

K57 Co-Reaction of Lactate/Lactate Dehydrogenase in an Enzymatic Ethanol Assay

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Learning Overview: After attending this presentation, attendees will understand how an enzymatic ethanol assay can produce false positives or falsely elevated results for samples containing elevated levels of lactate and Lactate Dehydrogenase (LDH) due to trauma or illness. Attendees will also understand threshold levels of lactate and LDH necessary for interference to occur and the magnitude of potential effects on ethanol results.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by clarifying the potential for interference by lactate/LDH in an Alcohol Dehydrogenase (ADH) -based ethanol assay and providing an understanding of the physiological parameters that could reasonably cause such an interference. The results of this project can be used to facilitate the interpretation of enzymatic ethanol results by pathologists, medical personnel, and toxicologists.

Enzymatic assays are commonly used to determine blood alcohol content in clinical settings. In some instances, this data may be used as evidence in legal settings (e.g., Driving Under the Influence (DUI) cases). The enzymatic assay measures the amount of ethanol indirectly as Δ NADH. It is theoretically possible that interference from the oxidation of lactate to pyruvate could occur and produce a falsely positive or elevated result.¹ While addressed sporadically over the past 30 years, questions of the conditions under which such interference may occur remain unresolved, particularly in the LDH range of 4,000U/L–10,000U/L. While abnormally elevated levels of lactate and LDH are not routinely present in clinical samples, nor are erroneous “false positive” ethanol results considered to be a common problem, there has been persistent concern regarding the potential for such interference affecting some samples of legal significance.²⁻⁹ Further, no information regarding the combination of ethanol, lactate, and LDH in an ADH assay has been provided.

To determine if the presence of lactate and LDH in a serum sample could impact the ethanol level observed in the assay, varying amounts of lactate and/or LDH were added to an enzymatic ethanol assay. The concentrations of lactate and LDH utilized were chosen to reflect elevated levels reasonably observable in living individuals (normal lactate: 0.5mM–2.2mM, elevated lactate: 5mM–50mM; normal LDH: 122U/L–225U/L, elevated LDH: 200U/L–10,000U/L) suffering from a combination of trauma, shock, hypoxia, hepatic disease, or other conditions producing leakage of hepatic enzymes and lactic acidosis.^{5-10,11}

The results indicate that elevated levels of lactate and LDH can result in false positive and falsely inflated results from the ADH assay, most notably at higher concentrations. In a system with no ethanol, the lower threshold of response was detected with a combination of 4,000U/L LDH and 50mM lactate. This indicates that interference occurs far above physiologically normal levels of LDH and explains the rarity of this interference. On the upper end, 10,000U/L LDH and 50mM lactate produced a signal of 92mg/dL, which may have significant legal implications.

When lactate and LDH were added to a system with ethanol present, a signal increase of up to 350% was observed, with greater effects noted with low (30mg/dL) and medium (60mg/dL) levels of ethanol in the system. These results indicate that lactate/LDH interference, while rare, can conceivably cause a significant false positive or falsely inflated “ethanol” result.

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Enzymatic Ethanol Assay, Lactate Interference, False Positive

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