CATACHEM

ETHYLENE GLYCOL FasTox® TEST KIT
PRODUCT NO. C504-0C

Intended Use
For ***in vitro*** diagnostic use in the qualitative determination of Ethylene Glycol in serum or plasma.

Clinical Significance [1-3]
Early identification of the presence of Ethylene Glycol presents a diagnostic challenge in cases of suspected ingestion, often dependent on the availability of sophisticated chromatographic techniques. Ethylene Glycol ingestion may result in severe metabolic acidosis, central nervous system depression, cardiopulmonary compromise and renal insufficiency. Laboratory features of Ethylene Glycol poisoning, often used as surrogates in diagnosis, include increased anion gap, increased osmolal gap, and calcium oxalate crystaluria.

Method Principle [4, 5]
Catachem Ethylene Glycol procedure is based on the affinity of a Glycerol Dehydrogenase enzyme from a specific bacterial species to catalyze the oxidation-reduction reaction of Ethylene Glycol in the presence of NAD. The reaction is monitored as a two-point kinetic assay on a standard spectrophotometer and the rate of increase in absorbance at 340nm is directly proportional to the concentration of Ethylene Glycol in the sample.

$$\text{Ethylene Glycol} + \text{NAD} \xrightarrow{\text{GDH}} \text{NADH} + \text{Glycoaldehyde} + H^+$$

SAMPLE DILUENT REAGENT
Each liter contains:

Buffer	
Glycerol Dehydrogenase	≥1000 Units
Stabilizer and nonreactive ingredients.	

ACTIVATOR REAGENT
Each liter contains:

NAD	6.0 mmoles
Stabilizer and nonreactive ingredients.	

KIT CONTENTS

Each kit, Product Number C504-0C, contains all of the materials, including controls, needed to perform three individual patient tests.

C504-34 Ethylene Glycol Sample Diluent Reagent (3 x 4 mL) (dry powder to be reconstituted).

C504-33 Ethylene Glycol Activator Reagent (3 x 750 µL) (liquid ready for use).

C504-13 "Negative" Control (3 x 100 µL) (liquid, ready for use) at approximately 200 mg/L (3.2 mmol/L) ethylene glycol.

C504-14 "Positive" Control (3 x 100 µL) (liquid, ready for use) at approximately 2,500 mg/L (40.0 mmol/L) ethylene glycol.

Each vial of reagent and control is for ***a single use only***. Opened vials should be discarded after use.

Precautions
Handle reagents in accordance with good laboratory practices. **DO NOT PIPETTE BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area(s) with copious amounts of cold water. Contain and clean spills immediately. Disposal should be in accordance with local regulations and laws. Refer to SDS for additional information.

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

Materials required but not provided

Spectrophotometer with a 340 nm filter
1.5 mL semi-micro cuvettes (1 cm path length)
10 µL pipette
150 µL pipette
1 mL pipette

PROCEDURE ON A MANUAL SPECTROPHOTOMETER
Reconstitute Ethylene Glycol Sample Diluent Reagent vial with 4 mL of deionized water using a volumetric pipette. Wait until powder has visibly dissolved and solution is clear.
With a volumetric pipette, dispense 1.0 mL of this solution into each of three labeled cuvettes, for the negative control, positive control and patient sample.

Add 10 µL of "Negative" Control to one cuvette, 10 µL of "Positive" Control to the second cuvette and 10 µL of the Patient Sample to the third cuvette. Gently mix all cuvettes.

Read the INITIAL OD at 340 nm of each cuvette on a spectrophotometer and record in the chart provided (Attachment A).

Add 0.15 mL (150 µL) of Ethylene Glycol Activator Reagent to each cuvette (at approximately 15 second intervals). Gently mix all cuvettes.

Incubate all cuvettes for 5 minutes at Room Temperature (or at 37°C) - It is important to handle all cuvettes similarly.) Read the FINAL OD at 340 nm for each cuvette (using the same approximate 15 second intervals) and record in the chart provided. (Attachment A).

Determine patient result compared to the controls as outlined in Attachment A.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.8 OD, 1 cm light path, 340nm
- Incorrect results on the provided kit controls as determined under results section in Attachment A.

If these reagent characteristics are observed, contact Catachem Technical Service at service@catacheminc.com

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Specimen Collection and Stability [4, 6]

To maintain sample integrity and avoid changes in Ethylene Glycol concentrations, venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied.

Serum specimens should be collected in plain tubes or in silicone barrier collection tubes.

Plasma specimens should be collected in tubes with EDTA, lithium heparin, sodium fluoride, 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 until analysis can be performed.

Materials Required But Not Provided

A spectrophotometer equipped with 340nm wavelength, volumetric pipettes, and a timer.

Calibration

The manner in which this test is run eliminates the need for further calibration.

Reference Values [2,7-11]

Any level of ethylene glycol in a sample is not normal. As this format of the test is a qualitative format and not designed for quantitative measurement, the reference values noted below are provided for informational purposes only.

None detected	≤ 50 mg/L (0.8 mmol/L)
Potentially Toxic	≥ 200 mg/L (3.23 mmol/L)*

*Treatment indication level based on general consensus, published literature and clinical guidelines. Ethylene glycol is rapidly converted *in vivo* to its major toxic metabolite, glycolic acid which itself is not measured in this assay.

Low or undetected levels of ethylene glycol should therefore not be interpreted unequivocally to assume that no ingestion of ethylene glycol has occurred, but should be carefully evaluated with other clinical evidence.

Interfering Substances

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

- Hemoglobin ≤ 200 mg/dL (2.0 g/L)
- Triglycerides ≤ 1000 mg/dL (11.3 mmol/L)
- Bilirubin ≤ 2.2 mg/dL (37.7 µmol/L)

The following substances have no significant effect on the interpretation of this assay up to a concentration of 100 mmol/L: acetone, ethanol, isopropanol, glycerol, lactate, methanol, propanediol and propylene glycol [4, 14].

Fomepizol (Antizol®) has no significant effect at therapeutic levels [12].

The following substances have no significant effect up to a concentration of 300 mg/dL in various combinations: 1-butanol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, formic acid, glycolic acid, glyoxal solution, glyoxylic acid, 1-octanol, oxalic acid, polyethylene glycol and n-propanol [12].

Other substances are known to influence the Ethylene Glycol values. Propylene glycol and DOT brake fluid (mixture of di- tri- and tetra ethylene glycols) at concentrations exceeding 1000 mg/L, do affect this chemistry although they do not affect the interpretation of results. 2, 3-butanediol has no significant effect up to a concentration of 250 mg/L (2.8 mmol/L). For concentrations exceeding this level, results can be affected [12, 14].

There have been reports in scientific literature that false positives can occur in samples with LDH activity >12 fold of the upper reference limit with concomitant lactate concentrations ≥10 fold of the upper reference limit [1, 8, 13].

Additional substances or drugs may influence ethylene glycol values [16, 17]

Method Performance Characteristics

Linearity: This procedure is linear over the qualitative equivalent of the ethylene glycol range 22 - 2600 mg/L (0.3 - 41.6 mmol/L).

Precision and Accuracy: This was determined by taking 21 patient samples from patients with an increased anion gap metabolic acidosis of which seven samples were known to contain ethylene glycol at levels > 100 mg/L and the others no ethylene glycol. This method correctly identified the seven samples containing ethylene glycol which had previously been determined using the quantitative Catachem Ethylene Glycol assay (C504-0A) on a Roche Cobas 8000 analyzer.

Bibliography

1. Eder AF, et al. Ethylene glycol poisoning: toxicokinetic and analytical factors affecting laboratory diagnosis. Clin Chem 1998; 44:168-177.
2. Wu AHB, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. Clin Chem 2003; 49:357-379.
3. McQuade DJ, Dargan PI, Wood DM. Challenges in the diagnosis of ethylene glycol poisoning. Ann Clin Biochem 2014; 51:167-178
4. Standefer J, Blackwell W. Enzymatic method for measuring ethylene glycol with a centrifugal analyzer. Clin Chem 1991; 37:1734-1736
5. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol.12, No. 4
6. Scherk JR, et al. Preliminary evaluation of a quantitative ethylene glycol test in dogs and cats. J Vet Diagn Invest 2013; 25:219-225.
7. Keyes DC, Kagalwalla AA. Ethylene glycol toxicity treatment and management. American Association of Poison Control Centers, National Poison Control Data System, 2009.
8. Fraser AD. Clinical toxicologic implications of ethylene glycol and glycolic acid poisoning. Ther Drug Monit 2002; 24:232-238.
9. Long H, Nelson LN, Hoffman RS. A rapid qualitative test for suspected ethylene glycol poisoning. Acad Emerg Med 2008; 15:688-690.
10. Scalley RD, Ferguson DR, Smart ML, Archie TE. Treatment of ethylene glycol poisoning. Am Fam Physician 2002; 66:807-812.
11. Barceloux DG, Krenzelok EP, Olson K, Watson W. American Academy of Clinical Toxicology practice guidelines on the treatment of ethylene glycol poisoning. Ad Hoc Committtee. J Toxicol Clin Toxicol 1999; 37:537-560.

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12. Juenke JM, et al. Adaptation of a commercial enzymatic assay to automated chemistry analyzers. Am J Clin Pathol 2011; 136:318-324.
13. Eder AF, et al. Elevated serum lactate and lactate dehydrogenase interfere in an enzymatic assay for ethylene glycol [Tech Brief]. Clin Chem 1996; 42:1489-1491.
14. Porter WH. Ethylene glycol poisoning: quintessential clinical toxicology; analytical conundrum. Clinica Chimica Acta 2012; 413:365-377.
15. Hardy L, et al. Ethylene glycol screening: adapting the Catachem enzymatic assay to minimize false positives. [Abstract] Clin Chem 2010; 56:A70 [Supplement].
16. Malandian H, Cano Y. Interferences of glycerol, propylene glycol and other diols in the enzymatic assay of ethylene glycol. Eur J CLin Chem BioCHem 1996; 34: 651-654.
17. Young DS, Pestaner LC, Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 1975; 21: No 5.

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C504-0C-EU/ Applic/ Rev D /2018 Apr25 /dtJRH

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