

Title: The Effects of Sodium Fluoride on Ethanol Stability in Vacutainer Gray Stopper Tubes

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Introduction: In 2019, Becton Dickinson (BD) issued a recall for their Vacutainer Gray Stopper Tubes (GSTs), lot number 8187663, due to absence of additives meant to preserve the sample. Each tube should have contained 100 mg of sodium fluoride, an anti-microbial, and 20 mg of potassium oxalate, an anti-coagulant, but it was confirmed that 300 tubes were missing additives. BD stated that the missing additives would not affect the Blood Alcohol Concentration (BAC) of a collected sample if stored at room temperature for no more than two days. However, their recall notice stated that, for samples stored at room temperature for longer than two days, the BAC determined may be falsely high or falsely low. Specific wording in the recall mentioning the “preservative” additive led to speculation that potassium oxalate may have been included in some GSTs while sodium fluoride was not included. Potassium oxalate would prevent the blood from clotting but would not prevent any microbial growth within the sample during long-term storage. Other manufacturers, such as American Molecular (AM), also produce GSTs with the same ratio of additives included.

Objectives: This study evaluates the effects of sodium fluoride on BAC stability under different experimental conditions using BD and AM GSTs. These conditions include GSTs containing sodium fluoride, GSTs containing in-date sodium fluoride, and GSTs containing expired sodium fluoride. All experimental conditions contain potassium oxalate, which was added during the blood collection process.

Methods: An ethanol stock solution (1.00 g/100 mL) was created by dispensing 10 g of absolute ethanol into a beaker, diluting with DI water, and transferring with repeated washing into a 1 L volumetric flask. Human whole blood containing potassium oxalate was purchased from ZenBio. This was used to create blood stock solutions at 0.08% and 0.15% concentrations, respectively, by pipetting 8 mL and 15 mL of the ethanol stock solution into a 100 mL volumetric flask and diluting with whole blood. GSTs were filled with 7 mL of stock solution, capped, and inverted approximately five times to ensure proper mixture of the additives. Six experimental conditions were evaluated. Each condition was analyzed in duplicate on a dual-column Headspace/Gas Chromatograph with a Flame Ionization Detector on days 0, 7, 14, 30, 60, and 90. Between analyses, GSTs were stored in a cooler at 4°C.

Results: Specimens were analyzed on Day 0 to determine initial ethanol concentrations. New tubes were opened and analyzed on subsequent days to prevent ethanol evaporation from taking place due to continuous use. Only negative conditions were reopened during each analysis. All results were reprocessed using the Day 0 calibration method to ensure continuity across results. No ethanol increases were noted throughout the experiment. Some ethanol decreases were noted, with amounts varying based on initial concentration and experimental condition. The inclusion or lack of sodium fluoride, as well as the manufacturer, did not contribute to any significant variation among conditions.

Discussion: This study has demonstrated that the inclusion of, or lack of, sodium fluoride will not affect ethanol stability in a sample collected in a sterile environment and stored at 4°C. Any loss in BAC is minimal. BAC does not increase during storage when sodium fluoride is expired, or in-date, or even

absent. This study has demonstrated that BAC remains stable at both 0.08% and 0.15% concentrations under different experimental conditions. This study also demonstrated that tubes created by BD and AM are suitable for use under these experimental conditions.