K23 Calculation of Potential Lactate/Lactate Dehydrogenase (LDH) Interference With Alcohol-Dehydrogenase (ADH)-Based Ethanol Assay

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Learning Overview: After attending this presentation, attendees will have a greater understanding of the potential for lactate/LDH interference in an ADH-based ethanol assay, an approach for consideration of the possibility of such interference in a particular case sample based on clinical parameters, and calculation of the magnitude of interference based on estimated, extrapolated, or determined lactate and LDH concentrations.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an approach for the evaluation of potential lactate/LDH-based interference in an ADH-based ethanol assay, and for the determination of the magnitude of such interference in specific cases.

Determination of serum alcohol is a common and routine clinical test, most often performed by an enzymatic assay based on the ADH-catalyzed oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD+, with the rate of NADH formation being the monitored variable (A340). Interference with an ADH-based alcohol assay by the presence of variable concentrations of lactate and LDH was demonstrated experimentally. Theoretically, the presence of adequate quantities of both lactate and LDH in a sample could result in a competitive, and interfering, reduction of NAD+, with the potential for falsely elevated or false positive alcohol results.[1,2] The question of the potential occurrence and magnitude of this phenomena has been the subject of a number of papers. Recently, several well-documented examples of the lactate/LDH interference with an alcohol result have demonstrated the significance such results can have with regard to forensic casework. Interference was found to be a function of variable lactate and LDH combinations, and minimum concentrations required to produce a forensically significant result (e.g., >0.0g/dL) were determined. A scaled-up version of an automated analyzer method, with volumes adjusted for manual analysis in a Ultraviolet/Visible (UV/Vis) spectrophotometer, was utilized with 0–50mM lactate (normal range ~0.5–2.2mM) and 0–10kU/L LDH (normal range ~0.12–0.22kU/L). Each lactate/LDH combination was run in triplicate, with the "Ethanol (EtOH) reading determined from a 6-point ethanol calibration curve. To generate an apparent alcohol result of 0.02g/dL at the maximal lactate concentration (50mM), an LDH of at least 4kU/L was required in the sample, and similarly, at maximal LDH concentration (10kU/L), a lactate concentration of at least 10mM was required. These results are consistent with the uncommon nature of this phenomena, suggesting that significant lactate and LDH levels are required in a sample to cause an interference. Perhaps more relevant, a clinical picture that would be expected to result in a persistently elevated lactate level, combined with significant leakage and presence in the blood of hepatic enzymes (e.g., LDH) would not be common (but can clearly occur as a consequence of disease or trauma).[3] The apparent EtOH readings in the lactate/LDH amended samples was linearly related to lactate concentration at specific LDH levels. Similarly, the slopes of those lines were linearly related to LDH concentration. Using these relationships (expected to be generally applicable, but assay-specific), an equation was derived that relates lactate, LDH, and expected "false-positive" ethanol readings, and describes the potential application of this approach to other assays. Finally, this equation was applied to the analysis of well-documented clinical cases, along with the consideration of relevant clinical parameters and assay results.

Reference(s):

1. Badcock, N.R. and D.A. O'Reilly. 1992. False-Positive EMIT-st Ethanol Screen with Post-Mortem Infant Plasma. *Clinical Chemistry*, 3: 434.
2. Powers, R.H. and D.E. Dean. 2009. Evaluation of Potential Lactate/Lactate Dehydrogenase Interference with an Enzymatic Alcohol Analysis. *Journal of Analytical Toxicology*, 33: 561-563.
3. Bishop-Freeman, S.C. and R.H. Powers. 2017. False Positive Enzymatic Alcohol Results: Two Cases From 2014. *Proceedings of the American Academy of Forensic Sciences*, 69th Annual Scientific Meeting, New Orleans, LA. 2017.

Lactate, Lactate Dehydrogenase, Alcohol Dehydrogenase