Alcohol (ETOH) – Serum, Plasma, Urine
Beckman UniCel DxC Systems

Technical Procedure 3126

Principle

Intended Use

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

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. Ethanol acts on cerebral functions as a depressant similar to general anesthetics. This depression causes most of the typical symptoms such as impaired thought, clouded judgment, and changed behavior. As the level of alcohol increases, the degree of impairment becomes progressively increased. Measurements obtained are used in the diagnosis and treatment of alcohol intoxication and poisoning. Toxic concentration is dependent on individual tolerance and usage although levels greater than 300-400 mg/dL can be fatal due to respiratory depression.

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

$$\text{Ethanol} + NAD^+ \longrightarrow \text{Acetaldehyde} + NADH + H^+$$

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers
Urine samples should be sent to the laboratory in a 13 X 75 Clear Cap BD tube.

Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Type of Specimen

Freshly drawn serum, plasma or freshly collected urine are the preferred specimens. Whole blood is not recommended for use as a sample. (1,3)

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

Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma should be physically separated from contact with cells within two hours from the time of collection.(4)

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.

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the *Primary Tube Sample Template*.

Reagents

Contents

Each kit contains the following items:

Two ETOH Reagent Cartridges (2 x 150 tests) **Kit Reorder # 474947**

Volumes per Test

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

Also non-reactive chemicals necessary for optimal system performance.

> CAUTION
> Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

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

Materials Needed But Not Supplied With Reagent Kit

SYNCHRON® Systems ETOH Calibrator
At least two levels of control material

Reagent Preparation

No preparation is required.
Date and initial reagent container(s) and document in reagent log before loading each new cartridge.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

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.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

SYNCHRON® Systems ETOH Calibrator Kit **Reorder #474994**

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 unless the expiration date is exceeded or when calibration and quality control recoveries have shifted.

DO NOT FREEZE.

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)

Calibration Information

The system must have a valid calibration in memory before controls or patient samples can be run.

Under typical operating conditions the ETOH assay must be calibrated every 30 days or with each new lot of reagent, and also with certain parts replacement or maintenance procedures, as defined in the UniCel DxC800 System *Instructions for Use* (IFU) manual.

This assay has within-lot calibration enabled. Refer to the UniCel DxC 800 System *Instructions for Use* (IFU) manual for information on this feature.

For detailed calibration instructions, refer to the UniCel DxC800 System *Instructions for Use* (IFU) manual.

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 DxC800 System *Instructions for Use* (IFU) manual.

Traceability

The measurand (ETOH) in this calibrator is traceable to Manufacturer's Working Calibrator. Working Calibrator is prepared gravimetrically from USP grade 200-proof ethanol. The traceability process is based on prEN ISO 17511.

The values were verified using representative samples from this lot of calibrator and are specific to the assay methodologies of the SYNCHRON systems. Values determined by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Cal1 = 0 (zero) mg/dL Ethyl Alcohol in Tris buffer with 0.09% Sodium Azide

Cal2 = 100 mg/dL (22 mmol/L) Ethyl Alcohol in Tris buffer with 0.09% Sodium Azide

Quality Control

A minimum of two levels of control material will be analyzed each shift.

In addition, controls should be run under the following circumstances:
Upon loading a new reagent cartridge.
Following each new calibration.
Following specific maintenance or troubleshooting procedures as detailed in the UniCel DxC800 System *Instructions For Use* manual.

More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	2°C to 8°C*
MAS ChemTrak 3	2°C to 8°C*

*Controls are received frozen and stored at –15°C to –25°C.
Bottles of controls in use are thawed and stored at 2°C to 8°C and are good for 14 days.

Testing Procedure

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration is required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operation.

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

Calculations

UniCel DxC Systems perform 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.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Reporting Results

Equivalency between the SYNCHRON LX and UniCel DxC 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 have been reported to cause coma, and levels of ≥ 400 mg/dL may cause death.(1,6) These values are intended to act only as a guide.

Critical Values

An ethanol result is considered a critical value as defined by the age ranges in the following table and should be called immediately to the attending physician or charge nurse.

Critical Values

Age	Critical Value
< 5 years	**Any Detectable Result**
5 - 15 years	**> 100 mg/dL**
> 15 years	**> 250 mg/dL**

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:

Acceptable Anticoagulants

Anticoagulant	Level of Anticoagulant Tested	Deming Regression Analysis
Lithium Heparin	14 Units/mL	Y = 0.998X - 1.44; r = 0.999
Potassium Oxalate/ Sodium Fluoride	2.5 / 2.0 mg/mL	Y = 0.983X + 0.71; r = 0.998
Sodium Heparin	14 Units/mL	Y = 0.996X - 1.25; r = 0.999

Limitations

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)

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

The following substances were tested for interference with this methodology:

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
	LDH and Lactate[c]	Porcine NA[d]	1890 U/L 14 mM	+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 mg/dL @ 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.

Increased levels of lactic acid and LDH in post mortem samples may cause elevated alcohol results.

Refer to References (9,10) for other interferences caused by drugs, disease and preanalytical variables.

Performance Characteristics

Analytical Measurement Range

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

Analytical measurement Range (AMR)

Sample Type	Conventional Units
Serum, Plasma or Urine	5 - 600 mg/dL

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units
Serum, Plasma or Urine	5 - diluted result mg/dL

Samples with concentrations below the AMR and CRR (5 mg/dL) will be reported as "**Negative**".

Samples with concentrations greater than the AMR should be diluted with ETOH Calibrator Level 1 and reanalyzed. *If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol*.

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.

Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods and documented by Beckman.

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

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

Equivalency assessed by Deming regression analysis of patient samples to accepted clinical methods.

As determined at UCDMC.

Serum or Plasma (in the range of 7.2 to 493.4 mg/dL):

Y (UniCel DxC800-4118)	= 1.038X - 1.58
N	= 31
MEAN (UniCel DxC800-4118)	= 138.46
MEAN (UniCel DxC800-1805)	= 134.88
CORRELATION COEFFICIENT (r)	= 0.9989

Serum or Plasma (in the range of 7.2 to 493.4 mg/dL):

Y (UniCel DxC800-4427)	= 1.025X - 1.37
N	= 31
MEAN (UniCel DxC800-4427)	= 136.92
MEAN (UniCel DxC800-1805)	= 134.88
CORRELATION COEFFICIENT (r)	= 0.9989

Serum or Plasma (in the range of 7.2 to 493.4 mg/dL):

Y (UniCel DxC800-4449)	= 1.040X - 1.84
N	= 31
MEAN (UniCel DxC800-4449)	= 138.39
MEAN (UniCel DxC800-1805)	= 134.88
CORRELATION COEFFICIENT (r)	= 0.9979

Serum or Plasma (in the range of 7.7 to 515.3 mg/dL):

Y (UniCel DxC800-4427)	= 0.988X + 0.19
N	= 31
MEAN (UniCel DxC800-4427)	= 136.92
MEAN (UniCel DxC800-4118)	= 138.46
CORRELATION COEFFICIENT (r)	= 0.9995

Serum or Plasma (in the range of 7.7 to 515.3 mg/dL):

Y (UniCel DxC800-4449)	= 1.001X - 0.25
N	= 31
MEAN (UniCel DxC800-4449)	= 138.39
MEAN (UniCel DxC800-4118)	= 138.46
CORRELATION COEFFICIENT (r)	= 0.9994

Serum or Plasma (in the range of 6.1 to 512.7 mg/dL):

Y (UniCel DxC800-4449)	= 1.014X - 0.44
N	= 31
MEAN (UniCel DxC800-4449)	= 138.39
MEAN (UniCel DxC800-4427)	= 136.92
CORRELATION COEFFICIENT (r)	= 0.9989

Precision

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

As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD	Changeover Value[a]	%CV
		mg/dL	mg/dL	
Within-run	Serum/Plasma/Urine	2.4	80.0	3.0
Total	Serum/Plasma/Urine	3.6	80.0	4.5

[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.

Precision established at UCDMC

Type of Precision	Sample Type	n	Mean (mg/dL)	1 SD (mg/dL)	%CV
DxC800-4118 Within-run	AMM/ETOH 1	20	44.61	1.56	3.5
	AMM/ETOH 3	20	395.99	2.81	0.7
DxC800-4427 Within-run	AMM/ETOH 1	20	49.43	1.10	2.2
	AMM/ETOH 3	20	376.34	4.87	1.3
DxC800-4449 Within-run	AMM/ETOH 1	20	45.62	1.75	3.8
	AMM/ETOH 3	20	379.08	2.91	0.8

Type of Imprecision	Sample Type	No. Systems	No. Data Points	Test Mean Value (mg/dL)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	1	360	19.8	1.10	5.6
	MAS ChemTrak 3	1	358	165.4	5.95	3.6
DxC800- 4427 Day to Day	MAS ChemTrak 1	1	372	18.9	1.07	5.7
	MAS ChemTrak 3	1	388	160.2	8.95	5.6
DxC800-4449 Day to Day	MAS ChemTrak 1	1	361	19.3	1.27	6.6
	MAS ChemTrak 3	1	370	160.4	8.30	5.2

Comparative performance

Comparative performance data for the system evaluated using the NCCLS Approved Guideline EP5-A appears in the table below.(12)

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

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 *Instructions for Use* and *Reference* manual.

References

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			10/25/2004	S. Devaraj
			11/28/2005	G. Kost
5/04/2006	Critical value update	M.Inn	05/16/2006	S. Devaraj
			09/26/2006	G. Kost
			11/05/2007	G. Kost
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			10/12/2010	G. Kost
6/09/2011	Reformat and general update	M.Inn	07/18/2011	G. Kost
			11/16/2011	G. Kost
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