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SHORT COMMUNICATION

Impact of pH on Urine Chemistry Assayed on Roche Analyzers

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SUMMARY

Background: **The pH may impact the concentration of certain urinary parameters, making urine pre-treatment questionable.**
Methods: **1) Determining the impact of pH *in vitro* on the urinary concentration of chemistry parameters assayed on Roche Modular analyzers. 2) Evaluating whether concentrations depended on pH in non-pretreated urines from patients.**
Results: **1) The optimal urinary pH values for each measurement were: 6.3 ± 0.8 (amylase), < 5.5 (calcium and magnesium), < 6.5 (phosphorus), > 6.5 (uric acid). Urinary creatinine, sodium and urea concentrations were not pH-dependent. 2) In urines from patients, the pH was negatively associated with the concentration of some urinary parameters. However, concentrations of all the parameters were strongly and positively correlated with urinary creatinine, and relationships with pH were no longer evidenced after creatinine-normalization.**
Conclusions: **The need for urine pH adjustment does not seem necessary when considering renal function. However, from an analytical and accreditation standpoint, the relationship between urine pH and several parameters justifies its measurement.**
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INTRODUCTION

Urine analysis is essential for the diagnosis of many diseases, in particular for urolithiasis with cristalluria [1]. The main urinary chemical analytes routinely measurable on laboratory analyzers and involved in crystal formation are calcium, phosphate, magnesium, and uric acid. Because of their pH-dependent solubility, urine acidification is recommended for an optimal measurement of calcium, phosphate, and magnesium, while alkalinisation is advisable before uric acid measurement [2-5]. In this way, and as mentioned in the technical sheets, Roche Diagnostics recommends acidifying urine for calcium, magnesium (pH ~1), phosphorus (pH < 3), and alkalinizing urine for uric acid if it cannot be measured immediately. Roche Diagnostics also recom-

mends alkalinizing urine before an amylase assay (pH ~7), whereas it is recommended to measure creatinine and sodium on urine collected without additives. However, pre-treatment of urine with HCl or NaOH/KOH is a time-consuming step which may be perceived as somewhat complicated for healthcare workers. Furthermore, conclusions about the need for pH-adjustment are not consensual depending on studies [6-9], making these recommendations debatable and probably not strictly followed by healthcare personnel. According to the ISO 15189 standard, manufacturer recommendations about urinary pH-adjustment should be enforced for the certification of urinary assays. In order to verify whether Roche Diagnostics' recommendations are necessary, we evaluated the impact of pH *in vitro* on the concentration of urinary chemistry parameters, over a wide and precise pH range. Then, based on a laboratory data extraction, we sought if there was a link between the pH and concentrations of chemistry parameters in non-pretreated urine from patients.

MATERIALS AND METHODS

1) *In vitro* impact of pH on urine chemistry

Twenty-three urines were tested for pH influence, collected from 17 volunteers among the laboratory staff, *plus* 6 patients with cristalluria [10]. Each fresh urine (volume ~50 mL, collected in sterile pot) was immediately sampled into 8 aliquots of 5 mL in tubes, with homogenization between each sampling, in order to prepare a pH range from about 1 to 14 according to the following subranges: < 2; 2 - 4; 4 - 6; 6 - 7.5; 7.5 - 8.5; 8.5 - 10; 10 - 12, and > 12. The urine pH was adjusted with solutions of HCl or KOH 6M prepared in sterile water for injection. Then, for each tube, the pH was measured using a CyberScan pH510 pH-meter (Eutech Instruments, The Netherlands) characterized by a pH range of 0.00 to 14.00, an automatic temperature compensation, and inter-assay coefficients of variation of 0.71% and 0.37% for mean pHs 4.03 and 7.01, respectively. Before assays, urine tubes were centrifuged (10 minutes, 1885 g, 17°C), then the following urinary parameters were assayed in duplicate on Modular P800® analyzers (Roche Diagnostics, Germany): amylase, calcium, creatinine, magnesium, phosphorus, sodium, urea, and uric acid (Supplemental Table 1). For each tube, concentrations were corrected for the dilution factor due to the additional volumes of HCl or KOH added to the initial 5 mL urine sample. Overall, one to three urines per day were processed and assayed within 2 hours after collection. For all analytes, results from each pH-adjusted urine were expressed as % change from the baseline concentration measured in non-pretreated urine. The profiles of % change were plotted and the optimal pH was estimated graphically by considering the analytical change limit (ACL, in %) as the allowable limit of variation, calculated as $1.96*\sqrt{2}*CVa$, wherein CVa was the analytical coefficient of variation (%) calculated from cumulated values of the Urine Chemistry® quality control (Bio-Rad Unity™ Interlab Report; Lot #66710).

2) Relationship between pH and concentrations in non-pretreated urines from patients

Since urinary pH is systematically measured for diagnosis of cristalluria in the nephro-urology department (Pitié-Salpêtrière Hospital, Paris, France), we extracted all the urinary data from patients followed in this department, from October 2014 to February 2016 (Laboratory Information System: GLIMS® software, MIPS-CliniSys, UK). The urine pH and concomitant concentrations of calcium, creatinine, phosphorus, potassium, sodium, urea, and uric acid were measured in non-pretreated urine from a second micturition, collected in a sterile pot (~50 mL) and processed within 2 hours or kept at +4°C until processing within the day. The association degree between urine pH and analyte concentrations was tested using the Spearman's rank correlation test (non-parametric data distribution). This correlation test was also applied between urine pH and analyte values normalized for urinary creatinine concentration to account for differences in renal excretory function. Statistical analysis was performed using MedCalc® software (MedCalc, Mariakerke, Belgium). Normality of data distribution was assessed by the d'Agostino-Pearson test, and a p-value less than 0.05 was considered significant.

RESULTS

1) *In vitro* impact of pH on urine chemistry

The urine pH-concentration relationship profiles are depicted for all parameters in Figure 1A - H. The mean baseline urinary pH was 5.94 ± 0.78. The crystalline species observed in urines from the six patients were: brushite (dicalcium phosphate dihydrate) *plus* uric acid (2 patients), whewellite (calcium oxalate monohydrate) *plus* weddellite (calcium oxalate dihydrate) (1 patient), weddellite (1 patient), uric acid (1 patient), and cystine (1 patient). Overall, the profiles were similar between volunteers and patients with cristalluria. For all subjects taken together, the optimal pH was: 6.3 ± 0.8 for amylase (i.e., maximal enzymatic activity), < 5.5 for calcium and magnesium, < 6.5 for phosphorus, and > 6.5 for uric acid. The urinary concentrations did not vary with pH for creatinine, sodium and urea, i.e., their variations remained within the ACL.

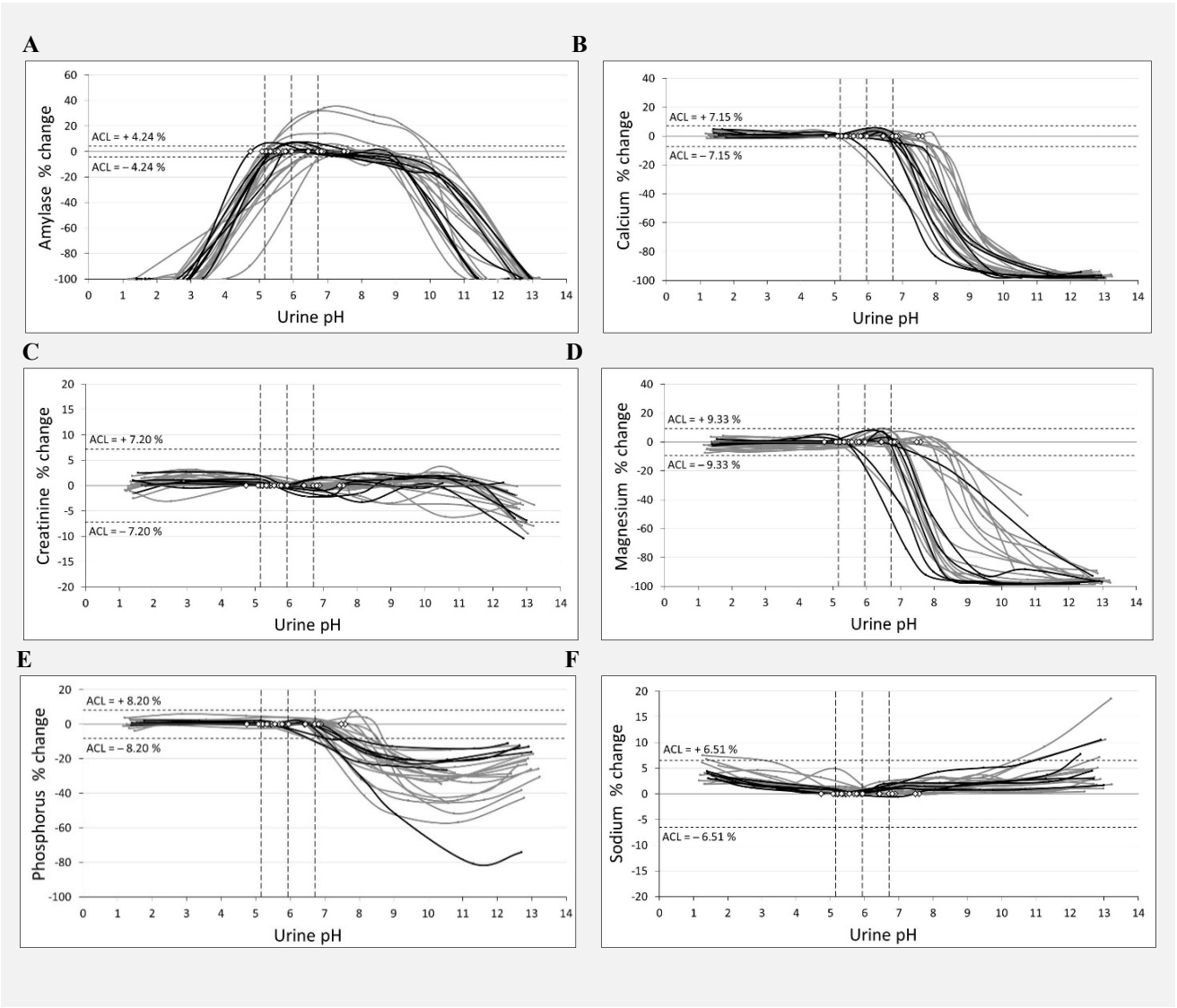
2) Relationship between pH and concentrations in non-pretreated urines from patients

Over the 17-month period, the data extraction led to approximatively 86 to 88 biological files associating pH results with concomitant results of urinary analytes assayed on non-pretreated urines, except for uric acid which was assayed along with pH in 55 biological files. The correlation analysis (Table 1) showed a significant negative relationship between the urine pH and concen-

Table 1. Correlations between pH and analyte concentrations in non-pretreated urines from patients.

Urine analyte	n	pH		Creatinine		Urine analyte	pH	
		ρ_s	p	ρ_s	p		ρ_s	p
Cr	88	-0.261	0.014					
Ca	86	-0.158	0.146	0.562	< 0.0001	Ca/Cr	0.029	0.792
K	88	-0.266	0.012	0.597	< 0.0001	K/Cr	-0.024	0.825
Na	87	-0.061	0.575	0.378	< 0.001	Na/Cr	0.185	0.087
P	88	-0.334	0.002	0.785	< 0.0001	P/Cr	-0.115	0.286
UA	55	-0.330	0.014	0.807	< 0.0001	UA/Cr	-0.126	0.360
Urea	88	-0.386	< 0.001	0.823	< 0.0001	Urea/Cr	-0.053	0.627

Legend: Urinary data were collected over a 17-month period from patients monitored in the Nephro-Urology Department. Abbreviations: Ca - calcium, Cr - creatinine, K - potassium, Na - sodium, P - phosphorus, UA - uric acid, ρ_s - Spearman's rank correlation coefficient.



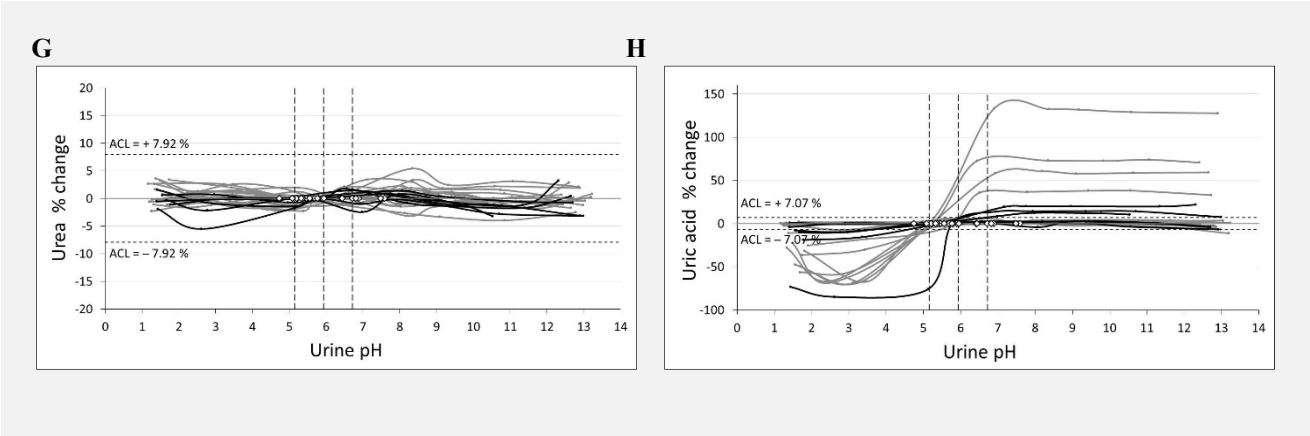


Figure 1.

Legend: The solid grey curves represent the variation in urinary concentration depending on pH for each healthy subject (n = 17), and the solid black curves are for the patients with cristalluria (n = 6), all expressed as % change from baseline concentration of non-treated urines (white dots on the horizontal zero line). The two short-dashed horizontal lines on either side from the zero line represent the analytical change limit equal to 1.96*√2*CVa, wherein CVa is the analytical imprecision. The three middle-dashed vertical lines correspond to the mean pH (central line) and its standard deviations, equal to 5.94 ± 0.78 for the 23 urines. Abbreviations: ACL - analytical change limit.

trations of creatinine, potassium, phosphorus, uric acid, and urea, but no significant correlation was observed between pH and calcium or sodium. However, for all the parameters, urinary concentrations were positively and strongly correlated with urinary creatinine, but none of them remained associated with pH when expressed as creatinine-ratio.

DISCUSSION

First, we confirmed that *in vitro* urinary pH impacts the concentration of amylase, calcium, magnesium, phosphorus, and uric acid, thus supporting the Roche Diagnostics recommendations. Second, the observational sub-study showed that, for some of these parameters, results may be considered as varying with pH from a statistical standpoint, but not when concentrations are creatinine-normalized.

Given the results from our first *in vitro* pH-based substudy, one could have expected higher calcium and phosphorus concentrations in the most acid urines from patients, as well as higher uric acid levels in the most alkaline ones, which was not the case. Besides the phenomenon of phosphate-dependent precipitation [11], calcium concentration has been shown to decrease with time in non-treated urines stored at 4°C, whereas it remained stable in acidified urines [5]. Also in a time- and concentration-dependent manner, uric acid decreases in non-treated urines but not in alkalinized urines [5]. Our goal was to determine the specific impact of pH on urinary parameters, which has never been assessed in such a detailed way. Therefore, we did not evaluate the influence of time and/or temperature of storage, nor to what extent the presence and/or type of cristalluria may influence urinary concentrations.

Overall in our study, the *in vitro* relationships between pH and concentrations were not those expected on patients' results, suggesting a minor impact of pH with regard to the renal function or other potential influencing factors related to the patient and/or treatment, as suggested elsewhere [6,7,9]. However, although a pH adjustment does not seem necessary for chemistry measurement in view of our results, its knowledge remains useful for correctly identifying urinary crystals. Indeed, the precipitation of uric acid, amorphous urates or cystine crystals is favored at pH ≤ 5.8, that of mono- or bihydrated calcium oxalate or cholesterol crystals favored at pH 5.4 to 6.7, and that of calcium phosphate or amorphous phosphate crystals at pH ≥ 7.0 and, in addition, crystallization of some drugs may occur at certain levels of pH, like indinavir, amoxicillin or ciprofloxacin [12]. Furthermore, the measurement of urinary pH, along with urinary parameters could help determine - and thus prevent and treat - the risk of urinary stone-formation, as recently proposed through nomogram-derived mathematical equations, which can be parameterized in the laboratory information system [13]. Ideally, the next generations of analyzers should integrate a systematic measurement of urine pH for any urinary chemistry assay, as done on the Cobas® 6500 urine analyzer, while keeping in mind the present results as support for expertise rules to be set up in the middleware.

CONCLUSION

The absence of a relationship between the urine pH and chemistry parameters when considering renal function does not support its adjustment in clinical practice. However, from an analytical standpoint, several urinary parameters depend on the pH, which should therefore be measured to validate the result. Altogether, our findings support the need for clear guidelines, mentioning whether or not urine pH adjustment is necessary, and should encourage manufacturers to develop an integrated system for systematic measurement of urine pH on their future analyzers.

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Declaration of Interest:
None declared.

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Supplementary Table:

Table 1. Principles of methods and references of Roche Diagnostics reagents.

Analyte	Principle of methods/technology	Wavelength (nm)	Reagent references
Amylase	IFCC/G7PNP enzymatic/Spectrophotometry	415 - 700	11876473316
Calcium	NM-BAPTA complex/Spectrophotometry	340 - 376	05061431190/05061458190
Creatinine	Jaffé Alkaline picrate-kinetic rate blanked, IFCC-IDMS Standardized/Spectrophotometry	505 - 570	11875663216/11929941216
Magnesium	Xylidyl blue colorimetry/Spectrophotometry	600 - 505	11929615216/11929623216
Phosphorus	Phosphomolybdate colorimetry/Spectrophotometry	340 - 700	11875949216/11875965216
Sodium	Ion Selective Electrodes/Indirect potentiometry	na	11183974216
Urea	Urease enzymatic/Spectrophotometry	340 - 700	11929470216/11929488216
Uric acid	Uricase enzymatic/Spectrophotometry	546 - 700	11929429216/11929437216

Abbreviations: IFCC - International Federation of Clinical Chemistry, na - not applicable.