How False Positive Serum Ethanol Results Occur Using Enzyme-Assay Hospital Tests

by Stefan Rose, M.D.
University Medical and Forensic Consultants, Inc. 10130 Northlake Boulevard
Suite 214-300, West Palm Beach, Florida 33412 Phone 561-795-4452

In order to understand how false-positive serum ethanol results may occur using a hospital based enzyme assay test, please refer to figures 1 and 2.

ADH (alcohol dehydrogenase) is an enzyme that catalyzes the metabolism of ethanol to acetaldehyde, an intermediate breakdown product of ethanol metabolism. NAD is a co-enzyme necessary for ADH to work. Both are found inside liver cells, the primary site of ethanol metabolism.

When ADH converts ethanol to acetaldehyde, NAD must accept a hydrogen atom (H) as part of the reaction, forming NADH. The increase in the concentration of NADH may be measured by ultraviolet absorption spectrophotometry at a wavelength of 340 nanometers. Figure 3 on the next page compares the difference in ultraviolet absorption between NAD and NADH. The increase in NADH concentration is directly related to the amount of ethanol present. This is a similar technique used in breath alcohol testing, except longer wavelengths of light, infrared, are used to measure ethanol.

The hospital enzyme assay test uses a sample of serum (centrifuged whole blood) to which is added a known amount of ADH and NAD. The formation of NADH is measured in a clinical chemistry analyzer at 340 nm wavelength, and the concentration of ethanol is calculated based on the amount of absorption compared to a negative sample with no ethanol.

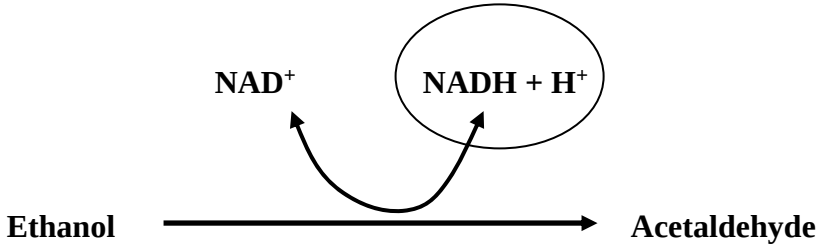
The enzyme assay test is subject to false positive ethanol results when both lactate (a compound formed as a result of trauma and hypoxia) and LDH (lactate dehydrogenase) are present in the serum sample. Normally LDH is located inside muscle cells (to metabolize lactate formed during anaerobic exercise) but is released into the blood stream after crush trauma, such as a car crash.

If lactate is administered in an intravenous solution (Ringer's lactate, other formulations) and/or formed as a result of decreased oxygen to the tissues, and LDH is released into the bloodstream, the serum sample collected for ethanol analysis will contain lactate and LDH as well.

When the enzyme assay test is performed, the NAD reacts with BOTH ethanol AND lactate to form more NADH than if lactate and LDH were not present.

The consequence is a falsely elevated serum ethanol result!

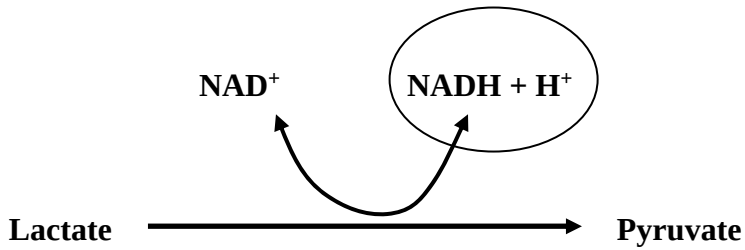
Serum ethanol testing in the hospital laboratory with a normal serum sample



Alcohol dehydrogenase (ADH)

Figure 1

Following crush trauma LDH and Lactate are found in the serum sample



Lactate dehydrogenase (LDH)

Figure 2

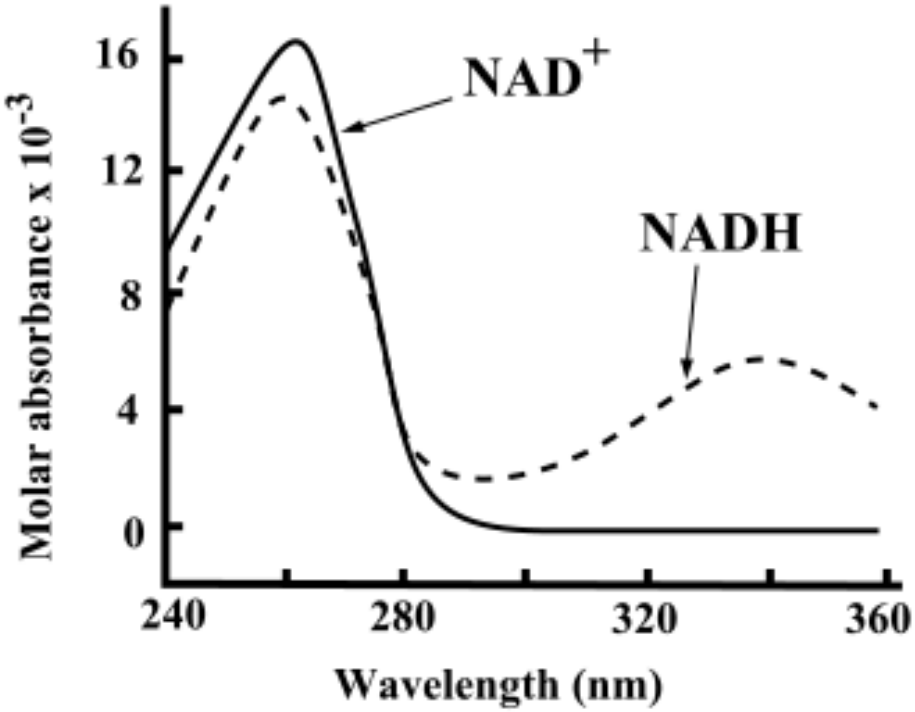


Figure 3

When the NAD is converted to NADH in the conversion of ethanol to acetaldehyde, the increase in the concentration of NADH is measured by the change in spectrophotometric absorption of ultraviolet light at 340 nm.

The higher the concentration of ethanol in the sample, the higher the NADH concentration will be.

Since the test is measuring NADH, a higher NADH concentration will result in a higher ethanol result.

Any other cause of increased NADH in the sample will cause a
<u>FALSE POSITIVE RESULT</u>