Screening Assay for Ethylene Glycol in Serum

To the Editor:
The recent paper by Eder et al. (1) recalls, among other things, the problems that a clinical STAT laboratory has to face in the case of ethylene glycol poisoning. As an alternative to the precise and accurate gas chromatographic technique, the enzymatic assay with glycerol dehydrogenase (EC 1.1.1.6) and NADH as tracer has been proposed by various authors [see, e.g., (2–4)]. The relative nonspecificity of glycerol dehydrogenase from Enterobacter aerogenes toward diols and triols allows interference with this test by propylene glycol, glycerol, 1,2-butanediol, and 2,3-butanediol (2, 5, 6). Nonetheless, its usefulness in emergency screening for ethylene glycol has been stressed, particularly for its technical simplicity (2).

Rynder et al. (2) used an analyzer, the Du Pont aca® SX (presently distributed by Dade Behring Inc., Deerfield, IL), that is particularly suited for emergency situations. Presently this instrument, together with the more recent version, the aca STAR, is still widely used in laboratories both in this country and in the United States. Unfortunately, the product that, following the method of Rynder et al., we were using to avoid interference from endogenous triacylglycerols and glycerol (triglyceride test kit for Monarch, cat. no. 35190, Instrumentation Laboratory) is no longer available in our country.

Because skilled personnel are not available at all times in our emergency laboratory and because cases of ethylene glycol poisoning are infrequent but not uncommon, I decided to continue to use the aca method whenever the gas chromatographic technique was not available. The modification I make is trivial: I cut compartment no. 2 (ingredients: lipase and stabilizers) of the aca triglyceride analytical test pack (Du Pont, cat. no. 702230901), wash it with deionized water, and firmly reseal the hole with transparent adhesive tape. I calibrate the test with a 16 mmol/L solution of ethylene glycol in pooled sera that are free of ethylene glycol, as assessed by gas chromatographic analysis, and use the same pooled sera as a blank. Linearity was studied by serial dilutions of the calibrator in ethylene glycol-free pooled sera down to the concentration of 1.6 mmol/L. The linear regression analysis of the results gave the following statistics: dose = 0.955 × response − 0.04 mmol/L, n = 10, r = 0.995, $S_{\text{dev}} = 0.41$ mmol/L. The test is recalibrated every month.

In our laboratory, we are aware that the results obtained in this way need cautious interpretation and gas chromatographic confirmation; however, this test permits us to screen economically for ethylene glycol with a typical turnaround time <30 min.

References

Why is Troponin T Increased in the Serum of Patients with End-Stage Renal Disease?

To the Editor:
Haller et al. (1) have strengthened the argument that the increased serum cardiac troponin T (cTnT) found in many patients with end-stage renal disease (ESRD) originates in heart muscle and not regenerating skeletal muscle, as suggested by others (2). We recently measured serum cTnT using the Elecsys 2010 immunoassay system (Boehringer Mannheim), a method that is specific for cTnT (3), and creatine kinase MB isoenzyme (CK-MB) mass (Dade Stratus II immunoassay system) in 71 stable patients with ESRD. The serum cTnT was >0.1 μg/L in 37 (52%) of them, and interestingly, cTnT and CK-MB were significantly correlated (Fig. 1); in all 14 patients with serum CK-MB >4 μg/L, the serum cTnT was >0.1 μg/L. This is consistent with a common source of these proteins, although of course it does not indicate whether the common source is heart or skeletal muscle.

Francesco Zoppi
Laboratorio di Biochimica Clinica ed Ematologia
Ospedale Niguarda Ca’ Granda
Piazza Ospedale Maggiore 3
20162 Milan, Italy
Fax 39-2-6442901
E-mail marla.melotti@galactica.it

The Director of Regulatory Affairs and Quality Assurance for the Dade Behring Chemistry Group replies:

To the Editor:
As manufacturer of the aca® discrete clinical analyzer and the TGL test pack described in this Letter to the Editor, Dade Behring appreciates the opportunity to comment. As described in our labeling, the intended use of the TGL test pack is to measure quantitatively the triglycerides in serum. Dade Behring cannot support non-standard use of the TGL test pack for ethylene glycol testing because the TGL test pack has not been optimized for this use. Furthermore, because we have not filed a premarket notification with the US Food and Drug Administration for use of TGL test packs in ethylene glycol testing, we are restricted from promoting or endorsing the product for this use.

Kay Maher
Chemistry Group
Dade Behring
Newark, DE 19714-6101
Fax 302-631-6299
We think that the most important question in the cTnT-ESRD debate is the prognostic importance of this biochemical abnormality, and particularly whether these patients require some modification of their treatment. We have followed 81 hemodialysis patients over a 20-month period and have found no increase in mortality in those with increased cTnT (7 deaths from 43 patients) compared with those with cTnT within reference values (7 deaths from 38 patients). Other researchers have found otherwise, with increased serum cTnT (4) or CK-MB activity (5) predicting myocardial infarction or death in uremic patients. The results of studies using current methods on large numbers of patients are eagerly awaited.

References


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Alan R. McNeil1*
Mark Marshall2
Chris J. Ellis3
Robert C. Hawkins4
Departments of 1 Molecular Medicine and 3 Medicine
University of Auckland
Auckland, New Zealand
2 Department of Nephrology
Auckland Hospital
Auckland, New Zealand
4 Department of Pathology and Laboratory Medicine
Tan Tock Seng Hospital
Singapore

*Author for correspondence. Fax 64-9-3074939; e-mail Alanmcn@ahsl.co.nz.

Serum Ubiquinone-10 in a Pediatric Population

To the Editor:
Ubiquinone-10 is a lipid implicated in several biological functions. In tissues, it has an important role in electron transport and ATP synthesis related to the mitochondrial respiratory chain. The reduced form of ubiquinone protects cells from peroxidative damage (1). In blood, ubiquinone-10 is transported by lipoproteins, is one of the antioxidants within LDL, and prevents free radical damage caused by neutrophils and oxidative injury by endothelial cells in ischemia-reperfusion (1). Adequate serum concentrations of ubiquinone seem necessary to prevent peroxidative damage.

Various procedures to measure total ubiquinone-10 have been described, with HPLC with ultraviolet (UV) detection being the most common (2). Reference intervals for adults (3) and newborns (4) have been reported, but to our knowledge, no data are available for children.

Our aim was to establish serum reference values for a pediatric population in our geographical area with our working conditions.

Specimens were collected from apparently healthy children (by history and analytical data) before minor surgical intervention (n = 102; 38 females and 64 males; ages, 1 month–18 years), in accordance with the Helsinki Declaration of 1975, as revised in 1983. We collected 1.5 mL of 12-h fasting blood samples in Venoject silicone-coated gel tubes (Terumo Corp.), protected the samples from light and centrifuged them (2000g for 10 min at 4 °C). The serum was separated, frozen at −40 °C, and analyzed within 2 weeks. The total ubiquinone-10 (reduced plus oxidized) concentration was measured by HPLC with UV detection (275 nm) according to Zierz et al. (2), with slight modifications (ubiquinone-7 was used instead of ubiquinone-9 as the internal calibrator). Briefly, we added ubiquinone-7 (Sigma Chemical Co.) to 400 µL of serum or calibrator [375 µL of 154 mmol/L sodium chloride + 25 µL of ubiquinone-10 (Sigma Chemical Co.)]. The final concentration of both calibrators was 1.25 µmol/L. They were dissolved in 200 mL/L n-hexane–800 mL/L ethanol. Samples and calibrators were added to 1 mL of methanol containing 10 g/L pyrogallol and were saponified with 50 µL of 500 g/L potassium hydroxide for 10 min at 56 °C. The ubiquinone was extracted with 1 mL of n-hexane, mixed thoroughly for 1 min, desiccated under nitrogen, and dissolved in 200 µL of ethanol.

Chromatographic conditions were as follows: a Perkin-Elmer Integral 4000 HPLC system with a Perkin-Elmer Turbochrom Data Analysis module, a Nucleosil C18 column (150 × 4 mm, 5 µm particle size), and a C18 precolumn. The eluting solvent was 7 g of NaClO3.H2O in 1000 mL of 700 mL/L ethanol–300 mL/L methanol–1 mL/L HClO4; the flow rate was 1.3 mL/min. The equilibration time was 3 min, and the total chromatographic time was 8 min.

The results for ubiquinone-10 were expressed as molar concentration (µmol/L) and related to serum cholesterol concentrations (µmol/mol cholesterol). Cholesterol was measured by standard procedures (Cobas Integra Analyzer, Roche Diagnostic Systems).

The within-run imprecision (CV;