CATACHEM

VetSpec™
ETHYLENE GLYCOL QUALITATIVE REAGENT TEST KIT (C504-0B) FOR VETERINARY USE ONLY

Intended Use
For *in vitro* diagnostic use in the manual, **QUALITATIVE** determination of ethylene glycol in serum or plasma in the veterinary laboratory.

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)
The Catachem Ethylene Glycol procedure is based on the affinity of the enzyme Glycerol Dehydrogenase (GDH) from bacteria to catalyze the oxidation-reduction reaction of ethylene glycol in the presence of NAD. NADH couples with a tetrazolium salt with a color change from clear to purple. The following reaction scheme (Fig 1) depicts the steps that occur in this ethylene glycol method.

FIGURE 1

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

$$\text{NADH} + \text{Tetrazolium Salt} \xrightarrow{\text{Diaphorase}} \text{Formazan Dye (Purple Color)}$$

ETHYLENE GLYCOL REAGENT
Each liter contains:
Buffer
Glycerol Dehydrogenase ≥1000 Units
Diaphorase ≥5000 Units
Cofactor
Tetrazolium Salt
Stabilizer and nonreactive ingredients

ETHYLENE GLYCOL REAGENT DILUENT
Each liter contains:
Water
Surfactant
Stabilizer and nonreactive ingredients

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 copious amounts of cold water. Clean up spills immediately.

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

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 plasma immediately from the cells and analyze promptly or store at 2-8°C if not assayed immediately.

Directions for Use
These instructions are for manually performing the Catachem Ethylene Glycol qualitative assay.

Materials Provided
Catachem Vetspec™ Ethylene Glycol Kit-B (C504-0B)
Kit Includes:
Catachem Ethylene Glycol Reagent C504-30
6 vials – (3 Red Cap, 3 Blue Cap)
Catachem Ethylene Glycol Reagent Diluent C504-31
(1x7mL vial - Black Cap)
Qualitative Ethylene Glycol Control C504-32
in Bovine serum albumin 20 mg/dL
U-100 Syringe, 1mL (100 Units) (3 syringes per Kit)

Materials Required, But Not Provided
Timer

Interfering Substances

No interference was found in this assay from 4 methyl pyrazole (Fomepizole) the common antidote which was tested to a level of 3,000 mg/dL.

The following substances if present in the serum/plasma sample may produce falsely positive results:

- Propylene glycol
- Propanediol
- Glycerol
- Sorbitol
- Thimerasol

Other substances and certain drugs are also known to influence the ethylene glycol test results.

Procedure:

1.) For each sample to be tested, use one Reference Vial (**blue cap**), one Test Vial (**red cap**) and one dispensing 1mL (100 Units) U-100 syringe from the reagent kit. Also take out the Catachem Ethylene Glycol Reagent Diluent Vial (**black cap**). Allow vials to reach room temperature before proceeding with the assay (about 10-15 minutes).

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2.) Carefully open the Catachem Ethylene Glycol Reagent Diluent Vial (**black cap**). Using the dispensing 100 Units (1mL) syringe (remove protective shields for needle and plunger) slowly draw **exactly 100 Units (1 mL)** of Catachem Ethylene Glycol Reagent Diluent (see Fig. 2). With the syringe needle, **carefully** pierce rubber septum of Catachem Ethylene Glycol Reagent Test Vial (**red cap)** and **gradually** inject Diluent into the Reagent Vial until syringe is completely empty.

Carefully withdraw needle from Reagent Vial. Mix Reagent Vial by inverting 2-3 times, gently, to prevent foaming, until Working Reagent is completely in solution (about 15 seconds).

3.) Repeat the same process to reconstitute the Reference Reagent Vial (**blue cap).**

4.) Using the **same** syringe as in Step 2, slowly draw **exactly 10 units** (0.1mL) of patient sample from serum/plasma container and inject sample into the Test Vial (**red cap**) containing the newly re-constituted Ethylene Glycol Working Reagent. Withdraw needle from Reagent Vial and using the same needle immediately draw exactly 10 units (0.1ml) of ethylene glycol control from the Control vial (**black cap**-C504-32) and inject this into the Reference Vial (**blue cap**) - replace protective shield on the needle. Quickly mix vial contents by inverting 2-3 times. Immediately set timer for 5 minutes. Carefully watch and note the color development in both the test and reference control vials. Compare color development in the Test Vial (**red cap**) to that of the Reference Vial (**blue cap – after control sample added**) which will develop a metered amount of color.

Interpretation of Results

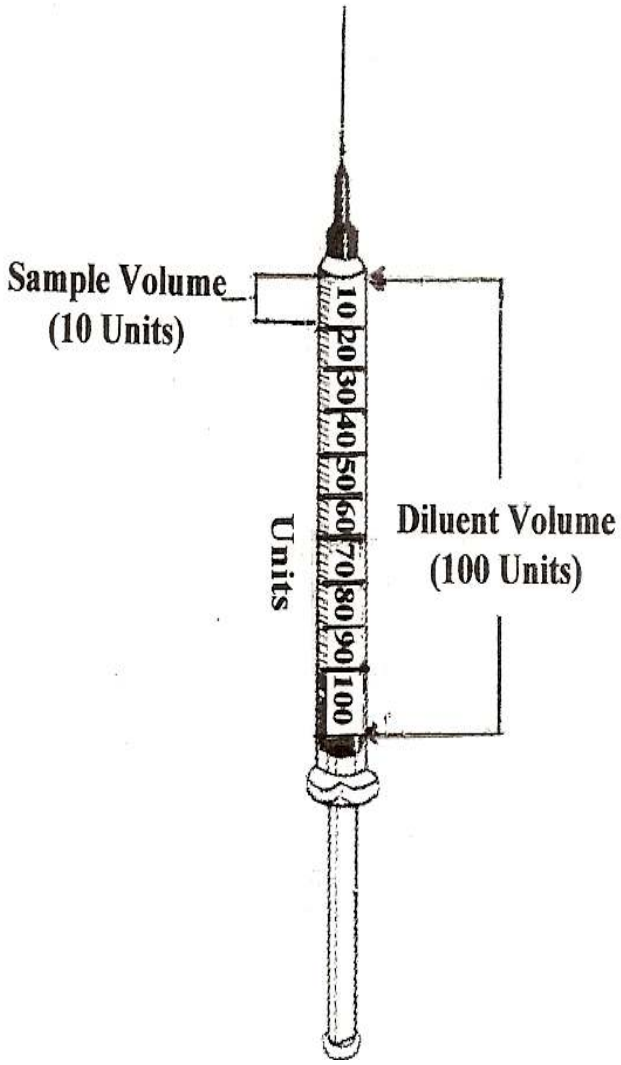
If after 5 minutes the color in the test vial is a darker, more intense purple than the color in the control vial then the animal has most likely ingested ethylene glycol.

Toxicity and Reference Values (5, 6)
No trace of ethylene glycol in a living organism is normal.

Toxic Levels (mg/dl)
Ethylene glycol levels in dogs peak 2 hours after ingestion. The half-life in the blood is 3-4 hours.[6] Values below are approximate and dependent on the size and weight of the animal.

Cats	20 mg/dL
Dogs	50 mg/dL

Fig. 2) 100 Units (1ml) Syringe



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