period, higher concentrations of CsA metabolites can arise from a slower rate of metabolism because of temporary liver congestion.

In conclusion we found EMIT to be a rapid, viable, and specific assay for measuring CsA concentrations in transplant recipients. Obviously, however, an adjustment of the CsA therapeutic range for the EMIT is necessary.

References


Availability of Assays for Definitive Diagnosis of Primary Hyperoxaluria Types 1 and 2

To the Editor:

We read with interest the paper by Dietzen et al. [1] on improving the extraction and therefore the quantification of urinary glycerate and glycolate. Since this paper went to press, there have been further developments in the field of primary hyperoxaluria (PH) that may be of interest to your readers. Dietzen et al. correctly state that normal glycolate excretion does not exclude PH1. How- ever, we now know that increased glycolate excretion cannot always confirm a diagnosis of this disease [2] and personal observations, so measurement of urinary metabolites cannot be conclusive. In addition, although l-glycerate is a marker of PH2, most methods cannot distinguish between the d and l isomers and, because of the small numbers studied, we do not yet know whether excretion of the l-isomer is unequivocally increased in all cases of PH2.

There is therefore a requirement for definitive diagnosis of these diseases by measurement of enzyme activity—not only because of the implications for treatment (hepatic vs renal transplantation) but also for counseling of families with respect to risk of recurrence. Methods are now available for the measurement of the relevant enzymes for PH1 and PH2 in a single needle liver biopsy [3, 4].

References


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Cautionary Note Regarding Urinary Calculi Analysis with the Merckognost® Kit

To the Editor:

Our laboratory has been using the Merckognost® (E. Merck) urinary calculi analysis kit for renal calculi analysis. A review of results from
1994 (performed with lot nos. 03053, 03050, 07092, 16124, 10034, 14085, 16124, and 12036) showed the presence of calcium oxalate in all stones except one that was pure uric acid. Although calcium oxalate because of its high insolubility is the most common component of renal calculi, its presence in 99.8% of the stones analyzed was much higher than reported in literature (Table 1). In a large series of 10,438 stones analyzed by infrared spectroscopy with microsampling [1], calcium oxalate was reported present in 86% of stones. In addition, the absence of pure calcium phosphate stones was unusual, because most studies report 5–21% of stones as pure calcium phosphate [2–4].

The reagent kit uses chemical methods for determinations: titrimetric assay for calcium (disodium EDTA, calcium oxalate, and colorimetric assays for oxalate) and phosphates, magnesium, ammonium, uric acid, and cystine. A ruler is provided to help estimate the percentages of calcium oxalate, calcium phosphate (either brushite or apatite), struvite, and ammonium urate.

Review of our quality-control data showed that results for the kit control were within stated ranges for all components, with the results for calcium and oxalate being on the high ends of the ranges. However, blank testing with acidified deionized water gave the result 10% oxalate and 5% calcium. Water from various sources in the laboratory (distilled, deionized grade for liquid chromatography work; deionized and filtered, 18 mOhm resistance) and from two other laboratories in Ontario gave similar results. The low background positivity of the solvent likely explains the high frequency of calcium oxalate found in the stones studied here. If the background calcium and oxalate values were subtracted before calculation of stone composition, the reported percentage of calcium oxalate as a component would fall to 86.7%, which compares well with data from the literature [1]. However, the percentage of pure stones would increase to 52% (calcium oxalate 42.0%, calcium phosphate 4.2%, uric acid 4.3%, cystine 1.3%). The high percentage of pure calcium oxalate may be explained by the lesser ability of chemical analysis to detect phosphate and uric acid. Furthermore, a larger proportion of calcium phosphate stones would be reported as brushite (CaHPO$_4$$\cdot$2H$_2$O) instead of the more chemically stable apatite [Ca$_{10}$PO$_4$$\cdot$5(OH)$_2$]. Finally, indiscriminate blanking could result in reports of stones with only anionic or cationic components.

This error in analysis carries important clinical implications. For calcium phosphate and purine-containing stones, it is important to know what the major component is. Calcium phosphate in trace amounts is common, as brushite is supersaturated at the higher pH and filtrate concentration usually occurring at the loop of Henle, and crystals formed may act as heterogeneous nuclei for calcium oxalate stones [5]. Hence, calcium oxalate stones with small amounts of calcium phosphate are managed the same as calcium oxalate stones, by hydration and reduction of existing hypercalciuria, hyperoxaluria, hyperuricosuria, and (or) hypocitraturia [6], whereas predominantly calcium phosphate calculi, which occur in renal tubular acidosis or hyperparathyroidism [5], may require definitive management. Similarly, pure uric acid and combination urate–calcium oxalate stones, which have different etiologies, must be distinguished [7]. Uric acid stones, formed when hyperuricosuria occurs in association with low urine pH, are managed with urinary alkalinization, whereas calcium oxalate–monosodium urate stones are the result of hyperuricosuria in a neutral or alkaline medium and are managed the same way as calcium oxalate stones [7]. Finally, tissue and drug calculi could be mistakenly reported as calcium oxalate.

We do not have a solution for this situation. Blanking raises many problems. Reporting the percentages of each component, accompanied by the warning that calcium oxalate in low concentrations may be an artifact, may help reduce clinical misinterpretation of results.

### References


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A spokesperson from Merck responds:

To the Editor:

We have not claimed that our semi-quantitative analysis is comparable with a high-sensitivity infrared spectroscopic analysis. Nevertheless, Merck will evaluate the determination of oxalate to check whether it is necessary to modify the oxalate color scale to prevent erroneous interpretations (background). Blanking with water is absolutely not allowed because all reactions work correctly only under acid conditions; the urate calculi must be dissolved in concentrated sulfuric acid and water, and all tests are optimized for these conditions.

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Cross-Reactivity of Fosphenytoin in Four Phenytoin Immunoassays

To the Editor:

Fosphenytoin, 5,5-diphenyl-3-[phosphonoxy)methyl]-2,4-imidazolidinedione disodium salt (Cerebyx®; Parke-Davis), is a phosphorylated form of the anticonvulsant drug phenytoin. Fosphenytoin itself has no pharmacological activity but is dephosphorylated in vivo by phosphorylases to the active drug, phenytoin. The elimination half-life of fosphenytoin in plasma is 8–15 min in healthy subjects [1, 2]. Fosphenytoin can be used for parenteral or intramuscular administration, where its superior aqueous solubility results in less severe side effects than does phenytoin [2, 3]. Few data, however, are available on the interference of fosphenytoin in phenytoin immunoassays, which are currently the most common method used to monitor patients’ phenytoin concentrations. In an abstract [4], Kugler et al. reported that the TDx® Phenytoin assay (Abbott Labs.) was interfered with by fosphenytoin. We report here the results of detailed cross-reactivity studies for fosphenytoin in four phenytoin immunoassays: ACS:180® Automated Chemiluminescent System (Chiron Diagnostics), TDx Phenytoin and Phenytoin II, and AxSym® Phenytoin II (also from Abbott Labs.).

A therapeutic total plasma phenytoin concentration (>10 mg/L) is attained within 10–30 min after administration of fosphenytoin. Conversion of fosphenytoin to phenytoin is reported to be complete in 2–4 h, depending on mode and rate of administration [5]. The plasma concentration of fosphenytoin depends on the route of administration and length of time between administration and patient sampling. The ordinary half-life of fosphenytoin is reduced by ~50% in patients with hepatic or renal diseases, apparently because of less protein binding of the prodrug [5].

A stock solution of fosphenytoin (a gift from Parke-Davis) in methanol was added to two separate serum pools—one without phenytoin (pool A), and the other containing 12.4 mg/L of phenytoin (pool B)—to give final fosphenytoin concentrations of 52, 39, 26, and 13 mg/L. Apparent phenytoin concentrations in both sets of samples were measured by all four assays according to the manufacturers’ directions.

The TDx and AxSym assays use homogeneous fluorescence polarization (FPIA) technology and were run on the TDxFlex® and AxSym automated analyzers, respectively. The TDx Phenytoin (TDx) assay uses a polyclonal sheep anti-phenytoin antiserum, whereas the TDx Phenytoin II (TDx-II) and AxSym Phenytoin II (AxSym-II) assays use murine monoclonal antisera. The ACS:180 Phenytoin (ACS:180) is a heterogeneous chemiluminescent immunoassay that uses murine monoclonal antibody; it was run on the automated, random-access ACS:180 chemiluminescent system [6–8]. The analytical range (0.5–40 mg/L) and detection limit (1 mg/L) for the TDx and ACS:180 assays are similar [8], but the AxSym-II assay has a lower detection limit (0.5 mg/L). Results of the ACS:180 assay agree well with both TDx and TDx-II assays [7, 8], whereas the AxSym-II assay correlates with the TDx-II assay (AxSym-II package insert).

The results for fosphenytoin cross-reactivity in pool A for the four phenytoin assays (Table 1) show that the TDx-II cross-reacted very strongly (>250%) with fosphenytoin; such cross-reactivity would result in seriously high TDx-II assay results, even in samples containing very low concentrations (<5 mg/L) of fosphenytoin. Fosphenytoin cross-reactivity in the absence of phenytoin for the other three assays exhibited the following order: ACS:180 > TDx > TDx-II > AxSym-II. However, although the ACS:180 cross-reactivity was independent of fosphenytoin concentration, that of TDx and AxSym-II decreased with increasing concentrations of fosphenytoin.

Table 1 also presents our data on the interference of fosphenytoin in the four assays in serum pool B, containing 12.4 mg/L phenytoin. The fosphenytoin cross-reactivity in TDx and AxSym-II assays was ~2- and 3-fold greater, respectively, in the presence of phenytoin than in its absence. Given that the rate of fosphenytoin metabolism may differ from person to person or in different disease states, the higher cross-reactivity in presence of phenytoin is of concern. Cross-reactivity in the ACS:180 assay, however, was indepen-