Letter to the editor

Rethinking the limit of detection for serum ethanol: evidence from a clinical laboratory

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Abstract

Ethanol is a widely consumed psychoactive substance. Serum concentrations above 100 mg/dL can cause cognitive impairment and confusion, while levels exceeding 300 mg/dL may be fatal. Thus, rapid and accurate determination of blood ethanol concentration is crucial for diagnosing and managing acute intoxication. Ethanol measurement also has important legal implications, such as in traffic accidents or criminal proceedings.

Gas Chromatography-Mass Spectrometry (GC-MS) is the reference method for ethanol determination due to its accuracy and specificity. In routine clinical laboratories, however, ethanol quantification is usually performed using enzymatic spectrophotometric assays. Alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde with NAD as coenzyme, which is simultaneously reduced to NADH. The increase in NADH absorbance at 340 nm allows indirect quantification of ethanol.

Although analytical characterization and method optimization are the manufacturers' responsibilities, literature indicates these processes are not standardized, and specific procedures are generally not disclosed. Consequently, clinical laboratories may need to verify these values, particularly during implementation of new methods, internal validation, or when analytical results conflict with clinical presentation.

We report four cases in our laboratory with positive ethanol results of 11–14 mg/dL that, after reprocessing, were reported as "not detected". This highlights the need to determine the smallest analyte quantity detectable with certainty, commonly expressed as the limit of detection (LoD).

In this study, we evaluated the LoD of the Emit II Plus Ethyl Alcohol assay (Siemens Healthineers, Dublin, Ireland) in human serum using the AU[5800] analyzer (Beckman Coulter, Brea, CA, USA), following CLSI EP[17]-A[2] recommendations for LoD verification. According to CLSI EP[17]-A[2], LoD is defined as the measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a measurand in a material is β, given a probability α of falsely claiming its presence, and LoB is the highest measurement result that is likely to be observed (with a stated probability α) for a blank sample. LoQ is defined as the lowest amount of a measurand in a material that can be quantitatively determined with stated accuracy, expressed as total error or as independent requirements for bias and precision, under stated experimental conditions. Because our study did not assess predefined accuracy goals for quantitative determination at low ethanol concentrations, it should be interpreted as a local LoD verification study rather than a LoQ evaluation. In this context, the kit insert reports a quantification range of 10–600 mg/dL, with a LoD < 10 mg/dL and reported precision of 0.8%–4.1%. The study was conducted in Spain between April and May 2025, using two instrument systems and two reagent lots to ensure adequate measurements.

An initial estimated LoB was obtained from 20 replicate measurements of ethanol-free serum, defined as the highest observed value (4 mg/dL). This preliminary LoB was then used to guide the selection of low-level samples, targeting concentrations approximately within 1–5 times the LoB, resulting in the preparation of five serum samples (4–20 mg/dL). The experimental LoB was then determined from five ethanol-free serum samples analyzed in four replicates per day over three consecutive days on each instrument. The 95th percentile (α = 0.05) was calculated using the rank-position formula (Rank Position = 0.5 + B·0.95, where B is the total number of replicates). In both analysers, interpolation between adjacent values was required, yielding LoB values of 3 mg/dL for instrument 1 and 9 mg/dL for instrument 2. To minimize false positives, the higher LoB (9 mg/dL) was used for subsequent calculations.

Each low-level serum sample was analyzed in four replicates per day over three days on each instrument, and the LoD for each system was calculated using the formula:

LoD = LoB + cp × SDL,

where SDL represents the pooled standard deviation of the low-level samples, calculated using the following formula:

$$SD_L = \sqrt{\frac{\sum_{i=1}^{J}(n_i - 1)\,SD_i^2}{\sum_{i=1}^{J}(n_i - 1)}}$$

SDi = standard deviation of results for the i-th low-level sample

ni = number of results for the i-th low-level sample

J = number of low-level samples

The correction factor cp is defined as:

$$cp = \frac{1.645}{1 - \frac{1}{4 \times (L - J)}}$$

L denotes the total number of low-level results per instrument/lot (L = 60), J the number of low-level samples (J = 5).

The LoD was 12 mg/dL in instrument system 1 and 14 mg/dL in instrument system 2, calculated using the SDL values of 1.68 and 3.04 mg/dL, respectively.

The discrepancy between the manufacturer-reported LoD (10 mg/dL) and the experimentally determined values (12 and 14 mg/dL) suggests that results may vary with instrument system, reagent lot, and calibration. Therefore, reagent manufacturers should conduct performance studies that incorporate analytical variability, in accordance with CLSI EP[17]-A[2] (6). Yet, standardized protocols are often lacking, and procedures to establish analytical characterization are generally undisclosed. Literature addressing these assay limitations also remains scarce.

The results highlight the importance of evaluating the LoD of enzymatic ethanol assays to minimize false positives. To ensure a conservative threshold, the highest experimentally determined LoD should be adopted. This is particularly relevant given the ethical implications of false-positive results, especially in vulnerable populations such as children or pregnant women. Adopting a higher, experimentally verified LoD does not modify established clinical or legal decision limits but improves the reliability of reporting at very low concentrations by reducing false-positive classifications below the locally verified detection capability of the assay.

In conclusion, for clinical laboratories using the Emit II Plus Ethyl Alcohol assay (Siemens Healthineers, Dublin, Ireland) on the AU[5800] analyzer (Beckman Coulter Inc., Brea, CA, USA), LoD verification is recommended. Consequently, results below the locally verified LoD should not be reported as positive quantitative ethanol results. When discrepancies are clinically or legally significant, the presence or absence of ethanol should be confirmed using a reference method such as GC-MS.

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Author contributions

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References

1. Tian, G., Zhang, XQ., Zhu, MS. et al. Quantification of ethanol in plasma by electrochemical detection with an unmodified screen printed carbon electrode. Sci Rep 6, 23569 (2016).
2. Xiao HT, He L, Tong RS, Yu JY, Chen L, Zou J, Li JQ, Bian Y, Zhang Y. Rapid and sensitive headspace gas chromatography-mass spectrometry method for the analysis of ethanol in the whole blood. J Clin Lab Anal. 2014;28(5):386-390
3. Kristoffersen L, Skuterud B, Larssen BR, Skurtveit S, Smith-Kielland A. Fast quantification of ethanol in whole blood specimens by the enzymatic alcohol dehydrogenase method. Optimization by experimental design. J Anal Toxicol. 2005;29(1):66-70.
4. Gegenschatz SA, Chiappini FA, Teglia CM, Muñoz de la Peña A, Goicoechea HC. Binding the gap between experiments, statistics, and method comparison: A tutorial for computing limits of detection and quantification in univariate calibration for complex samples. Anal Chim Acta. 2022;1209:339342. doi: 10.1016/j.aca.2021.339342. Epub 2021 Dec 3. PMID: 35569842.
5. Qian Sun, Kerry J Welsh, David E Bruns, David B Sacks, Zhen Zhao, Inadequate Reporting of Analytical Characteristics of Biomarkers Used in Clinical Research: A Threat to Interpretation and Replication of Study Findings, Clinical Chemistry, Volume 65, Issue 12, 1 December 2019, Pages 1554–1562,
6. Clinical and Laboratory Standards Institute. (2012). Protocols for determination of limits of detection and limits of quantitation. CLSI-EP-17-A. Clinical and Laboratory Standards Institute.
7. Winek CL, Wahba WW, Windisch RM, Winek CL Jr. Serum alcohol concentrations in trauma patients determined by immunoassay versus gas chromatography. Forensic Sci Int. 2004;139(1):1-3.