Dehydrogenase Interference with Enzymatic Ethanol Assays: Forgotten but Not Gone

To the Editor:

We have observed a positive interference affecting the Siemens (previously Bayer) Advia 1650® ethanol assay in samples collected from 3 patients with marked hepatocellular necrosis. During a recent 2-month period, we encountered 3 cases of acetaminophen-induced hepatocellular necrosis at our institution that demonstrated this effect (Table 1). The spurious ethanol results were in the 30–40 mmol/L range. Gas chromatography subsequently demonstrated that all 3 samples contained no detectable ethanol (<2 mmol/L). Additionally, ethanol was undetectable (<2 mmol/L) on the Dade Behring RXL MAX® platform using the Dade Behring Dimension® Flex® reagent cartridge. As it happened, we were alerted to a potential problem by the unusually large negative osmolar gaps in all 3 patients. Calculated osmolality was determined with this equation:

\[
\text{Osmolality} = 2[\text{Na}] + [\text{urea}] + [\text{glucose}] + 1.25[\text{ethanol}] \text{ (all concentrations in mmol/L).}
\]

The 1.25 multiplication factor for the concentration of ethanol is empirically derived and accounts for ethanol’s larger-than-expected contribution to measured osmolality (1).

It is known that alcohol dehydrogenase (ALD)-based ethanol assays that quantify NADH (or an NADH analog, in this case) spectrophotometrically can produce spuriously increased results in the presence of high concentrations of lactate dehydrogenase (LDH) and lactate (2, 3). This phenomenon is a result of the production of NADH by LDH according to

\[
\text{lactate + NAD}^+ \rightarrow \text{pyruvate + NADH.}
\]

The interference by LDH/lactate can be eliminated by measuring ethanol in protein-free ultrafiltrates of affected samples (4).

Based on their own experiments spiking LDH and lactate into ethanol-free serum specimens, Siemens informed us that to generate a spurious ethanol result >80 mg/dL (17.4 mmol/L), LDH would have to be >100 000 U/L (by a lactate-to-pyruvate methodology) in the presence of a lactate concentration above the normal reference interval (correspondence from Siemens). Based on this information, none of our patients had LDH/lactate combinations high enough to generate the observed spurious ethanol results. Therefore, we suspect that endogenous dehydrogenases and substrates other than LDH and lactate may be implicated in NADH production, leading to the false ethanol increases.

| Table 1. Initial laboratory investigations for the 3 patients with hepatocellular necrosis secondary to acetaminophen ingestion. |
|--------------------|------------------|------------------|------------------|
| Test               | Patient 1 | Patient 2 | Patient 3 |
| AST, U/L           | 8330      | 7080      | 18 012    |
| ALT, U/L           | 3540      | 12 286    | 4119      |
| LDH, U/L           | 8075      | 4871      | 10 147    |
| Lactate, mmol/L    | 22.5      | 1.2       | 5.1       |
| Sodium, mmol/L     | 139       | 138       | 133       |
| Urea, mmol/L       | 9.1       | 5.2       | 7.8       |
| Glucose, mmol/L    | 0.8       | 6.0       | 5.6       |
| Acetaminophen, μmol/L | 1218   | <66       | 78        |
| ETOH, mmol/L       |           |           |           |
| Advia 1650         | 33        | 33        | 34        |
| RXL MAX            | <2        | <2        | <2        |
| Gas chromatograph  | <2        | <2        | <2        |
| Measured osmolality, mmol/kg | 306 | 286 | 278 |
| Osmolal gap        |           |           |           |
| Calculated using ETOH from Advia 1650 | -23 | -42 | -44 |
| Calculated using ETOH from gas chromatograph | 18 | -1 | -1 |

*Acetaminophen concentrations were measured at approximately 24, 96, and 48 h after ingestion for patients 1, 2, and 3, respectively. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ETOH, ethyl alcohol.
This issue has ramifications for patient safety. For example, our third patient presented to the emergency department with epigastric abdominal pain. After the spurious ethanol was reported, a presumptive diagnosis of alcoholic gastritis was made, and the diagnosis of acetaminophen toxicity and therapy with N-acetylcysteine was substantially delayed.

Until the manufacturer can address this issue, we have implemented a policy requiring alanine aminotransferase (ALT) to be measured in all patients with ethanol concentrations >5 mmol/L. Out of concern that hepatocellular necrosis may cause spurious ethanol elevation, when the ALT is >500 U/L, we refer the specimen to another local hospital for ethanol analysis using their Dade Behring RXL MAX to get a rapid preliminary confirmation and by gas chromatography (GC) for a final confirmation. Our experience serves as a reminder that laboratories should inquire how the manufacturers of their ethanol assays have addressed the issue of dehydrogenase interference.

Grant/Funding Support: None declared.
Financial Disclosures: None declared.
Acknowledgments: We thank Morris Pudek of Vancouver General Hospital (Vancouver, British Columbia, Canada) for confirmatory ethanol analysis by Dade Behring RXL MAX and GC.

References

Angineh Gharapetian1
Daniel T. Holmes2*
Nadine Urquhart2
Frances Rosenberg2

1 University of British Columbia
Undergraduate Program
Faculty of Medicine
The University of British Columbia
Gordon & Leslie Diamond Health Care Centre
Vancouver, BC, Canada
2 St. Paul’s Hospital
Department of Pathology and Laboratory Medicine
The University of British Columbia
Vancouver, BC, Canada

* Address correspondence to this author at:
St. Paul’s Hospital
Department of Pathology and Laboratory Medicine
1081 Burrard St.
Vancouver, BC, V6Z 1Y6 Canada
Fax 604 806 8815
E-mail dholmes@interchange.ubc.ca

DO: 10.1373/clinchem.2008.103853

Rapid Detection of Intact FGF-23 in Tumor Tissue from Patients with Oncogenic Osteomalacia

To the Editor:

Oncogenic osteomalacia (OOM)1 is a rare tumor-induced disease characterized by hypophosphatemia due to a decreased renal threshold of phosphate reabsorption, low 1,25-dihydroxyvitamin D concentrations, and osteomalacia (1). Determining the location of OOM tumors, which often produce excess amounts of the phosphaturic hormone fibroblast growth factor-23 (FGF-23), can be difficult, and confirmation of successful tumor removal may require prolonged postoperative observation until the return of serum indicators to reference-interval concentrations (2). Here, we report the modification of a commercially available intact FGF-23 assay (3), which enabled us to rapidly document high FGF-23 content in OOM tumor extracts. The assay takes <30 min to complete, and visual inspection of the test plate is sufficient to distinguish positive from negative samples, therefore allowing fast intraoperative assessment of FGF-23 content in OOM tumor extracts.

Tumor tissue from 6 patients with OOM was used in this study. Tumor 1 tissue was from a 63-year-old woman with biochemical abnormalities characteristic of OOM. Ten years before the current study, this patient underwent resection of a small mesenchymal tumor in the maxilla, and thereafter her blood and urine chemistry indicators returned to reference intervals. The patient again developed hypophosphatemia, however, and underwent a second operation. During this procedure, tissue from the regrown tumor was obtained for this study. We also obtained tissue from 5 tumors that have previously been described (4): tumor 2 from the mandible (mixed connective tissue tumor), tumor 3 from the thigh (angiodysplastic tumor), tumor 4 from the nose (hemangioendothelioma), tumor 5 from the thigh (hemangiondysplasia), and tumor 6 from the foot (hemangiopericytoma). All tumors had been immediately frozen after surgical removal and stored at −80°C. In healthy individuals, FGF-23 is predominantly produced by bone, and therefore bone was chosen as a