

Intended Use

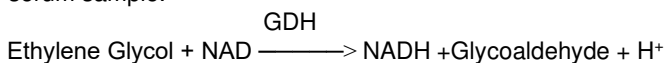
For **In Vitro Diagnostic** use in the automated, quantitative, determination of Ethylene Glycol in serum or Plasma.

Clinical Significance (1-2)

An important toxicological problem in clinical diagnosis is Ethylene Glycol poisoning. When ingested in the form of antifreeze or other automotive products, Ethylene Glycol results in central nervous system depression, cardiopulmonary compromise, and renal insufficiency. Laboratory features of Ethylene Glycol poisoning include increased anion gap and increased osmolal gap, calcium oxalate crystaluria, and detectable Ethylene Glycol in serum.

Method Principle (3-4)

Catachem Ethylene Glycol procedure is based on the affinity of a specific Glycerol Dehydrogenase enzyme from bacteria to catalyze the oxidation-reduction reaction of Ethylene Glycol in the presence of NAD. This two-point kinetic procedure is read at 340nm and the increase in absorbance is directly proportional to the concentration of Ethylene Glycol in the serum sample.

**ENZYME REAGENT**

Each liter contains:

Buffer

Glycerol Dehydrogenase ≥ 1000 Units

Stabilizer and nonreactive ingredients.

ACTIVATOR REAGENT

NAD 5.0 mmol

Stabilizer and nonreactive ingredients.

ETHYLENE GLYCOL KIT A C504-0A contains:

Ethylene glycol sample diluent 2 x 10 mL

Ethylene glycol activator reagent 1 x 5 mL

Ethylene glycol calibrator 1 x 3 mL

Ethylene glycol control I 1 x 3 mL

Ethylene glycol control II 1 x 3 mL

Precautions

Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability

Store the Ethylene Glycol reagents at 2-8°C. When stored as directed, the reagents are stable until expiration date stated on the label.

Working Reagent Preparation

The Ethylene Glycol sample diluent reagent is reconstituted with the addition of 10 mL deionized water. Once reconstituted this reagent is stable for 30 days at 2-8°C. The Activator reagent is used as is.

Reagent Indications of Deterioration

- Turbidity
- Absorbance > 0.8 OD, 1 Cm light path, 340nm
- Quality control values out of assigned ranges.

If these reagent characteristics are observed contact Catachem technical service.

Specimen Collection and Stability (1)

To maintain sample integrity and avoid changes in Ethylene Glycol concentrations care should be taken to collect the sample specimens:

Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied. Plasma specimens should be collected in tubes with heparin, sodium fluoride EDTA, citrate or oxalate as anticoagulants. Separate immediately from the cells and analyze promptly or store well sealed at 2-8°C for up to 24 hours.

Procedure

These instructions are outlined for performing the Ethylene Glycol assay using an automated/manual analyzer. Read the entire original instrument manufacturer's instructions before performing this automated procedure.

Materials Provided

Ethylene Glycol Reagents

Ethylene Glycol Calibrator material with assigned value.

Ethylene Glycol Quality Control material with assigned values.

Materials Required but Not Provided

Automated Analyzer/Spectrophotometer equipped with 340nm wavelength.

Calibration

Catachem's protein-based calibrator which contains a known Ethylene Glycol value is recommended.

Calibration Schedule

Calibration should be performed when this method is implemented on the automated analyzer/spectrophotometer for the first time. Recalibration is best performed prior to the assay of any patient sample and within any 48-hour period.

Calibration Procedure

Instructions for calibrating the automated analyzer/spectrophotometer are provided by the specific instrument manufacturer. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control

To monitor the quality performance of the procedure used, Catachem Ethylene Glycol Control Level I and Control Level II should be included in the assay procedure when the assay is run.

Directions for Use

Catachem's Ethylene Glycol method requires two reagents.

Procedure Limitations

Samples with Ethylene Glycol values greater than 300 mg/dL should be diluted 1:2 with physiological saline and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Materials Required (Not Provided) Manual procedure

Spectrophotometer
Cuvettes 1 cm light path
Timer to time incubation time
Pipette 0.5 ml and 0.1 ml for reagents
Pipette 0.006 ml for sample

Materials Provided

Enzyme Reagent
Activator Reagent Calibrator
Q.C. Controls I and II

Analytical Parameters

Wavelength 340 nm
Temperature 37°C
Pathlength 1 cm Reaction Mode - Rate
Reaction Time 5-10 minutes
Reaction Volume (R1) 0.50 ml
Reaction Volume (R2) 0.100 ml
Sample Volume 0.006 ml
Total Volume 0.606 ml

Assay Procedure

- Bring the Ethylene Glycol Working Reagents to room temperature.
- Set spectrophotometer wavelength at 340 nm and zero the instrument with the cuvette containing water.
- Pipette 0.5 ml of R1 Reagent into each of four cuvettes marked: "sample", "calibrator", "control 1," "control 2".
- Pipette 0.006 ml of calibrator, controls, and sample(s) into their respective cuvettes. Mix all cuvettes well.
- Incubate cuvettes for 3.0 minutes at 37°C.
- Pipette 0.100 ml of Activator Reagent into all cuvettes. Mix all cuvettes well and incubate for 2 minutes. After this 2 minute period take an initial read (A1) and then after an additional 2 minutes take a second read (A2) to obtain a Δ -absorbance (OD @ 340 nm)
- Read the Δ -absorbances for "calibrator", "controls" and "sample".
- Calculate the Ethylene Glycol concentration (mg/dL) in the sample(s), as shown in calculations and results.

Calculations and Results

$$\text{Ethylene glycol (mg/dL)} = \frac{\Delta \text{ OD sample (A2 - A1)}}{\Delta \text{ OD calibrator (A2 - A1)}} \times \text{cal. (mg/dL)}$$

Example:

$$\begin{aligned} \text{Calibrator} &= 150 \text{ mg/dL} \\ \text{Ethylene Glycol (mg/dL)} &= \frac{0.13 - 0.01}{0.15 - 0.05} \times 150 \text{ mg/dL} \\ &= 90 \text{ mg/dL} \end{aligned}$$

Reference Values (5)

None detected ≤ 5 mg/dL
Potentially Toxic > 100 mg/dL

Interfering Substances

The following substances have no significant effect on the accuracy of this Ethylene Glycol procedure at the concentrations stated.

- Glycerol ≤ 47 mg/dl
- Hemoglobin ≤ 200 mg/dl
- Triglycerides ≤ 1000 mg/dl
- Bilirubin ≤ 20 mg/dl
- Propylene glycol ≤ 100 mg/dl
- Ethanol ≤ 350 mg/dl
- Lactate (200 mM) + Lactate dehydrogenase (LDH at 12,000 U/L)
- Acetaminophen $\leq 1,000$ $\mu\text{g/mL}$
- Fomepizole (4 methyl pyrazole) ≤ 120 mg/L

Other alcohols and associated products have been evaluated to determine potential interferences with this assay and are referenced below.(6) Certain drugs are also known to influence the Ethylene Glycol values (1-2).

Method Performance Characteristics

Sensitivity: Using a pathlength of 1 cm, a Δ -absorbance of 0.0016-0.0020 per mg/dL should be obtained.

Linearity: This procedure is linear over the range of 0-300 mg/dL.

Precision: Precision data was obtained using five levels of protein based controls and following the NCCLS EP5-T2 procedure on an automated analyzer (4). The following results were observed:

Precision

Ethylene Glycol	Within-Run Precision		Total Precision	
	Mean	SD	SD	CV
mg/dL	mg/dL	%	mg/dL	%
6	0	0	0	0
88	0	0	3.339	3.940
155	2.920	1.886	3.879	2.502
224	2.610	1.165	4.903	2.188
255	2.619	1.027	9.577	3.755

Bibliography

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