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Effect of interference from hemolysis, icterus and lipemia on routine pediatric clinical chemistry assays

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ABSTRACT

Background: : Clinical laboratory assays can be affected by interferents like hemoglobin (Hb), lipids and bilirubin. We evaluated the effect of these interferences on pediatric samples for different chemistry assays. Further we established cut-off indices above which these interferences confound sample results.
Methods: : Three separate serum pools were spiked with increasing concentrations of hemolysate or intralipid or bilirubin and different analytes were analyzed. The Hemolysis-(H), Lipemia-(T) and Icterus-(I) indices were measured on Vitros 5600. Analytes affected by lipemia were treated with LipoClear ® and re-analyzed. All the measured analytes were compromised by gross hemolysis (H-Index >1000).
Results: : Except lipase and magnesium (Mg^{++}), all other analytes were affected by moderate (H-Index >250) and significant hemolysis (H-Index >500). Low estradiol levels showed a significant effect at severe icterus (I-Index >20.0). C3, C4, Ceruloplasmin, Haptoglobin, Immunoglobulins (Ig) and Vitamin D were significantly affected by moderate (T-Index >100) and severe (T-Index >500) lipemia. LipoClear ® treatment significantly attenuated the lipemic interference on the above analytes except for C3, C4, and IgG.
Conclusions: : Accurate reporting of pediatric samples for the analytes affected by common interferences will lead to better clinical interpretation.

1. Introduction

Results of chemical assays can be affected by either endogenous or exogenous substances [1]. Endogenous interference occurs from substances found naturally in the patient sample. The most common and important endogenous compounds that can interfere and are encountered in the clinical laboratory include Hb, lipids, bilirubin, autoantibodies, and heterophile antibodies [2]. The presence of one or more of these substances in a serum or plasma sample can bias the sample result leading to inaccurate conclusions.

We evaluated the effect of hemolysis, lipemia and bilirubin on the routinely analyzed samples on the laboratory instruments including Vitros 5600, Prospec, Xpand, Architect i1000SR, and Centaur respectively. Only the Vitros 5600 analyzer provides what is termed as an index value based on the mathematical calculations from measuring the absorbance of the sample at different wavelengths.

2. Materials and methods

The present study was conducted at Texas Children's Hospital Laboratory, Houston, Texas to evaluate the effect of Hb, bilirubin and lipids on routine analytes. This included analyses of the most common analytes reported by manufacturer to be affected by these interferents as shown in Table 1. We chose the most common, established interferents based on the manufacturer's previously reported studies; in addition, we tested different levels of interferents and provide a comprehensive report on the effect of these interferents on the analytes mentioned in Table 1.

We studied the effect of hemolysis on K^+, AST, LDH, TBIL, ALT, CK, Mg^{++}, ALB, TP, ALKP, Fe, Lipase, NH_3 and Phos. Similarly, the effect of lipemia interference was evaluated on HIV-1/2, Testosterone, Progesterone, Ceruloplasmin, and Haptoglobin, C3/C4, IgA/IgM/IgG, AFP, and aHBC, and HCG, CK-MB, TSH, VIT D, ALB, and Ferritin and Glucose. Lastly, for icteric interference, we analyzed Estradiol, Folate, ALB, TP, ALT, GGT, Glucose, Na^+, Cortisol, C4, Haptoglobin and HbA1c. For all the analytes studied, pooled patient serum samples without detectable concentrations of free Hb, bilirubin or a known amount of triglycerides were used. All the experiments were carried out using 3 different serum pools for each interferent (total of 9 separate pools) which were spiked with increasing concentrations of hemolysate, commercially available bilirubin and commercially available 20% Intralipid. These were then

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Table 1
Analytes and the different platforms used for the study.

Analyte	Vitros 5600	Architect i1000 SR	Prospec	Xpand	Centaur
AFP	XXX				
aHBC	XXX				
Albumin	XXX				
ALKP	XXX				
ALT	XXX				
AST	XXX				
TBIL	XXX				
C3				XXX	
C4				XXX	
Cerulo			XXX		
CK	XXX				
CK-MB	XXX				
Cortisol	XXX				
ESTR					XXX
Fe (iron)	XXX				
Ferritin	XXX				
Fol		XXX			
GGT	XXX				
Gluc	XXX				
Hapto			XXX		
HbA1c	XXX				
HCG	XXX				
HIV-1/2		XXX			
IgA				XXX	
IgG				XXX	
IgM				XXX	
K^+	XXX				
LDH	XXX				
Lipase	XXX				
Mg^{++}	XXX				
Na^+	XXX				
NH_3	XXX				
Phos	XXX				
Proges					XXX
Testo		XXX			
TP	XXX				
TSH	XXX				
Vitamin D		XXX			

analyzed on the various instruments in the laboratory respectively as shown in Table 1. The Hemolysis-(H), Lipemia-(T) and Icterus-(I) indices were measured and documented on Vitros 5600. Samples with analytes affected by lipemia were treated with LipoClear ® (lipid clearing reagent suspended in cyclodextrin tubes), and re-analyzed.

To study the effect of hemolysis interference, Hb was added as erythrocyte hemolysate prepared by osmotic disruption by 3 repeat cycles of freezing and thawing [3]. The free Hb concentration of the hemolysate was measured spectrophotometrically at 450 nm. The samples were spiked with hemolysate to obtain a final free Hb concentration of 0.75 g/l (normal), 1.5 g/l, 3 g/l and 6 g/l to designate the corresponding degree of hemolysis i.e. slight hemolysis (SHEM;H-index >100), moderate hemolysis (MHEM;H-index >250) and significant hemolysis (SIHEM;H-index >500), respectively. Gross hemolysis was defined as H-index >1000. The samples were then analyzed to establish the baseline values for the assigned analytes on Vitros 5600. The hemolysis index was then established taking into consideration the total allowable error (TAE) for the parameters which showed interference. Three different pools of sera were used to perform experiments for each of the 3 interferents.

To study the effect of lipemia interference, we obtained 3 different pools of sera and measured to establish baseline values for the assigned analytes on their respective analyzers. 20% Intralipid was used to mimic the effect of lipemia. Intralipid which closely resembles endogenous lipemia, has been used in previous studies to test interference from lipemia possibly due to the deficiency of VLDL and chylomicrons lipid–lipoprotein complexes [4–7]. Commercially available 20% Intralipid (Pharmacia,) [4] was used to spike the pool sera to make final triglyceride concentrations of 400, 1000 and 2000 mg/dl. The assigned analytes were measured on their respective analyzers. For all the analytes analyzed by nephelometry, the degree of lipemia was established using a comparison with semi-qualitative tube method representing different triglyceride concentrations i.e.; negative, 1+ (mild), 2+ (moderate) and 3+ (severe).

The analytes showing a significant bias (±10%) were further treated with LipoClear ® and re-analyzed. Briefly, to 100 μl of LipoClear ® reagent (StatSpin®), 500 μl of sample was added and left at room temperature for 5 min, centrifuged for 20 min at 2000 g/3300 rpm and then the clear supernatant was re-analyzed and all results were multiplied by the 1.2 dilution factor.

To study the effect of bilirubin interference, 3 different pools of sera were obtained and measured for total bilirubin to ensure that minimal concentrations were present. Five milligrams of commercially available bilirubin (Acros organics; 99% unconjugated bilirubin) was weighed out. For simplicity, only the unconjugated salt was used for icteric interference evaluation. Previous studies indicate that the chemical property of bilirubin whether conjugated or unconjugated does not contribute to the differing interferences [5,8]. A 10 mmol/l bilirubin stock solution was prepared and stored protected from light at −20 °C [4]. The sample pools were spiked with 10 mmol/l bilirubin stock solution to get a final concentration of 500, 250 and 100 μmol/l respectively and immediately measured for the analytes listed above. The original sample pool was treated with 0.1 mol/l NaOH to create a similar matrix as the bilirubin spiked sample. The sample pool was then analyzed for the analytes listed above to establish baseline values. Analytically significant interference was defined using the total allowable error (TAE) for the laboratory analytes according the CAP and CLIA guidelines as shown in Supplement Table 1.

3. Results

The evaluation of different interferents (hemolysis, lipemia and bilirubin) on various analytes was tested to establish the degree of interference. Effect of hemolytic interference on K^+, AST, LDH, TBIL, ALT, CK, Mg^{++}, ALB, TP, ALKP, Fe, Lipase, NH_3 and Phos was tested on Vitros 5600 (Table 2). At Gross hemolysis (H-index >1000), the sample integrity was compromised with all analyte values beyond the clinical reportable range. All the analytes tested except Lipase and Mg^{++}, were affected by moderate (H-index >250) and significant hemolysis (H-index >500). K^+ showed a positive bias of 5%–10% at MHEM and 15%–20% at SIHEM whereas CK and Iron values were significantly increased at MHEM (10–30%) and SIHEM (20–60%) respectively. For all these analytes we recommend to report the percentage bias with the result. On the other hand, ALT and ALKP showed a negative bias of 20–30% at MHEM and >30% at SIHEM. In addition, there was a significant positive bias in slightly hemolyzed specimens (H-index >100) when tested for Fe (>20% bias), NH_3 (>45% bias) and LDH (>75% bias). The LDH assay showed >45% bias even with H-index of approximately 100.

Lipemia interference was evaluated on HIV-1/2, Testosterone, Progesterone, Ceruloplasmin, Haptoglobin, AFP, and aHBC, HCG, CK-MB, TSH, VITD, ALB, Ferritin and Glucose on different instruments as listed above (Table 3a). Out of these Ceruloplasmin, Haptoglobin, C3, C4, VIT D, IgG/IgM/IgA and ferritin were significantly altered at a triglyceride concentration of 2000 (T-index >500). Ceruloplasmin and Haptoglobin showed a significant positive bias at moderate (TG ≥ 1000; T-index >250) and severe lipemia (TG ≥ 2000; T-index >500), with almost a fourfold increase from baseline values. C3 and C4 showed a negative bias of 10% and 15% respectively at moderate (TG ≥1000; T-index >250) and severe lipemia (TG ≥ 2000; T-index >500). VIT D showed a negative bias of 10%–13.5% at moderate lipemia and at severe lipemia (TG ≥ 2000; T-index >500), the variation was in the range of 15%–20%. IgG and IgA levels were significantly increased at moderate (TG ≥1000; T-index >250) and severe lipemia (TG ≥ 2000; T-index >500).