A saliva urea test strip for use in feline and canine patients: a pilot study

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Abstract. We evaluated a saliva urea test strip (Kidney-Chek; SN Biomedical), as a rapid, noninvasive method to screen for azotemia. The test is a semiquantitative method that assesses 7 levels of saliva urea concentration, and indirectly serum urea, from <3 to >17 mmol/L. Ninety-two dogs (14 azotemic) with serum urea of 1.3–37 mmol/L and 56 cats (16 azotemic) with serum urea of 4.1–89.3 mmol/L were enrolled. A positive correlation was found for saliva urea against serum urea in each species (dogs: r = 0.30, p < 0.005; cats: r = 0.50, p < 0.001). After turning the semiquantitative data into continuous data by attributing to each level the midpoint of the described range, a receiver operating characteristic curve analysis showed good performance for detecting serum urea above the upper limit of the laboratory RI (dogs: 2.1–11.1 mmol/L; cats: 5–12.9 mmol/L), with an area under the curve of 0.81 in dogs and 0.83 in cats. We recommend that the test be used as an exclusion test, given that it cannot confidently confirm azotemia with higher test results. Additional investigations are recommended for dogs with a test strip reading of ≥9–11 mmol/L and for cats with a test strip reading of ≥12–14 mmol/L.

Keywords: azotemia; cats; dogs; reagent strip; renal insufficiency; saliva; serum urea; urea.
saliva test strip, as well as a relatively large saliva volume requirement (40 µL), which is generally too large a volume to obtain feasibly from a small dog or cat.

We attempted to evaluate the effectiveness of a saliva urea test strip customized for dog and cat use (Kidney-Chek; SN Biomedical) as a measure of serum urea. The Kidney-Chek test strips are designed to be used directly in the mouth of both dogs and cats with no special equipment and with only 3–4 µL of saliva required. The fact that the test strips require no laboratory equipment or trained personnel allow for them to be used as both an in-clinic and at-home option. At-home monitoring provides significant possibilities for improving health outcomes. For example, the long-term use of NSAIDs to treat older, osteoarthritic animals can result in gastrointestinal ulceration and potentiate renal ischemia by inhibition of the cyclooxygenase (COX) pathway and the subsequent synthesis of prostaglandin. At-home monitoring of saliva urea may provide another tool for veterinarians and pet owners to screen for gastrointestinal bleeding, hypovolemic episodes, and renal failure caused by long-term NSAID use. Most importantly, we evaluated the use of saliva urea test strips in cats, in which kidney disease is highly prevalent. To our knowledge, the use of saliva urea test strips to detect azotemia in cats has not been reported.

Our aims were to: 1) evaluate levels of saliva urea, using a saliva test strip, in a wide population of dogs and cats, including healthy, sick, and those with acute and chronic kidney disease; 2) determine the correlation and level of agreement between a saliva urea test strip reading and serum urea; and 3) to evaluate the ability of a saliva urea test strip as a rapid, noninvasive method to screen for azotemia, in canine and feline patients.

Materials and methods

Canine and feline patient enrollment

Our study was a cross-sectional survey of 92 canine and 56 feline patients with a spectrum of health histories, including healthy as well as sick patients with acute and chronic renal disease. All canine and feline patients undergoing venipuncture for blood biochemical assessment were eligible for participation. Four veterinary clinics (Currents Veterinary Centre, Edmonton, AB, Canada; Alberta Helping Animals Society, Edmonton, AB, Canada; Tri-Municipal Veterinary Clinic, Spruce Grove, AB, Canada; Meridian Veterinary Centre, Stony Plain, AB, Canada) were selected as the study sites, and collection took place from February to July 2021. The clinics were chosen based on convenience and a willingness to participate and follow study protocols. The study protocol was approved by the Institutional Animal Care & Use Committee (IACUC) of Chinook Contract Research (approval 20050-001).

Saliva and blood sampling and analysis

All owners gave permission, via a consent form or verbally given limitations of in-person interactions as a result of COVID-19 restrictions, for veterinarians or veterinary technologists to perform sampling and for the results to be published. All patients were fasted for at least 2 h prior to sample collection.

Saliva urea was measured using a saliva test strip (Kidney-Chek; SN Biomedical). The test strip holds 2 sample pads, a test pad, which contains the reactive ingredient urease, the pH indicator bromothymol blue, and a buffer, as well as a control pad, which contains no urease and compensates for variation in saliva pH (Fig. 1). According to the manufacturer, verification of test strip quantitative results used a urea assay test kit (QuantiChrom urea assay kit; BioAssay Systems), which uses an adaptation of a published method, and synthetic dog and cat saliva with urea concentrations of 0–20 mmol/L. The 7 reference color indicators for the test pad, and 3 reference color indicators for the control pad, were determined via 10 replicate tests in 1-mmol/L increments from 0–20 mmol/L urea in synthetic saliva samples of various pHs, and selected ranges that ensured a 90% CI to separate the concentration range associated for each color swatch. Note that the published method is a direct method of measurement for urea and different from the indirect method that was used to analyze urea in serum at Antech Diagnostics Canada, as discussed below.

Following the manufacturer’s protocol, the strip was placed under the tongue or gently rubbed on the patient’s gums for several seconds to ensure that both the test and control pads were fully wetted by saliva. If the sample pads were not fully wetted, the strip was exposed a second time for several seconds. The test strip was then removed from the animal’s mouth, excess saliva removed by dabbing the side of the test strip on a paper towel or tissue, and then allowed to incubate for 2 min to allow the color change to occur. The test strip was then imaged via a smartphone and the color of the test pad and control pad recorded manually by visually comparing with 7 reference color indicators for the test pad,
and 3 reference color indicators for the control pad (Fig. 1). The test and control pad scores were then summed to achieve a final score that was correlated to a certain range of saliva urea concentrations: <3, 3–5, 6–8, 9–11, 12–14, 15–17, and >17 mmol/L. Each veterinary practitioner registered for an account on a custom web-based application (https://snbio-dev.web.app/#/), in order to save images of the test strips. The name of the animal, as well as a time and date stamp, were recorded with the image in the web application. Any animal that participated in the study but did not have a saved image of the test strip after use was eliminated from the study. Following the study, an individual anonymized to the serum urea results then recorded the score of the test pad and control pad from each image by comparing with the reference color indicators. In addition, the test strip was also analyzed without consideration of the control pad. This deviation from the manufacturer’s original protocol was analyzed because it made wetting of the test strip in an animal’s mouth, especially cats, much easier and more consistent given the size of the strip and spatial constraints of the animal’s mouth.

Within 30 min of saliva testing and prior to any other treatments or fluid therapy, a blood sample was taken from the same patient via standard venipuncture collection techniques into a serum separator tube, for serum urea determination. Blood was held at room temperature for 30 min to allow complete clot formation and retraction. Samples were then centrifuged at 3,400 × g for 5 min in a serum separator tube and serum sent for testing in a red-top serum tube to Antech Diagnostics Canada (Adult Chem Panel CSA665; Mississauga, ON, Canada). Serum urea was measured by Antech using an adaptation of a published method22 and a clinical chemistry analyzer (AU680; Beckman Coulter). All saliva urea and serum urea concentrations were recorded in a data collection sheet for each patient along with additional patient information including: species, sex, age, breed, dental condition score (1 = good to 4 = poor), hydration status score (1 = good to 4 = poor), concurrent medical conditions, the reason for blood work, any current medications, as well as International Renal Interest Society staging of kidney disease (http://www.iris-kidney.com/guidelines/staging.html), if present.

**Statistical analysis**

All data were recorded in a spreadsheet (Excel; Microsoft) for analysis. Statistical analysis was completed using a statistical software package (OriginPro 2018; OriginLab). The saliva urea concentration was converted to the midpoint of the described range, used for the visual color scale, so that a continuous variable could be used for statistical analysis. Descriptive statistics of serum urea and saliva urea across the various demographics were tabulated separately for dogs and for cats.

The test correlation was measured using a Spearman rank correlation coefficient between saliva urea and serum urea tests in dogs and in cats. Test performance for detecting serum urea above the upper limit of the laboratory RI was analyzed by measuring sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and by performing a receiver operating characteristic (ROC) curve analysis for various saliva urea test concentrations. Area under the curve (AUC) was calculated for the saliva urea test strip considering the test pad score and the inclusion or exclusion of the control pad score in both dogs and in cats. The upper limit of the RI for determining high serum urea levels (the gold standard) was set at ≥11.1 mmol/L for dogs (RI: 2.1–11.1 mmol/L) and ≥12.9 mmol/L for cats (RI: 5–12.9 mmol/L), as stated and used by Antech Diagnostics Canada.

The correlation between test error and dental score was measured using a Spearman rank correlation coefficient to see if the dental health of the animal had any effect on the error between saliva and serum measurements. The difference between saliva urea and serum urea was measured against a dental score of 1–4.

A Bland–Altman plot was used to visualize the quantitative test agreement between serum urea and saliva urea in dogs and in cats. The difference between the saliva urea and serum urea, in mmol/L, was plotted against the mean of the 2 measurements. The limits of agreement were set to the mean of the differences between saliva urea and serum urea across the population±1.96SD of the differences between saliva urea and serum urea. Normality of the differences was confirmed using a Kolmogorov–Smirnov test (dogs: p=0.57; cats: p=1). Note that if the serum urea was >20 mmol/L, the data point was eliminated from the Bland–Altman analysis because the saliva urea test strips cannot read above 17 mmol/L.

**Results**

**Dogs**

**Descriptive statistics.** We enrolled 92 canine patients in our study, including 14 azotemic patients. The ages of the dogs averaged 9.8±3.7 y (median: 10 y; range: of 0.5–18 y). The serum urea concentration average was 8±5.1 mmol/L (median: 6.8 mmol/L; range: 1.3–37 mmol/L). Non-azotemic dogs had a serum urea average concentration of 6.4±1.8 mmol/L (median: 6.4 mmol/L; range: 1.3–10.9 mmol/L). Azotemic dogs had a serum urea average concentration of 17.3±7.5 mmol/L (median: 14.8 mmol/L; range: 11.2–37 mmol/L). A plateau was observed from 3–11 mmol/L saliva urea, with an increase in serum urea observed at 12–14 mmol/L saliva urea and higher (Fig. 2; Table 1).

**Test performance.** A positive correlation between the saliva urea and serum urea tests was found, with Spearman correlation coefficient r=0.30 (p<0.005). The ROC AUC, which corresponds to the probability that a randomly selected
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individual with high serum urea (≥11.1 mM) has a greater test value than that of a randomly selected individual with normal serum urea, was 0.77 (SE = 0.08; 95% CI = 0.62–0.92) when using the test pad only and 0.81 (SE = 0.06; 95% CI = 0.69–0.94) when using the test pad in conjunction with the control pad (Fig. 3; Table 2). The prevalence of high serum urea (≥11.1 mM) in the study population was 15% (14 azotemic of 92 dogs).

No correlation was found between dental score and the difference between the saliva urea and serum urea concentrations, with a Spearman correlation coefficient of −0.13 (p=0.20), showing that dental condition had no effect on the error between saliva urea and serum urea concentrations in dogs.

Test agreement. The saliva urea generally underestimates the concentration when the mean urea is low and overestimates the concentration as the mean urea increases above ~7 mmol/L (Fig. 4). The largest average differences between the saliva urea and serum urea were found for the saliva urea range of <3 mmol/L.

Cats

Descriptive statistics. We enrolled 56 feline patients in our study, including 16 azotemic patients. The ages of the cats averaged 11.7 ± 4.8 y (median: 12 y; range: 3 mo to 21 y). The serum urea concentration average was 13.5 ± 12.7 mmol/L (median: 10.4 mmol/L; range: 4.1–89.3 mmol/L). Non-azotemic cats had a serum urea average of 9.4 ± 1.8 mmol/L (median: 8.9 mmol/L; range: 4.1–12.7 mmol/L). Azotemic cats had a serum urea average of 23.9 ± 20.6 mmol/L (median: 16.5 mmol/L; range: 13.2–89.3 mmol/L). Generally, a plateau was observed from 3–8 mmol/L saliva urea, with an increase in serum urea observed at 9–11 mmol/L saliva urea and higher (Fig. 5; Table 3).

Test performance. A positive correlation between the saliva urea and serum urea was found, with Spearman correlation coefficient r =0.50 (p<0.001). The ROC AUC, which corresponds to the probability that a randomly selected individual with high serum urea (≥12.9 mmol/L) has a greater test value than that of a randomly selected individual with normal serum urea, was 0.83 (SE=0.07; 95% CI=0.70–0.96) when using the test pad only and 0.73 (SE=0.08; 95% CI=0.57–0.89) when using the control pad in conjunction with the test pad (Fig. 6; Table 4). The prevalence of high serum urea (≥12.9 mmol/L) in the study population was 29% (16 of 56 cats were azotemic).
No correlation was found between dental score and the difference between saliva urea and serum urea concentrations, with Spearman correlation coefficient of 0.05 ($p=0.73$), suggesting that dental condition also did not influence test error in cats.

### Test agreement.

The saliva urea, again, generally underestimates the concentration when the mean urea is low and overestimates the concentration as the mean urea increases above $~11$ mmol/L (Fig. 7). The largest average differences between the saliva urea and serum urea were found for the saliva urea ranges of $<3$ mmol/L and $>17$ mmol/L.

No common error found during the study was the incorrect initial reading of the saliva urea test strips. The test strips frequently showed darker edges, leading to incorrectly high results when compared to the result based on the color in the middle of the strips, or the majority of the strip color (Fig. 8).

### Discussion

The saliva urea test strip showed significant positive correlations against serum urea in both canine and feline patients, with the observed average difference minimally lower for saliva urea ($-0.2$ mmol/L for dogs and $-0.7$ mmol/L for cats). The control pad, which is intended to compensate for variability of saliva pH, improved the performance of the test for dogs (AUC=0.81 with control pad vs. 0.77 without the pad). However, the control pad...
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Table 3. Summary statistics for serum urea across saliva urea levels (mmol/L) in 56 cats.

| Saliva urea (mmol/L) | Frequency | Frequency | Mean | SD | Median |
|---------------------|-----------|-----------|------|----|--------|
| <3                  | 8         | 8.2       | 1.8  | 8.7|        |
| 3–5                 | 6         | 9.2       | 1.6  | 9.0|        |
| 6–8                 | 8         | 9.8       | 3.3  | 8.6|        |
| 9–11                | 7         | 13.7      | 4.7  | 12.0|        |
| 12–14               | 12        | 11.9      | 4.8  | 10.4|        |
| 15–17               | 4         | 14.1      | 1.5  | 14.2|        |
| >17                 | 11        | 24.0      | 25.9 | 14.7|        |

The Bland–Altman plot showed limits of agreement of –8.5 to 8.1 mmol/L in dogs and –10.9 to 9.5 mmol/L in cats. The limits of agreement between the 2 methods appear to be too broad to recommend using the saliva test strip as the sole measure of serum urea levels in making a diagnosis. However, the clinical utility of this test is in its use as an exclusion test and setting up a clinical classification, not as a confirmation test. This modality of testing is similar to the urine cortisol:creatinine ratio (UCCR) test for the screening of hyperadrenocorticism. In dogs, we suggest the following interpretation strategy with normal clinical presentation:

- <3–11 mmol/L: the test strip does not correlate well with serum urea; <3 mmol/L: azotemia can a priori be excluded; 3–5 and 6–8 mmol/L: azotemia is unlikely (NPV of 93%) but cannot be completely ruled out; 9–11 mmol/L: the drop in Se prevents good performance of this test of exclusion; azotemia cannot be ruled out, and further investigation is recommended. The majority of clinically normal dogs are expected to have serum urea within the RI.
- 12–14 mmol/L: azotemia cannot be excluded, and blood testing is recommended; the dog may have serum urea within the RI.
- 15–17 mmol/L: azotemia cannot be excluded, and blood testing is recommended; the cat may still have serum urea within the RI.
- >17 mmol/L: azotemia cannot be ruled out, and blood testing is recommended; the cat may still have serum urea within the RI.

Moreover, in cases in which renal insufficiency is suspected clinically, no strip result can rule out azotemia, and blood testing
is recommended. Although we found that the threshold for recommendation of further testing matches the upper limits of the serum RIs (dogs: saliva 9–11 mmol/L, serum 2.1–11.1 mmol/L; cats: saliva 12–14 mmol/L, serum 5–12.9 mmol/L), if ever the upper limits of RI of the method used by DVMs in various clinics are different, the threshold for recommendation of further testing in dogs and cats should remain 9–11 mmol/L in dogs and 12–14 mmol/L in cats with this saliva test.

An important result found in our study was that dental condition had no effect on the error between saliva urea and serum urea. There were 29 dogs and 8 cats with moderate-to-severe dental disease (rating of 3 or 4 on dental score); however, no correlation was found between the dental score and the difference in saliva urea versus serum urea. It was previously thought that animals with severe dental disease may have significant populations of urease-producing bacterial flora and possibly lead to incorrectly low saliva urea results17; however, our findings suggest that this is not an issue.

We found a lower correlation of saliva urea to serum urea, \((r_s = 0.30, p < 0.005)\) versus \((r_s = 0.63, p < 0.0001)\), than found previously in dogs.17 The sampling method is suspected to be a reason for the lower correlation, given that previous methods used a fixed volume of saliva (40 µL) extracted from the animal’s mouth using a sponge and then centrifuged and pipetted onto the test strip. Simply exposing the test strip to the animal’s gums, as was done in our study, allowed for easier and faster sampling; however, a variable volume was exposed to each test strip. Despite the lower correlation in dogs, the saliva test strip was found to be useful for detecting elevated serum urea with an AUC of 0.81, showing a good-to-very-good accuracy by this

### Table 4. Summary of performance for determining high serum urea (≥ 12.9 mmol/L) in 56 feline patients with various saliva urea cut-points.

| Saliva urea (mmol/L) | Se (%) | Sp (%) | PPV (%) | NPV (%) | True | False | Classified correctly (%) |
|----------------------|--------|--------|---------|---------|------|-------|--------------------------|
| <3                   | 100    | 0      | 29      | NA      | 16   | 0     | 29                       |
| ≥3–5                 | 100    | 20     | 33      | 100     | 16   | 8     | 43                       |
| ≥6–8                 | 100    | 35     | 38      | 100     | 16   | 14    | 54                       |
| ≥9–11                | 94     | 53     | 44      | 95      | 15   | 21    | 64                       |
| ≥12–14               | 81     | 65     | 48      | 90      | 13   | 26    | 70                       |
| ≥15–17               | 63     | 88     | 67      | 85      | 10   | 35    | 80                       |
| ≥17                  | 44     | 90     | 64      | 80      | 7    | 36    | 77                       |

NA = not applicable.

Figure 7. Bland–Altman plot showing the limits of agreement between the saliva urea and serum urea tests for feline subjects. The dotted gray lines are the upper and lower limits of agreement, 9.5 mmol/L and −10.9 mmol/L, respectively; the solid gray line is the observed average difference between the saliva urea and serum urea (−0.7 mmol/L).

Figure 8. An example test strip in which the edges are significantly darker than the center, leading to a falsely high reading if read from the edge. In this example, the attending veterinarian read the test pad as 3 and the control pad as +1, yielding a result of 4 (12–14 mmol/L). When a separate individual, anonymized to the serum urea result, read the strip using the majority of the pad color rather than the edge, the test pad score was 0 and the control pad score was +1, yielding a result of 1 (3–5 mmol/L). The serum urea concentration was 4.6 mmol/L.
standard, and the ability to be used as an exclusion test for clinically normal animals. Previous work in humans determined that serum urea must meet a minimum threshold level before it diffuses into the saliva. This may explain why the correlation coefficient was found to be moderate, but the test strip still performed well in detecting serum urea above the upper limit of the RI. Once the threshold is reached, urea begins to diffuse significantly into the saliva where it is detected by the saliva test strip to signal that azotemia is present.

A limitation of our study was that we did not look at intra- and inter-reliability of test strip reading. We recommend that careful instruction be provided on the use of test strips to prevent incorrect reading of darker edges; standardization via an automated strip reader or phone application could further minimize these errors. An additional limitation was the sample size and number of azotemic dogs enrolled. To achieve a 90% CI width of no wider than 10%, we estimated a population size requirement of 117 dogs and 51 cats. Even though there was reduced power, our study enrolled 92 dogs and 56 cats, and therefore would only result in a slightly reduced statistical power for dogs from that originally calculated.

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Declaration of conflicting interests
MR Nickel and HM Sweet are salaried members of SN Biomedical. MR Nickel took part in data analysis and interpretation. MR Nickel and HM Sweet took part in the writing and editing of the draft manuscript. All other authors had no conflict of interest.

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