Protein Interference with Common Laboratory Tests

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ABSTRACT

Objective: Protein as an interferent in under appreciated. Therefore, we evaluated interference by proteins on clinical laboratory tests. Methods: Base Pool was prepared using a serum pool. A High Pool was prepared by spiking proteins into the Base Pool (Concentrated human albumin and gamma-globulins 1:1; ASSURANCE™ Interference Test Kit, Sun Diagnostics, New Gloucester, ME). The Base Pool and High Pool were intermixed to create five levels of total protein (3.5, 6.9, 9.4, 12.4, and 15.3 g/dL). Multiple analytes were measured on the Beckman AU5800. Results: Minimal effects were seen with ALP, AMY, DBIL, TBIL, CK, GGT, GLU, LDH, LIP, PHOS, BUN, UA, LPA, sdLDL-C, hsCRP, ferritin, LDL-C, HDL-C, K, CL, TRIG, and CHOL. Positive bias with increasing protein concentration was seen with ALT, AST, CA, CRE, FE, MG, homocysteine (HCY), and non-esterified fatty acids (NEFA). Because proteins bind analytes such as calcium, magnesium, iron, HCY, and NEFA, we believe these increases were artifactual. Negative bias was seen with CO2, apo AI, and apo B. Conclusion: Manufacturers and laboratorians need to pay more attention to proteins as a potential interferent. The mechanism may be photometric or a volume depletion effect, whereby a very high protein concentration will reduce the available water so that the analyte concentration is artifactually low.

INTRODUCTION

Proteins as potential interferents are overlooked. The assumption is that protein interference is caused by paraproteins (monoclonal immunoglobulins) [1,2] but this is an oversimplification. Elevated proteins, including albumin and gamma-globulins may affect many assays, including bilirubin, phosphate, HDL cholesterol, GGT, CRP, and glucose [1]. The manufacturer may note on the package insert that monoclonal gammopathies may result in errors, but rarely do they perform a comprehensive study of protein interference. The Clinical Laboratory Standards Institute (CLSI) recommends testing with both albumin and gamma-globulins at a total protein concentration of 12 g/dL [3]. The interference may be due to physical or chemical alteration in the signal (e.g. increased light scattering) or it may be due to a volume displacement effect [4]. The practical effect of an increased concentration of proteins is a decrease in serum water or volume depletion: the aspirated sample is diluted with solid (protein and/or lipid) so that the analyte concentration is artifactually low. Viscosity is also a common problem with specimens containing monoclonal immunoglobulins, especially IgM, and this may affect the accuracy of sample delivery.

METHODS

A Base Pool was prepared using leftover, de-identified patient sera. A Test Pool was prepared by adding 1 part concentrated human proteins *ASSURANCE™ Test Kit, Sun Diagnostics, LLC, New Gloucester, ME) to 1 part Base Pool. The concentrated proteins are ~50% human albumin and 50% human gamma-globulins. A Control Pool was prepared by mixing 1 part Base Pool with 1 part saline to maintain equal analyte concentrations. The Test Pool and Control Pool were intermixed to create five levels of protein interference pools. Multiple analytes in the five levels were measured in duplicate on the Beckman AU5800 at HDL, Inc. (Richmond, VA). Bias was calculated for levels 2 to 5 versus level 1. Bias < ½ the total allowable error was considered acceptable.

RESULTS

- Minimal interference with total protein concentrations up to 15.5 g/dL were seen for alkaline phosphatase (ALP), amylase (AMY), direct bilirubin (DBIL), total bilirubin (TBIL), chloride (CL), total cholesterol (CHOL), creatine kinase (CK), ferritin (FER), gamma-glutamyltransferase (GGT), glucose (GLU), high density lipoprotein cholesterol (HDL-C), HDL3-C, high sensitivity C-reactive protein (hsCRP), lactate dehydrogenase (LDH), lipase (LIP), lipoprotein(a) (LPA), low density lipoprotein cholesterol (LDL-C), phosphorus (PHOS), potassium (K), small, dense LDL cholesterol (sdLDL-C), triglycerides (TRIG), urea nitrogen (BUN), or uric acid (UA).
- Positive bias was seen with increasing protein concentrations for alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (CA), creatinine (CRE), homocysteine (HCY), iron, nonesterified fatty acids (NEFA), and magnesium (MG).
- Negative bias was seen with carbon dioxide (CO2), apolipoprotein AI (APOAI), and apolipoprotein B (APOB).
- Because human proteins bind calcium, homocysteine, iron, nonesterified fatty acids, and magnesium we measured these analytes in the concentrated protein interference material and subtracted this from the measured concentrations. Positive bias remained for all analytes except NEFA, which converted to a negative bias.

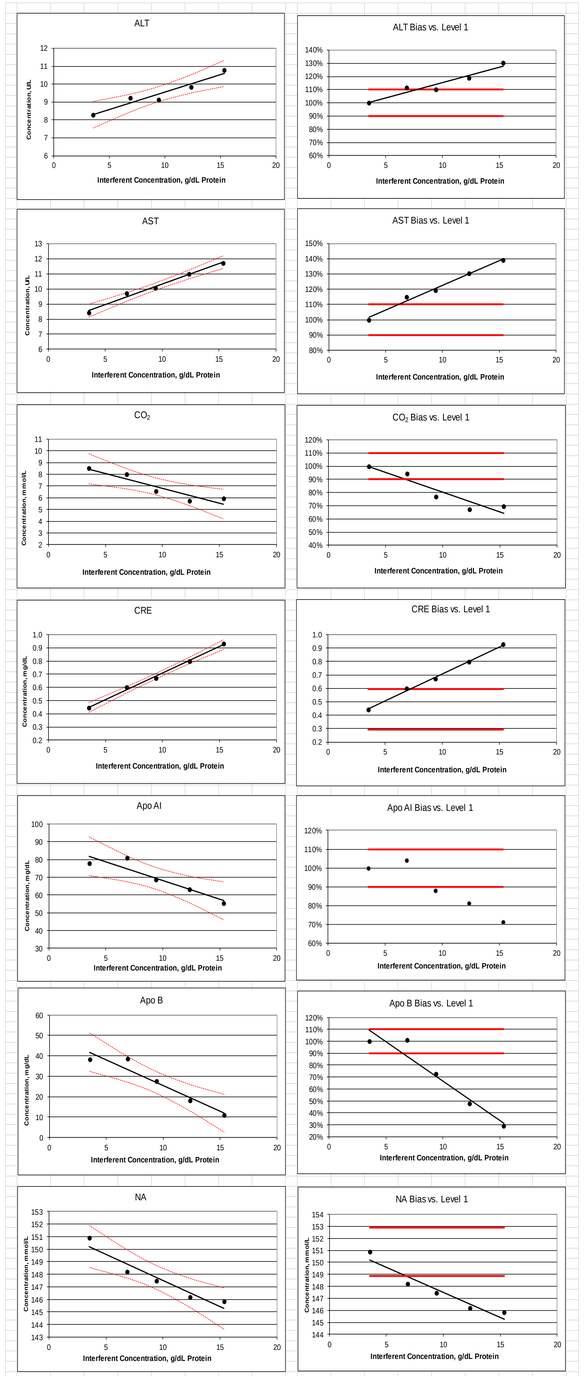
Table 2: Analyte Concentrations, TEa, and Bias

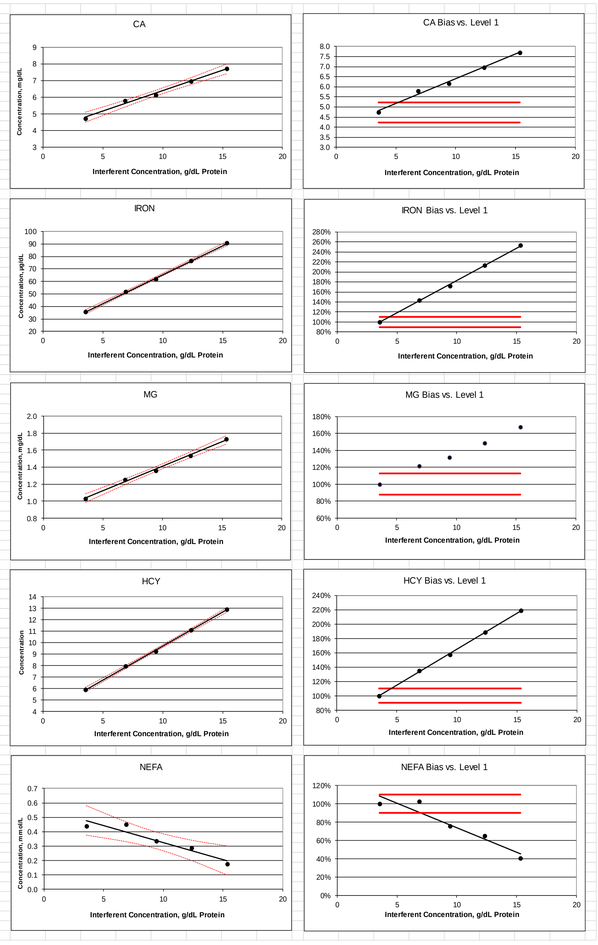
Analyte	Units	Conc	½ TEa	Bias Level 5 (Level 4) v. Level 1
ALP	U/L	35.2	15%	0; 0%
ALT	U/L	8.28	10%	>10%
AMY	U/L	21.5	15%	0.1; 0.3%
APOAI	mg/dL	77.8	10%	>10%
APOB	mg/dL	38.1	10%	>10%
AST	U/L	8.43	10%	>10%
BUN	mg/dL	9.54	4.5%	0.6; 5.8% (0.2; 2.0%)
CA	mg/dL	4.74	0.5 mg/dL	>0.5 mg/dL
CHOL	mg/dL	87.3	4.5%	4.4%
CL	mmol/L	130.3	5%	-5.6; -4.3%
CK	U/L	48.1	15%	-5.0; -11.2%
CO2	mmol/L	8.51	10%	>10%
CRE	mg/dL	0.44	7.5%/0.15 mg/dL	>7.5%
DBIL	mg/dL	0.03	0.2 mg/dL	-0.16 mg/dL
IRON	µg/dL	35.8	10%	>10%
FER	ng/mL	72.1	10%	1.1; 1.5%
GGT	U/L	16.7	10%	0.05; 0.3%
GLU	mg/dL	51.4	5%/3 mg/dL	1.2; 2.3%
HCY	µmol/L	5.88	10%	>10%
HDL-C	mg/dL	26.8	6.5%	-0.6; -2.2%
HDL3-C	mg/L	15.0	10%	0.7; 4.9%
hsCRP	mg/L	3.12	10%	0.04; 4.9%
K	mmol/L	2.40	0.25 mmol/L	0.11 mmol/L
LDH	U/L	58.7	10%	-2.8; -4.8%
LDL-C	mg/dL	57.0	6%	0.3; 0.6%
LIP	U/L	12.4	15%	2.5; 19.6%; (1.2; 9.7%)
LPA	nmol/L	19.3	10%	-0.1; -0.5%
MG	mg/dL	1.03	12.5%	>12.5%
NA	mmol/L	150.9	2 mmol/L	> 2 mmol/L
NEFA	mmol/L	0.44	10%	>10%
PHOS	mg/dL	1.82	10%	0.19; 10.4%; (0.12; 6.6%)
sdLDL-C	mg/dL	9.12	10%	0.51; 5.6%
TBIL	mg/dL	0.16	0.2 mg/dL	0.07 mg/dL
TRIG	mg/dL	61.2	7.5%	3.8; 6.2%
UA	mg/dL	2.89	8.5%	-0.12; -4.2%

RESULTS

Table 1: Measured Protein Concentrations of Test Pools, g/dL

Level	Total Protein	Albumin	Globulins
1	3.5	2.1	1.4
2	6.9	4.0	2.8
3	9.4	5.4	4.0
4	12.4	7.0	5.4
5	15.3	8.6	6.8





CONCLUSIONS

- **Human proteins (albumin and gamma-globulins) appear to interfere with a number of common laboratory tests.**
- **Protein interference is not described by most manufacturers, except to note possible interference by monoclonal paraproteins.**
- **Laboratorians and manufacturers need to pay greater attention to proteins as a source of interference with chemistry assays.**

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