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ORIGINAL ARTICLE

Assessment of Hemolysis, Icterus, and Lipemia Interference in 14 Clinical Chemistry and 5 Immunoassay Parameters

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SUMMARY

Background: **Preanalytical interferences caused by hemolysis, icterus, and lipemia (HIL) remain major sources of diagnostic error in clinical laboratories. Manufacturer-declared cutoffs are often based on limited validation data and may not reflect local analytical performance, particularly across heterogeneous assay systems. This study aimed to determine experimentally derived HIL interference thresholds for a broad panel of clinical chemistry and immunoassay tests and to compare them with manufacturer specifications.**
Methods: **Nineteen routinely used assays (14 clinical chemistry and 5 immunoassays) were evaluated on the Abbott Architect c16000 and Siemens Advia Centaur XP platforms following CLSI EP07-A2 guidelines. Serum pools were spiked with graded levels of hemolysate, conjugated bilirubin, or lipid emulsion. Bias was calculated at each interference level, and thresholds were defined as the point exceeding the lower of the desirable bias or total allowable error (TEa) limits or the manufacturer's declared cutoff.**
Results: **Hemolysis produced significant deviations in several assays. Potassium interference began at 0.85 g/L hemoglobin versus the declared 1.25 g/L limit, and HDL-cholesterol exceeded the 5% desirable bias at 2.5 g/L, well below the 7.2 g/L manufacturer threshold. Bilirubin interference affected creatinine and glucose from 124 µmol/L, despite declared limits above 340 µmol/L, consistent with spectral absorption in the Jaffé reaction. Lipemic interference was observed at 2.2 mmol/L for sodium and HDL-cholesterol, nearly fivefold lower than the stated cutoffs. Among immunoassays, ferritin showed a positive bias, TSH a negative shift, and vitamin B12 a bidirectional pattern under hemolytic conditions; fT4 displayed concentration-dependent bias under lipemia.**
Conclusions: **Manufacturer-declared HIL limits may be overly permissive, masking clinically relevant bias. Locally verified thresholds revealed earlier interference onset for several analytes. The immunoassay platform used lacked an automated HIL index analyzer, highlighting the need to integrate such detection systems for optical and matrix-related interference monitoring. Routine local validation and continuous index surveillance are essential to ensure analytical accuracy and patient safety.**
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KEYWORDS

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INTRODUCTION

Preanalytical errors are among the most frequent causes of laboratory test inaccuracy and can compromise patient safety [1]. Endogenous interferences - hemolysis, icterus, and lipemia (HIL) - particularly affect photo-

metric assays [2]. Hemolysis is the rupture of erythrocytes with release of intracellular contents, notably hemoglobin; icterus denotes serum bilirubin > 20.0 µmol/L; and lipemia is serum turbidity from elevated triglycerides and other lipids, mainly chylomicrons and very low-density lipoprotein [3]. Because these factors distort biochemical results, early detection and management are essential for reliable diagnosis and treatment [4].

Spectrophotometric and automated analyzers provide sensitive, objective assessments but are cost-intensive and require technical expertise. The Working Group for the Preanalytical Phase (WG-PRE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), which serves as the federation's expert body for preanalytical quality improvement, recommends the use of automated hemolysis indices whenever feasible [3,5].

HIL index systems compare sample interference against predefined thresholds; in an EFLM survey, 43% of laboratories reported using HIL indices with heterogeneous evaluation methods [6]. Manufacturer cutoffs, however, are often derived under limited conditions and may not reflect local variability, necessitating laboratory validation to ensure result reliability and minimize diagnostic errors [7].

Accordingly, this study aimed to determine interference thresholds for hemolysis, icterus, and lipemia in 19 frequently ordered, HIL-susceptible analytes - including clinical chemistry tests and immunoassays - using prepared serum pools.

MATERIALS AND METHODS

Selection of test parameters

Nineteen assays with documented susceptibility to hemolysis, icterus, and lipemia (HIL) were included (Table 1). Serum pools were prepared from residual clinical samples free of visible HIL, anonymized before pooling. Baseline HIL indices were: hemolysis < 0.04 g/L hemoglobin, icterus < 17 µmol/L, and lipemia < 0.11 mmol/L.

Sample preparation

Pool preparation and target concentration ranges followed Clinical and Laboratory Standards Institute (CLSI) EP07-A2 guideline [8]. Where feasible, two levels (low and high/normal) were established; calcium (Ca^{+2}) and sodium were tested at a single level due to practical constraints. Because of the limited serum pool volume, lipemic interference was assessed at a single concentration level for both Serum Iron and UIBC, with UIBC likewise examined at a single level in the hemolysis experiments. Pools were homogenized and aliquoted into 15 equal-volume tubes per level.

Interferent spiking

Interference was simulated by adding increasing amounts of *in-vitro* hemolysate generated by freeze–thaw cycles [9], conjugated bilirubin solution (BILIRUBIN 97%, 250 mg, BLD PHARMA, Shanghai, China), or a 20% lipid emulsion (OLIVIA 20% I.V., POLIFARMA, Istanbul, Türkiye). To limit matrix effects, added volumes were ≤ 5% of pool volume, per CLSI EP07 [8]. Interferents were applied across a semi-quantitative 0 - 4+ scale (0: none; 4+: maximum), with maxima of 9.30 g/L hemoglobin, 410 µmol/L bilirubin, and 8.40 mmol/L lipids (Table 2). Given their high hemolysis sensitivity per manufacturer inserts [10,11], Potassium (K^+) and Aspartate Aminotransferase (AST) were additionally assessed on a six-level hemolysis scale (0 - 5 g/L Hb; Table 2).

Analysis and statistics

HIL indices were measured on an Abbott Architect c16000 using absorbance pairs 500/524, 572/604, 628/660, and 524/804 nm with predefined calculations. For repeatability, each sample (both levels) was run in quintuplicate on the Abbott Architect c16000 and Siemens Advia Centaur Xp (Siemens Healthcare Diagnostics Inc., USA). For each analyte, the interference-onset concentration and persistence at higher levels were determined. bias was computed as:

BIAS (%) = [(Mean Measured - Mean Baseline) / Mean Baseline] × 100.

Here, Mean Measured refers to the average result of interferent-spiked samples, while Mean Baseline denotes the mean of the unspiked control pool. Thus, bias represents the relative deviation introduced by the interferent. The acceptability of this deviation was judged against analytical performance specifications. Desirable bias and Total Allowable Error (TEa) values were obtained from the EFLM Biological Variation Database, which provides biologically based quality goals. Desirable bias reflects the largest systematic deviation still compatible with reliable clinical interpretation, whereas TEa defines the overall permissible analytical error, combining both bias and imprecision. Interference was considered significant when the observed bias exceeded the lower of the Desirable bias, TEa, or the manufacturer's declared threshold (Table 1) [12].

RESULTS

Hemolytic interference

Across both concentration levels, hemolysis affected Albumin, HDL-Cholesterol (HDL-C), Glucose, Total Bilirubin (T-Bil), Free Thyroxine (fT4), Ferritin, and vitamin B12. Hemolysis caused elevated results for albumin, vitamin B12, and ferritin at both concentration levels (Table 3A). In contrast, Alanine Aminotransferase (ALT) and Triglycerides (Trig) showed minor increases limited to Level 1, while Total bilirubin showed a consistent negative bias across all hemolysis levels,

Table 1. Test parameters, measurement units, evaluated concentration ranges (Level 1 and Level 2), desirable values, EFLM-derived desirable Total Error limits, and manufacturer-reported thresholds.

Assay	Unit	Assay Range		Desirable BIAS (%)	Manufacturer BIAS (%)	EFLM desirable total error
		Level 1	Level 2			
Albumin	g/L	39.59 - 41.01	42.76 - 44.24	1.2	10	3.3
ALT *	U/L	18.2 - 20.2	147.2 - 167.8	8.1	10	18.5
AST †	U/L	18.4 - 20.4	75.2 - 77.2	5.3	10	12.2
Ca^{+2} ‡	mmol/L	2.31 - 2.40	-	0.7	10	2.2
T-Chol §	mmol/L	4.31 - 4.49	5.08 - 5.31	4.0	10	8.6
Creatinine ‖	µmol/L	61.9 - 70.7	176.8 - 194.5	4.2	10	7.8
HDL-C ¶	mmol/L	0.94 - 0.99	1.08 - 1.15	5.5	5	10.7
Glucose	mmol/L	5.3 - 5.6	10.2 - 10.5	2.3	6	6.2
Na^{+} **	mmol/L	138 - 142	-	0.2	10	0.7
Trig ††	mmol/L	1.34 - 1.38	2.64 - 2.7	9.9	10	26.2
K^{+} ‡‡	mmol/L	4.50 - 4.60	5.47 - 5.63	1.7	10	4.9
Iron	µmol/L	9.13 - 9.49	13.96 - 14.14	9.6	10	32.4
UIBC §§	µmol/L	50.6 - 52.0	54.6 - 57.8	–	10	–
TSH ‖‖	mIU/L	0.426 - 0.474	22.56 - 23.43	10	10	24.7
fT_4 ¶¶	pmol/L	11.0 - 11.5	22.5 - 23.5	2.3	–	6.3
B12 ***	pmol/L	123.3 - 170.1	427.7 - 472.7	9.4	–	15.4
Ferritin	ng/mL	8.76 - 9.06	605.7 - 675.2	33.2	10	43.8
T-Bil †††	µmol/L	10.2 - 20.5	52.5 - 53.3	8.0	10	24.7
TnI ‡‡‡	ng/mL	0.033 - 0.037	1.514 - 1.63	9.5	10	18.2

* Alanine Aminotransferase, † Aspartate Aminotransferase, § Cholesterol Total, ‖ Serum Creatinine, ¶ Cholesterol, HDL, ** Sodium, †† Triglycerides, ‡‡ Potassium, §§ Unsaturated Iron Binding Capacity, ‖‖ Thyroid Stimulating Hormone, ¶¶ Free Thyroxine, *** Vitamin B12, ††† Bilirubin Total, ‡‡‡ Troponin-I.

Table 2. Semi-quantitative scale for assessing hemolysis, icterus, and lipemia (HIL) interference, expressed as mean and range values for each interference level.

Scale	Hemolysis (Hgb g/L) mean (min - max)	Lipemia (mmol/L) mean (min - max)	Icterus (µmol/L) mean (min - max)	Hemolysis (Hgb g/L) * mean (min - max)
0	0.07 (0.04 - 0.10)	0.60 (0 - 0.11)	23.00 (17.1 - 29.0)	0.05 (0 - 0.10)
1+	2.50 (2.26 - 2.77)	2.20 (2.10 - 2.37)	124.00 (111.1 - 136.8)	0.85 (0.75 - 1.00)
2+	4.90 (4.38 - 5.40)	4.40 (4.18 - 4.52)	214.00 (205.2 - 222.3)	1.75 (1.50 - 2.00)
3+	7.20 (6.48 - 7.95)	6.50 (6.22 - 6.67)	308.00 (299.2 - 316.3)	2.75 (2.50 - 3.00)
4+	9.30 (8.55 - 10.12)	8.40 (8.14 - 8.70)	410.00 (401.8 - 418.9)	3.75 (3.50 - 4.00)
5+	-	-	-	4.75 (4.50 - 5.00)

*** The right-most column represents the semi-quantitative scale used to evaluate hemolysis interference for potassium and AST. Because these assays are sensitive even to mild hemolysis, the assessment was performed across six intervals within the 0 - 5 g/L hemoglobin range.**

and Thyroid-Stimulating Hormone (TSH) was affected only at the higher hemolysis degree (Figure 1A - B). Serum Iron was highly sensitive, showing marked positive bias even with minimal hemolysis. Troponin-I (TnI) exhibited concentration-dependent bidirectional bias – falsely elevated at lower hemolysis and decreased at higher degrees (Figure 1C). Potassium and AST increased from mild hemolysis onward, irrespective of

Table 3A. Hemolysis-induced (%) for all analytes measured in two concentration pools (L1: low/normal, L2: high).

Parameter (L1/L2)	H1	H2	H3	H4	Target BIAS (%)
Albumin L1	-0.82	1.89	3.55	8.45	1.20
Albumin L2	-0.07	2.13	4.32	6.52	1.20
ALT L1	11.5	21.3	30.4	37.6	8.10
ALT L2	0.70	2.20	1.70	2.30	8.10
Calcium L1	0.29	0.04	-0.64	-0.30	0.70
Creatinine L1	0.59	1.39	3.58	3.99	4.20
Creatinine L2	-1.40	1.60	2.30	2.40	4.20
Ferritin L1	14.4	26.5	30.7	46.9	10.0
Ferritin L2	1.45	4.28	8.64	21.3	10.0
fT4 L1	-4.37	-4.60	-8.51	-9.20	2.30
fT4 L2	-0.42	-3.54	-4.25	-3.12	2.30
Glucose L1	-0.18	-0.68	-1.93	-3.33	2.30
Glucose L2	-0.24	-0.81	-1.28	-2.32	2.30
HDL-C L1	-1.10	-4.24	-3.96	-7.20	5.00
HDL-C L2	-3.54	-4.75	-6.25	-9.82	5.00
Iron L1	61.3	114.5	165.8	222.7	9.60
Iron L2	36.2	71.3	99.5	136.4	9.60
Sodium L1	0.06	-0.28	-0.72	-0.97	0.20
T-Bil L1	-3.25	-9.12	-11.2	-16.1	8.00
T-Bil L2	-4.51	-8.69	-12.5	-15.4	8.00
T-Chol L1	0.31	1.04	0.94	1.13	4.00
T-Chol L2	0.20	-0.20	-0.10	0.10	4.00
Triglycerides L1	9.63	18.7	28.7	37.9	9.90
Triglycerides L2	2.7	4.7	7.3	9.4	9.90
TnI L1	16.3	48.1	110.9	174.5	9.50
TnI L2	-7.08	-7.12	-11.8	-23.0	9.50
TSH L1	5.13	5.87	1.47	-3.05	10.0
TSH L2	-1.75	-9.06	-20.1	-23.0	10.0
UIBC L1	2.31	-1.68	-8.73	-15.1	10.0
Vitamin B12 L1	8.53	11.7	18.0	29.7	9.40
Vitamin B12 L2	-15.7	-13.8	-17.9	-16.4	9.40

	H1	H2	H3	H4	H5	Target BIAS (%)
Potassium L1	5.80	12.32	24.64	36.23	49.28	1.60
Potassium L2	3.57	7.14	11.07	15.71	20.00	1.60
AST L1	36.84	73.68	110.53	157.89	215.79	5.30
AST L2	25.93	58.61	86.06	113.07	150.11	5.30

Hemolysis-induced BIAS (%) for all analytes measured in two concentration pools (L1: low/normal, L2: high). Columns H1-H4 denote increasing hemoglobin concentrations according to the 4-level hemolysis scale (see Table 2; approximate means 2.50, 4.90, 7.20, and 9.30 g/L). Cells exceeding the Target BIAS threshold are emphasized; shading indicates the degree of deviation (light gray > 1 × threshold; dark gray > 2 × threshold). Negative values indicate decreases versus baseline. At the bottom of the table, expanded hemolysis assessment for potassium and AST across five graded hemolysis levels (H1+ - H5+) is shown (approx. means 0.85 - 4.75 g/L hemoglobin). BIAS (%) is presented for both concentration pools (L1, L2). All deviations are positive, demonstrating proportional increases with rising hemolysis index.

Table 3B. BIAS (%) under icteric (I1 - I4) and lipemic (LI1 - LI4) interference for each analyte at two concentration pools (L1, L2).

Parameter (L1/L2)	I1	I2	I3	I4	LI1	LI2	LI3	LI 4	Target BIAS (%)
Albumin L1	-3.31	-5.28	-4.50	-8.78	1.49	1.49	1.99	3.00	1.20
Albumin L2	-0.93	-0.47	-4.19	-2.79	0.92	1.83	2.29	2.75	1.20
ALT L1	4.44	4.44	6.67	6.67	0.00	-4.04	-6.06	-14.1	8.10
ALT L2	-1.88	-2.57	-2.34	-2.80	0.13	-3.31	-4.58	-7.25	8.10
AST L1	-4.08	0.00	-7.14	-10.2	0.18	-0.86	-1.01	-2.34	5.30
AST L2	0.18	-0.86	-1.01	-2.34	-0.48	-1.05	-5.52	-6.29	5.30
Calcium L1	-1.04	-2.78	-2.43	-2.08	0.22	-0.65	0.65	0.43	0.70
Creatinine L1	-6.67	-8.24	-15.5	-17.3	-1.09	-3.80	-5.98	-6.79	4.20
Creatinine L2	-6.18	-8.51	-11.9	-15.1	0.75	-2.80	-4.39	-4.76	4.20
Ferritin L1	-1.40	-3.60	-3.90	-8.40	-2.60	-5.60	-4.80	-3.70	10.0
Ferritin L2	8.72	8.04	8.34	3.98	-5.18	-2.41	-6.24	-4.97	10.0
fT4 L1	0.00	4.76	3.08	5.60	0.61	0.77	2.00	5.23	2.30
fT4 L2	4.69	2.75	4.55	4.69	2.36	2.92	6.40	9.10	2.30
Glucose L1	-0.79	-1.58	-3.16	-5.26	-0.20	-2.00	-3.41	-4.61	2.30
Glucose L2	-0.87	-2.13	-1.05	-1.95	-0.85	-2.86	-3.60	-4.45	2.30
HDL-C L1	-1.62	-8.30	-8.30	-9.99	-17.4	-27.6	-33.8	-37.8	5.00
HDL-C L2	-4.16	-2.58	-5.60	-8.86	-14.7	-27.0	-33.1	-37.1	5.00
Iron L1	0.96	1.91	0.96	1.91	11.3	24.6	38.6	50.0	9.60
Iron L2	0.56	2.22	0.56	1.67	-	-	-	-	9.60
Potassium L1	0.57	1.49	-0.80	-0.80	-0.43	-2.13	-2.98	-4.26	1.70
Potassium L2	-1.25	-1.25	-1.25	-1.25	-1.79	-2.50	-3.93	-5.36	1.70
Sodium L1	-0.25	-0.25	-0.67	-0.67	-1.14	-2.28	-3.14	-3.85	0.20
T-Bil L1	-	-	-	-	-0.99	-1.98	-2.03	-4.65	8.00
T-Bil L2	-	-	-	-	-1.71	-2.46	-2.78	-5.24	8.00
T-Chol L1	-7.02	-12.4	-21.0	-16.9	0.83	-0.47	-0.59	0.83	4.00
T-Chol L2	-5.86	-10.9	-13.2	-16.3	1.31	2.01	2.41	3.62	4.00
Triglycerides L1	-7.68	-18.2	-36.7	-37.7	-	-	-	-	9.90
Triglycerides L2	-6.14	-15.4	-22.4	-28.1	-	-	-	-	9.90
TnI L1	-3.88	-9.71	-9.71	-10.6	-4.11	4.06	-5.39	-4.21	9.50
TnI L2	-3.63	-7.56	-12.2	-16.1	-1.22	-0.92	-3.31	-4.44	9.50
TSH L1	-0.98	-1.44	-1.90	1.61	-3.10	-6.10	-4.03	-8.89	10.0
TSH L2	-2.33	0.83	-0.06	-2.69	-1.05	-1.27	-3.11	-4.81	10.0
UIBC L1	4.23	4.58	4.85	9.64	-	-	-	-	10.0
UIBC L2	7.98	7.54	11.0	12.9	0.42	-7.49	-5.41	-9.89	10.0
Vitamin B12 L1	-3.75	-9.06	-7.08	-3.44	4.11	5.90	-5.55	-2.33	9.40
Vitamin B12 L2	-5.80	-1.44	-1.60	-5.30	-2.98	-4.13	-2.29	-4.02	9.40

BIAS (%) under icteric (I1 - I4) and lipemic (LI1 - LI4) interference for each analyte at two concentration pools (L1, L2). Icterus levels correspond to bilirubin ≈ 124, 214, 308, and 410 µmol/L; lipemia levels correspond to lipid indices ≈ 2.20, 4.40, 6.50, and 8.40 mmol/L (see Table 2). Values exceeding the Target BIAS threshold are emphasized; shading indicates deviation magnitude (light gray > 1 × threshold; dark gray > 2 × threshold). Negative values indicate decreases relative to baseline.

analyte concentration (Figure 1D; Table 3A). Glucose and UIBC exceeded the acceptable bias threshold only at the highest hemolysis, while sodium (single range, 138 - 142 mmol/L) showed significant interference from moderate hemolysis. Calcium, Total Cholesterol (T-Chol), and Serum Creatinine were unaffected.

Table 4. Head-to-head comparison of manufacturer-declared versus experimentally observed HIL interference thresholds by analyte and concentration pool (L1, L2).

Parameter	Hemolysis (Hb g/L)				Icterus (µmol/L Bilirubin)				Lipemia (mmol/L Lipid)			
	Manufact. Claim *		Observed		Manufact. Claim *		Observed		Manufact. Claim *		Observed	
	L-1 †	L-2 ‡	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2
Glucose	> 20		> 9.30		> 1,000		> 308.00	> 214.00	> 22.6		> 4.40	> 6.50
Albumin	> 7.50		> 4.90		> 1,000		> 124.00	> 308.00	> 33.9		> 2.20	> 4.40
Sodium	> 20		> 4.90	N/A §	> 1,000		> 124.00		> 22.6		> 2.20	
Calcium	> 20		N/E ‖		> 1,000		> 124.00		> 8.48		N/E	
Creatinin	> 10		N/E		> 342	> 684	> 124.00		> 8.48	> 11.3	> 6.50	
HDL-C	> 20		> 9.30	> 7.20	> 1,000		> 214.00	> 308.00	> 11.3	> 22.6	> 2.20	
Potassium	> 1.25		> 0.85		> 1,000		N/E		> 22.6		> 4.40	> 2.20
Total Bilirubin	> 20		> 4.90		N/A				> 8.48	> 11.3	N/E	
Triglycerides	> 5.00		> 2.50	N/E	> 70		> 214.00		N/A			
fT4	> 3.00		> 2.50	> 4.90	> 340		N/E	> 124	> 11.3		> 8.40	> 2.20
Ferritin	> 9.00		> 2.50	> 9.30	> 1,000		N/E		> 22.6		N/E	
Troponin-I	> 5.00		> 2.50	> 7.20	> 340		> 214.00	> 308.00	> 11.3		N/E	

For each interferent - hemolysis (hemoglobin, g/L), icterus (bilirubin, µmol/L), and lipemia (lipid index, mmol/L) - the table lists the manufacturer's claim (*) and the corresponding observed threshold at which interference was identified.
*** manufacturer-declared threshold, † Level-1 pool, ‡ Level-2 pool, § not applicable, ‖ no effect detected within the tested range. Discrepancies between claimed and observed thresholds are highlighted; no material differences were noted for vitamin B12, TSH, total cholesterol, AST, iron, ALT, and UIBC.**

Icteric interference

Total cholesterol and creatinine were sensitive to icterus at both levels from the lowest detectable interference; sodium and calcium (single levels) were likewise affected. TnI was impacted only at more pronounced icterus. Triglycerides decreased progressively with increasing icterus (Table 3B). Low-concentration albumin crossed thresholds earlier than high-concentration albumin, which was affected only at advanced stages. Glucose and AST at the lower level were affected under severe icterus, with no effect at the higher level. UIBC showed interference only at advanced stages and only at the higher concentration (Figure 2A - C). No significant icteric effects were observed for ALT, Iron, Potassium, TSH, vitamin B12, or Ferritin (Table 3B).

Lipemic interference

Lipemia highly affected albumin, creatinine, fT4, glucose, HDL-C, iron (single level), potassium, and sodium (single level); ALT and AST showed slight effects (Table 3B). Calcium, Total bilirubin, total cholesterol, UIBC, TSH, vitamin B12, Ferritin, and Troponin-I were not significantly influenced at any tested lipemia level (Figure 2D - F).

Manufacturer vs. empirical cutoffs

Discrepancies between manufacturer-reported and empirically determined HIL cutoff values were identified for several assays (Table 4). No statistically significant differences were found for vitamin B12, TSH, Total cholesterol, AST, Iron, ALT, or UIBC.

DISCUSSION

This study demonstrated that several analytes showed interference thresholds that differed from the manufacturer's claims under local laboratory conditions. Albumin, sodium, and HDL-C were affected by all three types of interference, showing clinically relevant bias below the declared limits and highlighting the need for laboratory-specific verification.

Hemolysis produced analyte-specific effects in both direction and magnitude. One of the most notable findings was obtained from cardiac Troponin-I measurements. Hemolysis affected TnI results in opposite directions: low levels were falsely elevated, whereas high levels were falsely decreased. Similarly, other studies have also reported this bidirectional effect [13,14]. Proteases released from red blood cells may degrade antigenic sites on high-sensitive cardiac TnI, reducing detectability, while free hemoglobin can attenuate luminescent signals, leading to falsely low results [15]. In patients with persistently elevated TnI, a change of less than 20% generally indicates chronic myocardial injury, provided this finding aligns with the clinical presentation

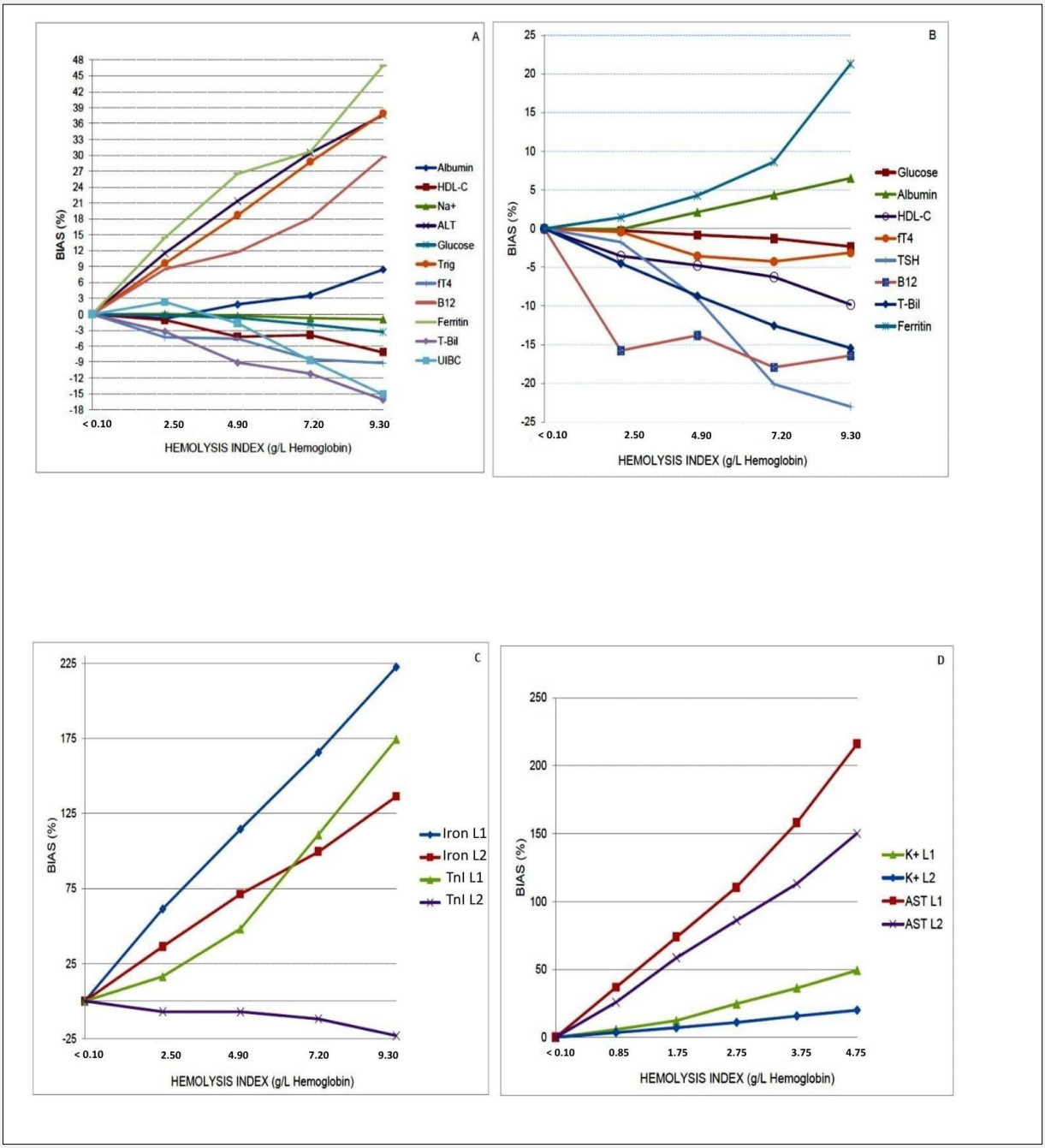

Figure 1. A Analytes affected by hemolysis at the Level 1 concentration range. B Analytes affected by hemolysis at the Level 2 concentration range. C A dedicated graph is presented for the Serum Iron and Troponin-I assays, as their calculated values were found to be substantially higher compared to those of other analytes. D A separate graph is provided for potassium and AST, which were evaluated using five hemolysis levels due to their distinct sensitivity. As both showed consistent positive bias at Level 1 and Level 2, the results are displayed together.

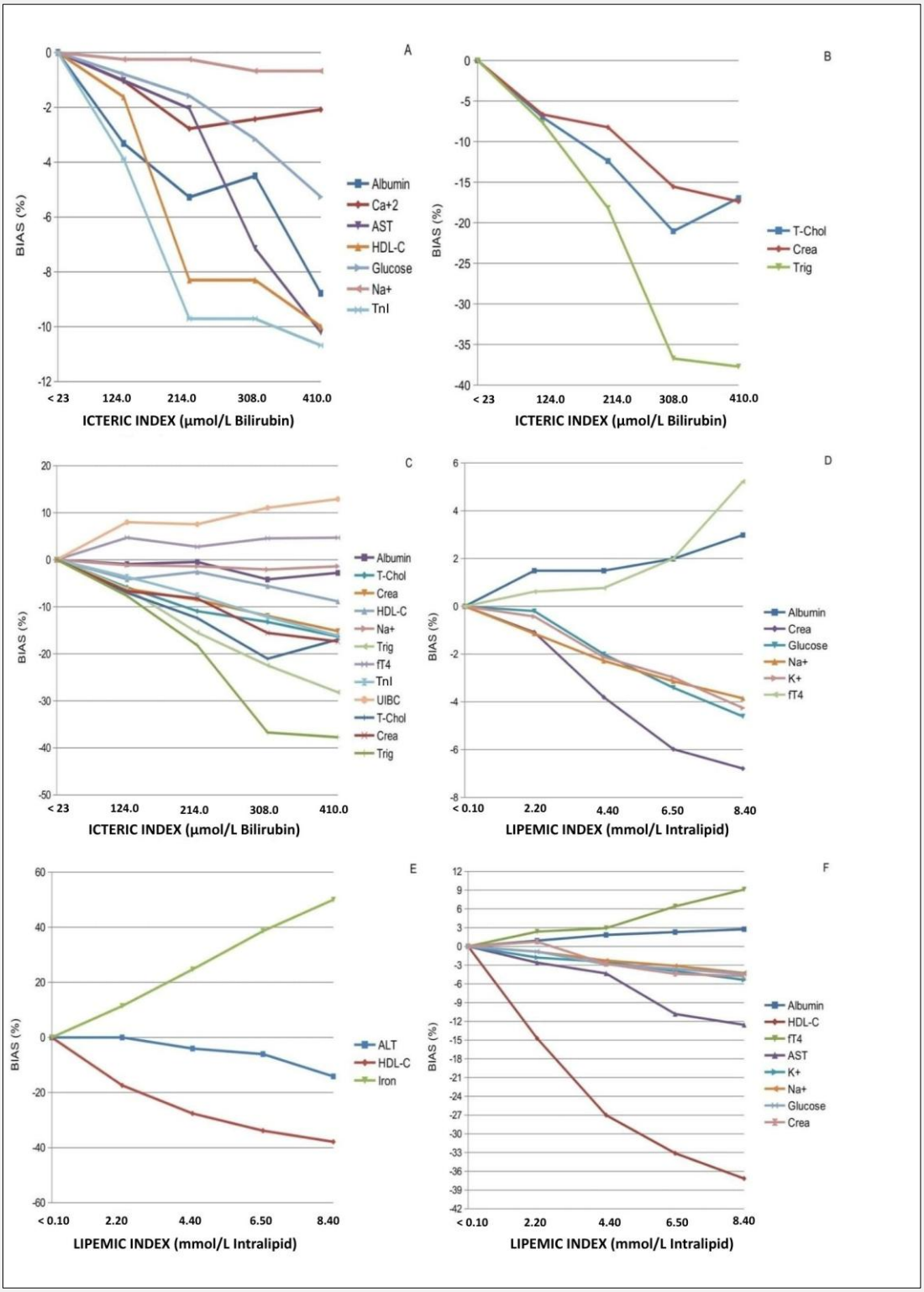

Figure 2. A Dose-response plots for analytes affected by icteric interference at the Level 1 concentration range. B Dose-response plots for analytes affected by icteric interference at the Level 1 concentration range. C Dose-response plots for analytes affected by icteric interference at the Level 2 concentration range. D Analytes affected by lipemic interference at the Level 1 concentration range. E Analytes affected by lipemic interference at the Level 1 concentration range. F Analytes affected by lipemic interference at the Level 2 concentration range.

[16]. Importantly, under hemolytic conditions, a patient with initially low TnI may show a falsely high baseline and a falsely low follow-up value, potentially masking the true rise associated with myocardial infarction (MI). If the overall change remains below 20%, the diagnosis of MI may be overlooked.

The observed deviations, primarily attributable to hemolysis, revealed that total bilirubin, fT4, glucose, and HDL-C showed negative bias, whereas albumin showed a mild positive bias at higher hemolysis levels following an initial minimal decrease. HDL-C exceeded the desirable bias limit (5%) at 2.5 g/L hemoglobin, and sodium also showed earlier interference beginning at 0.85 g/L, both below their respective manufacturer thresholds. Similar early-onset negative bias has been reported, attributed to optical interactions of hemoglobin with ion-selective electrode measurements [4,9]. As expected, both AST and potassium levels increased proportionally with the extent of hemolysis, reflecting leakage of intracellular constituents. Notably, potassium interference began at 0.85 g/L, earlier than the manufacturer's declared limit of 1.25 g/L, emphasizing the potential for clinical misinterpretation - particularly in critically ill patients, where even minor electrolyte shifts can influence therapeutic decisions [17]. Ferritin demonstrated a positive bias at both levels, whereas TSH showed a negative deviation only at the higher hemolysis level. Vitamin B12 exhibited a bidirectional pattern-positive at the lower and negative at the higher concentration. Comparable findings have been reported in independent investigations using the Abbott Architect i2000 system, where ferritin and TSH demonstrated similar positive shifts, but vitamin B12 showed consistent negative bias under hemolytic conditions [18]. Clinically, hemolytic deviations are most critical for analytes interpreted near decision thresholds, where even minor analytical biases in electrolytes, thyroid hormones, or bilirubin can lead to misjudgment. The HIL index should therefore be regarded not merely as a pre-analytical marker but as a key indicator of patient safety, ensuring reliable results and sound clinical decisions.

Bilirubin interference was observed at considerably lower concentrations than those specified by the manufacturer. For creatinine, a significant negative bias emerged from 124 µmol/L bilirubin, despite the declared limit of 342 µmol/L. Similar early interference has been reported in previous studies, attributed to spectral interactions between bilirubin and the Jaffé reaction used in creatinine measurement [4,19], glucose and HDL-C were likewise affected well below their stated cutoffs (> 1,000 µmol/L and 214 µmol/L, respectively), exhibiting clear negative bias beginning near 124 µmol/L, consistent with the same mechanism. Calcium also demonstrated a shift in bias direction-with a non-significant negative deviation at low and a slight positive deviation at higher bilirubin levels-reflecting the bidirectional absorption phenomenon described in earlier studies [19].

Lipemic interference was observed at substantially lower lipid concentrations than those specified by the manufacturer. Sodium and HDL-C displayed significant bias already at 2.2 mmol/L, despite declared limits exceeding 11.3 mmol/L, reflecting nearly a five-fold difference in analytical sensitivity and supporting previous observations on variable manufacturer-defined thresholds [20]. fT4 also demonstrated a concentration-dependent shift, showing a slight positive bias under mild lipemia and a clear negative bias at higher lipid levels, consistent with lipid-induced disruption of microparticle binding mechanisms described in recent immunoassay evaluations [7]. Creatinine and potassium likewise showed earlier than expected bias, in agreement with prior reports attributing such effects to optical light scattering caused by elevated triglyceride concentrations [4].

Desirable bias and TEa are commonly used to evaluate analytical significance. Some studies have also incorporated the Reference Change Value (RCV) [7,21,22]. However, RCV includes within-subject biological variation (CV_i) [23]. Therefore, we believe that bias or TEa limits are more suitable for interpreting interference experiments.

Overall, manufacturer-declared HIL cutoffs may be overly permissive and mask clinically significant effects. Several analytes demonstrated earlier interference onset than declared, emphasizing the importance of local verification. In our study, the immunoassay platform used (Siemens Advia Centaur XP) lacked an automated HIL index analyzer, and notable deviations were observed across multiple assays compared with the manufacturer's limits, emphasizing that reliable detection of HIL effects must be an inherent feature of modern immunoassay platforms. As overlapping interferences are common in pediatric and lipemic samples, future work should explore these combined effects under real-world conditions. Establishing analyzer-specific limits and continuous bias-based monitoring will further enhance result integrity and patient safety.

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The authors declare that they have no conflicts of interest.

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