

Comparison of automated colorimetric reagent strip method and sulfosalicylic acid (SSA) precipitation method for detection of urine protein



Proteinuria has been shown to be a valuable prognostic indicator of morbidity and death in dogs and cats with kidney disease. Early and accurate detection of renal proteinuria has important implications for staging, prognosis, and management of kidney disease in dogs and cats.

The semiquantitative protein result included in a urinalysis is considered a screening test for identifying patients who may have protein-losing kidney disease. If protein is identified, then a urine protein:creatinine (UPC) ratio is recommended to evaluate the clinical significance of the proteinuria, including persistence and magnitude, and to determine the treatment and monitoring recommendations. Because the urine protein result determines whether further investigation by UPC ratio is pursued, it is important that the method used has a high sensitivity.

Historically, the colorimetric reagent strip method included on a urine dipstick was considered to have both a high rate of false negatives and of false positives. As a result, IDEXX Reference Laboratories has previously provided urine protein measurement by sulfosalicylic acid (SSA) precipitation measurement. However, in recent years the colorimetric reagent strip method has improved significantly. Recent studies have shown superior performance of the colorimetric reagent strip method as compared to the SSA method. The use of automated analyzers to read the colorimetric reagent strips also removes the element of subjectivity to reading of results, with the potential to further improve accuracy. Automated analyzers are available both in the reference laboratory setting and for in-house use (e.g., IDEXX VetLab® UA™ Analyzer).

IDEXX Reference Laboratories recently performed an internal study to confirm that the same improvement in urine protein accuracy reported in the literature would be seen with the colorimetric reagent strips and automated analyzers used in our laboratories.

As a result of these studies, IDEXX Reference Laboratories will be discontinuing the use of the SSA method for protein in our urinalyses effective October 2, 2018.

Study design

A total of 256 canine and feline urine specimens submitted for urinalysis at IDEXX Reference Laboratories were evaluated for urine protein twice by automated read of the colorimetric reagent strips using the two automated analyzers currently in use at IDEXX Reference Laboratories (CLINITEK Novus® and CLINITEK Atlas®), by SSA read manually by two different urinalysis technicians, and by quantitative measurement of micro total protein (MTP) on the Beckman chemistry analyzer. The MTP, which is the urine chemistry methodology also used in measurement of the UPC ratio, was considered the gold-standard method for comparison.

Correlation of Novus to Atlas automated analyzer colorimetric results

The colorimetric reagent strips utilized by the CLINITEK Novus and the CLINITEK Atlas automated analyzers are identical. Therefore, it was anticipated that results from the two analyzers would be comparable, allowing the colorimetric results from the two analyzers to be combined for the comparison to MTP (see table 1).

Frequency		Novus					Total
		0	1	2	3	4	
Atlas	0	110	3	1	0	0	114
	1	6	44	3	0	0	53
	2	0	5	54	9	0	68
	3	0	0	2	11	1	14
	4	0	0	1	3	3	7
Total		116	52	61	23	4	256

Table 1. Comparison of Novus and Atlas colorimetric results.

A weighted Kappa test statistic of 0.934 95% CI (0.910, 0.958) confirmed near perfect agreement between the two analyzers. The symmetry test statistic ($S = 8.955$) with $Pr > S$ of 0.536 showed that the minor disagreements in results seen were consistent with random variation within instruments.

Intra-assay variability in SSA results across paired measurements

The SSA method for reading urine protein requires a subjective manual read of turbidity, which may introduce an element of variability to results. For each urine specimen, the SSA urine protein was measured by two different urinalysis technicians to assess the degree of variability between readings (see table 2). Assignment as technician 1 or technician 2 varied randomly between each specimen.

Frequency		SSA_2					Total
		0	1	2	3	4	
SSA_1	0	179	15	2	0	0	196
	1	3	13	6	4	0	26
	2	0	4	2	4	3	13
	3	0	0	0	4	12	16
	4	0	0	0	0	5	5
Total		182	32	10	12	20	256

Table 2. Comparison of paired SSA urine protein results.

Agreement between the two SSA readings was not as strong as that between the CLINITEK Novus® and CLINITEK Atlas®, but it was still excellent with a weighted Kappa statistic of 0.876 95% CI (0.836, 0.916). Unlike with the automated colorimetric results, there were more values that differed between paired SSA measurements by greater than one result bucket (e.g., 2+ versus 4+). Differences in results between paired measurements were not symmetric, with the second measurement tending to be higher than the first measurement. This was not a reflection of technician bias, since the technician performing the first versus second measurement was randomized with each specimen.

Disagreement between colorimetric and SSA results

Comparisons were also performed between SSA urine protein and the automated colorimetric results provided by the CLINITEK Atlas and by the CLINITEK Novus. In both cases, there was only fair to moderate agreement between SSA and the CLINITEK Atlas and the CLINITEK Novus colorimetric results (Kappa weighted statistics of 0.453 and 0.390 respectively). The disagreement was asymmetrical, with colorimetric results consistently giving higher urine protein results than those obtained by SSA. In no cases did the colorimetric results report negative protein with a positive SSA result.

Correlation to quantitative urine protein levels

The semiquantitative colorimetric and SSA urine protein results were compared to the quantitative MTP urine protein level as measured on the Beckman Coulter chemistry analyzer (see figure 1). The colorimetric urine protein results showed a stronger correlation (Spearman's rank correlation [ρ] of 0.87 for CLINITEK Atlas and 0.89 for CLINITEK Novus) with the actual quantitative urine protein results than that seen by SSA (Spearman's rank correlation [ρ] of 0.61). Of particular concern is the wide range of quantitative urine protein results greater than 100 mg/dL that may be reported as normal (N: negative or trace) by SSA as compared to what is seen with the colorimetric method.

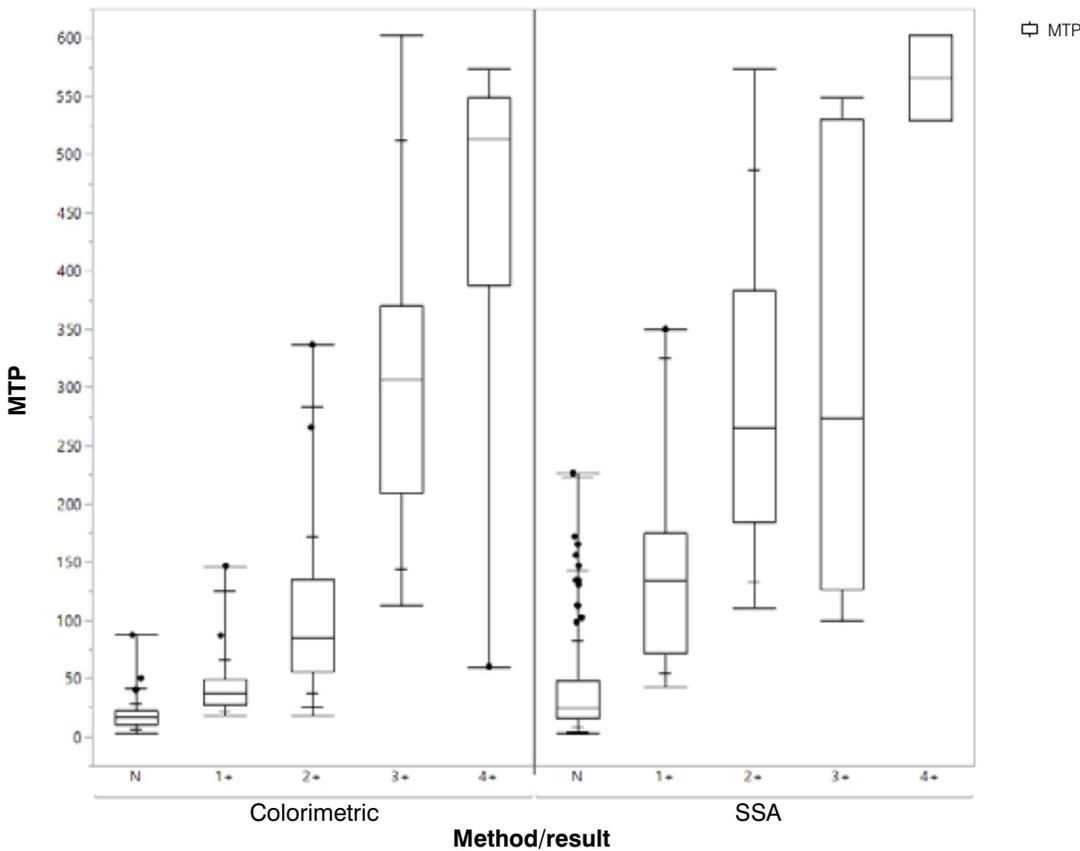


Figure 1. Urine protein results by colorimetric and SSA methods compared to quantitative MTP urine protein.

The sensitivity and specificity of the SSA method and the colorimetric method (CLINITEK Novus and CLINITEK Atlas) for detection of a quantitative urine protein of 30 mg/dL was calculated by comparison to MTP results (see tables 3 and 4). Positive SSA or colorimetric results were defined with a result of 1+, 2+, 3+, or 4+, and positive urine MTP results were any urine protein results > 30 mg/dL as measured on the Beckman Coulter chemistry analyzer. The SSA method showed 100% specificity (result within normal limits when quantitative MTP < 30 mg/dL) but very low sensitivity (66%) for urine protein at this level. Increasing the positive urine protein cutoff to 100 mg/dL only increased the sensitivity of SSA to 76%. In contrast, the colorimetric method showed an excellent sensitivity of 99% for detection of 30 mg/dL urine protein, while still maintaining a very good specificity of 87%.

Quantitative urine protein	Method	Semiquantitative results reported as	Specificity
< 30 mg/dL	Colorimetric	Within normal limits (negative to trace*)	87%
	SSA		100%

Table 3. Specificity of colorimetric versus SSA methods for urine protein levels < 30 mg/dL.

Quantitative urine protein	Method	Semiquantitative results reported as	Sensitivity
≥ 30 mg/dL	Colorimetric	Trace* to 4+	99%
	SSA		66%
≥ 100 mg/dL	Colorimetric	1+ to 4+	100%
	SSA		76%

Table 4. Sensitivity of colorimetric versus SSA methods to detect urine protein ≥ 30 mg/dL and ≥ 100 mg/dL.

*Trace values are considered within the reference interval for urine protein. These low levels of protein may or may not be clinically significant and should be interpreted in light of urine specific gravity, patient history, and presence of other evidence of kidney disease (e.g., high SDMA).

Analysis of interfering factors for urine protein measurement

A multiple linear regression model was performed to determine what other factors might have affected the correlation of SSA or colorimetric urine protein results with the quantitative MTP urine protein levels. Factors assessed included urine color, clarity, presence of blood, bilirubin, and specific gravity. Urine color was the only factor that showed an interfering influence on the colorimetric method for urine protein measurement. For the SSA method, urine clarity, specific gravity, and blood were contributing factors to the poor correlation with quantitative urine protein results.

It should be noted that although pH was not included in this analysis, urine pH is known to have an impact on both colorimetric methods (false positives may rarely occur in highly buffered alkaline urine pH > 7.5) and SSA protein methods (false negatives in highly buffered alkaline urine pH > 7.0).

Summary

Our internal studies showed that the colorimetric method had an excellent sensitivity (99%) for detection of even very low levels of urine protein (≥ 30 mg/dL) while still maintaining a very good specificity (87%). SSA protein measurement, although of high specificity, had too low a sensitivity (66% for ≥ 30 mg/dL and 76% for ≥ 100 mg/dL urine protein) to be useful as a screening test for proteinuria. In addition, the relative degree of proteinuria (1+, 2+, 3+) reported by the colorimetric “reagent” method showed better correlation to the actual quantitative urine protein measurements than those reported by SSA.

The results of this internal study provide confidence that the use of the colorimetric method for detection of urine protein on IDEXX urinalyses will reduce missed diagnoses and discordancies between urinalysis and UPC results, resulting in earlier and more consistent detection of proteinuria and better management of kidney disease in dogs and cats.

Learn more about the diagnosis and management of renal proteinuria at idexx.com/urineprotein.