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Short Communication

Urine protein detection by dipstick: No interference from alkalinity or specific gravity☆

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A B S T R A C T

Tetrabromphenol blue dye based methods are used to detect proteinuria using urinalysis dipsticks. Manufacturers have claimed that alkalinity leads to false positive proteinuria, and that high specific gravity leads to false negative protein results. However, published reports describing this phenomenon remain equivocal. This study aimed to determine whether pH and/or specific gravity affect protein detection in patient urine using three different tetrabromophenol blue dye-based dipsticks.

Patient urine pools were divided into individual aliquots with varied pH or specific gravity, and measured for protein in triplicate using iChem 10SG, iChem Velocity, and Multistix 8SG dipsticks. The pH experiment involved progressive alkalinization of urine aliquots with either 1M NaOH, Na_2CO_3, or $NaHCO_3$; pH was recorded by electrode. The specific gravity experiment involved mixing aliquots with NaCl and spiking with human albumin. Urine electrolytes and total CO_2 were measured (Roche cobas 8000). Fresh patient urines (N = 35) were analyzed for physiological urine pH and total CO_2.

Urine protein results were not affected by NaOH alkalinization up to pH 10.9. False positive protein occurred at pH 9.9 and > 97 mmol/L total CO_2 (Na_2CO_3 alkalization; $P < .05$). Moreover, false positive protein occurred at pH 7.6 when total CO_2 exceeded 137 mmol/L ($NaHCO_3$ alkalization; $P < .05$). Fresh patient urines did not exceed pH 8.5 or 86 mmol/L total CO_2. NaCl elevated specific gravity and caused false negative protein detection when urine ionic strength was > 1100 mmol/L ($P < .05$).

Tetrabromphenol blue dipsticks provide robust detection of proteinuria when human urine is within physiological pH, total CO_2 and ionic strength.

1. Introduction

Urinalysis dipsticks embedded with pH indicator dyes, such as tetrabromphenol blue, are widely used for screening of proteinuria and renal pathology. Anionic albumin selectively extracts bound hydrogen ions to elicit a colour change from yellow to blue. This method offers rapid, inexpensive, semi-quantitative detection of urine proteins, however package inserts and published studies caution that alkalinity, specific gravity, and dyes/detergents interfere with tetrabromphenol blue protein detection [1–3]. Therefore, laboratories may communicate possible interferences to clinicians, or maintain procedures to identify and acidify urines to confirm a protein result. These practices cause clinical uncertainty and additional laboratory costs.

Gyure [1] described several interferences with protein dipstick methods, including false positive protein detection in alkaline urine after Na_2CO_3 addition, and false negative protein in high specific gravity urine after NaCl/KCl addition [1]. However, a critical observation that was not interrogated was the absence of these interferences when pH 10 borate buffer was used to adjust pH, and when glucose or urea were used to adjust specific gravity. Moreover, the authors of the current study are unaware of a single report describing these interferences in patient urine [2]. The purpose of this investigation was to determine whether pH and specific gravity do indeed affect semi-quantitative detection of protein dipsticks using tetrabromphenol blue dye methods in clinical urine specimens.

2. Materials and methods

2.1. Materials

Urine protein was measured using the following methods: iChem 10SG (0.2% tetrabromphenol) measured by Iris iChem 100 (Beckman Coulter, Mississauga, ON); iChem Velocity strips (0.2% tetrabromphenol) measured by iChem Velocity (Beckman Coulter,

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Mississauga, ON); Multistix 8SG (0.3% tetrabromphenol) measured by Clinitek Status + (Siemens Diagnostics, Oakville, ON). All dipsticks report three protein categories (0.3, 1, 5 g/L), and the Clinitek Multistix also report 0.15 g/L. The Roche Cobas 8000 c702 was used to measure urine sodium, chloride, potassium, total calcium, total CO_2, and total protein (Roche Diagnostics, Laval, QC). The Iris iChem 100 claim that pH and specific gravity are interferences in protein detection, the iChem Velocity claims interference with specific gravity, and the Clinitek does not report interference from specific gravity or pH. The pH was measured by standard hydrogen electrode (PHM220, Radiometer Analytical, London, ON). Specific gravity was measured using a refractometer (Amercian Optical Corp, MA). Crystalline NaCl, Na_2CO_3, $NaHCO_3$, and NaOH was reagent grade from Sigma Aldrich (Oakville, ON). Glacial acetic acid was from Fisher Scientific (Ottawa, ON). Use of patient urines throughout these experiments were classified as a quality assurance evaluation activity used for clinical laboratory assessment, management and/or improvement, and was exempt from ethics review by the University of Calgary Conjoint Health Research Ethics Board.

2.2. Urine protein detection with NaOH addition

Random urine specimens negative for all pathological findings on urine dipstick were alkalinized by adding 1 M NaOH dropwise to stirring urine while monitoring pH. Aliquots of urine were sampled into urine sediment tubes as pH levels became incrementally greater. These aliquots were measured in triplicate using each of Multistix, iChem 10 SG, and iChem Velocity test strips (Supplemental Fig. 1A).

2.3. Urine protein detection with carbonate or bicarbonate addition

Random urine samples that were negative for protein and had unremarkable (i.e. negative) results by urine dipstick were pooled (N = 3) and divided into a “carbonate specimen pool” and a “bicarbonate specimen pool”. Graded levels of crystalline Na_2CO_3 were added to the carbonate specimen pool while stirring the urine and monitoring pH. At random pH intervals, 7 mL of the carbonate specimen pool was sampled into urine sediment tubes after recording the pH and the amount of Na_2CO_3 that was added until there were 5 samples with graded levels of Na_2CO_3 from each pool. The same procedure was repeated for the bicarbonate specimen pool using crystalline $NaHCO_3$ in place of Na_2CO_3. The remaining carbonate and bicarbonate specimen pools, containing excess Na_2CO_3 and $NaHCO_3$, were acidified by adding glacial acetic acid to achieve pH 5–6.5. The acidified specimens were sealed for further analysis.

Each urine aliquot was measured in triplicate for protein by the Multistix, iChem 10 SG, and iChem Velocity test strips (Supplemental Fig. 1B).

2.4. Urine pH and total CO_2 from patient samples

Leftover urine from patient specimens from a large urban tertiary care centre, as well as community samples, were analyzed within the clinical laboratory by pH meter and the total CO_2 assay (Roche Cobas 8000). All specimens were measured within 24 h of collection, and the samples were sealed and stored at 4 °C between testing times.

2.5. Specific gravity

Five random urine samples that were negative for protein and other dipstick analytes were aliquoted (4.5 mL) into 5 separate urine sediment tubes containing graded levels of crystalline NaCl. The salt was vortex mixed, and the specific gravity of each tube was measured by refractometer. Urine specimens were then spiked with 12 µL of 25% human albumin (Grifols Therapeutics, NC, USA) and mixed by gentle inversion. Dipstick specific gravity and protein were measured in triplicate with Multistix, iChem 10SG, and iChem Velocity test strips (Supplemental Fig. 1C). Urine sodium, chloride, potassium, total calcium and total protein levels were measured with Roche Cobas 8000. Ionic strength (I) was calculated as $I = \frac{1}{2}\Sigma cz^2$, where c is concentration and z is charge.

2.6. Statistics

Urine protein levels were plotted relative to total CO_2 and pH using GraphPad Prism 8.0 (San Diego CA), and when indicated, they were analyzed by linear and non-linear regression analysis in order to interpolate the pH, total CO_2, specific gravity, and ionic strength required to interfere with tetrabromphenol blue dyes. False positive protein was considered a single pad level change, i.e. from negative to 0.3 g/L for pH experiments. False negative protein was considered a single pad category drop from 1 g/L to 0.3 g/L for specific gravity experiments. Mean concentrations and standard deviation (SD) were determined.

3. Results

The addition of Na_2CO_3 led to false positive protein detection, which was strongly associated with total CO_2 for iChem 10SG, iChem Velocity, and Multistix strips (Table 1, Supplemental Fig. 2), and also with elevated pH for each type of strip (Table 1, Supplemental Fig. 3). The addition of $NaHCO_3$ affected total CO_2 without drastically changing pH (Table 1, Supplemental Fig. 4), but it did cause false positive protein with iChem 10SG, iChem Velocity, and Multistix strips (Table 1, Supplemental Fig. 5). Further, addition of 1 M NaOH to urine did not cause false positive protein up to pH 10.9 (data not shown), which exceeds the physiological range of pH in human urine. However, it is notable that pH 12.5 NaOH stock was positive for protein on all dipsticks. Acidification of specimens that had Na_2CO_3 or $NaHCO_3$ added to

Table 1
Interpolated pH, total CO_2, specific gravity, and ionic strength required to change dipstick protein value after adding crystalline Na_2CO_3, $NaHCO_3$, or NaCl to pooled urines with known levels of protein.

Urinalysis strip	Na_2CO_3 addition			
	Interpolated pH values[a]		Interpolated total CO_2[a]	
	pH	R^2	Total CO_2 (mmol/L)	R^2
iChem 10 SG	10.0	0.472	104.9	0.828
iChem velocity	9.9	0.438	97.1	0.805
Multistix 8SG	10.1	0.525	115.3	0.770
	$NaHCO_3$ addition			
	Interpolated pH values[a]		Interpolated total CO_2[a]	
	pH	R^2	Total CO_2 (mmol/L)	R^2
iChem 10 SG	7.8	0.343	160.8	0.819
iChem velocity	7.6	0.365	137.5	0.805
Multistix 8SG	7.9	0.201	174.2	0.628
	NaCl addition			
	Interpolated specific gravity[a]		Interpolated ionic strength[a]	
	Specific gravity	R^2	Ionic strength	R^2
iChem 10 SG	1.038	0.384	1308.6	0.560
iChem velocity	1.035	0.419	1156.0	0.519
Multistix 8SG	1.049	0.218	1448.0	0.393

[a] Interpolated pH, total CO_2 and ionic strength computed using non-linear regression analysis for a single level change, i.e. from negative to 0.3 g/L for pH experiments or from 1 g/L to 0.3 g/L for specific gravity experiments.

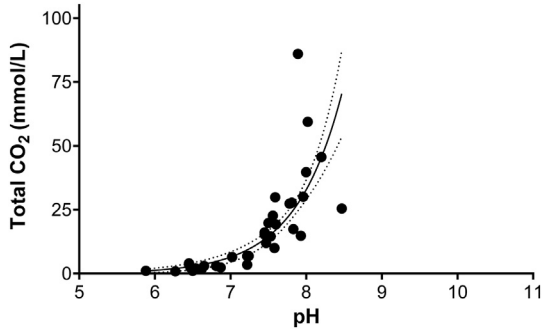

Fig. 1. The relationship between urine pH and Total CO2 levels in random patient urines.

achieve pH < 6.5 caused gaseous CO_2 release, and a reduction by one pad/category on the dipstick for protein detection. It did not, however, result in complete reversal of the interference. Mean protein concentration of urine pools was 0.08 ± 0.02 g/L throughout the experiment. A sampling of patient urines (N = 35) was completed for pH and total CO_2, and there was a non-linear relationship between total CO_2 and pH (R^2 = 0.84) (Fig. 1). The maximum observed pH was 8.5 and total CO_2 was 86.0 mmol/L, and based on the regression it was not possible to interpolate potentially interfering pH and total CO_2 values with confidence. There were no observed false positive protein dipstick results in patient urines.

Elevated urine specific gravity due to NaCl addition caused a single pad/category reduction in protein level for the iChem 10SG, iChem Velocity, and Multistix strips (Table 1, Supplemental Fig. 6). However, this relationship was better associated with calculated urinary ionic strength for iChem 10SG, iChem Velocity, and Multistix strips (Table 1, Supplemental Fig. 7). Prior to NaCl addition, the mean concentration of sodium, chloride, calcium, and potassium was 58.2 ± 52.0, 48.9 ± 31.8, 1.7 ± 1.3, 29.3 ± 25.8 mmol/L, respectively. Mean protein concentration of albumin spiked urine pools was 0.77 ± 0.06 g/L.

4. Discussion

False detection of proteinuria can have serious consequences leading to unnecessary testing and patient anxiety. Accordingly, the investigation of false protein readings with tetrabromphenol blue dye binding methods on urine dipsticks due to alkalinity or specific gravity is important. It was observed that pH alone or specific gravity alone did not affect protein dipstick results under physiological conditions.

The observation that protein interferes with pH measurement when using indicator dyes was first described as the “Protein error of indicators” [2], whereby anionic albumin extracts hydrogen ions bound to dye that elicits a colour change without substantially altering the pH of a solution. This led to the rational expectation that variable urine pH values would affect protein quantitation using tetrabromophenol blue dye. However, tetrabromophenol blue concentration is ≤0.3% in the urinalysis strips, with the remainder predominately composed of strong buffer. Indeed, only 1 M NaOH (pH 12.5) resulted in false-positive protein, at a pH well beyond what could be expected in human urine (Fig. 1) [2]. Whereas addition of anionic carbonate and bicarbonate, with a more modest pH elevation, led to false positive dipstick protein. Carbonate/Bicarbonate buffering is greatest at pH of 6.33 and 10.35, which is equal to pKa of these acid/base pairs, and this attenuates the change in pH that would otherwise accompany the addition of the salts to the urine. Thus, greater interference is seen with a more modest increase in pH.

This study provides an initial description of urinary total CO_2 in patient urines that was non-linear with respect to urinary pH (Fig. 1). No false positive protein results were observed in patients with total CO_2 of up to 86 mmol/L and pH up to 8.5 (Fig. 1). Total CO_2 levels of 97–174 mmol/L, as achieved with sodium carbonate or bicarbonate addition, were necessary to elicit false positive protein detection using three different urinalysis strip types.

Clinical scenarios where greater urine total CO_2 concentrations are expected include renal tubular acidosis (RTA), Fanconi syndrome, and in patients taking carbonic anhydrase inhibitors (eg. acetazolamide) or receiving IV bicarbonate. However, literature does not support that physiological total CO_2 would reach the required threshold identified in this study during these conditions [4–6]. It is acknowledged that the human kidney can produce urine with > 160 mmol/L bicarbonate, however that was achieved during prolonged IV bicarbonate infusions and under meticulous research control setting in healthy men where urine pH remained ≤7.5 [7]. Regardless, the pH and total CO_2 levels required to cause false positive protein using tetrabromphenol dye appear non-physiological (Fig. 1).

The detection of specific gravity by urinalysis strips commonly use pH-sensitive dyes, and thus urine with high specific gravity was expected to interfere with tetrabromphenol blue dye. Indeed, protein dipstick detection was underestimated when specific gravity was elevated by NaCl addition. However, further scrutiny into this phenomenon demonstrates that calculated ionic strength explains the effect of NaCl addition. This observation corroborates other investigations into tetrabrompophenol blue methods, which demonstrated no interference by extremely turbid urines [2], or when specific gravity was adjusted using glucose or urea [1]. The effects of other sources of specific gravity on protein detection was not explored here, and could be considered in future investigations.

Under experimental conditions, since NaCl was added to urines in solid form, it would be expected that other electrolytes such as magnesium, phosphate, and calcium would remain constant while the increase in ionic strength could be attributed to NaCl addition. Na^+ and Cl^- contributions to ionic strength may thus underestimate the total ionic strength in patient urine. In random patient urines, other electrolytes such as potassium, magnesium, phosphate, and calcium would need to be measured to get an accurate estimate of total ionic strength. Ionic strength may act by two mechanisms, whereby excess cations may neutralize anionic albumin or excessive anions may occupy dye binding sites for albumin [8]. Indeed, the Multistix are 0.3% tetrabromophenol blue, rather than 0.2% in other two strips, and were most resistant to ionic strength interferences. Regardless, false negative protein did not occur reliably until sodium and chloride concentrations were > 800 mmol/L. It is important to recognize that specific gravity measured by dipstick or refractometer were poor indicators of this interference.

Elevated CO_2 and ionic strength interfered with tetrabromophenol blue dipstick methods. Urine pH was not suggestive of false positive protein results caused by CO_2, and direct measurement of urine total CO_2 is not routinely performed or validated in most clinical laboratories. Similarly, specific gravity measurement was not suggestive of false negative protein results caused by ionic strength, and this interference is best detected by measuring urine electrolytes. Importantly, this report demonstrates the CO_2 and ionic strength levels required interfere with protein dipstick detection are unlikely to be encountered in patient urine specimens. In conclusion, there is an overall excellent correlation between urine dipstick and urine total protein across the range of physiological specific gravity and pH seen in the clinical laboratory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.07.005.

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