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Research Article

Validation of The Accu-Chek® Aviva Glucometer for Richardson's Ground Squirrel, *Urocitellus richardsonii*

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Abstract

The ability to take repeated, in situ blood glucose readings using a hand-held glucometer has considerable utility for researchers. These devices are small, affordable, and impose minimal stress on test subjects, yet their reliability is uncertain. We assessed the precision and accuracy of the Accu-chek® Aviva glucometer in measuring blood glucose concentrations from Richardson's ground squirrels, *Urocitellus richardsonii*, by taking repeated Accu-chek® test strip readings and comparing those to readings from Manitoba Agriculture's Veterinary Diagnostic Services Laboratory VITROS®250 chemistry analyzer. Samples were collected at the Assiniboine Park Zoo in Winnipeg, Manitoba, Canada from 15 female ground squirrels. Our results show a relatively strong positive correlation between the blood glucose concentrations obtained using the Accu-chek® Aviva glucometer and the VITROS®system, with differences between those falling within the Canadian Diabetes Association bounds of acceptable error. While not significantly different, portable glucometer values were, on average, 0.852 mmol/L lower, and exhibited roughly twice the range of variation of the VITROS®-derived blood glucose concentrations. We recommend that researchers employing this glucometer in the field conduct a similar validation to optimize the number of test-strip readings taken, and to calibrate their blood glucose measurements relative to those obtained from more accurate lab-based instruments.

INTRODUCTION

Glucose measurement is of great importance in physiological, ecological and behavioural research, in that glucose constitutes the body's primary fuel source. Traditional glucose analysis methods require time-consuming laboratory assays [1], and rely on field-collected blood samples that are prone to clotting or spoilageowing to adverse conditions [2]. Transporting the organism to a lab or location where blood can besampled and stored reliably, or assayed immediately, is complicated by stress-induced elevation of blood glucose [3]. The use of a hand-held glucometer in the field alleviates such concerns and may provide immediate and reliable blood glucose concentrations.

Hand-held glucometers are readily available and relatively inexpensive, often being provided at no cost with purchase of test strips. Portable glucometers require only a small droplet of blood to measure blood glucose concentration, with some brandsallowing blood to be added to the test strip where the initial sample proves insufficient [4]. The currentAccu-chek® Aviva handheld glucometer (Roche Diagnostics, Laval, Quebec) requires only 0.6 µl of blood per test and provides a result in approximately 5 seconds.This meter is describedby Roche as having more than 150 system integrity checks to avoid unreliable results, and being"ready to use right out of the box" with no calibration required before meter use(https://www.accu-chek.ca/en/meter-systems/aviva, accessed 27 August 2017; https://www.accu-chek.com/meters/aviva-meter, accessed 25 July 2017). Though a control solution is available, it is not included with the device or with the disposable test strips, and thus results may be unreliable. In a study of five commercially available glucometers, only three (the GlucoPlus™, OneTouch® Horizon™, and Accu-chek® Active) fell within the acceptable performance range set by the International Standardization Organization (ISO)of < 20% variation from lab-derived values [5].

Studies involving humans [6], cats and dogs [7,8], ferrets [9], grey seals [10], andparrots [11] have reported issues with both the accuracy and precision of portable glucometer readings. While falling within the ISO's accepted error range, the glucometers tested rarely produced results representative of the glucose values obtained from lab analysis.Given that these glucometers are marketed primarily as a medical point-of-care device, there should be little room for error in readings. Portable glucometers, including the Accu-chek® Aviva, are also routinely used and are considered reliable in diabetes research employing rodents, including both laboratory mouse and rat lines [12,13,14, 15,16].

While the Accu-chek® Aviva meter has been validated for use with humans, it has not been validated for squirrels [6,17]. No validation of a portable glucometer has been conducted to date for Richardson's ground squirrels, *Urocitellus richardsonii*. These squirrels are semi-fossorial, obligate hibernators that occupy shortgrass prairie and human-modified areas across Western Canada and the Northern United States of America [18]. These and

other ground squirrel species are subject to intensive research exploring sociality [19,20,21], communication [22,23,24], life history, and ecology [25,26,27]. They are listed as being of least concern on the International Union for Conservation of Nature's (IUCN) Red List, however they are an important prey species [26,28,29], and play a pivotal role in structuring pastureland plant communities [30]. Here, we assess the accuracy and precision of the Accu-chek® Aviva glucometer for use in field research with Richardson's ground squirrels.

METHODS

Research was conducted under Protocol F16-002 approved by the University of Manitoba's Fort Garry Campus Animal Care Committee, Protocol 2014-A002 approved by the Assiniboine Park Zoo, and Manitoba Conservation Wildlife Scientific Permit WB18473. Fifteen sexually mature (≥ 1 year-old) female Richardson's ground squirrels were sampled from the population located at the Assiniboine Park Zoo in Winnipeg, Manitoba(49°52'11.0"N 97°14'30.0"W). This is a well-established population that has been studied extensively and is located in close proximity to an on-site veterinary hospital, minimizing stress on the squirrels due to transport [27,31,32]. Squirrels were captured using Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter (No Name™ Brand, Loblaw Companies Ltd., Toronto, ON) and transported on foot within 5 min of capture to the veterinary hospital in apillowcase-covered live-trap, thereby reducing stress on the squirrel [32,33]. Inside a procedure room at the veterinary hospital, the captured squirrel was coerced into a cloth handling bag, weighed to the nearest 5 g with a Pesola® spring balance (PesolaAG, Schindellegi, Switzerland), and then extracted from the handling bag with a gloved hand and manually restrained on its back on a towel-covered surgical table. Up to 1 ml of blood was drawn from each unanaesthetized squirrel from the medial saphenous vein of the left or right hindleg by veterinary staff using a 29-gauge insulin syringe. Upon removal of the needle, the vein was held off for a period of roughly 1 min to ensure coagulation and to prevent hematoma formation.

Droplets of blood from that sample wereapplied in immediate succession via the needle tip to 6 Accu-chek® Aviva test strips, to obtain 6 glucometer-based blood glucose concentration measurements from each sample. For each blood sample, a strip was loaded into the glucometer, blood was applied, then replaced with the next strip as soon as a reading was obtained. Once all six strip readings were obtained, values were retrieved from the device's built in memory.The remaining blood sample was then ejected via the syringe tip (with the needle removed) into additive-free Monoject™ Covidien red stopper blood collection tubes (non-silicone coated tube) (Covidien Ilc, 15 Hampshire Street, Mansfield, MA USA). We chose to use plain red-top tubes over serum separation tubes as they are commonly on hand and routinely used for serum chemistry in a typical clinical setting. These tubes have also been shown to yield results comparable to those of sodium fluoride-treated tubes, which, owing to their additive, may also dilute low volume samples, an important consideration given the small volume of blood that could safely be drawn from each squirrel [34]. After sitting at room temperature for approximately 20 minutes (until the clot was well formed), samples were centrifuged at 3400 rpm for 15 min to separate serum from the clot. Serum was then pipetted off and kept refrigerated overnight at 4 °C before being sent to the Provincial Veterinary Laboratory forcolorimetric glucose concentrationquantification in duplicate using a VITROS®250 Chemistry Analyzer (Ortho Clinical Diagnostics, Raritan, NJ). Serum glucose concentrations from the VITROS® 250 chemistry analyzer have been reported to be well-correlated with those from other biomedical-grade instruments in previous studies [35,36], and thus were adopted as the standard for comparison in our study.Subsequent to sampling, squirrels were transported back to the field site in a pillowcase-covered live-trap, and released at their point of capture.

Major axis regression explored the relationship between the mean glucose concentrations of each of the 15 blood samples obtained using the VITROS® and Accu-chek® systems, while the correlation between those paired concentration measurements was calculated using a Spearman's rho test. Further, mean ranks from the two systems for each of the 15 samples obtained were compared using the Wilcoxon paired-sample test, in that the distribution of differences obtained from those samples was unlikely to have been drawn from a normally-distributed distribution of differences. Coefficients of variation for glucose concentration measurements were calculated for the first two samples measured with the VITROS® and the Accu-chek® systems, and for each of the subsequent concentration measurements made with the Accu-chek® glucometer. This assessed not only how variability was affected by the method employed to measure glucose concentration, but also how it changed with repeated sampling using the Accu-chek® glucometer. Further, we calculated Spearman's rank-order correlation coefficients (rho) between concentrations measured from the 15 samples with the mean VITROS®- versus Accu-chek®-derived glucose concentration values independently for the first through the sixth test strip readings, to assess how repeated glucometer readings affect accuracy.

The Wilcoxon paired-sample test was performed using Statview® 5.0.1, while all other inferential statistical tests were performed in R (R version 3.4.0, R Core Team 2013) using functions from the lmodel2 [37] package and then plotted using functions from the ggplot2 [38] package. Differences were considered statistically significant where $P \leq 0.05$.

RESULTS

Major axis regression revealed a positive relationship between the mean Accu-chek® and VITROS®blood glucose readings (R^2 = 0.341; Figure 1). The Spearman's rho test confirmed thatblood glucose concentrations measured with the Accu-chek® Aviva portable glucometer were well correlated with those obtained from the VITROS® chemistry analyzer (Table 1). Further, a Wilcoxon paired-sample test contrasting the mean rank of Accu-chek®-obtained blood glucose concentrations with those from the VITROS® system for the 15 samples detected no significant difference between the ranks of those readings (W_{14} = 88, Z = -1.590, P = 0.112). The mean ± SE difference between the Accu-chek® and VITROS®concentrations within samples was 0.852 ± 0.100 mmol/L, though concentrations measured using the Accu-chek® glucometer were more variable than those

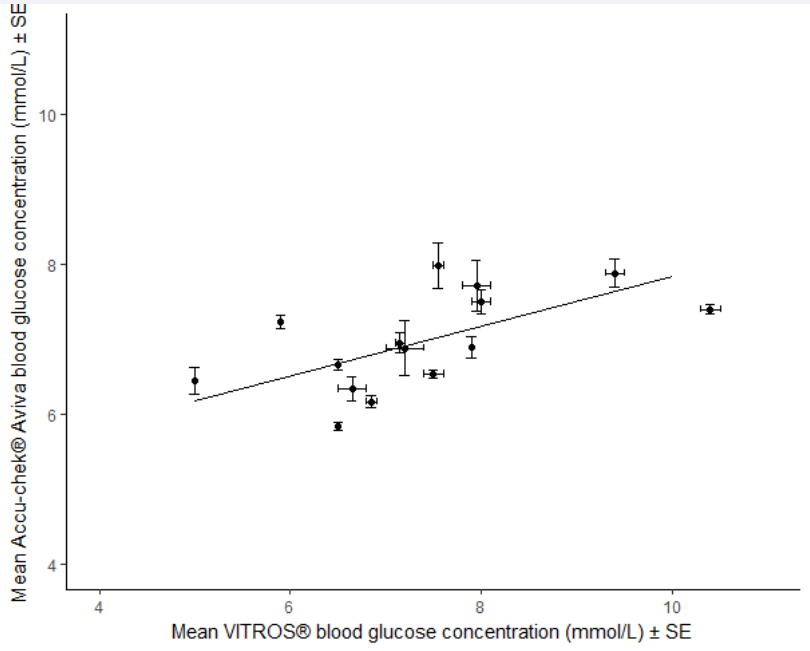

Figure 1 Mean blood glucose concentrations measured with the Accu-chek® Aviva versus the VITROS® systems. Major axis regression resulted in a best-fit linear relationship of $y = 0.334x+4.506$, $R^2 = 0.341$.

obtained from the VITROS® system, with standard errors of 0.159 and 0.07 mmol/L respectively. The coefficient of variation for the Accu-chek® was greater than that for the VITROS® system based upon two measurements from each underlying sample, and increased more or less linearly with successive measurements through the sixth measurement made using the Accu-chek® on each blood sample (Table 1). Based on the mean ± SE difference between the Accu-chek® and VITROS® concentrations within samples, accuracy did not stabilize until after 5 samples and peaked at 6 samples, with a mean difference of 0.852 ± 0.100 mmol/L (Table 2). Concentrations measured at this point using the Accu-chek® glucometer were more variable than those obtained from the VITROS® system, with standard errors of 0.159 and 0.07 mmol/L respectively.

DISCUSSION

Our findings suggest that the Accu-chek® Aviva glucometer can be used to obtain reliable blood glucose concentration measurements from Richardson's ground squirrels. However, due to the sizable but non-significant difference between the Accu-chek® glucometer and VITROS® glucose concentration readings, we recommend that Accu-chek®-derived concentrations be adjusted relative to those obtained from known concentration standards, or by adjusting measured concentrations according to their relationship to results obtained from a calibrated instrument such as the VITROS® system employed in our study. Here, more accurate blood glucose concentrations for each subject squirrel can be obtained by inserting the Accu-chek® Aviva-derived measurement into the regression equation defining the relationship between the Accu-chek®-derived and VITROS®-derived concentration measurements.

Further compounding problems encountered with theAccu-chek®, thevariability of the measurements derived from it (as measured by the Standard Error of the Mean) were more than double that for repeated concentration measurements derived from the VITROS® system. In that coefficients of variation for measurements derived from the Accu-chek® Aviva increased in an essentially linear fashion up to and including 6 samples, without any notable asymptote being reached, it is apparent that repeated sampling up to and including six samples does not resolve the problem of extensive variability in the glucose concentration measured with the portable glucometer. Repeated sampling with the Accu-chek® Aviva does, however, improve accuracy, as is evident from the increasing correlation between Accu-chek® Aviva and VITROS®-based measurements with an increasing number of test-strip measurements per sample. For blood samples obtained from the medial saphenous vein of Richardson's ground squirrels, our study reveals that while variability does not diminish with repeated measurement up to and including 6 test strips, accuracy does not appear to stabilize and peak until results from 5 test strips have been obtained. We thus recommend that future research involving Richardson's ground squirrels employ at least 5 repeated Accu-chek® Aviva test strips for each sample to ensure that the concentrations obtained are accurate. A validation study such as that reported here would prove prudent, however, to determine the optimal number of test strips required to obtain a representative mean blood glucose concentration for individuals of a given species at a particular point in time.

The main drawbacks of this study relate back to the differences between laboratory studies and field studies of wild animals. One of the problems encountered was the smaller sample size of 15 squirrels. Since we were trapping individuals from a wild population, we were limited by the number of squirrels that could be trapped and sampled within the time available. Another possible drawback is the potential influence of the health of the individuals studied. By collaborating with veterinarians that have

Table 1: Spearman's Rho values revealing the correlation between Accu-chek® Aviva versus VITROS®-derived blood glucose concentrations for increasing numbers of tests run on each sample alongside the corresponding mean coefficients of variation for concentrations derived from the two instruments.

		Number of tests					
		1	2	3	4	5	6
Spearman's RHO	σ	0.631	0.680	0.618	0.606	0.686	0.681
	P	0.012	0.005	0.014	0.017	0.005	0.005
Accu-chek® AVIVA	CV		0.030611	0.036034	0.037345	0.044397	0.054763
VITROS®	CV		0.012841				

Table 2: Mean difference between Accu-chek® and VITROS® concentration readings and standard errors within samples for increasing numbers of tests run on each sample.

Mean Difference Between Accu-chek® and VITROS® Readings		Number of Tests					
		1	2	3	4	5	6
Difference in concentration	mmol/L	0.903333	0.854444	0.885556	0.863333	0.855333	0.852222
Difference in SE	mmol/L		0.116667	0.10158	0.095625	0.095544	0.099812

experience with local wildlife or experts on the organism, such as we have, the risks of making generalizations about a population based upon specimens in poor condition can be minimized but there will still be variation between individuals. One of the novel aspects of utilizing a hand-held glucometer in the field is the ability to obtain a reading on site using whole blood. We compared these readings however with those obtained from the VITROS® system using spun down serum from the same samples. While whole blood glucose and serumglucose are correlated, there may still be a significant difference between the two [39]. We also did not test the Accu-chek® Aviva at the limits of its range, as our goal was to examine its applicability to the naturally occurring blood glucose range of Richardson's ground squirrels [27].

In general terms, our findingsparallel those of Mahmoodpoor et al. (2016) in their validation of the Accu-chek® Aviva with diabetic humans, though their glucometer measurements were consistently higher than the laboratory-measured values [6], while ours fell both above and below laboratory-determined concentrations. This difference may be attributable to the differences in hematocrit (the volume of red blood cells in a blood sample) values of humans and ground squirrels [40], although Roche reports that the Accu-chek® Aviva is designed for a hematocrit range from 10% to 65% (https://www.accu-chek.ca/en/meter-systems/aviva, accessed August 27, 2017). Thus, the measurements obtained with this glucometer should be robust to variation in hematocrit concentration since ground squirrel hematocrit concentrations fall within this range [41,42,43].Despite this, the Accu-chek® Aviva has been found to be sensitive to variations in hematocrit in humans [44]. Future studies may want to look into variation in hematocrit ranges within Richardson's ground squirrels. While such variability raises concerns about the comparability of concentration measurements among units, batches of test strips, and the potential applicability of this handheld glucometer model across species, it does appear that with the benefit of validation results, these portable glucometers can prove a valuable research tool.

CONCLUSION

Our validation data reveal that the Accu-chek® Aviva portable glucometer provides reasonably accurate and precise blood glucose concentration readings for blood samples drawn from free living Richardson's ground squirrels, particularly where at least 5 repeated test strip readings are taken for each blood sample. That proven reliability, combined with relatively low cost, minimal blood volume required to obtain a glucose concentration measurement, and convenience of use, should render the Accu-chek® Aviva a useful tool for researchers requiring blood glucose concentration data.

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