

The use of dry reagent strips (dipsticks) is an essential component of a complete urinalysis, in addition to determination of physical properties and analysis of urinary sediment. Dipsticks are easy to use and provide immediate, semiquantitative results. Interpretation of the results provides information regarding urinary tract health as well as systemic health of an animal.

Most commercially available urine dipsticks include test pads for bilirubin, blood, glucose, ketones, leukocytes, nitrites, pH, protein, urobilinogen, and urine SG. Of these tests, results for leukocytes are unreliable, and urobilinogen and nitrites are of little clinical relevance in dogs and cats. Dipstick methods for approximation of SG of dogs and cats are considered unsatisfactory.

**ABBREVIATIONS**

SG  Specific gravity  
UPCR  Urine protein-to-urine creatinine ratio

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**OBJECTIVE**

To evaluate effects of blood contamination on dipstick results, specific gravity (SG), and urine protein-to-urine creatinine ratio (UPCR) for urine samples from dogs and cats.

**SAMPLE**

Urine samples collected from 279 dogs and 120 cats.

**PROCEDURES**

Urine pools were made for each species (dogs [n = 60] and cats [30]). Blood was added to an aliquot of a pool, and serial dilutions were prepared with the remaining urine. Color and dipstick variables were recorded, and SG and UPCR were measured. For cats, 1 set of pools was used; for dogs, 2 sets were used. Comparisons were made between undiluted urine and spiked urine samples for individual colors. Repeated-measures ANOVA on ranks was used to compare dipstick scores and UPCR results; χ² tests were used to compare proteinuria categorizations (nonproteinuric, borderline, or proteinuric).

**RESULTS**

Any blood in the urine resulted in significantly increased dipstick scores for blood. In both species, scores for bilirubin and ketones, pH, and SG were affected by visible blood contamination. No significant difference for the dipstick protein reagent results was evident until a sample was visibly hematuric. The UPCR was significantly increased in dark yellow samples of both species. Proteinuria categorizations differed significantly between undiluted urine and urine of all colors, except light yellow.

**CONCLUSIONS AND CLINICAL RELEVANCE**

Any degree of blood contamination affected results of dipstick analysis. Effects depended on urine color and the variable measured. Microscopic blood contamination may affect the UPCR; thus, blood contamination may be a differential diagnosis for proteinuria in yellow urine samples. (Am J Vet Res 2018;79:525–531)
change in sample color, blood contamination adds constituents (eg, protein) to the urine. Studies conducted to evaluate the effect of blood contamination on the UPCR in urine samples from dogs have yielded conflicting results. Investigators of 1 study concluded that blood contamination increases the UPCR. However, the protein quantification method used (trichloroacetic acid precipitation) is less sensitive, and the reference range for the UPCR (<2.0) in that study was higher than currently accepted UPCR reference ranges of <0.5 in dogs and 0.4 in cats. In contrast, investigators in another study determined that blood contamination should be considered a differential diagnosis for albuminuria only when the urine is red. However, the effect on the UPCR was not evaluated directly; it was inferred. Furthermore, only 3 urine samples were included, and the samples all had undetectable protein concentrations before blood was added, which would not represent the variety of samples collected clinically. Thus, the effect of blood contamination on the UPCR as measured with a sensitive assay, by use of current UPCR reference ranges, and in a large number of randomly collected urine samples from dogs has not been evaluated. Furthermore, to our knowledge, no studies have been conducted to evaluate the effects of blood contamination on the UPCR in urine from cats.

Therefore, the purposes of the study reported here were to determine the effect of blood contamination on results of urine dipstick evaluation and urine SG for samples obtained from dogs and cats as well as the effect of blood contamination on the UPCR for urine samples obtained from dogs and cats. Our hypotheses were that the dipstick results for glucose, bilirubin, blood, ketones, pH, and protein and the urine SG measured with a refractometer would be significantly altered by the presence of blood in the urine of both species and that microscopic blood contamination would significantly increase the UPCR in urine samples obtained from dogs and cats.

**Materials and Methods**

**Sample**

Urine samples submitted consecutively to the Clinical Pathology Laboratory of the Auburn University Veterinary Teaching Hospital for complete urinalysis were used. Urine samples from 120 dogs and 120 cats collected from January 1 to March 31, 2012, were used for evaluation by dipstick analysis and for measurement of UPCR in cats. Urine samples from 159 dogs collected from September 1 to December 31, 2014, were used for measurement of UPCR in dogs. Signalment, method of urine collection, and reason for hospital visit were not evaluated. Free-catch samples were collected into clean vessels by personnel of the veterinary medical teaching hospital. Samples that were visibly red, orange, brown and samples that contained >5 RBCs/hpf or >5 WBCs/hpf (or both) during evaluation of the urine sediment were excluded. Urine was centrifuged at 1,300 X g at room temperature (21°C) for 5 minutes. Supernatants were collected and stored frozen at -20°C until use.

**Procedures**

The day of dipstick analysis and SG measurement, urine supernatants were thawed and centrifuged. Supernatants collected after the second centrifugation were pooled to provide a final volume of ≥7 mL/pool; 30 pools were evaluated for each species. Canine or feline blood was added to an aliquot of each pool at a 1:20 dilution (1 part blood:19 parts urine). The remainder of each pool was used in serial dilutions (1:2), with a final dilution of 1:5,120. Blood that was added to the urine had been collected into EDTA-containing glass tubes. Canine blood was collected the morning of use; feline blood included samples that had been submitted to a clinical pathology laboratory within the previous 24 hours and that had been stored at 4°C. The PCV and total solids concentration of blood were determined before use; results were within reference intervals for the clinical pathology laboratory.

The undiluted pooled urine and dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:1,280, and 1:5,120 were evaluated further; dilutions of 1:640 and 1:2,560 were not evaluated further. For the dipstick analysis, diluted and undiluted samples were maintained at room temperature for 3 hours to replicate a delay between sample collection and dipstick evaluation. Urine samples were evaluated in an arbitrary order. One investigator (HPL) recorded the color of the urine sample as light yellow, dark yellow, light pink, dark pink, or red and measured the SG with a handheld refractometer. The investigator then submerged a dipstick in the sample, tapped the dipstick to remove excess urine, and handed the strip to a trained examiner (who was not aware of the color of the urine sample) to assess the results. Sediment examinations were performed on every dilution of 3 pooled samples for both species to obtain the mean number of RBCs per dilution. A color was assigned for each dilution (Appendix).

To ensure that the presence of EDTA did not affect dipstick performance, EDTA was added to urine at the same concentration that would have been achieved had the tubes of blood been filled with only half their volume. Although the tubes of blood used to create blood contamination appeared to be maximally filled, 2 times the EDTA concentration was used to account for underfilling. Three urine pools for each species were used for this analysis. Serial dilutions for the EDTA-only samples were prepared in the same manner as for urine samples to which blood was added.

Dipsticks were scored in accordance with manufacturer’s instructions. Results for the clinically relevant variables of glucose (scale, 0 to 5), bilirubin (scale, 0 to 3), ketones (scale, 0 to 4), protein (scale, 0 to 4), blood (scale, 0 to 3), and pH were recorded. For scores of protein and ketones, a result of trace was assigned a score of 0.5. For blood scores, results of nonhemolyzed trace, hemolyzed trace, and moderate nonhemolyzed trace were assigned a score of 0.25, 0.5, and 0.75, respectively.
The UPCR measurements in feline urine were performed by use of the same set of pools. After the dipstick analysis and SG measurements, the samples were centrifuged and supernatants were collected and stored at 4°C for a maximum of 20 hours. For UPCR measurement in canine urine, a second set of pools was created; but no dipstick analysis was performed or SG measured. Urine protein and urine creatinine concentrations were determined by use of an automated clinical chemistry analyzer via a turbidimetric method with benzethonium chloride and the Jaffe method with picric acid, respectively. The UPCR was calculated by dividing the urinary protein concentration by the urinary creatinine concentration.

Statistical analysis
Color was recorded; therefore, dilutions with blood were further referred to by their color. Owing to subjectivity of color determination, results for light yellow and dark yellow were used to calculate a mean value (combined yellow samples); results for light pink, dark pink, and red were also used to calculate a mean value (combined red samples). A repeated-measures ANOVA on ranks was used to compare the dipstick scores, SG, and UPCR among individual colors and the undiluted sample. A repeated-measures ANOVA on ranks was used to compare the dipstick scores, SG, and UPCR between the undiluted, combined yellow, and combined red samples. Post hoc comparisons were performed with the Tukey test.

The UPCR results were classified on the basis of proteinuria before and after addition of blood. For cats, the classifications were proteinuric (> 0.4), borderline (0.2 to 0.4), or nonproteinuric (< 0.2). For dogs, the classifications were proteinuric (> 0.5), borderline (0.2 to 0.5), or nonproteinuric (< 0.2). A χ² test was used to compare the degree of proteinuria (nonproteinuric, borderline, or proteinuric) among colors. Only pooled urine samples that had an initial value for each color (nonproteinuric, borderline, or proteinuric) among the undiluted, combined yellow, and combined red samples. Post hoc comparisons were performed with the Tukey test.

The UPCR results were classified on the basis of proteinuria before and after addition of blood. For cats, the classifications were proteinuric (> 0.4), borderline (0.2 to 0.4), or nonproteinuric (< 0.2). For dogs, the classifications were proteinuric (> 0.5), borderline (0.2 to 0.5), or nonproteinuric (< 0.2). A χ² test was used to compare the degree of proteinuria (nonproteinuric, borderline, or proteinuric) among colors. Only pooled urine samples that had an initial UPCR < 0.5 for dogs (n = 19) and < 0.4 for cats (21) were included in the comparison; pooled samples with a UPCR > 0.5 for dogs and > 0.4 for cats were already classified as proteinuric, and the addition of blood could not change that classification (ie, from nonproteinuric or borderline to proteinuric). Significance was set at values of P < 0.05 for all tests.

Results

Effects of EDTA on dipstick results for urine obtained from dogs and cats
The presence of EDTA at any dilution did not change the dipstick results for any variable in urine obtained from dogs or cats (data not shown).

Effects of blood contamination on SG and dipstick results for urine obtained from dogs
The presence of any blood contamination did not significantly affect glucose scores for any individual color of urine or combined color group. In comparison, regardless of the color of the sample, the presence of blood significantly increased scores for blood; once blood was added, most scores were 3 (scale, 0 to 3; Table 1).

For individual colors of urine, significant (P < 0.001) differences were found among scores for bilirubin, ketones, and protein; pH; and SG as determined with a refractometer. Dipstick scores were significantly higher, compared with scores for undiluted urine, for bilirubin in dark pink and red samples, ketones in red samples, and protein in light pink, dark pink, and red samples. Dipstick values for pH were significantly lower in red samples than in undiluted urine. Refractometer values for SG were significantly higher in dark pink and red samples than in undiluted urine.

Specific gravity and scores for bilirubin, ketones, and protein were not significantly different between undiluted and combined yellow samples, but values for the combined red samples were significantly different from values for both the undiluted and combined yellow samples. There was no significant difference in pH among the undiluted, combined yellow, and combined red samples.

Table 1—Median (range) results for variables measured by use of a dipstick or refractometer in urine samples obtained from dogs (n = 30 pooled urine samples at all dilutions).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Undiluted</th>
<th>Light yellow</th>
<th>Dark yellow</th>
<th>Light pink</th>
<th>Dark pink</th>
<th>Red</th>
<th>Combined yellow</th>
<th>Combined red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0 (0–4.0)</td>
<td>0 (0–4.0)</td>
<td>0 (0–5.0)</td>
<td>0 (0–4.0)</td>
<td>0 (0–4.0)</td>
<td>0 (0–4.0)</td>
<td>0 (0–4.0)</td>
<td>0 (0–4.0)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0 (0–2.0)</td>
<td>0 (0–1.0)</td>
<td>0 (0–3.0)</td>
<td>1.0 (0–3.0)</td>
<td>2.0* (0–3.0)</td>
<td>2.5* (1.0–3.0)</td>
<td>0.3 (0–1.0)</td>
<td>2.0 (1.0–2.5)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.1 (0–3.0)</td>
<td>3.0 (1.5–3.0)</td>
<td>3.0 (1.0–3.0)</td>
<td>3.0* (1.0–3.0)</td>
<td>3.0 (1.0–3.0)</td>
<td>3.0* (1.0–3.0)</td>
<td>3.0* (1.0–3.0)</td>
<td>3.0* (1.0–3.0)</td>
</tr>
<tr>
<td>Ketones</td>
<td>0.0 (0–0.5)</td>
<td>0.0 (0–0.5)</td>
<td>0 (0–0.5)</td>
<td>0 (0–1.0)</td>
<td>0 (0–1.0)</td>
<td>0.5 (0–1.3)</td>
<td>0 (0–0.0)</td>
<td>0.3* (0–1.0)</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 (5.0–8.0)</td>
<td>7.0 (6.0–8.0)</td>
<td>7.0 (6.0–8.0)</td>
<td>7.0 (5.5–8.5)</td>
<td>7.0 (6.0–8.0)</td>
<td>6.9 (5.5–8.0)</td>
<td>7.0 (6.3–8.0)</td>
<td>6.4 (5.9–8.1)</td>
</tr>
<tr>
<td>Protein</td>
<td>1.0 (0–3.0)</td>
<td>1.0 (0–3.0)</td>
<td>1.0 (0–3.0)</td>
<td>2.0* (1.0–4.0)</td>
<td>2.0* (1.0–4.0)</td>
<td>4.0 (1.5–4.0)</td>
<td>1.0 (0–3.0)</td>
<td>2.8†* (1.8–3.5)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0 (0–2.0)</td>
<td>0 (0–1.0)</td>
<td>0 (0–3.0)</td>
<td>1.0 (0–3.0)</td>
<td>2.0* (1.0–3.0)</td>
<td>2.5* (1.0–3.0)</td>
<td>0.3 (0–1.0)</td>
<td>2.0 (1.0–2.5)</td>
</tr>
</tbody>
</table>

Glucose was scored on a scale of 0 to 5, bilirubin was scored on a scale of 0 to 3, blood was scored on a scale of 0 to 4, and protein was scored on a scale of 0 to 4. For scores of protein and ketones, a result of trace was assigned a score of 0.5. For blood scores, results of nonhemolyzed trace, hemolyzed trace, and moderate nonhemolyzed trace were assigned a score of 0.25, 0.5, and 0.75, respectively. The SG was measured with a refractometer.

Within a row, value differs significantly (P < 0.05) from the value for the undiluted sample. Within a row, value differs significantly (P < 0.05) from the value for the combined yellow sample.

See Appendix for description of urine dilutions.
Table 2—Median (range) results for variables measured by use of a dipstick or refractometer in urine samples obtained from cats (n = 30 pooled urine samples at all dilutions).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Undiluted</th>
<th>Light yellow</th>
<th>Dark yellow</th>
<th>Light pink</th>
<th>Dark pink</th>
<th>Red</th>
<th>Combined yellow</th>
<th>Combined red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.5)</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.0)</td>
<td>0 (0-4.0)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.5 (0-3.0)</td>
<td>3.0 (1.5-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>3.0 (2.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
</tr>
<tr>
<td>Ketones</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>pH</td>
<td>6.5 (5.0-7.5)</td>
<td>6.5 (5.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
<td>6.5 (6.0-7.5)</td>
</tr>
<tr>
<td>Protein</td>
<td>1.0 (0.5-2.0)</td>
<td>1.0 (0.5-2.5)</td>
<td>1.0 (0.5-4.0)</td>
<td>1.0 (1.0-3.0)</td>
<td>2.0 (0-3.0)</td>
<td>3.0 (1.5-4.0)</td>
<td>1.0 (0.7-2.7)</td>
<td>2.5 (1.5-3.3)</td>
</tr>
<tr>
<td>SG</td>
<td>1.020 (1.00-1.030)</td>
<td>1.020 (1.00-1.030)</td>
<td>1.020 (1.015-1.030)</td>
<td>1.025 (1.015-1.030)</td>
<td>1.025* (1.0135-1.035)</td>
<td>1.028* (1.013-1.030)</td>
<td>1.021 (1.012-1.030)</td>
<td>1.025*† (1.019-1.030)</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Table 3—Median (range) results for UPCR measured by use of a refractometer for urine samples obtained from dogs and cats (n = 30 pooled urine samples at all dilutions for each species).

<table>
<thead>
<tr>
<th>Species</th>
<th>Undiluted</th>
<th>Light yellow</th>
<th>Dark yellow</th>
<th>Light pink</th>
<th>Dark pink</th>
<th>Red</th>
<th>Combined yellow</th>
<th>Combined red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>0.37 (0.06-2.31)</td>
<td>0.39 (0.07-2.39)</td>
<td>0.47* (0.12-2.39)</td>
<td>0.54* (0.18-5.79)</td>
<td>0.71* (0.30-2.63)</td>
<td>1.21* (0.61-9.57)</td>
<td>0.42* (0.06-2.39)</td>
<td>0.95*† (0.44-5.21)</td>
</tr>
<tr>
<td>Feline</td>
<td>0.29 (0.11-2.60)</td>
<td>0.30 (0.13-2.62)</td>
<td>0.46* (0.19-2.72)</td>
<td>0.54* (0.29-2.89)</td>
<td>0.77* (0.42-3.20)</td>
<td>1.51* (0.83-5.19)</td>
<td>0.35* (0.14-2.72)</td>
<td>0.95*† (0.29-5.19)</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Table 4—Classification of the UPCR results in canine urine samples that had a UPCR < 0.5 before the addition of blood to the urine samples (n = 19).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Undiluted</th>
<th>Light yellow*</th>
<th>Dark yellow*</th>
<th>Light pink*</th>
<th>Dark pink*</th>
<th>Red*</th>
<th>Combined yellow*</th>
<th>Combined red*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonproteinuric</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Borderline</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>14</td>
<td>9</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Proteinuric</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td>19</td>
<td>1</td>
<td>19</td>
</tr>
</tbody>
</table>

*The categorization of samples differs significantly (P < 0.05) from the categorization for the undiluted sample.

Effects of blood contamination on SG and dipstick results for urine obtained from cats

Similar to results for urine from dogs, the presence of any blood contamination did not significantly affect glucose scores for any individual color of urine or combined color group. Once blood was added, most scores for blood were 3 (Table 2).

For individual colors of urine, significant (P < 0.001) differences were found among scores for bilirubin, ketones, and protein; pH; and SG as determined with a refractometer. Dipstick scores were significantly higher, compared with scores for undiluted urine, for bilirubin in dark pink and red samples, ketones in red samples, and protein in dark pink and red samples. Dipstick values for pH were significantly lower in red samples than in undiluted urine. Refractometer values for SG were significantly higher in dark pink and red samples than in undiluted urine.

In the grouped samples, scores for bilirubin, ketones, and protein; pH; and SG as determined with a refractometer were not significantly different between the undiluted and combined yellow samples, but values for the combined red samples were significantly different from values for both the undiluted and combined yellow samples.

Effects of blood contamination on the UPCR in urine obtained from dogs

The UPCR in undiluted samples was significantly (P < 0.001) lower, compared with the UPCR in the combined samples and all individual colors of urine, except light yellow (Table 3). In samples that had a nonproteinuric or borderline UPCR prior to the addition of blood (n = 19), the frequency for each classification of UPCR (nonproteinuric, borderline, or proteinuric) was significantly different when comparing results for undiluted samples with results for combined yellow, combined red, and all individual colors of urine, except light yellow (Table 4). The percentage of samples that changed categorization when comparing results for undiluted samples with results for the combined colors was 16% and 100% for combined yellow and combined red, respectively.

Effects of blood contamination on the UPCR in urine obtained from cats

The UPCR in undiluted samples was significantly (P < 0.001) lower, compared with the UPCR in combined samples and all individual colors of urine, except light yellow (Table 3). In samples that had a nonproteinuric or borderline UPCR prior to addition of blood (n = 21), the frequency with which addition of blood changed the classification of samples regarding UPCR classification was significantly dif-

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The percentage of samples that changed categorization when comparing results for undiluted samples with results for each individual color of urine was 14%, 76%, 90%, 100%, and 100% for light yellow, dark yellow, light pink, dark pink, and red, respectively. The percentage of samples that changed categorization when comparing results for undiluted samples with results for the combined groups was 48% and 100% for combined yellow and combined red, respectively.

**Discussion**

Currently, there is a gap in the information about the ways that blood, which commonly enters urine in vivo as a result of a disease process or during sample collection, affects dipstick values or measured UPCR and the importance or impact of those effects. Results of the study reported here indicated that blood significantly affected the dipstick values for all variables, except glucose, in both species; the degree of interference depended on the variable and degree of blood contamination. Visible blood contamination increased the SG values for both dogs and cats. Importantly, the addition of exogenous blood significantly increased the UPCR and affected the classification of proteinuria for both species, even in samples that were not visibly hematuric.

The dipstick score for blood in urine of both species was strongly positive in all diluted samples, regardless of urine color. Given the mean cell counts per hpf, these results were expected. Approximately 5 to 20 RBCs/μL are required to cause a positive reaction.14

The bilirubin score was significantly higher in urine from cats when the urine was dark pink and red. Dogs have a low renal threshold for bilirubin, and bilirubin can be present in the urine of clinically normal dogs.14 However, because cats have a higher renal threshold for bilirubin, a dipstick result that is positive at any level indicates the presence of a hepato-biliary or hemolytic disease process and a need for further diagnostic investigation. In the present study, some undiluted feline urine samples that had a negative bilirubin score subsequently had a positive score for the dark yellow sample. Thus, a positive bilirubin score in cats should be interpreted cautiously when there is blood contamination, even if the contamination is only evident microscopically.

**Table 5**—Classification of the UPCR results in feline urine samples that had a UPCR < 0.4 before the addition of blood to the urine samples (n = 21).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Undiluted</th>
<th>Light yellow</th>
<th>Dark yellow*</th>
<th>Light pink*</th>
<th>Dark pink*</th>
<th>Red*</th>
<th>Combined yellow*</th>
<th>Combined red*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonproteinuric</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Borderline</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Proteinuric</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>11</td>
<td>21</td>
<td>21</td>
<td>4</td>
<td>21</td>
</tr>
</tbody>
</table>

*Classifications were proteinuric (> 0.4), borderline (0.2 to 0.4), or nonproteinuric (< 0.2).

See Table 4 for key.

Compared with results for undiluted urine, pH was significantly lower in red urine samples of dogs and cats and combined red samples of cats. The change in pH in feline samples may have been related to blood pH that was lower than physiologic values as a result of blood storage. The pH in canine blood decreases significantly after storage for 48 hours, likely as a result of ongoing glycolysis and anaerobic cellular metabolism that produce acids.16 Canine blood was collected the day of use; therefore, storage was unlikely a factor that caused the addition of blood to result in a lower urine pH. A falsely low pH may result from improper technique if the acid buffer from the protein reagent pad flows into the pH reagent pad.7 However, because there was a significant change for urine of only 1 color, improper strip use was considered unlikely. Regardless, pH measurements obtained by use of reagent strips should be considered approximations because they are less accurate and less reproducible than are results for pH meters.18 Furthermore, the change in pH was of a small magnitude, so it would be of less clinical importance.

Urine SG, as measured by use of a refractometer, was significantly affected by the presence of blood in both species. Each increase in protein in the urine (1 g of protein/dL of urine) will add approximately 0.003 to 0.005 to the SG.14 However, although this results in significant differences in urine SG, the clinical importance would overall likely be low regardless of the amount of blood or sample color because of the small magnitude of change (0.008). Thus, renal concentrating ability can be evaluated in most animals even in the presence of blood contamination of urine. Possible caveats include a patient with azotemia, visible blood contamination, and an SG slightly < 1.030 for dogs or 1.035 for cats in urine samples without blood contamination; visible blood contamination could increase the SG sufficiently for a patient to be judged as having adequate renal concentrating ability.

The addition of exogenous blood did not result in a positive dipstick score for glucose in any of the canine or feline urine samples. Whether contamination with blood with high glucose concentrations would affect dipstick results was not determined. Scores for ketones were significantly higher only for red and combined red samples of dogs and cats, compared with scores for undiluted samples, which likely was attributable to greater colorimetric interference.
The dipstick protein score was significantly higher in canine urine samples that were light pink, dark pink, or red. To our knowledge, investigators in previous studies have not assessed the effect of blood on the dipstick protein results. For cats, the dipstick protein score was not significantly higher until samples were dark pink.

In comparison with the dipstick score for protein, the effect of blood contamination on the UPCR was detected in samples that were not visibly pink or red. In dog and cat samples, the UPCR was significantly higher in dark yellow and combined yellow samples, compared with the UPCR in undiluted samples. The UPCR and urine albumin concentration-to-urine creatinine concentration ratio reportedly are not affected in urine from dogs until a sample is visibly hematuric.\textsuperscript{10,13} Investigators of a study\textsuperscript{13} concluded that blood contamination may not cause a significant increase in urine albumin concentration until the sample is red; the same relationship was inferred for proteinuria measured by use of the UPCR. However, the effects of blood contamination in that study\textsuperscript{13} were assessed on only 3 pooled samples that had undetectable protein concentrations and to which only small amounts of blood were added. Investigators of another study\textsuperscript{10} used an insensitive method for protein detection and added large amounts of blood. Furthermore, the reference range for the UPCR used in that study\textsuperscript{10} was different than the reference range used for the present study (< 2.0 was considered a normal value for dogs, compared with the value of < 0.5 for the present study). In contrast, results of the present study suggested that the UPCR can be significantly increased before a sample has visible evidence of blood contamination.

Clinical importance of blood contamination is further indicated by the effect on categorization of the degree of proteinuria. Even when there was no visible blood contamination (ie, dark yellow samples), approximately one-third of canine samples and three-fourths of feline samples had a change in categorization after the addition of blood. In the combined yellow group, approximately 48% of the feline samples had a change in category. Classification of a sample is important because it can influence decisions made by clinicians in terms of indication for further diagnostic testing, course of treatment, and prognosis.\textsuperscript{5,12,19} Classification of proteinuria was significantly different for samples included in the present study, even in urine lacking any visible pink or red color.

The study reported here had limitations. First, urine dipsticks were interpreted visually. An automated dipstick analyzer could have provided a more objective interpretation. However, manual inspection is the method most commonly used in clinical practice, and automated analyzers may not be accurate for analysis of urine from cats.\textsuperscript{20} The dipstick examiners were not aware of the color of the urine sample; therefore, bias could not have been introduced because of knowledge of sample color. Second, urine samples used to make the pools for evaluation were collected via a variety of methods (cystocentesis, free catch, and catheterization); all of these methods are used in clinical practice and may introduce some contaminants. Third, patients may have been receiving medications (eg, cephalixin or enrofloxacain) that could have affected dipstick results.\textsuperscript{21} Given the retrospective nature of the study, it was not possible to determine the medication history of all patients. However, because the drug would have been present in all dilutions of that pooled sample, effects of blood contamination could still be determined. In addition, it was likely that only a small number of dogs or cats were receiving medications that would change dipstick results. Fourth, differences in results for the UPCR can be obtained by use of different assays. The analyzer for the study reported here involved use of a turbidimetric method to quantify precipitated protein. Thus, results for the present study may not be applicable when protein concentrations are measured by use of a dye-binding method.

It is important to recognize that the dipsticks were used after addition of blood and without additional centrifugation. The manufacturer's instructions stipulated that urine should be centrifuged before use of dipsticks; however, when centrifugation is not performed before dipstick analysis, results for the present study should be applicable. Because changes in dipstick results were attributable to colorimetric interference and not to contents of RBCs, results for the present study would also likely apply to samples that could not be cleared by centrifugation and in which a pink or red color remained; additional studies would be necessary for confirmation. In contrast, the UPCR was measured after the contamination was removed by centrifugation and did not reflect simple colorimetric interference.

In the present study, blood contamination had significant effects on all urine dipstick results, except for glucose score, for urine samples obtained from both dogs and cats. Even small amounts of blood caused significant alterations in dipstick results. Urine concentrating ability can be assessed in hematuric samples, except for urine samples with an SG of slightly < 1.030 in dogs and 1.035 in cats. Importantly, blood contamination had a greater effect on assessment of protein concentrations than has been reported previously.\textsuperscript{10,13} The UPCR was significantly higher in dark yellow and combined yellow samples, compared with the UPCR in undiluted samples, for both dogs and cats. Similarly, categorization of proteinuria was significantly different, even when blood contamination was not visible. Urine samples with ≥ 250 RBCs/hpf can have a false diagnosis of proteinuria.

Footnotes

a. Clinical Pathology Laboratory, College of Veterinary Medicine, Auburn University, Auburn, Ala.
b. TS 400, Reichert, Depew, NY.
c. MultiStix 10 SG reagent strips, Siemens, Washington, DC.
d. Hitachi 911, Boehringer Mannheim, Indianapolis, Ind.
References

Appendix
Characterization of urine samples used to evaluate effects of blood contamination on dipstick results, SG measured by use of a refractometer, and UPCR.

<table>
<thead>
<tr>
<th>Group</th>
<th>Individual color</th>
<th>Dilution</th>
<th>Mean No. of RBCs/hpf</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined red</td>
<td>Red</td>
<td>1:20</td>
<td>1,147</td>
<td>1,990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:40</td>
<td>920</td>
<td>1,503</td>
</tr>
<tr>
<td></td>
<td>Dark pink</td>
<td>1:80</td>
<td>817</td>
<td>957</td>
</tr>
<tr>
<td></td>
<td>Light pink</td>
<td>1:160</td>
<td>586</td>
<td>843</td>
</tr>
<tr>
<td></td>
<td>Dark yellow</td>
<td>1:320</td>
<td>267</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>Light yellow</td>
<td>1:1,280</td>
<td>153</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:5,120</td>
<td>108</td>
<td>84</td>
</tr>
<tr>
<td>Combined yellow</td>
<td>NA</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td></td>
</tr>
</tbody>
</table>

Undiluted urine samples were spiked with blood (1:20 blood-to-urine dilution) and were then serially diluted. Sediment examinations were performed on all dilutions made from 3 pooled samples of urine for both species; a mean value for the counts of each dilution was obtained for the number of RBCs per hpf. Photographs are representative examples of a urine sample for the various dilutions. NA = Not applicable.