Original Study

Journal of Veterinary Emergency and Critical Care **18**(5) 2008, pp 477–479
doi: 10.1111/j.1476-4431.2008.00343.x

Preliminary validation of a point-of-care ethylene glycol test for cats

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Abstract

Objective – To evaluate the sensitivity and specificity of a newly available, semi-quantitative, cage-side test for the detection of ethylene glycol (EG) toxicosis in cats.
Design – Prospective, laboratory study.
Setting – University teaching hospital.
Animals – This study utilized samples from 57 cats, whose blood had been anticoagulated with EDTA and submitted to the hospital's laboratory for a complete blood count. Samples were centrifuged, and the plasma separated, aliquoted, and immediately frozen at −30 °C.
Interventions – Samples were randomly divided into 2 primary groups (Group 1: no EG added, Group 2: EG added). Twenty microliters of plasma from each of the Group 1 samples was applied directly to the test strip. Plasma samples from Group 2 had EG added at different concentrations to achieve approximate final concentrations of 20, 60, or 80 mg/dL. These samples were then applied to the test strip.
Measurements – Two readers who were blinded to the sample preparation procedure and isolated from each other were asked to categorically interpret the colorimetric reaction on the randomly presented test strips.
Main Results – The agreement of the 2 reviewers at the 3 different levels of EG concentrations (20, 60, 80 mg/dL) were 0.7, 0.7, and 0.5, respectively. Thus, the readers demonstrated substantial agreement while reading the 2 lower concentrations, while at 80 mg/dL the level of agreement was moderate. Overall, the sensitivity of the assay increased as the concentration of EG increased (reviewer 1: 67%, 67%, 86%; reviewer 2: 56%, 89%, 100%), while the specificity of the assay decreased with increasing concentrations of EG (reviewer 1: 77%, 45%, 50%; reviewer 2: 77%, 53%, 25%).
Conclusions: Because of the likelihood for false negatives and false positives, results from this test must be viewed in light of clinical data and should not be relied upon as a lone diagnostic test.

(J Vet Emerg Crit Care 2008; 18(5): 477–479) doi: 10.1111/j.1476-4431.2008.00343.x

Keywords: antifreeze, feline, poisoning, toxicology

Introduction

Ethylene glycol (EG) ingestion is a common small animal toxicosis and a leading cause of acute renal failure in companion animals.[1–3] Although EG historically has been found in industrial solvents, lacquers, pharmaceuticals, polishes, and cosmetics,[4,5] exposure to automobile antifreeze is the cause of most animal exposures.[6] While the willingness of animals to consume this toxic product has often been blamed on its sweet flavor[5,6] and warming sensation,[7] at least 1 study demonstrated that dogs are not particularly attracted to its taste.[8] This may also be true for cats.

Suspicion of EG toxicosis is based on history, clinical signs, and compatible biochemical changes. In the first 12 hours following ingestion, affected patients often display signs consistent with alcohol intoxication,[7,9] while later signs are associated with acute kidney injury.[7] Metabolic acidosis,[10] increased anion gap,[11] hypocalcemia,[11,12] hyperglycemia,[11] and azotemia[13] are commonly detected biochemical abnormalities. Calcium oxalate monohydrate crystalluria, which begins to appear 3 hours after ingestion, is also common.[11] Rapid diagnosis of suspected EG toxicosis is critical, as

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Conflicts of interest: None

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successful treatment is dependent on timely intervention.[14] In cats, definitive diagnosis of EG toxicosis has been hindered by the lack of a suitable diagnostic test. Currently available cage-side veterinary diagnostic tests accurately detect blood EG levels in excess of 50 mg/dL,[a] which is greater than the feline toxic dose, increasing the chance for false negative test results.

Recently, a semiquantative veterinary EG test kit has been introduced.[b] This test, which requires a single drop (20 μL) of plasma, is designed to detect EG at concentrations as low as 20 mg/dL. This test, has been promoted for its ability to detect potentially fatal EG plasma levels in cats; however, there are no published studies to support this claim. The goal of this study is to determine if this test demonstrates sufficient sensitivity and specificity for clinical use when utilized on feline plasma containing EG at concentrations of 20, 60, and 80 mg/dL.

Methods and Materials

According to the manufacturer's directions, whole blood should be collected and anticoagulated with EDTA or heparin. The sample should then be centrifuged for 5 minutes (speed unspecified) and 20 μL of the resulting plasma placed on the test strip. If EG is present, a colorimetric reaction will occur and the degree of color change is commensurate with the plasma EG concentration. The strip is to be read after 8 and before 10 minutes.[b]

In this study, we collected feline blood samples that had been anticoagulated with EDTA and submitted to the hospital's diagnostic laboratory for a CBC. In all cases, the samples were no longer needed for diagnostic purposes. Samples were excluded from analysis if the patient's serum chemistry analysis revealed azotemia, increased anion gap, hypocalcemia, hyperglycemia, if a matching chemistry was unavailable or if the patient had received any medication containing propylene glycol, glycerol, or ethanol. Samples were centrifuged for 5 minutes at 500 *g* and the plasma was separated, aliquoted, and immediately frozen at −30 °C. Samples that demonstrated evidence of hemolysis were discarded.

Fifty-seven feline plasma samples were available for analysis. Plasma samples were thawed, warmed to 37 °C, and assigned to either a control group (Groups 1, 3, 5), or an experimental group (groups 2, 4, 6) via a random number generator.[c] For each sample assigned to Group 1, 3, or 5, 20 μL of unadulterated plasma was directly applied to the test strip. Pure ethylene glycol[d] with a density of 1.113 g/mL was diluted in phosphate-buffered saline to a concentration of 8.95 μg/μL. For samples assigned to Group 2, 2.3 μL of the diluted EG was added to 97.7 μL of the plasma sample and mixed by rapid pipetting to obtain an EG concentration of approximately 20 mg/dL. Samples assigned to Group 4 had 6.9 μL of the diluted EG added to 93.1 μL of the plasma sample and mixed by rapid pipetting to obtain a final concentration of approximately 60 mg/dL. For samples assigned to Group 6, 9.2 μL of the diluted EG was added to 90.8 μL of the plasma sample and mixed by rapid pipetting to obtain an EG concentration of approximately 80 mg/dL. Twenty microliters of each sample was pipetted onto a test strip. The test strips were prepared and presented to the readers in the order provided by the random number generator. Only the individual mixing the samples (M.J.A.) had knowledge of the ordering of the samples. Two readers who were blinded to the sample preparation process (M.E.J., V.F.S.) were placed in separate work areas and were not allowed to communicate. In accordance with the manufacturer's directions, the first reader examined the strip at 8 minutes and the other evaluated the same strip at 9 minutes. Each had 30 seconds to interpret the colorimetric reaction. They were only asked to record if the sample was positive or negative for EG, not the level. In order to ensure that the samples were prepared correctly, 2 samples from Group 2, which the readers failed to identify as positive, were sent to a diagnostic laboratory for quantitative analysis.[e] Both samples were found to contain 24 mg/dL EG.

The 95% confidence intervals (CI) were calculated for binomial proportions where appropriate. Cohen's test was used to measure the level of agreement (precision) between the 2 individuals screening the assays.[15,16] The test sensitivities and specificities were estimated for each reviewer at each EG level.

Results

The statistics comparing the 2 observers at the 3 different levels of EG (20, 60, 80 mg/dL) were 0.7, 0.7, and 0.5, respectively. Based on the suggestions of Landis and Koch,[15] the assays with 20 and 60 mg/dL of EG were considered to have substantial agreement, while at 80 mg/dL the level of agreement was moderate. The test sensitivities and specificities for each reviewer at the 3 different concentrations of EG are reported in Table 1.

Discussion

EG exposure is a common cause of small animal poisoning and a leading cause of acute kidney injury and death. Cage-side test kits, which are often used to confirm EG exposure, are designed to detect alcohols in the blood and can provide false positive results in animals

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Table 1: Test sensitivity and specificity by ethylene glycol concentration

	Sensitivity (20 mg/dL)	Sensitivity (60 mg/dL)	Sensitivity (80 mg/dL)
Reviewer 1 (%)	67	67	86
Reviewer 2 (%)	56	89	100
	Specificity (20 mg/dL)	**Specificity (60 mg/dL)**	**Specificity (80 mg/dL)**
Reviewer 1 (%)	77	45	50
Reviewer 2 (%)	77	53	25

that have received drugs containing propylene glycol or those exposed to ethanol or sorbitol. Nevertheless, a positive result may quickly lead to emergency treatment or euthanasia while a negative result leads to a search for an alternate diagnosis. The authors are unaware of any published study demonstrating the accuracy or precision of these tests. In this study, 2 blinded readers were given EG test strips that were exposed to either nonadulterated feline plasma or plasma to which a known concentration of the toxicant had been added. No attempt was made by the readers to utilize the test in a semiquantitative manner. Only positive or negative results were recorded.

With any colorimetric reaction, there is always the possibility that 2 or more reviewers could interpret the results differently. Nevertheless, analysis shows that the readers had a substantial level of agreement at the 20 and 60 mg/dL levels of EG concentration, but only a moderate level of agreement at 80 mg/dL.

Sensitivity of the test at the lowest level of detection (20 mg/dL) ranged from 56% to 67%. Thus, as many as 40% of all tested samples containing EG were incorrectly interpreted as not poisoned. Although sensitivity significantly increased with the concentration of EG, the specificity decreased. At the highest concentration of EG (80 mg/dL), between 50% to 75% of all non-EG-containing samples were interpreted by the readers as poisoned.

One possible explanation for the problems with sensitivity and specificity demonstrated by this study is that the test was designed to detect both EG and its metabolites. However, communications with the manufacturer before the commencement of this study confirmed that the test strip reacts only with EG. Another possible explanation could be the test strip readers were inexperienced; however, both have had extensive experience in the veterinary field. The manufacturer does provide control samples for high and low (20 mg/dL) concentrations of EG that can be used to train observers in private practice. Initial training using these standards may have improved sensitivity and specificity of the test; however, these were unavailable at the time of this study.

The diagnosis of EG toxicity is one that must be made on the basis of clinical signs, history, and laboratory data. The EG test examined in this study produced a large number of false negatives and positives at various EG concentrations. At lower EG concentrations, the high rates of false negatives will lead to missed opportunities for treatment. False positive tests may subject patients to costly and unnecessary medical intervention or euthanasia. Therefore, results of this test must be viewed in light of the clinical data and should not be used alone for a definitive diagnosis. The fact that the specificity of the assay was never $>80\%$ suggests that this test has limited value as a stand-alone screening test.

Footnotes

[a] EGT test kit, Allelic Biosystems, Kearneysville, WV.
[b] Kacey ethylene glycol test, Kacey Inc, Asheville, NC.
[c] GraphPad Prism 5.0 for Mac, GraphPad Software, San Diego, CA.
[d] Ethylene Glycol Reagent Plus, Sigma-Aldrich, St Louis, MO.
[e] Louisiana Animal Disease Diagnostic Laboratory, Baton Rouge, LA.

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