mmol/L aqueous KSCN (Merck), corresponding to 12.5 μmoles of KSCN, to 10 mL of ISE solution 1, which contains 115 mmol/L NaCl. Because of the high sensitivity of the chloride electrode for KSCN, this solution mimics a chloride concentration of ~120 mmol/L. A loss of selectivity should lead to markedly increased values.

After placement of a new electrode, results for the selectivity control solution gradually increased with a final peak at 2–6 weeks (Fig. 1). After replacement of the electrode, selectivity control solution results returned to the expected concentration range (Fig. 1). Results for control A showed this effect less well and could not demonstrate unambiguously deterioration of the electrode (Fig. 1). Control B varied statistically around the target value (Fig. 1) and therefore was not a suitable indicator.

The time dependence of the deterioration was consistent with an “aging” of the electrode, which could be the reason for the emerging loss of selectivity. One possible explanation for this effect could be the coating of the membrane with protein, particularly fibrinogen. Daily preventive maintenance performed according to the manufacturer’s instructions did not improve our results sufficiently. After such a procedure the selectivity of a worsening electrode was reconstituted for some hours only. We believe that this short-lived reconstitution was attributable to partial peeling of the electrode surface. After the maintenance procedure, falsely increased results for patient samples were detected more frequently than in the first hours after reconstitution. Repeat analyses with the coulometric reference method revealed significantly lower chloride concentrations [mean (SD) difference, 3 (3.7) mmol/L] in most cases. In 3% of those patient samples, the differences were >10 mmol/L.

Replacing the chloride electrode whenever the result for selectivity control solution exceeded its 3 SD limit reduced the rate of falsely high (difference for ISE minus coulometry >10 mmol/L) results to 0.3%. The mean difference between ISE and coulometry has been halved [mean (SD) difference, 1.7 (1.75) mmol/L]. Thus the introduction of this selectivity control solution offers a novel and inexpensive way to achieve a maximum useful lifetime of the chloride electrode.

Roche Diagnostics supported our work by substitution of defective electrodes free of charge.

References
and 10.3 mmol/L. It also be beneficial if the manufacturer is conservative in urine samples. It would indicate the effect of acetic acid as a pre-treatment of urine aliquots, using a 1:21 dilution according to manufacturer’s recommendations.

The mean relative error for samples acidified with acetic acid was 17.3% with a range of 7.4–36.7%. Samples acidified with HCl yielded a mean relative error of 3.0% with a range of −2.6% to 10.6%.

Further investigation of the effect of acetic acid included titration of three urine samples with an initial pH of 7 and initial creatinine concentrations of 2.3, 4.0, and 10.3 mmol/L. To 1-mL aliquots we added 10, 20, 30, and 35 μL of 50% glacial acetic acid. Creatinine and pH were measured on each aliquot as described above, with creatinine concentrations corrected for dilution. The results, summarized in Fig. 1, demonstrated that relative error is dependent on creatinine concentration and pH, with greater positive interference occurring at lower pH.

We would therefore caution users of the Vitros CREA reagent to evaluate the effect of acetic acid as a preservative in urine samples. It would also be beneficial if the manufacturer’s reagent instructions were more specific with regard to conditions of interference testing.

Reference

Fig. 1. Error in creatinine measurements for urine samples acidified with acetic acid.
Initial creatinine concentrations at pH 7 were 2.3 (●), 4.0 (■), and 10.3 mmol/L (▲).

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Representatives of Ortho-Clinical Diagnostics respond:

To the Editor:

In their letter, Lambert et al. describe a positive interference in creatinine measurements in urine specimens acidified to pH 3.0 with glacial acetic acid when assayed with VITROS Chemistry Products CREA Slides. The authors state that these findings are inconsistent with the VITROS Creatinine Instructions for Use document (1). According to the authors, the VITROS Instructions for Use indicates that both 12 mol/L HCl and glacial acetic acid have an effect of <2% on VITROS creatinine results.

The VITROS Instructions for Use statement (1) is limited to standard use of glacial acetic acid or HCl only as a urine preservative and does not address the situation described by Lambert et al., where the objective was to adjust sample pH to 3.0 or lower to optimize conditions for measuring vanillylmandelic acid (VMA) and homovanillic acid (HVA). The Instructions for Use, the reference cited by Lambert et al., states: “Urine: The following preservatives have been tested and demonstrated an effect of <2% on creatinine results: thymol, toluene, boric acid, glacial acetic acid, 12 N HCl, NH4OH, bromide, iodide, 5% NaOH” (1). This statement is based on studies designed to simulate conditions for use of the concentrated acids as preservatives in a 24-h urine collection jug, consistent with recommendations given in standard clinical chemistry textbooks. One example of such recommendations is provided in J.B. Henry’s Clinical Diagnosis and Management by Laboratory Methods (2). Typically, 15 mL of glacial acetic acid or 12 mol/L HCl is added to standard collection jugs for 24-h urine samples before initiation of urine collection, leading to typical volume fractions of the respective acid in the final collected urine in the range of 0.5–1% for the majority of samples.

To investigate the authors’ findings, we collected 10 random urine samples and acidified an aliquot of each sample with either glacial acetic acid or 12 mol/L HCl to a pH of ~3. After pH adjustment, the samples were diluted 21-fold and analyzed on a VITROS 950 Chemistry System. In our studies, the amount of glacial acetic acid required to adjust to pH 3.0 varied from a low of 1.9% (by volume) up to as much as 9% (by volume). The amount of concentrated HCl added to achieve pH 3.0 ranged from 0.1% to 0.7% (by volume). The differences in measured creatinine concentrations were highly variable when glacial acetic acid was added, with measured concentrations ranging from ~40% to ~155% of the expected. After addition of glacial acetic acid to achieve pH 3.0, the mean measured creatinine concentration was 117% of the expected, consistent with the mean difference reported by Lambert et al. In specimen aliquots treated with HCl, the difference was <2% for all
samples tested, consistent with the VITROS document (1) and the findings of Lambert et al.

Our investigation indicated that the volume fraction of glacial acetic acid required to achieve a pH of 3.0 was substantially different from the volume fraction originally studied to establish the information in our labeling, in some cases well beyond what might be characterized as “minimal” dilution of the sample. In an additional experiment, glacial acetic acid was added to a urine pool at 0.25%, 0.50%, 1.0%, and 2.0% (by volume). The results of this experiment demonstrated that the differences in measured creatinine generated by addition of glacial acetic acid to volume fractions consistent with its use as a urine preservative (0.5–1.0% by volume) are within the range described in the VITROS document (1). However, at a volume fraction of 2% glacial acetic acid, a difference of ~10% was observed, consistent with the findings of Lambert et al. and with our own current studies. We have not systematically studied the effect of larger volume fractions of glacial acetic acid, but based on these results and all of the data now available, we would expect incremental differences with larger volume fractions of this acid.

We thank the authors for this new information, and we will update the Instructions for Use for VITROS CREA Slides to include a precaution concerning the use of glacial acetic acid when acidifying specimens to achieve a particular low pH.

References
