CATACHEM

ETHYLENE GLYCOL FasTox Quant KIT
PRODUCT NO. C504-FQ
[IN THE USA, FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES]

Intended Use
For ***in vitro*** diagnostic use in the quantitative 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 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-FQ, contains 12 x 1 cm cuvettes with Caps and all the materials, including calibrator and controls, needed to perform three individual patient tests in duplicate.

C504-70 Ethylene Glycol Sample Reagent 3 x 67.5 mg (Powder reagent.) R1 reagent.

C504-71 Ethylene Glycol Reagent diluent (3 x 4.5 ml) (Liquid diluent). R1 Diluent.

C504-72 Ethylene Glycol activator reagent (1 x 4 mL) Dropper bottle

C504-80 Calibrator (1 mL) (liquid, ready for use) at 25 mmol/L, (155 mg/dL, or 1,550 mg/L) ethylene glycol in a serum base.

C504-81 Control (1 mL) (liquid, ready for use) range 36-50 mmol/L (223-310 mg/dL) ethylene glycol in a serum base.

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 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

Volumetric Pipette capable of pipetting 10 µL accurately
Volumetric Pipette capable of pipetting 1 mL accurately

Procedure on StatFax to perform one patient test in duplicate.

1. Switch on the StatFax machine and allow the temperature in the wells of the heating block to increase to 37°C (36.5°-37.5°C) – approximately 5 minutes.
2. Pour entire contents of the C504-71, Ethylene Glycol (EG) R1 Diluent vial (clear glass) into a vial of C504-70, EG reagent powder R1 (amber glass).
3. Replace black cap and gently invert vial until all powder is dissolved and solution clear.
4. Pipette 1 mL of this reconstituted R1 solution into each of 4 plastic cuvettes and place these into the heating block.
5. *Note: Four cuvettes are provided to allow one patient sample to be run in duplicate.*
6. Wait approximately 5 minutes to ensure solutions have reached 37°C.
7. Press **Run Test** on the StatFax screen and follow prompts as below.
8. Select the **Ethylene glycol** test. The screen will read **Referencing Air** while the test loads.
9. When **Print Full Header** appears, select **yes** to print the test parameters.
10. The machine will ask: **Use stored blank**; Press **no**.
11. The machine will prompt: **Insert blank**.
12. Insert any of the incubating R1 cuvettes into the reading well to act as a blank.
13. The machine will prompt: **Remove Tube**
14. A graph will appear on screen with options to **accept** or **discard**; press **discard** since you are recalibrating.
15. The machine will prompt: **Insert Standard**
16. Prepare the "Standard" by pipetting 10uL of C504-80, EG Calibrator (small glass vial, black cap), into one cuvette, then add 5 drops of C504-72, EG activator reagent R2 (dropper bottle, white cap) into the same cuvette.
17. Firmly place a black stopper on the "Standard" cuvette and gently invert 3-4 times to mix contents.
18. Place the "Standard" cuvette into the reading well on the analyzer. *Note: the clear sides point right-left (< — >).*
19. Once the read is complete, the machine will prompt: **Remove tube**; remove the "Standard" cuvette. The machine is now calibrated.
20. Press **Accept**; the machine is now ready to accept the other cuvettes.

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ●catachem@catacheminc.com

21. Prepare "patient cuvette 1" by pipetting 10uL of patient sample into one of the three remaining cuvettes. Add 5 drops of R2 C504-72, EG Activator Reagent, to the same cuvette and gently invert 3-4 times to mix.
22. Insert "patient cuvette 1" into the reading well of the StatFax. The machine will automatically begin the read once the cuvette is inserted into the reading well.
23. When the read is complete, the machine will prompt: **Remove tube**; remove "patient cuvette 1".
24. Prepare the "control" cuvette by pipetting 10uL of C504-81, EG control (small glass vial, blue cap), into one of the two remaining cuvettes. Add 5 drops of C504-72, EG Activator Reagent, to the same cuvette and mix well.
25. Insert the "control" cuvette into the reading well of the StatFax. The machine will automatically begin the read once the cuvette is inserted into the reading well.
26. When the read is complete, the machine will prompt: **Remove tube**; remove the "control" cuvette.
27. Prepare "patient cuvette 2" by pipetting 10uL of patient sample into the last remaining cuvette. Add 5 drops of R2 C504-72, EG Activator Reagent, to the same cuvette and mix well.
28. Insert "patient cuvette 2" into the reading well of the StatFax. The machine will automatically begin the read once the cuvette is inserted into the reading well.
29. When the read is complete, the machine will prompt: **Remove tube**; remove "patient cuvette 2".
30. Test results are printed automatically.
31. Verify that the result for the "control" is within specification (36 – 50 umol/L).
32. If the "control" is within specification, the average of the patient sample results should be used to determine the most accurate Ethylene glycol level in the sample.
33. If the "control" reads outside the 36-50 umol/L range repeat the test with new reagents from the beginning. (*if value reads outside specification this suggests an unsuccessful calibration.)*
34. Once all analysis is completed, press **Quit** and turn off the machine.

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.

Calibration

The test is freshly calibrated each time it is performed.

Reference Values [2,7-11]

Any level of ethylene glycol in a sample is not normal.

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

Fomepizole (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 by evaluating the altered reaction curve these interferences are easy to detect. 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 Lactate dehydrogenase (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 shows a linear response over the ethylene glycol range 0.3 – 50 mmol/L. (22 - 3100 mg/L).

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.
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.

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ●catachem@catacheminc.com