases Surveillance (IWDS) program, conducted by the Israel Nature and Parks Authority. The bats were netted at dusk when leaving their roosting sites, placed in covered wire cages with pieces of cloth to hang from. The bats were immediately transported to the laboratory, where the testing was performed, within 1 to 4 hours from capture in order to minimize iatrogenic effects on their hydration and urine production (1). A physical examination was performed by a veterinarian for each bat, and the overall health status, sex and body weight were recorded.

Blood sampling and BUN analysis

Blood samples were obtained from the proptagial vein using a flushed pre-heparinized 1.0 mL syringe, with a 25-27G 0.5 x 16 mm needle, and placed into a lithium-heparin tubes (BD Microtainer, Becton Dickinson, Franklin Lakes, NJ, USA).

Whole-blood chemistry analysis was performed immediately using a dry chemistry POC analyzer (iSTAT®, Chem8, Abaxis Veterinary Diagnostics, Union City, CA), chosen as it was previously used in wildlife species, requiring a small volume of whole blood sample (~100µL) and provides immediate results (within ~2min) (11). Whole blood BUN was also measured immediately (within <5 minutes of sampling) using a commercially available reagent test strip (Azostix, Siemens Healthcare Diagnostics, Tarrytown, NY), according to the manufacturer's instructions.

The BUN reagent test strips

This reagent test strip is a semi-quantitative test for determining whole blood BUN concentration. It has a rectangular, yellow-colored reagent pad area, permeable to urea but not to other blood pigments. The strip reagent area is impregnated with both urease and bromothymol blue. The urease acts as a catalyst for blood urea hydrolysis, converting it to carbon dioxide and ammonium hydroxide. With an increase in ammonium hydroxide concentration, an alkaline pH shift occurs, which then generates a range of green-color changes (19). The degree of color change is designed to correlate with the blood sample BUN concentration. The result is assigned one of four BUN concentration categories by the manufacturer, as following: 1 (BUN 5-15 mg/dL); 2 (BUN 15-26 mg/dL); 3 (BUN 30-40 mg/dL) and 4 (BUN 50-80 mg/dL) (19).

For accurate results the reagent pad must be completely covered by a large fresh whole blood drop, and rinsed with water after exactly 60 seconds. When the color change following the reaction does not fully match the green-color scale provided by the manufacturer, the BUN category is assigned the nearest higher category (19). All the BUN reagent strip results were read under white-florescent lighting by the same operator (DE), who was blinded to the chemistry analyzer BUN results.

Statistical methods

For each blood sample, the chemistry analyzer-generated BUN concentration (ABUN) was assigned a BUN con-

centration category based on the above-mentioned Azostix instructions and this was later compared to the BUN concentration category read using the Azostix.

Descriptive statistics were calculated using standard software (Microsoft Excel, Version 2010, Microsoft Corporation, Redmond, WA, USA). The ABUN and Azostix BUN were compared using Student's paired *t*-test. The agreement between paired BUN results was examined using the Lin's concordance correlation coefficient (<https://services.niwa.co.nz/services/statistical/concordance>) (12). Sensitivity and specificity for the reagent strips were calculated using standard equations.

RESULTS

The study included 41 EFBs (32 males and nine females) with an average body weight of 140 ± 23 g (Mean \pm SD).

The ABUN results were 5 ± 3 mg/dL (Mean \pm SD) (Figure 1). The Azostix BUN results were classified as Category 1 in all bats. The accuracy, agreement, sensitivity and specificity of the Azostix BUN were therefore 100%.

DISCUSSION

The purpose of this study was to evaluate the clinical use of a commercial reagent test strip as an accurate method of estimating BUN concentration and azotemia in Egyptian fruit bats. The study results suggested that the tested BUN reagent test strip correctly assigned whole blood BUN concentration to a correct concentration category as determined by the ABUN results. However, the assignment of all ABUN results to a single category had limited the prognostic ability of the Azostix to indicate azotemic animals.

The distribution of the ABUN results for bats in this study (Figure 1) suggested the presence of three BUN outliers at concentrations (11–15mg/dL), all of which were still within Azostix category 1. It is possible that these outliers were truly abnormal and because of the inability of the Azostix to indicate them as azotemic, the 100% specificity (the proportion of non-azotemic animals that tested negative) may be overestimated. The lack of observed azotemia-positive animals by the Azostix precluded calculating a genuine sensitivity (the proportion of azotemic animals that tested positive) and predictive values (positive and negative), and likely provided a false negative in some of these cases. For this reason, future studies should also include animals

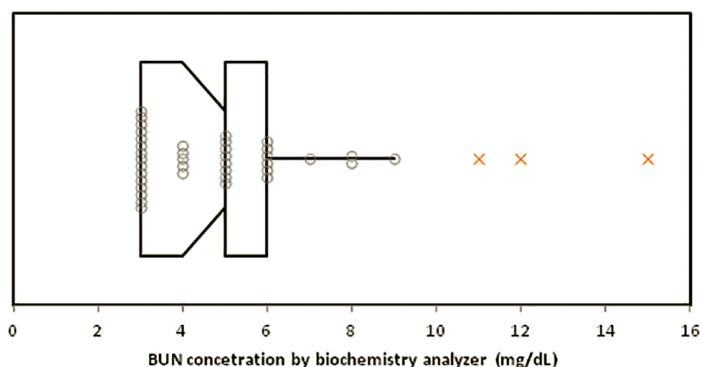


Figure 1: The distribution the BUN concentrations for Egyptian fruit bats (*Rousettus egyptiacus*) in this study. The “X” markers in this chart suggest BUN measurements identified as outliers by a post hoc Turkey test.

with confirmed azotemia (due to conditions such as renal or hepatic disease).

The BUN concentration for bats in this study was determined using the iSTAT POC analyzer. It is possible that this analyzer had artifactually provided lower BUN concentration results for bats in this study and future studies should compare the Azostix to BUN results obtained by different biochemistry analyzers. Alternatively, it is also possible that the Azostix tended to read lower BUN values in this species or malfunctioned in this study. In order to clear this point, reagent strips from the same batch were used to estimate the BUN in other mammalian species, and these showed variable results above category 1. However, studies (using different biochemistry analyzers) in this bat species (3) and in other *Pteropodid* bats, (20–25), have shown similar BUN ranges, suggesting that fruit bats species normally have low BUN concentrations, possibly because of their low-protein diet and lower levels of protein breakdown products (20, 25). As the Azostix reagent strip cannot read an exact BUN concentration within a low and narrow BUN range, its use in fruit bats may be of limited value, and other tests, designed for better precision within low BUN measurements should be investigated.

CONCLUSION

The present data suggests that the Azostix reagent strips correctly assigned all BUN results within a single predetermined category, compared to a dry chemistry POC analyzer. The naturally low BUN of this species leading to the inclusion of all test results within a single Azostix category, suggest

that this testing methodology may not be a useful screening test for azotemia in the EFB, and therefore cannot be recommended as a surrogate to BUN testing by chemistry analyzers. Further studies for the potential utilization of POC BUN testing in fruit bats are still indicated.

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REFERENCES

- Studier, E. H. and Wilson, D. E.: Natural urine concentrations and composition in Neotropical bats. *Comp. Biochem. Physiol. A. Physiol.* 75:509-515, 1983.
- Fenton, M. B.: Science and the conservation of bats. *J. Mammal.* 78:1-14, 1997.
- Korine, C., Zinder, O. and Arad, Z.: Diurnal and seasonal changes in blood composition of the free-living Egyptian fruit bat (*Rousettus aegyptiacus*). *J. Comp. Physiol. [B]*. 169:280-286, 1999.
- Geva-Sagiv, M., Las, L., Yovel, Y. and Ulanovsky, N.: Spatial cognition in bats and rats: from sensory acquisition to multiscale maps and navigation. *Nat. Rev. Neurosci.* 16:94-108, 2015.
- Herrera, L. G. M., Cruz-Neto, A. P., Wojciechowski, M. S., Larrain, P., Pinshow, B. and Korine, C.: The relationships between food and energy intakes, salt content and sugar types in Egyptian fruit bats. *Mamm. Biol.* 80:409-413, 2015.
- Jones, M. E., Schuh, A. J., Amman, B. R., Sealy, T. K., Zaki, S. R., Nichol, S. T. and Towner, J. S.: Experimental inoculation of Egyptian rousette bats (*Rousettus aegyptiacus*) with viruses of the ebolavirus and marburgvirus genera. *Viruses*. 7:3420-3442, 2015.
- Eshar, D. and Weinberg, M.: Venipuncture in bats. *Lab. Anim.* 39:175, 2010.
- Korine, C. *Rousettus aegyptiacus*. The IUCN Red List of Threatened Species 2016: e.T29730A22043105. <http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T29730A22043105.en>. Downloaded on 06 January 2017.
- Olds, J. E., Burrough, E. R., Fales-Williams, A. J., Lehmkühl, A., Madson, D., Patterson, A. J. and Yaeger, M. J.: Retrospective evaluation of cases of neoplasia in a captive population of Egyptian fruit bats (*rousettus aegyptiacus*). *J. Zoo. Wildl. Med.* 46:325-332, 2015.
- Leone, A. M., Crawshaw, G. J., Garner, M. M., Frasca, Jr, S., Stasiak, I., Rose, K., Neal, D. and Farina, L. L.: A retrospective study of the lesions associated with iron storage disease in captive Egyptian fruit bats (*Rousettus aegyptiacus*). *J. Zoo. Wildl. Med.* 47:45-55, 2016.
- Stoot, L. J., Cairns, N. A., Cull, F., Taylor, J. J., Jeffrey, J. D., Morin, F., Mandelman, J. W., Clark, T. D. and Cooke, S. J.: Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv. Physiol.* 2:cou011, doi: 10.1093/conphys/cou011. eCollection 2014. 2014.
- Lindholm, C. and Altimiras, J.: Point-of-care devices for physiological measurements in field conditions. A smorgasbord of instruments and validation procedures. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol. A.* 202:99-111, 2016.
- Logan, J. E., Renton, H. M. and Eby, P. W.: Observations on diagnostic kits for the determination of urea nitrogen. *Clin. Chem.* 2:189-196, 1968.
- Eby, P. W. and Logan, J. E.: Some observations on the use of Azostix for the determination of blood urea nitrogen. *Can. Med. Assoc. J.* 100:125, 1969.
- Bold, A. M., Menzies, I. S. and Walker, G.: An assessment of the Azostix strip test. *J. Clin. Path.* 23:85-89, 1970.
- Wenk, R. E., Lustgarten, J. A. and Byrd, C.: An Evaluation of "Kits" for Determining Urea Nitrogen. *Am. J. Clin. Pathol.* 59:542-548, 1973.
- Watson, A. D. J. and McDonald, P. J.: Comparison of several laboratory tests for quantifying azotemia in dogs. *Aust. Vet. J.* 57:407-410, 1981.
- Hill, D., Correa, M. T. and Stevens, J. B.: Sensitivity, specificity, and predictive values of reagent test strip estimations of blood urea nitrogen. *Vet. Clin. Path.* 23:73-75, 1994.
- Berent, A. C., Murakami, T., Scroggin, R. D. and Borjesson, D. L.: Reliability of using reagent test strips to estimate blood urea nitrogen concentration in dogs and cats. *J. Am. Vet. Med. Assoc.* 227:1253-1256, 2005.
- Heard, D. J. and Whittier, D. A.: Hematologic and plasma biochemical reference values for three flying fox species (*Pteropus* sp.). *J. Zoo. Wildl. Med.* 28:464-470, 1997.
- Heard, D. J.: Medical management of megachiropterans. In: Fowler, M. E., Miller, R. E., (eds.). *Zoo and wild animal medicine, Volume 4, Current therapy*. Saunders, 1997.
- Hall, J., Rose, K., Smith, C., De Jong, C., Phalen, D., Austen, J. and Field, H.: Health assessment of the Christmas Island flying fox (*Pteropus melanotus natalis*). *J. Wildl. Dis.* 50:447-458, 2014.
- McMichael, L., Edson, D., Mayer, D., Broos, A., Kopp, S., Meers, J. and Field, H.: Physiologic Biomarkers and Hendra Virus Infection in Australian Black Flying Foxes (*Pteropus alecto*). *J. Wildl. Dis.* 53:111-120, 2017.
- McMichael, L., Edson, D., Mayer, D. and McLaughlin, A., Goldspink, L., Vidgen, M. E., Kopp, S., Meers, J. and Field, H.: Temporal Variation in Physiological Biomarkers in Black Flying-Foxes (*Pteropus alecto*), Australia. *Eco. Health.* 13:49-59, 2016.
- Selig, M., Lewandowski, A. and Kent, M. S.: Establishment of reference intervals for hematology and biochemistry analytes in a captive colony of straw-colored fruit bats (*eidolonhelvum*). *J. Zoo. Wildl. Med.* 47:106-112, 2016.