

Effect of Hemolysis, Icterus, and Lipemia on Chemistry Tests and Association between the Amount of Interfering Substances and LIH Indices

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INTRODUCTION

- Various endogenous substances (e.g., hemoglobin, bilirubin, lipids) can affect clinical laboratory assays.
- Automated chemistry analyzers can accurately detect interferences due to hemolysis, icterus, and lipemia.
- The aim of this study was to evaluate the effect of hemolysis, icterus, and lipemia on chemistry assays and determine the cut-off indices above which these interferences impact the results of analysis.

METHODS

- Three different serum pools were prepared and spiked with increasing concentrations of hemolysate, commercially available bilirubin, and triglyceride (human triglyceride-rich lipoproteins) (Table 1).

Table 1. Target Concentrations of Hemoglobin, Bilirubin, and Triglyceride in Each Serum Pool

Interferents	Target Concentrations (mg/dL)
Hemoglobin (Hemolysis)	25, 75, 150, 250, 400, 800
Bilirubin (Icterus)	1.0, 3.7, 7.5, 15, 30, 60
Triglyceride (Lipemia)	50, 300, 600, 1100, 1500, 2100

- A blinded visual inspection of the amount of hemolysis, icterus, and lipemia in each sample was conducted by three independent technologists.
- The amount of hemolysis, icterus, and lipemia were also measured semi-quantitatively using a photometric test on a Beckman Coulter AU5800 series analyzer and analyzed via the LIH reagent system (Table 3).
 - Values are assigned on an ordinal scale as six different qualitative flag levels: "N", "+", "++", "+++", "++++" and "+++++" (Table 2).

Table 2. Hemoglobin and Bilirubin Concentration Range at LIH Test and Associated Flag Levels

Hemoglobin Concentration (mg/dL) Range at LIH test	Bilirubin Concentration (mg/dL) Range at LIH test	Triglyceride Concentration (mg/dL) Range at LIH test (Established at MRL)	Flag Level
>500	>40	≥ 2016	+++++
300-500	20-40	≥1300-≤1979	++++
200-299	10-19.9	≥790-≤1255	+++
100-199	5-9.9	≥428-≤762	++
50-99	2.5-4.9	≥216-≤356	+
<50	<2.5	≤153	N

- Each sample was tested on the same instrument for 40 chemistry analytes for hemolysis, 38 for icterus, and 31 for lipemia interferences.
 - Results were compared to baseline values; observed bias was determined.
 - Analytes showing a bias more than ±10% for lipemia were airfuged (30 PSI for 10 min) and reanalyzed.
 - The concentration of interferences that impacted results was determined based on total allowable error limits according to CAP and CLIA guidelines.
- The final cut-off indices were determined for each analyte at the concentration level of hemolysis, icterus, and lipemia that affected the results of analysis.

SUMMARY OF RESULTS

Hemolysis

- Hemolysis (Table 4) affected 20 results out of 40 tested analytes.
- For most of the assays, the data agrees with manufacturer information in the package inserts. However, some assays are affected at higher hemolysis index values than inserts indicate.

Insert	Results
BUN results are not affected by hemolysis up to [Hb] of 500 mg/dL.	There is no interference by hemolysis up to [Hb] of ~800 mg/dL.
UIBC and free glycerol results are not affected by hemolysis up to [Hb] of 200 mg/dL and 250 mg/dL, respectively.	There is no interference until samples are grossly hemolysed at [Hb] of ~800 mg/dL.

- Significantly affected by hemolysis:**
 - LDH and K⁺ = results should not be reported if qualitative hemolysis flag levels are ≥2+ and ≥4+, respectively.
 - HDL-C measured by dextran sulfate precipitation and GGT = should not be reported if samples are grossly hemolysed with hemolysis flag levels of 5+.
- 50% of total tested analytes are not affected by hemolysis at any hemoglobin concentration levels, including at ~800 mg/dL.
- Visual inspection results (Table 3) for hemolysis showed good agreement between three technologists and when compared to corresponding flag levels. The three technologists agreed on 71.4% of results, while two technologists agreed on 28.6% of results.

Table 3. Visual Assessment

LIH	Pool	Spike	Technologist #1			Technologist #2			Technologist #3		
			Hemolysis	Icterus	Lipemia	Hemolysis	Icterus	Lipemia	Hemolysis	Icterus	Lipemia
N	1	Neat	N	S	N/A	N	N	N/A	N	S	N/A
N	1	Normal	N	N	N	S	N	N	N	S	N
+	1	1+	S	S	S	S	N	S	S	N	S
++	1	2+	S	S	S	S	N	M	S	M	M
+++	1	3+	S	S	M	M	S	M	M	M	G
++++	1	4+	M	M	G	G	S	G	G	M	G
+++++	1	5+	G	M	G	G	M	G	G	G	G
N	2	Neat	N	S	N/A	N	N	N/A	N	S	N/A
N	2	Normal	N	N	S	N	N	N	N	S	N
+	2	1+	S	S	S	S	N	S	S	S	S
++	2	2+	S	S	M	S	S	S	S	S	M
+++	2	3+	M	S	M	M	S	M	M	M	M
++++	2	4+	M	M	M	G	S	G	M	M	G
+++++	2	5+	G	M	G	G	M	G	G	G	G
N	3	Neat	N	N	N/A	N	N	N/A	N	N	N/A
N	3	Normal	N	N	N	N	N	N	N	N	N
+	3	1+	S	S	S	S	N	S	S	S	S
++	3	2+	S	S	S	S	N	M	S	S	S
+++	3	3+	M	S	M	G	S	M	M	S	M
++++	3	4+	M	M	G	M	S	G	G	G	G
+++++	3	5+	G	M	G	G	M	G	G	G	G

N: normal, S: slight, M: moderate, and G: gross
Neat and normal samples were the same for lipemia study; hence, results are listed "N/A" for neat samples.

Table 4, 5, 6 Notes: Results are listed as N/A if values undetermined due to instrument flags (e.g., OD of reaction is higher than the maximum OD range). Orange results indicate results outside the TaE limits.

Table 4. Hemolysis Interference

Flag Level	Actual [Hb] (mg/dL)	N						
		25	76	150	249	399	798	
ANALYTE	TaE	Bias (% or absolute)						
Albumin	±10.0%	-1.1%	-1.3%	-0.9%	0.7%	3.3%	10.2%	
Aldolase	±20.0%	22.6%	11.5%	24.1%	N/A	N/A	N/A	
ALP	±30.0%	-3.7%	-4.4%	-5.4%	-8.0%	-9.8%	-14.9%	
ALT	±20.0%	3.2%	10.0%	13.3%	23.2%	31.4%	58.2%	
Amylase	±30.0%	-0.2%	0.4%	-3.7%	-0.1%	15.6%	7.6%	
AST	±20.0%	8.1%	27.9%	50.6%	87.3%	131.6%	263.2%	
CO ₂	±21.6%	-1.3%	-2.7%	-3.6%	-4.4%	-3.3%	-1.0%	
Direct Bilirubin	±20.0%	-14.6%	-27.0%	-34.8%	-31.3%	-38.5%	-48.6%	
Total Bilirubin	±0.4mg/dL	0.02	0.01	0.02	0.02	0.05	0.11	
BUN	±9.0%	-0.6%	0.0%	0.8%	1.4%	2.4%	4.8%	
Calcium	±1.0 mg/dL	0.01	0.09	0.05	0.00	-0.05	0.01	
CK	±30.0%	2.4%	7.8%	17.7%	28.1%	44.5%	89.1%	
Creatinine-Jaffe	±0.3 mg/dL	0.00	0.01	-0.03	-0.05	-0.02	0.06	
Creatinine-Enzymatic	±0.3 mg/dL	0.00	-0.04	-0.09	-0.13	-0.21	-0.38	
Fructosamine	±9.9%	1.8%	5.8%	11.4%	13.7%	14.2%	0.4%	
GGT	±15.3%	1.7%	3.1%	3.7%	4.2%	13.1%	36.7%	
Glucose	±10.0%	-0.1%	-0.4%	-0.6%	0.1%	0.2%	1.8%	
LDH	±20.0%	18.3%	60.6%	113.8%	196.9%	276.0%	546.5%	
Lipase	±30.0%	-0.8%	1.4%	1.6%	3.4%	3.6%	6.7%	
Mg	±25.0%	0.5%	1.7%	4.2%	9.5%	15.7%	31.7%	
Phosphorous	±10.7%	0.3%	2.1%	4.0%	5.9%	9.4%	17.9%	
Total Protein	±10.0%	-0.3%	1.4%	2.0%	3.6%	6.1%	11.2%	
Uric Acid	±17.0%	-0.1%	-1.2%	-1.8%	-3.3%	-3.9%	-6.5%	
UIBC	±20.7%	-0.6%	-1.1%	-2.8%	-7.9%	-12.1%	-24.2%	
Cholesterol	±10.0%	-0.1%	1.9%	3.7%	9.1%	11.6%	26.1%	
Triglyceride	±15.0%	1.1%	-0.5%	-0.4%	-1.2%	-1.3%	-3.6%	
HDL-C direct	±13.0%	-0.1%	0.5%	-0.1%	0.0%	0.5%	0.0%	
HDL-Cppt	±13.0%	1.5%	8.4%	14.0%	23.3%	37.3%	81.7%	
Ethanol	Cut-off = 10 mg/dL	neg	neg	neg	neg	neg	pos	
Iron	±20.0%	0.2%	-0.4%	-0.4%	-2.0%	-2.9%	-22.2%	
Transferrin	±20.0%	0.3%	1.2%	-1.4%	-0.2%	-0.4%	-0.6%	
LDL	±12.0%	0.3%	1.7%	-0.1%	-0.1%	-0.2%	-0.4%	
Cholesterol	Free Glycerol	±20.0%	-2.2%	-6.0%	-9.4%	-15.4%	-19.8%	-33.0%
Lactate	±0.4 mmol/L	0.00	-0.01	-0.01	-0.01	-0.01	-0.04	
Lp(a)	±52.2%	-2.5%	2.3%	1.2%	1.3%	-3.0%	-9.0%	
Phospholipid	±20.0%	1.1%	0.7%	1.3%	1.9%	3.1%	5.5%	
Free Cholesterol	±20.0%	0.3%	1.8%	3.1%	5.3%	8.8%	18.1%	
K ⁺	±0.5 mmol/L	0.09	0.25	0.52	0.87	1.29	2.72	
Na	±4.0 mmol/L	-0.06	-0.89	-0.28	0.02	-0.09	0.17	
Cl	±5.0%	-0.2%	-0.6%	-0.2%	0.1%	0.4%	1.5%	

Table 5. Icteric Interference

Flag Level	Target [Bilirubin] (mg/dL)	N						
		1.0	3.7	7.5	15	30	60	
ANALYTE	TaE	Bias (% or absolute)						
Albumin	±10.0%	-0.4%	-0.8%	-1.6%	-1.4%	-0.4%	-0.4%	
Aldolase	±20.0%	12.2%	16.2%	9.4%	-1.5%	-6.9%	N/A	
ALP	±30.0%	0.1%	0.5%	-0.2%	0.4%	-0.3%	-0.2%	
ALT	±20.0%	7.1%	5.5%	5.5%	5.3%	5.9%	6.2%	
Amylase	±30.0%	-0.9%	-0.4%	-0.3%	-7.2%	-14.0%	-22.6%	
AST	±20.0%	4.6%	2.8%	4.0%	3.2%	3.4%	1.7%	
CO ₂	±21.6%	-5.2%	-5.9%	-6.1%	-5.2%	-2.1%	0.1%	
BUN	±9.0%	-0.7%	-0.5%	-0.6%	-0.2%	-0.3%	-0.5%	
Calcium	±1.0 mg/dL	-0.01	-0.02	-0.12	-0.11	0.13	0.08	
CK	±30.0%	4.5%	3.8%	3.9%	4.3%	4.5%	3.3%	
Creatinine-Jaffe	±0.3 mg/dL	0.01	-0.01	0.02	0.07	0.11	-0.09	
Creatinine-Enzymatic	±0.3 mg/dL	0.00	0.00	0.00	-0.02	-0.04	-0.11	
Fructosamine	±9.9%	0.7%	7.8%	17.8%	35.2%	71.8%	171.3%	
GGT	±15.3%	5.1%	4.1%	2.0%	-3.8%	-14.4%	-21.3%	
Glucose	±10.0%	0.8%	0.7%	0.4%	1.2%	0.8%	0.9%	
LDH	±20.0%	3.8%	3.7%	4.2%	3.8%	4.1%	4.3%	
Lipase	±30.0%	0.1%	-0.4%	-3.8%	-14.5%	-30.4%	-59.2%	
Mg	±25.0%	0.7%	1.4%	2.0%	3.6%	6.7%	14.0%	
Phosphorous	±10.7%	0.8%	-0.4%	-0.1%	1.5%	1.7%	1.2%	
Total Protein	±10.0%	-0.5%	-1.1%	-2.3%	-4.0%	-8.3%	-17.9%	
Uric Acid	±17.0%	-0.4%	-0.3%	-0.1%	0.0%	-0.6%	-2.2%	
UIBC	±20.7%	3.9%	2.7%	2.9%	2.2%	0.1%	-3.5%	
Cholesterol	±10.0%	-0.5%	-4.1%	-7.1%	-14.9%	-31.7%	-60.5%	
Triglyceride	±15.0%	1.5%	0.5%	0.6%	0.7%	-1.3%	-3.3%	
HDL-C direct	±13.0%	-1.7%	0.0%	-1.7%	-2.1%	-2.0%	-5.2%	
HDL-Cppt	±13.0%	-3.8%	-9.7%	-16.8%	-35.5%	-62.0%	-75.4%	
Ethanol	Cut-off = 10mg/dL	neg	neg	neg	neg	neg	neg	
Iron	±20.0%	-0.9%	-0.7%	-1.1%	-0.3%	-0.6%	-1.0%	
Transferrin	±20.0%	-0.9%	-0.4%	-1.6%	-1.2%	-0.5%	-1.3%	
LDL	±12.0%	-1.1%	-0.2%	-1.4%	-0.5%	-0.8%	-0.8%	
Cholesterol	Free Glycerol	±20.0%	-12.4%	-43.5%	-72.5%	-95.1%	-60.7%	-72.3%
Lactate	±14.4%	-0.8%	-2.4%	-2.6%	-7.4%	-17.1%	-34.7%	
Lp(a)	±52.2%	2.5%	0.4%	-2.9%	1.0%	-6.4%	0.2%	
Phospholipid	±20.0%	-0.1%	-0.7%	-1.3%	-2.6%	-6.0%	-13.7%	
Free Cholesterol	±20.0%	-1.6%	-4.9%	-10.0%	-13.1%	-8.3%	-8.7%	
K ⁺	±0.5 mmol/L	-0.01	-0.04	-0.02	-0.02	0.02	-0.02	
Cl	±5.0%	-0.6%	-0.7%	-0.9%	-0.2%	0.1%	0.0%	

Table 6. Lipemia Interference

Flag Level	Target [Trig] (mg/dL)	N					
		300	600	1100	1500	2100	
ANALYTE	TaE	Bias (% or absolute)					
Albumin	±10.0%	-0.5%	-0.5%	1.8%	4.0%	8.1%	
Aldolase	±20.0%	4.7%	8.9%	33.7%	67.7%	N/A	
ALP	±30.0%	1.3%	1.1%	-0.9%	0.5%	-6.5%	
ALT	±20.0%	5.7%	14.6%	31.1%	N/A	N/A	
Amylase	±30.0%	-2.1%	-2.8%	-4.7%	-7.4%	-5.8%	
AST	±20.0%	6.4%	24.2%	35.6%	N/A	N/A	
CO ₂	±21.6%	-7.1%	-7.0%	-8.8%	-10.5%	-20.4%	
Direct Bilirubin	±20.0%	-3.7%	-9.2%	-12.6%	-18.1%	-25.4%	
Total Bilirubin	±0.4mg/dL	0.00	-0.01	0.02	0.04	0.08	
BUN	±9.0%						