CONE HEALTH
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CHM.28850 Ethanol Specificity

Requirement:
If the laboratory tests for ethanol, the method has been evaluated for ethanol specificity.

Evidence of Compliance:
Records of ethanol specificity evaluation studies OR evaluation of information provided by the manufacturer OR evaluation of published literature

Note:
Elevated lactic acid and lactate dehydrogenase (LDH) may falsely elevate enzymatically determined ethanol levels.

Cone Health Evidence of Compliance:

Evaluation of Information Provided by Manufacturer:
In order to comply with CHM.28850, the Cone Health Laboratory Quality Assurance department reviewed manufacturer evaluation for interfering substances affecting Beckman Coulter Synchron System(s) ETOH Alcohol REF 474947.

Package Insert Revision Reviewed: A18490 AR October 2017

Beckman Coulter tested the following for interferences:

1. Hemoglobin (RBC hemolysate)
2. Bilirubin (porcine)
3. Lipemia (human)
4. Lactate Dehydrogenase (LDH) and Lactate (porcine)

Beckman Coulter defines significant interference as ± 4.8 mg/dL or 6%. Hemoglobin, bilirubin, and lipemia did not demonstrate significant interference.

Lactate dehydrogenase and lactate demonstrated a 4 mg/dL increase in ethanol recovery at concentrations of 1890 U/L and 14 mmol/L, respectively. Beckman Coulter also made note increased levels of lactic acid and LDH in post mortem samples may cause elevated alcohol results.

Manufacturer precision studies demonstrated total precision as 3.6 mg/dL or 4.5% (changeover value 80.0 mg/dL). Increased ethanol recovery of 4 mg/dL is negligible when compared assay total precision and not clinically significant.

Additionally, Beckman Coulter states that both lactate and LDH must be greater than, or equal to, the values tested for interference (1890 U/L and 14 mmol/L, respectively).

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Evaluation of Published Literature

Enzymatic alcohol assays utilize the reduction of NAD+ to NADH for spectrophotometric analysis at 340 nm. In the presence of ethanol and NAD+, this reaction is catalyzed by alcohol dehydrogenase (ADH), producing acetaldehyde and NADH. Similarly, lactate in the presence of NAD+ is catalyzed by lactate dehydrogenase into pyruvate and NADH. Due to the similarity between the reactions, arguments have been made stating elevated lactate and LDH levels may falsely elevate serum ethanol results due to increase production of pyruvate and, subsequently, NADH.

In a research study by N. Nacca et al, 37 patients with lactate values ranging from 2.4 – 24.2 mmol/L and LDH levels ranging from 242 – 8838 U/L were tested for ethanol. Of these 37 patients, only four had measurable ethanol concentrations, which were confirmed as elevated via gas chromatography. The other 33 patients did not have measureable ethanol concentrations. While this study was performed using Roche Diagnostics Ethanol Gen. 2 (ETOH2) reagent, the data may be applied to Beckman Coulter ETOH REF 474947. Cone Health correlated ETOH REF 474947 with ETOH2 during their initial DxC install at Annie Penn, MedCenter High Point, Moses Cone, Wesley Long, and Women's Hospital. While slight biases were present between the two methods, these were well within the limits of acceptable performance as defined by the College of American Pathologists (± 25%), and, therefore, the methods are considered clinically equivalent.

Using similar logic as Powers and Dean, the likelihood of interference can be predicted. Given the sample size for the assay is 10 µL, the amount of LDH in the reaction mix, considering a total LDH of twice of clinical reportable range (20,000 U/L), would only be 0.2 U, which when compared to the amount of ADH delivered to the assay mix is negligible. Additionally, the presence of elevated lactate alone is insufficient due to lactate being a poor substrate for ADH[2]. In order for significant interference to occur, the patient must have extremely elevated levels of both LDH and lactate, which are typically seen in perimortem/postmortem samples due to cellular degeneration[2]. Therefore, as stated by Beckman Coulter, ETOH REF 474947 may result in falsely elevated ethanol concentrations in postmortem samples.

Conclusion:

While Beckman Coulter Synchron System(s) ETOH Alcohol REF 474947 should not be used for postmortem sample analysis, routine analysis of patient samples will not demonstrate significant interference from elevated lactate and LDH concentrations. In the event a postmortem ethanol is requested, all elevated concentrations will be confirmed by non-enzymatic assays. Suggested assay for use is LabCorp Test 017996 CPT 80320 Ethanol, Whole Blood (Gas Chromatography). The laboratory will update appropriate autopsy procedures to include this confirmation.

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References:

1. Nicholas Nacca, Michael J. Hodgman, Kirselle Lao, Matthew Elkins & Michael G. Holland (2017): Can elevated lactate and LDH produce a false positive enzymatic ethanol result in live patients presenting to the emergency department?, Clinical Toxicology, DOI: 10.1080/15563650.2017.1357825.

2. Powers, RH, Dean DE. Evaluation of potential lactate/lactate dehydrogenase interference with an enzymatic alcohol analysis. J Anal Toxicol. 2009;33:561-563.

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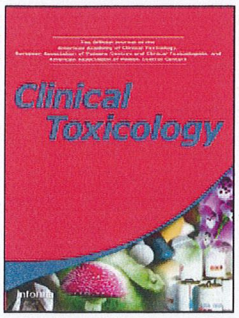

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CLINICAL RESEARCH

Can elevated lactate and LDH produce a false positive enzymatic ethanol result in live patients presenting to the emergency department?

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ABSTRACT

Background: There have been allegations in the courtroom that elevated serum lactic acid in trauma victims can yield a falsely elevated serum ethanol assay. Most hospitals utilize an indirect method of ethanol measurement where a serum sample is added to a mix of alcohol dehydrogenase and oxidized nicotinamide adenine dinucleotide (NAD+). This allows any ethanol in the patient's serum to be metabolized to acetaldehyde, and in the process results in the reduction of NAD + to NADH. NADH is then measured using spectrophotometry. The courtroom allegation stems from the concept that oxidation of lactate to pyruvate by lactate dehydrogenase (LDH) results in the same molar-for-molar reduction of NAD + to NADH, and could therefore theoretically cause patients with elevated lactate and LDH to have a falsely elevated ethanol concentration.

Methods: Patients with elevated lactic acid and LDH concentrations who presented to a university hospital from 20 April 2015 to 13 December 2015 were identified to provide possible test specimens. If a sufficient amount of serum was available, the sample was used to re-run the lactate and LDH concentration simultaneously with an enzymatic ethanol assay. Any samples that had elevated lactic acid and LDH concentrations on this retesting, and also yielded a positive ethanol concentration, were sent for confirmatory gas chromatography testing of ethanol concentrations. A control group of 20 samples with normal lactate and LDH were included.

Results: A total of 37 samples were included in the final analysis. Only 4 patients had an elevated enzymatic ethanol concentration, and all 4 also had a measurable GC ethanol concentration. The lactate in this dataset ranged from 2.4 to 24.2 mmol/L, with a mean of 6.53 mmol/L (normal value 0.5–2.2). The LDH ranged from 242 to 8838 U/L with a mean of 1695 U/L (normal value 122–225 U/L). Twenty control samples were run on patients with normal lactate and LDH, none of which yielded a positive enzymatic ethanol result.

Conclusions: This data does not support the contention that an elevated LDH and lactate can yield a false positive serum ethanol result as run by enzymatic ethanol assay in live patients presenting to the emergency department.

ARTICLE HISTORY

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KEYWORDS

Ethanol assay; alcohol assay; false positive ethanol; false positive alcohol; lactate interference; lactate; lactic acid; ethanol; lactate dehydrogenase

Introduction

There have been allegations in the courtroom that simultaneously elevated serum lactic acid (lactate) and lactate dehydrogenase (LDH) in trauma victims can result in a falsely elevated serum ethyl alcohol (ethanol) assay result [1]. Most hospitals utilize an indirect method of ethanol measurement where a serum sample is added to a mix of alcohol dehydrogenase (ADH) and oxidized nicotinamide adenine dinucleotide (NAD+). This allows any ethanol in the patient's serum to be metabolized to acetaldehyde, and in the process results in the reduction of NAD + to NADH. NADH is then measured using spectrophotometry, and the concentration is correlated to a serum ethanol concentration.

The courtroom allegation stems from the concept that oxidation of lactate to pyruvate by LDH results at the same molar-for-molar reduction of NAD + to NADH, and can therefore cause patients with elevated lactate and LDH to have a falsely-elevated ethanol concentration (Figure 1). The possibility of a false positive ethanol result by this mechanism is also commonly taught by toxicology educators in toxicology fellowships and conferences. Though this notion has led to *in-vitro* measurement and analysis which suggests the possibility of a false positive result, to the authors' knowledge, it has never been prospectively investigated in live patients.

$$\text{Ethyl alcohol} + \text{NAD+} \xrightarrow{\text{ADH}} \text{Acetaldehyde} + \text{NADH}$$

$$\text{Lactate} + \text{NAD+} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

Figure 1. Chemical reactions forming the basis of suggestion that false positive ethanol may result from patients with elevated lactate and LDH. ADH: alcohol dehydrogenase; LDH: lactate dehydrogenase.

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Methods

The institutional review board approved this study with waiver of consent. We sought to determine if a false positive ethanol result could be obtained from patients with elevated lactate and LDH, but negative ethanol by gas chromatography. Patients presenting to a single university hospital emergency department between 20 April 2015 and 13 December 2015 who had both a lactic acid and LDH concentration ordered as standard of care were eligible for inclusion. Patients with concomitant elevations of both lactic acid and LDH concentrations were identified by an automatically generated secure email system that notified the research staff of results on a daily basis. Research staff then identified and located samples which had extra serum available after all patient care oriented assays had been run. Samples were stored in a freezer regulated between 2 and 8 °C. LDH and ethanol were drawn in plastic serum separator tubes (BD vacutainer 367983), lactic acid was drawn in plastic sodium fluoride/potassium oxalate tubes 10mg/8mg (BD vacutainer 367922). If a sufficient amount of serum was available, the sample was used to re-run the lactate and LDH concentration and ethanol concentration by enzymatic assay for study purposes. These repeat tests were performed in order to control for degradation that may have occurred during sample storage resulting in an unmeasured confounding variable. All samples were run on a P800 Roche Modular Analyzer with commercially available kits (ethyl alcohol: Roche Diagnostics, reference number 11776312 190; LDH: Roche Diagnostics, reference number 03002209 122; lactate: Pointe Scientific Inc, reference number L7596-50). Any samples that had elevated lactic acid and LDH concentrations on retesting and yielded a measurable ethanol concentration were sent for confirmatory gas chromatography-flame ionization detector testing (GC-FID: Agilent 6890 series; headspace: G1888, column screen: Agilent DB-ALC2; quantitation on Agilent DB-ALC1). No study results were reported to the medical treatment team. A control group of 20 samples with normal lactate and LDH were identified and repeat lactate and LDH were performed simultaneously with an enzymatic ethanol assay. Patient age, sex, and admission diagnosis were recorded and all data were entered into an excel spread sheet.

Results

A total of 46 patients were identified as having concomitantly elevated serum lactate and LDH during the study period. For two patients there were no samples associated with the identified accession number. Two patients were incorrectly identified and did not have elevated lactate or LDH. In four patients repeat testing yielded a normal lactate result. In one other patient, there was an insufficient amount

Table 1. Results of serum ethanol, lactate, and LDH from patients included in analysis.

Age and gender	Admission diagnosis	Lactate (mmol/L)	LDH (U/L)	Enzymatic ethanol (g/dL)	GC ethanol (g/dL)
59 M	Smoke inhalation	7.1	335	0.29	0.25
62 M	Cellulitis	4.2	303	0.01	0.007
59 M	Ethanol intoxication	3.3	242	0.02	0.01
23 M	Gastrointestinal hemorrhage, NSTEMI	11.9	8119	0.07	0.05
84 F	Sepsis	5.48	5751	0	NP
55 M	Sepsis	2.9	258	0	NP
68 M	Sepsis	8.0	330	0	NP
81 F	Sepsis	6.1	526	0	NP
53 M	Sepsis	6.2	310	0	NP
72 M	Sepsis	4.5	590	0	NP
58 M	Sepsis	10	676	0	NP
30 M	Sepsis	4.0	3623	0	NP
85 M	Sepsis	9.0	2018	0	NP
62 F	Sepsis	9.5	554	0	NP
60 M	Metastatic prostate cancer	10	8160	0	NP
54 M	Metastatic lung cancer	4.6	1165	0	NP
67 M	Metastatic lung cancer	3.0	3405	0	NP
54 M	Metastatic unspecified cancer	5.6	1746	0	NP
54 F	Metastatic pancreatic cancer	3.6	297	0	NP
51 M	Acute myocardial infarction	10.7	8838	0	NP
20 M	Cardiac arrest	3.1	581	0	NP
67 F	Acute myocardial infarction	24.2	1071	0	NP
44 F	Acetaminophen toxicity	4.5	599	0	NP
85 F	Altered mental status	5.9	462	0	NP
67 F	Acute respiratory failure	4.3	313	0	NP
81 M	Autoimmune hemolytic anemia	3.7	903	0	NP
45 F	COPD exacerbation	7.4	289	0	NP
52 F	Gangrene	4.0	383	0	NP
26 M	Hemophagocytic syndrome	5.2	791	0	NP
92 F	Idiopathic thrombocytopenic purpura	3.1	615	0	NP
64 M	Ischemic colitis	4.1	496	0	NP
26 M	Neutropenic fever	20	1293	0	NP
70 F	Neutropenic typhlitis	8.6	260	0	NP
81 F	Peritonitis	4.9	323	0	NP
40 M	Pyelonephritis	2.4	1853	0	NP
66 F	Rhabdomyolysis	3.2	4866	0	NP
55 M	Spontaneous bacterial peritonitis	3.4	373	0	NP

LDH: lactate dehydrogenase; GC: gas chromatography; NP: not performed.

of blood available for analysis. This resulted in a total of 37 samples remaining to be included in the final analysis.

The mean age was 59 years, and 62% of the patients included were male. The most common admission diagnosis was sepsis, followed by metastatic cancer. Only 4 of the 37 patients had an elevated enzymatic ethanol concentration, and all 4 also had a measurable GC ethanol level (Table 1). Of those patients included in the analysis, the lactate ranged from 2.4 to 24.2 mmol/L, with a mean of 6.53 mmol/L (normal value 0.5–2.2). The LDH ranged from 242 to 8838 U/L with a mean of 1695 U/L (normal 122–225 U/L). Twenty control samples were run on patients with normal lactate and LDH, none of which yielded a positive enzymatic ethanol result. Of note, one patient included in the analysis was admitted with a diagnosis of ethanol intoxication. The initial ethanol level was 0.2 g/dL, however, the repeat assay as per study protocol was 0.02 g/dL, likely owing to volatilization in the time period in between assays.

Discussion

In 1992, while looking for biomarkers in Sudden Infant Death Syndrome, Australian researchers noted that a commercially available homogenous enzyme linked immunoassay for ethanol was positive in several postmortem infant plasma and vitreous humor samples. They noted that gas chromatography was negative for ethanol in these same samples, and concluded that use of this enzymatic assay is unreliable postmortem, presumably due to elevated LDH and lactate levels [2]. In 1994, Thompson et al. reported two cases of critically ill patients with severe lactic acidosis that were found to have profoundly elevated ethanol concentrations by enzyme linked assay testing. Rerunning these samples after protein free ultrafiltration (i.e., removal of LDH) resulted in normalization of the false positive ethanol [3]. The survival of these patients was not reported, and the enzymatic assay used to measure serum ethanol is not likely to be in use any longer. The observation that two post mortem pediatric cardiopulmonary arrest patients had positive ethanol concentrations by enzyme linked assay led another group to investigate this phenomenon. In 1995, Nine et al. used postmortem blood samples to test three commercially available enzymatic screening assays that utilize the enzymatic process of ethanol to acetalydehyde by the enzyme alcohol dehydrogenase. The conclusion was that one commercially available test, in particular, (Syva) led to more false positive ethanol concentrations than did other assays (Roche and Abott). Two critically ill patients were identified as having false positive ethanol results on the Syva, but not Roche or Abott. The survival of these patients was not reported. The same study confirmed that all three assays were subject to false positive results when the samples were spiked with exogenous lactate and LDH. The authors suggested that due to the possibility of false positive results, testing should be interpreted carefully [4]. A letter to the editor on this article pointed out that the conclusions are based on postmortem blood samples, and such an extrapolation to a live patient population is irresponsible given the potential medico-legal ramifications [5].

These observations showed potential clinical relevance in a case such as that presented by Powers and Dean in 2009. They described a case where a patient in a motor vehicle collision was found to have mild liver injury and an elevated ethanol concentration by enzyme linked testing. The defendant in the case claimed that minor liver injury and subsequent lactic acidosis (which was not measured by the medical team) resulted in false positive ethanol. The authors were able to indirectly refute this claim based on extrapolation from the available laboratory investigations which did not suggest an elevated LDH or anion gap metabolic acidosis sufficient to result in a lactic acid high enough to cause a false positive ethanol concentration.

To the authors' knowledge, no studies using unadulterated blood specimens from living adults hospitalized with an acute illness using modern assays have been performed to evaluate whether this phenomenon is clinically relevant. The only available cases in the literature suggesting the possibility of false positive were perimortem, postmortem, or performed on antiquated equipment.

In the present study, no false positive ethanol concentrations were identified in living patients with elevated LDH and lactate. Of note, the ethanol concentrations from GC/FID in the 4 patients with positive enzymatic ethanol results were slightly, though consistently, lower than the initial assay. It is possible, that the lactate and LDH in these samples contributed to the slightly higher result on the enzymatic assay. Interpreted in the context of the rest of the data set, it seems more likely the some ethanol volatilized between assays. There were several limitations to this study. There was no a priori power analysis performed due to the extremely low predicted false positive rate. The relatively small sample size of the study is a significant limitation. During the study period there were no trauma victims meeting inclusion criteria. This is likely the result of the rarity in which a treatment team would order a lactate and LDH concentration in a trauma victim. Trauma victims are known to have elevations in both LDH and Lactate that are thought to correlate with severity of illness. In a retrospective study of 75 abdominal trauma victims with known liver injury, the range of LDH was 106–2,577 IU/L [6]. Another retrospective study of 5995 general trauma patients identified a range of Lactate to be 0–40 mmol/L [7]. In the authors' opinion, it is unlikely that the mechanism leading to elevation of LDH and lactate has influence over the result of the enzymatic ethanol assay given that false positives have been demonstrated in vitro spiked blood samples. While this study only applies to the Roche chemistry analyzer used by our lab, it likely applies to the other available products. Data from the College of American Pathologists (CAP) lactate dehydrogenase proficiency testing statistical evaluation indicates that 21% of 3000 labs use the Roche analyzer, but there are 4 other analyzers in common use (Beckman, Siemen's, Vitros, Abbott). The CAP's serum ethanol proficiency testing event treats this assay similarly regardless of the analyzer manufacturer, because the assay is so similar across all platforms. Therefore, it is likely these results would be generalizable to the other chemistry analyzers. It is known that samples spiked with LDH and lactate, run on the same equipment used in this

study can result in false positive ethanol assay, and the minimum LDH required is 26,366 IU/L which must be associated with a lactate of at least 14mM. The minimum lactate required is 4mM which must be associated with an LDH of 43,991 IU/L [4]. No patients in this study had serum concentrations that matched those spiked samples. This suggests that obtaining these concentrations in a live patient is extremely unlikely, and when lactate and LDH concentrations are this high, it is usually a perimortem or postmortem finding. Repeat testing with GC/MS or GC/FID, or repeat enzymatic testing after ultrafiltration to remove LDH can accurately confirm or refute the presence of ethanol. The present study does not support the contention that an elevated LDH and lactate can yield a false positive serum ethanol result as run by enzymatic ethanol assay in live patients presenting to the emergency department.

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Disclosure statement

The authors of this manuscript have no conflicts of interest.

Disclaimer: The views expressed in the submitted article are his or her own and not an official position of the institution or funding source.

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Case Report

Evaluation of Potential Lactate/Lactate Dehydrogenase Interference with an Enzymatic Alcohol Analysis

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Abstract

The Connecticut Department of Public Safety laboratory recently addressed a legal challenge to a hospital alcohol dehydrogenase (ADH)-based serum ethanol determination based on the suggestion of interference by lactate dehydrogenase (LDH)-catalyzed oxidation of lactate. Both ADH- and LDH-oxidations require NAD^+ (present in excess in the assay). NADH produced by LDH-catalyzed lactate oxidation in the assay is interpreted as derived from ethanol. Hepatic trauma was suggested as the basis for elevated levels of lactate and LDH. Clinical laboratory results were evaluated, specifically serum hepatic enzymes, ions, and anion gap. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were 229 and 144 U/L, respectively (~ 8× and 4× reference range midpoint values). Na^+, K^+, Cl^-, and CO_2 levels were 143, 3.0, 112, and 20 meq/L, respectively, yielding an anion gap of 8 meq/L (ref. range 8–15). Serum lactate contributes to "unmeasured anions"; hence, the anion gap was inconsistent with a significant lactate elevation. Based on the slight elevation of ASAT and ALAT, LDH levels were estimated to be elevated to no more than 10-fold. Calculation of the amount of LDH and ADH present in the ethanol assay suggest an ADH/LDH ratio of 200:1. Hence, contribution by lactate oxidation to the serum ethanol concentration in this case would have been negligible.

Introduction

This laboratory was recently asked to provide an opinion regarding the validity of a hospital laboratory alcohol level in a criminal case. The test was an alcohol dehydrogenase (ADH)-based serum ethanol determination. A question was raised regarding the validity of the ethanol value due to the possibility of the assay reflecting a "false positive" result as a consequence of lactate dehydrogenase (LDH)-catalyzed oxidation of lactate present in the sample. In this case, neither lactate nor LDH levels were measured in the patient sample, which forced an evaluation and response based on other clinically measured parameters and information regarding the specific assay utilized for the ethanol analysis. This approach may be a useful model for other practitioners to consider in similar cases.

The enzymatic ethanol method used by the hospital (Roche Ethanol Gen.2), and typical of such assays, is an initial rate method based on the rate of appearance of reduced nicotinamide adenine dinucleotide (NADH; $\lambda_{max} \leftarrow 340$ nm) as a function of ethanol oxidation catalyzed by ADH (1). Similarly, oxidation of lactate to pyruvate by LDH also produces NADH (2) and, if generated in the ADH-assay mix, would be interpreted as ethanol oxidation and hence, as a false "ethanol" result. Although recognized as a potential confounder of the assay, neither lactate nor LDH is regularly present in the serum at levels that could affect the validity of the ADH assay.

LDH is primarily a hepatocellular, cytosolic enzyme not usually significantly present in the blood. LDH and other cytosolic enzymes may be released from the liver as a consequence of abdominal trauma and damage to hepatocytes or chronic liver injury or dysfunction [e.g., cirrhosis, hepatocellular necrosis, or inflammation (3)]. Hepatocellular damage may be assessed by evaluation of levels of such hepatic enzymes in serum. Typically, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) are used clinically to assess hepatic trauma (4). Therefore, the appearance of LDH, ASAT, and ALAT in serum levels in a trauma case are indicative of such injury (3,4).

Lactate (the anionic form of β-hydroxypropanoic acid) is a metabolic intermediate with blood reference levels being 0.9–1.7 mmol/L (5). Lactate is the end product of anaerobic glycolysis and also the glycolytic end product in red blood cells. Lactate is readily taken up from the blood by the liver and utilized in gluconeogenesis (6). Lactate blood levels may be elevated when local tissues are hypoxic (e.g., hypovolemic shock, myocardial infarction, pulmonary edema, or musculoskeletal trauma). Blood lactate may also be temporarily elevated because the patient received treatment in the form of Lactated Ringer's solution (which contains 28 mmol/L lactate). In this case, there was no indication in the medical record that the patient received Lactated Ringer's solution.

LDH normally functions as a reductase, producing lactate from the β-keto analogue pyruvate (as a gluconeogenic pre-

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cursor) and oxidized NAD^+. In the circumstance of elevated lactate and in the presence of excess NAD^+ (as would be found in an ADH-based EtOH assay), the reversible reaction oxidizes lactate to pyruvate with the concomitant reduction of NAD^+ to $NADH+H^+$ (Figure 1). Therefore, it is reasonable to suggest that a serum sample containing both LDH and lactate could produce a false-positive result in an ADH-based EtOH assay as the excess NAD drives the reaction towards pyruvate. The essential condition for a false positive contribution is that there be enough LDH and lactate to actually compete with the ADH-ethanol system. Thompson et al. (7) have shown that lactate-based interference with the assay disappears in ultrafiltered (hence LDH-free) serum samples, emphasizing the need for both enzyme (LDH) and substrate (lactate) to be present for the interfering reaction to proceed. Some assays determine the initial rate of NADH production prior to the addition of ADH as a baseline value. Then following the addition of ADH to the reaction mix, the new initial rate (minus the "baseline" rate) is reflective of ADH-catalyzed oxidation and, hence, ethanol concentration. In this manner, the assay can be "controlled" for the presence of lactate and LDH.

In our experience, the suggestion of lactate/LDH interference has been argued in court on a strictly theoretical basis or based only on the possibility of lactate being present. Lactate, a β-hydroxy carboxylic acid, is not a substrate for ADH, which catalyzes the oxidation of short-chain aliphatic alcohols (8). In the absence of LDH, the presence of lactate alone is not, therefore, an adequate basis for the suggestion of interference with ADH-based ethanol assays.

We had the opportunity with this case to present a numerically-based evaluation of the potential for LDH-based interference. Because the potentially competing reaction is dependent upon the amount of lactate and LDH in the assay, we evaluated clinical parameters to estimate reasonable maximal levels for both lactate and LDH. We then calculated the amount of each that would be present in the assay vessel based on the sampling aliquot.

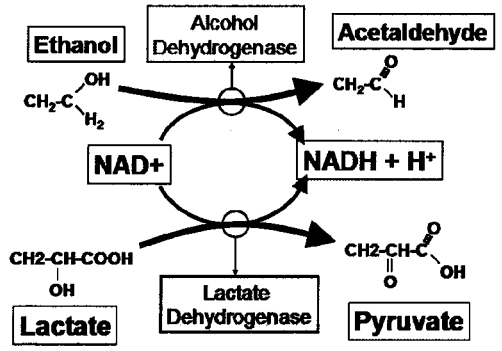

Figure 1. Oxidation of lactate and ethanol to pyruvate and acetaldehyde, catalyzed by LDH and ADH.

Table I. Pertinent Clinical Results

Analyte	Result	High (H)/Low(L)	Reference Range*
Sodium	143		138–145 mmol/L
Potassium	3.0	L	3.4–5.0 mmol/L
Chloride	112	H	98–107 mmol/L
CO_2 content	20	L	22–29 mmol/L
Glucose	161	H	65–99 mg/dL
ASAT	229	H	10–50 U/L
ALAT	144	H	10–50 U/L
Lipase	204	H	0–80 U/L
Amylase	184	H	28–100 U/L

* Laboratory-specific reference ranges.

Case History

The driver in this case (a 33-year-old male) was involved in a single motor vehicle collision by impacting a tree. He was transported to a local hospital emergency department, and blood was drawn shortly thereafter. There was neither radiological nor surgical evidence for intraabdominal organ injury; however, some of his measured serum hepatic and pancreatic enzyme levels were elevated, which was suggestive of some degree of trauma. The authors made the conservative assumption of minor liver injury based upon the approximate fivefold increase of ALAT and eightfold increase of ASAT as compared to the midpoints of the hospital reference ranges (Table I). Because of the rapidity with which hospital testing was performed, aging of samples was not considered to be a significant factor in this case. The treating hospital performed a standard panel of laboratory tests, including a serum alcohol determination using a method based on the rate of NAD reduction by ADH. Serum ethanol was reported as 200 mg/dL, yielding an approximate whole blood % ethanol of 0.17 g/dL. Hospital laboratory results were obtained by warrant, and results of the alcohol test were admitted into evidence during criminal proceedings. Pertinent clinical laboratory findings were as noted in Table I. Anion gap was determined to be 8 mmol/L ($[Na^+] + [K^+] - [Cl^-] - [CO_2]$). The reference range for anion gap is 8–15 (9); therefore, in this case, the anion gap was not elevated. The assay utilized by the hospital laboratory (Roche Ethanol Gen.2) mixes 50 µL of a 37 mU/µL ADH solution with 4-µL sample (1). The assay is an "initial rate" method using ΔA_{340} as the monitored variable.

Results and Discussion

This case was focused on the validity of the alcohol determination performed by the hospital laboratory. Although it had been suggested that lactate alone can interfere with the ADH-based assay by "cross-reaction," lactate, as noted previously (8), is not an effective substrate for ADH. It is, however, reasonable to suggest that the combination of high levels of both lactate and LDH can, in the presence of the excess NAD^+ in the assay, produce NADH and thereby cause a falsely elevated ethanol reading as reported by Nine et al. (10), who also

demonstrated the differential susceptibility of specific assays to this interference.

Serum lactate increases rapidly after death, even in the early postmortem period (11). In postmortem samples, it can be reasonably assumed that intracellular material, including lactate and LDH, have been released into the blood as cellular membrane integrity degrades. For this reason, enzymatic-based alcohol analyses are generally recognized as unreliable when used on postmortem samples. (Such determinations are normally performed using the more specific headspace gas chromatography methodology.)

We considered the (albeit indirect) evidence addressing the likelihood of both LDH and lactate being present in the assay mix from this specific case. First, anion gap is well-recognized as an indicator of lactic acidosis, or elevated lactate generated by metabolic processes. In the case noted previously, the anion gap was not elevated at 8 mmol/L, reference range 8–15 mmol/L (9), a result that is inconsistent with significantly elevated serum lactate. However, we did note that the chloride level was slightly elevated, and carbon dioxide was slightly low. We could not preclude the possibility of a correspondingly slightly elevated serum lactate level of ~ 2–8 meq/L based on the anion gap result. As noted previously, normal lactate levels are ~ 1 meq/L. In comparison, a 0.2 g/dL level of ethanol corresponds to ~ 40 meq/L ethanol, so in this case the ratio of ethanol to lactate was probably at least 5:1. So, in the hypothetical case of a 5:1 ethanol/lactate ratio and equivalent amounts of corresponding enzymes (ADH and LDH), we would expect that the rate of the ethanol/ADH reaction would be at least five times the rate of the lactate/LDH reaction.

The second essential element of consideration is the level of LDH. This enzyme may be released as a function of trauma. When the liver is injured, hepatocytes rupture, and their cytosolic contents (enzymes, etc.) spill into the local blood stream and then the general circulation. Trauma-induced enzyme leakage is non-selective; all the elements that had been contained in the once-intact hepatocytes are released into the blood. Because the mechanism of release is non-specific, one can use the levels of other hepatic enzymes as a general indicator of the extent to which hepatic LDH could reasonably have leaked into the blood. We reasoned that the hepatic enzymes ASAT and ALAT would provide a reasonable indication of the extent of LDH release. The usual LDH blood level is ~ 200 U/L (3). In this case, ASAT and ALAT were elevated ~ eight- and ~ fivefold, respectively. Therefore, we conservatively estimated that LDH was maximally elevated approximately 10-fold. This would yield a serum concentration of ~ 2000 U/L, or 2 mU/µL. The 4-µL sample volume used in the enzyme assay would therefore maximally include 8 mU of LDH in the mix. In contrast, the assay mix receives 1850 mU of ADH (this is an assay-specific parameter and would be expected to vary between manufacturers). This > 200-fold excess of ADH to LDH suggests that even with equivalent concentrations of ethanol and lactate (~ 40 meq/L, which we did not have in this case), the contribution of lactate to the final result would be 1 part in 200. In a serum alcohol result of 200 mg/dL correcting for lactate contribution would yield a 199 mg/dL. Combination of the two factors leads to our expectation that the ethanol level in the assay was at least five times the lactate level, and the ADH level was at least two hundred times the LDH level. Therefore, we concluded that there would be no significant contribution to the ethanol reading in the assay due to the oxidation of lactate.

Conclusions

A potentially legitimate challenge to the validity of an enzymatically based ethanol assay may be raised based on the presence of both lactate and LDH. Although that contention can be argued on a theoretical basis, evaluation of clinical values provides useful information regarding reasonable concentrations of both lactate and LDH that may be present in a specific case. Neither the presence of lactate nor LDH alone provides an adequate basis to suggest interference with typical ADH-based assays. Hence, clinical data indicating levels of lactate and LDH within reference ranges may be adequate to disprove the interference challenge. Actual calculation of the amount of ADH present in the reaction vessel in comparison to maximal estimates of the amount of LDH available (again based on clinical laboratory values) may provide an even stronger basis for the evaluation of interference potential from lactate and LDH.

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BECKMAN COULTER CE

SYNCHRON System(s)
Chemistry Information Sheet

ETOH
Alcohol
REF 474947

For *In Vitro* Diagnostic Use

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

ETOH reagent, when used in conjunction with UniCel DxC 600/800 System(s) and SYNCHRON Systems ETOH Calibrator, is intended for quantitative determination of ethyl alcohol concentration in human serum, plasma, urine, or treated whole blood.

CLINICAL SIGNIFICANCE

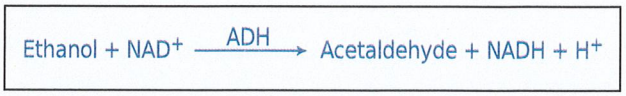
Testing for alcohol is common in medical/legal cases concerning toxic or abused substances.[1] Alcohol can be lethal by itself or can contribute to accidents of all types. Measurements obtained are used in the diagnosis and treatment of alcohol intoxication and poisoning.

METHODOLOGY

Alcohol reagent is used to measure ethyl alcohol concentration by an enzymatic rate method.[2] In the reaction, alcohol dehydrogenase (ADH) catalyzes the oxidation of ethanol to acetaldehyde with the concurrent reduction of Nicotinamide Adenine Dinucleotide (NAD) to NADH.

The SYNCHRON System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 27.5 parts reagent. The system monitors the rate of change in absorbance at 340 nanometers. The rate of change in absorbance due to NADH is directly proportional to the concentration of ethyl alcohol in the sample and is used by the System to calculate and express the ethyl alcohol concentration based upon a two-point calibration curve.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[1,3] Freshly drawn serum, plasma, whole blood collected in sodium fluoride/potassium oxalate tubes, or freshly collected urine are the preferred specimens. Acceptable anticoagulants for plasma are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Nonalcoholic germicidal solution should be used to swab the venipuncture site or to clean the equipment used to collect the specimen.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.[4]
2. Samples should be analyzed without delay and immediately after opening the sample tube. Precautions should be taken to prevent alcohol evaporation from calibrators, controls and samples.

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE PREPARATION

(For whole blood only):

1. Prepare a 6.25% aqueous solution of trichloroacetic acid (TCA).
2. Pipet 300 µL of 6.25% TCA into a labeled microcentrifuge tube.
3. Pipet 300 µL of a well mixed whole blood control or patient sample, into the TCA. Cap tightly.
4. Vortex at least 20 seconds to mix.
5. Centrifuge for 5 minutes at 1500 x g.
6. Using transfer pipets, transfer the supernatant from each tube to a sample cup.
7. Analyze the supernatant.
8. Multiply the result by 2.

Note: SYNCHRON Systems will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two ETOH Reagent Cartridges (2 x 150 tests)

VOLUMES PER TEST

Serum or Plasma	
Sample Volume	10 µL
Total Reagent Volume	275 µL
Cartridge Volumes	
A	200 µL
B	75 µL
C	– –

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Tris reaction buffer (0.2 M)	41 mL
Alcohol dehydrogenase (yeast) (35 KU/L), NAD (9 mmol/L) in Tris buffer	16 mL

Also non-reactive chemicals necessary for optimal system performance.

> ⚠ **CAUTION**
>
> **Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
> **To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

Avoid skin contact with reagent. Use water to wash reagent from skin.

GHS HAZARD CLASSIFICATION

Alcohol Reagent (Compartment A)	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Tris(hydroxymethyl)– aminomethane 1 - 5%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON Systems ETOH Calibrator
At least two levels of control material
Trichloroacetic acid (TCA)

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

ETOH reagent when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON Systems ETOH Calibrator

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON Systems ETOH Calibrator when stored unopened at +2°C to +8°C will remain stable until the expiration date printed on label. Opened calibrators that are recapped and stored at +2°C to +8°C are stable until the expiration date.

⚠ CAUTION

Urine is not known to transmit infectious disease such as Hepatitis or HIV. However, because this product contains material of human origin, it should be handled as though capable of transmitting infectious diseases. The United States Food and Drug Administration recommends such samples be handled as specified in the Centers for Disease Control's Biosafety Level 2 guidelines.[5]

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have valid calibration factors in memory before controls or patient samples can be run.
2. Under typical operating conditions the ETOH reagent cartridge must be calibrated every 30 days and also with certain parts replacements or maintenance procedures, as defined in UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. Load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

The SYNCHRON System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

NOTICE

Sample results are rounded up or down to a maximum of 2 decimal places. To obtain results in percent with 3 decimal places, divide results in mg/dL by 1000 (e.g., 76 mg/dL = 0.076%).

For whole blood calculations see "SAMPLE PREPARATION".

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

The pharmacological response to serum ethyl alcohol level is subject to considerable individual variation. Levels of 300 mg/dL (65.1 mmol/L) have been reported to cause coma, and levels of ≥ 400 mg/dL (86.8 mmol/L) may cause death.[1,6] These values are intended to act only as a guide.

Reference intervals as designated by this laboratory:

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired serum and plasma samples. Values of serum (X) ranging from 4.8 to 540 mg/dL were compared with the values from plasma (Y) yielding the following results:

Table 2.0 Acceptable Anticoagulants

ANTICOAGULANT	LEVEL OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS
Sodium Heparin	14 Units/mL	Y = 0.998X - 1.44; r = 0.999
Sodium Fluoride/Potassium Oxalate	2.5 / 2.0 mg/mL	Y = 0.983X + 0.71; r = 0.998
Lithium Heparin	14 Units/mL	Y = 0.996X - 1.25; r = 0.999

LIMITATIONS

1. Adulteration of the urine sample may cause erroneous results. Alteration of a urine specimen may be detected by checking the appearance, temperature, pH specific gravity, and creatinine levels of a sample.[7]
2. An effort should be made to keep pipetted samples free from gross debris. It is recommended that highly turbid specimens be centrifuged before analysis.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

Table 3.0 Interferences

SUBSTANCE		SOURCE	LEVEL	OBSERVED EFFECT[a]
Serum	Hemoglobin	RBC hemolysate	500 mg/dL	NSI[b]
	Bilirubin	Porcine	30 mg/dL	NSI
	Lipemia	Human	4+	NSI
	Lactate Dehydrogenase (LDH) and Lactate[c]	Porcine NA[d]	1890 U/L and 14 mmol/L	+4 mg/dL
Urine	Acetaldehyde	NA	2000 mg/dL	NSI
	Acetone	NA	2000 mg/dL	NSI
	n-Butanol	NA	2000 mg/dL	+22.1 mg/dL @ 7.6 mg/dL
	Ethylene Glycol	NA	2000 mg/dL	NSI
	Glycerol	NA	2000 mg/dL	NSI
	Isopropanol	NA	2000 mg/dL	+7.2 @ 7.6 mg/dL
	Methanol	NA	2000 mg/dL	NSI
	n-Propanol	NA	2000 mg/dL	+198.5 mg/dL @ 7.6 mg/dL

a Plus (+) or minus (-) signs in this column signify positive or negative interference.
b NSI = No Significant Interference (within ± 4.8 mg/dL or 6%).
c Both LDH and Lactate must be greater than, or equal to, the values listed for interference to occur.[8]
d NA = Not applicable.

2. Increased levels of lactic acid and LDH in post mortem samples may cause elevated alcohol results.
3. Refer to References (9,10) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The SYNCHRON System(s) method for the determination of ethyl alcohol provides the following analytical range:

Table 4.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum, Plasma or Urine	5 – 600 mg/dL	1.1 – 130 mmol/L
Treated Whole Blood	10 – 700 mg/dL	2.2 – 152 mmol/L

Serum, plasma or urine samples with concentrations exceeding the high end of the analytical range should be diluted with ETOH Calibrator Level 1 and reanalyzed.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 5.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for ETOH determination is 4 mg/dL (0.87 mmol/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods. The serum/urine study included 100 fortified serum samples and 97 urine samples (69 fortified). The whole blood study included 88 samples (66 fortified).

Serum/Urine (in the range of 10 to 600 mg/dL):

Y (SYNCHRON LX Systems)	= 0.968X - 0.34
N	= 197
MEAN (SYNCHRON LX Systems)	= 183
MEAN (Enzymatic)[a]	= 190
CORRELATION COEFFICIENT (r)	= 0.999

a A product of Microgenics, Inc., Fremont, CA.

Whole Blood (in the range of 16 to 581 mg/dL):

Y (SYNCHRON LX Systems)	= 1.039X + 4.08
N	= 88
MEAN (SYNCHRON LX Systems)	= 182
MEAN (Radiative Energy Attenuation)[a]	= 171
CORRELATION COEFFICIENT (r)	= 0.995

a A product of Abbott Laboratories, Abbott Park, IL.

Whole Blood (in the range of 15 to 599 mg/dL):

Y (SYNCHRON LX Systems)	= 1.050X + 8.14
N	= 88
MEAN (SYNCHRON LX Systems)	= 182
MEAN (Gas Chromatography)[a]	= 166
CORRELATION COEFFICIENT (r)	= 0.997

a A product of Perkin Elmer, Wellesley, MA.

Refer to References (11) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON System(s) should exhibit precision values less than or equal to the following:

Table 6.0 Precision Values

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[a]		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Within-run	Serum/Plasma/Urine	2.4	0.52	80.0	17.33	3.0
Total	Serum/Plasma/Urine	3.6	0.78	80.0	17.33	4.5
Within-run	Treated Whole Blood	3.2	0.70	80.0	17.33	4.0
Total	Treated Whole Blood	4.8	1.0	80.0	17.33	6.0

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the system evaluated using the NCCLS Approved Guideline EP5-A appears in the table below.[12] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 7.0 NCCLS EP5-A Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (mg/dL)	EP5-A Calculated Point Estimates	
						SD	%CV
Within-run	Aqueous	Control 1	1	80	49.8	0.98	2.0
	Aqueous	Control 2	1	80	102.3	1.32	1.3
	Aqueous	Control 3	1	80	464.4	6.38	1.4
Total	Aqueous	Control 1	1	80	49.8	1.29	2.6
	Aqueous	Control 2	1	80	102.3	2.21	2.2
	Aqueous	Control 3	1	80	464.4	8.85	1.9

a The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

Refer to References (12) for guidelines on performing precision testing.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REVISION HISTORY

Revision AF

Updated corporate address; updated OSHA precaution and removed EDTA as an Acceptable Anticoagulant claim.

Revision AG

Updated the table in the interference section under LDH and Lactate.

Revision AH

Added Revision History

Revision AJ

Added new language requirement: Czech, and Korean.

Revision AK

Removed references to CX and LX systems as they are discontinued effective 12/2013.

Added Beckman Coulter trademark statement and disclaimer.

Revision AL

Added GHS Classification information

Revision AM

Added GHS Classification information

Revision AN

Added new language requirement: Romanian

Revision AP

Updates to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AR

Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

SYMBOLS KEY

Table 8.0

REF	Catalogue Number	IVD	In Vitro Diagnostic
CONTENTS	Contents		Temperature limit
	Manufacturer		Expiration Date
LOT	Batch code	SDS	Safety Data Sheet
CE	CE Mark		Consult Instructions for Use
EC REP	Authorized Representative in the European Community		Date of Manufacture
	Caution		Do not reuse
WARNING	WARNING		
Made in USA of US and Foreign Components		Made in USA of US and Foreign Components	

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