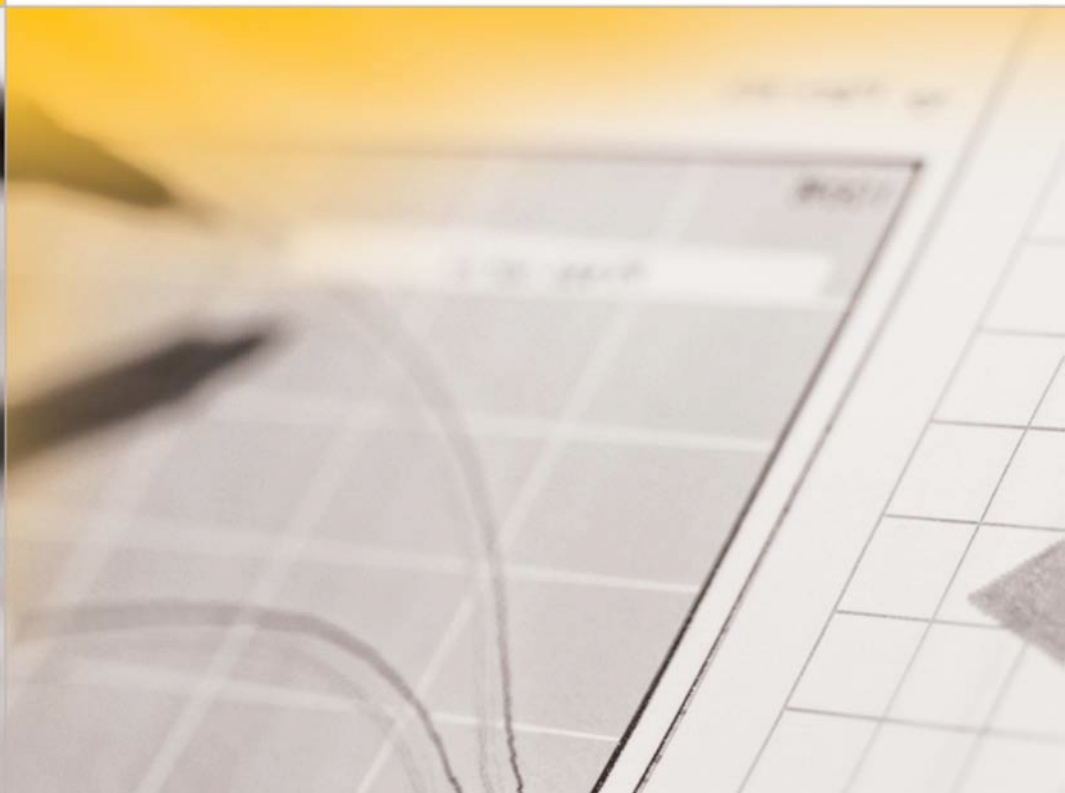


SCIENTIFIC POSTER

Effects of Hemolysis, Icterus, and Lipemia on Serum Osmolality Results using the Advanced® Model 3250 Single-Sample Osmometer

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ABSTRACT

Hemolysis, icterus, and lipemia may affect the results of routine serum chemistry tests and thereby lead to erroneous clinical interpretations and inappropriate actions regarding patient care. The accurate determination of serum osmolality in the clinical laboratory is of critical importance to the differential diagnosis of many disorders involving water balance. This study was conducted to quantify the influence of hemolysis, icterus, and lipemia on serum osmolality results using the Advanced® Model 3250 Single-Sample Osmometer. Normal human serum was spiked with varying concentrations of hemolysate, bilirubin, and Intralipid following NCCLS EP7-A dose-response guidelines in order to simulate hemolyzed, icteric, and lipemic specimens, respectively. Hemolysis, icterus, and lipemia were studied up to concentrations of 500 mg/dL hemoglobin ($\Delta_{\text{mOsm}} = -1.2$), 25.1 mg/dL bilirubin ($\Delta_{\text{mOsm}} = -1.2$), and 1557 mg/dL triglycerides ($\Delta_{\text{mOsm}} = 2.0$), respectively. The results of this study demonstrate that serum can be reliably tested for osmolality with considerable levels of hemoglobin, bilirubin, or triglycerides.

INTRODUCTION

ISO 14971 Section H.2.4.4 states that incorrect results of routine clinical chemistry tests can occur in normal use of IVD medical devices due to an unexpected influence of interfering factors in the sample matrix and can lead to a hazardous situation for an individual patient¹. Factors in patient specimens that have the potential to interfere with routine clinical chemistry tests include but are not limited to specimen abnormalities, prescription and over-the-counter drugs, abnormal biochemical metabolites, and specimen additives².

Hemolysis, icterus, and lipemia are common specimen abnormalities that interfere with routine serum chemistry tests^{3,4,5} (see Table 1). Hemolytic specimens are most often attributable to improper collection and handling. Less frequently, hemolytic specimens are due to *in vivo* hemolysis as a result of a variety of causes such as autoimmune hemolytic anemia, severe infections, or transfusion reactions⁶. Icteric specimens may result from patients with liver, hematologic, hemolytic, liver, and metabolic disorders including gallbladder obstructive disease and hepatitis. Lipemic specimens may be caused by diabetes mellitus, liver obstruction, nephrosis, other diseases involving lipid metabolism, and various endocrine disorders⁷.

Interferences present difficulty when they significantly affect the true results of a measurement. Results with a small measurement bias, on the other hand, may be considered acceptable and used in decision making. The goal of this study was to quantify the bias in serum osmolality results created by hemolysis, icterus, and lipemia using the Advanced® Model 3250 Single-Sample Osmometer.



Table 1. Reference intervals for three common specimen abnormalities

Specimen abnormality	Condition	Reference intervals (mg/dL)*
Hemolysis	Abnormal	≥ 3
Icterus	Abnormal	> 2.0 (Adult)
Lipemia	High	150 - 199
	Hypertriglyceridemic	200 - 499
	Very High	> 499



MATERIALS AND METHODS

Study Design

The influence of hemolysis, icterus, and lipemia on serum osmolality was studied using the dose-response method described in CLSI (NCCLS) document EP7-A.

Sample Preparation

Packed Red Blood Cells, Na Heparin (SeraCare; Milford, MA) were washed with isotonic saline. Tubes were inverted slowly 10 times and centrifuged for 10 minutes at 4,000 U/min using a Hettich EBA 3S Centrifuge (Hettich Centrifuges, USA; Beverly, MA). The saline wash was discarded and cells were washed an additional four times. Washed cells were diluted with an equal volume of Reagent Grade Water (NERL; East Providence, MA). Tubes were inverted slowly 10 times and frozen at -80°C overnight. Frozen cells were thawed and brought to room temperature. Cells were centrifuged for 30 min at 4,000 U/min to remove red cell debris. The hemolysate was sent to Quest Diagnostics® (Cambridge, MA) for hemoglobin analysis. A measured volume of hemolysate (12.8 g/dL hemoglobin) was added to normal human serum (SeraCare; Milford, MA) to make ~500 mg/dL hemoglobin. Dilutions were prepared from admixtures of the neat and spiked serum. Final hemoglobin concentrations were ~0, ~15.625, ~31.25, ~62.5, ~125, ~250, ~375, and ~500 mg/dL.

Bilirubin, Mixed Isomers (Sigma-Aldrich, St. Louis, MO) were dissolved in Sodium Hydroxide 0.1000N (VWR; West Chester, PA). Pooled normal human serum (SeraCare; Milford, MA) was spiked with the stock bilirubin solution. Dilutions were prepared from admixtures of the neat and spiked serum. Bilirubin concentrations were determined using a Lambda 20 UV/VIS spectrophotometer (Perkin Elmer; Waltham, MA) with its detecting wavelength set to 454 nm. A Bilirubin Standard Kit and Bilirubin Standard, Level F (Fisher Scientific; Waltham, MA) in combination with diluent from a Total Bilirubin

Test Kit (Advanced Instruments, Inc., Norwood, MA) were used to generate a standard curve. Bilirubin equivalents from the Total Bilirubin Test Kit (Advanced Instruments, Inc.; Norwood, MA) were used to validate the standard curve. Final bilirubin concentrations were 0.1, 1.3, 4.1, 9.1, 20.3, 25.1, and 36.0 mg/dL.

Pooled lipemic human serum (SeraCare; Milford, MA) was clarified by centrifugation at 6,000 U/min for 15 minutes. Intralipid, 20% Emulsion (Sigma-Aldrich; St. Louis, MO) was added to the clarified serum pool. Dilutions were prepared from admixtures of the neat and spiked serum. Final triglyceride concentrations, reported by Quest Diagnostics®, were 111, 194 (lipemic serum pool), 304, 499, 868, 1557, and 3030 mg/dL.

Measurements

Osmolality was determined on the Advanced® Model 3250 Single-Sample Osmometer (Advanced Instruments, Inc.) according to the manufacturer's instructions. The Model 3250 Osmometer uses the freezing point depression method for an accurate and reliable determination of the osmolality of a 0.2 to 0.25 mL sample in approximately two minutes. The instrument specifications include linearity that is less than $\pm 0.5\%$ from a straight line and repeatability with a standard deviation of ≤ 2 mOsm/kg H_2O between 0 and 400 and $\leq 0.5\%$ of value in mOsm/kg H_2O when operated between 20°C to 25°C and 40% to 60% relative humidity®. Samples were measured in quintuplicate and in order of increasing concentration.

Data Analysis

The data was analyzed by graphing the averages of the quintuplicate measurements in Microsoft® Office Excel 2007 using XY scatter plots. A significance criterion of 3 SD from the mean osmolality of the neat human serum was used to determine whether the results were clinically significant.

RESULTS

Mean osmolality results for hemolyzed, icteric, and lipemic serum samples (Figure 1, 2 and 3) fell within 3 SD of the mean osmolality of the neat serum up to a concentration of 31.25 mg/dL hemoglobin ($\Delta_{\text{mOsm}} = -1.2$), 25.1 mg/dL bilirubin ($\Delta_{\text{mOsm}} = -1.2$), and 1557 mg/dL triglycerides ($\Delta_{\text{mOsm}} = 2.0$) mg/dL, respectively.

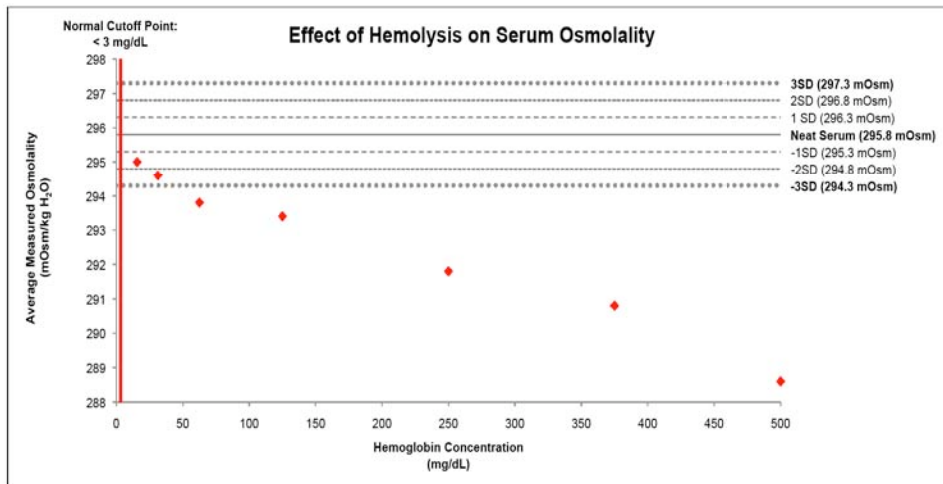


Figure 1. Effect of hemolysis on serum osmolality

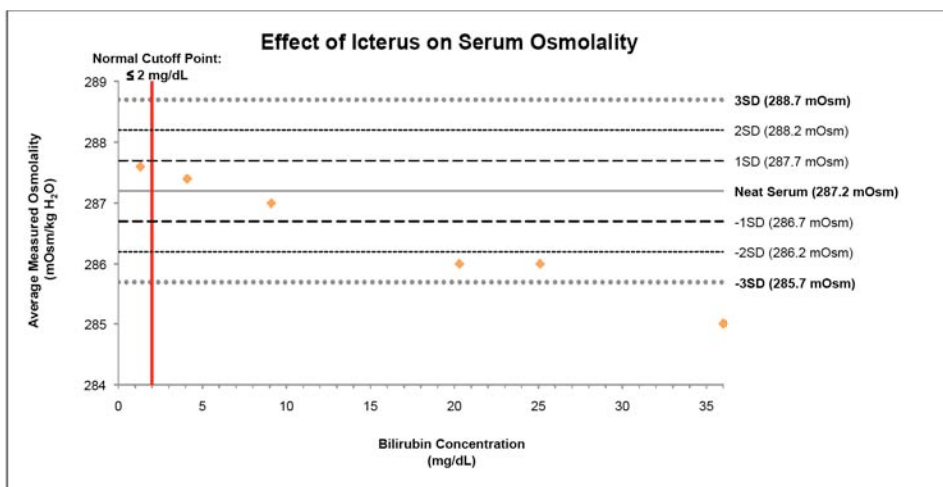


Figure 2. Effect of icterus on serum osmolality

“Serum osmolality measurements using the Model 3250 Single-Sample Osmometer were not significantly affected at clinically high concentrations of hemoglobin, bilirubin or triglycerides”

RESULTS (cont.)

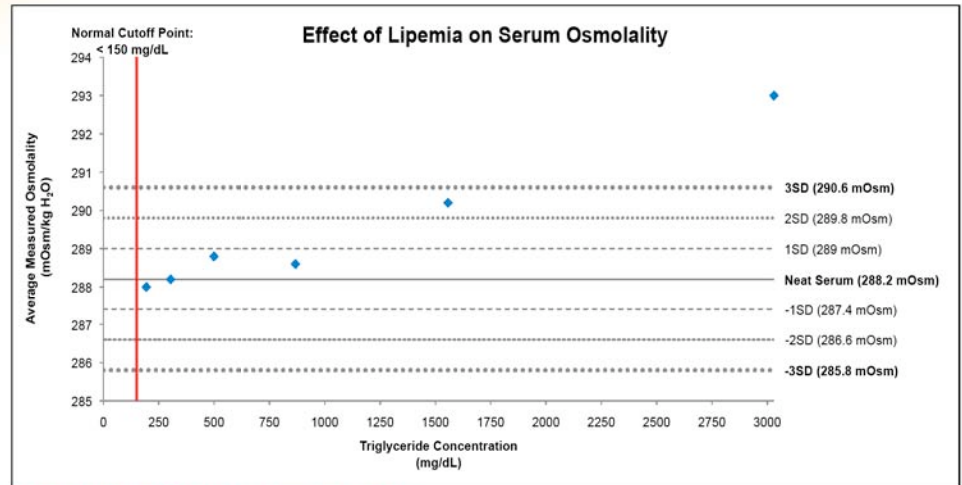


Figure 3. Effect of lipemia on serum osmolality

CONCLUSION

Hemolysis, icterus, and lipemia are important factors to consider when performing routine serum chemistry tests in the clinical laboratory. The results of this study were obtained with specific commercial reagents so variation may be seen with other reagents. In this study, hemolytic, icteric, and lipemic serum samples were reliably tested for osmolality with considerable levels of hemoglobin, bilirubin, or triglycerides using the Advanced Model 3250 Single-Sample Osmometer. Although the Advanced Model 3250 Single-Sample Osmometer was used as the measurement instrument in this study, other Advanced Model Osmometers should produce similar results because they use the same freezing point technology.



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