Clinica Chimica Acta 412 (2011) 1550–1553

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Evaluation of the interference of hemoglobin, bilirubin, and lipids on Roche Cobas 6000 assays

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ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 27 April 2011
Accepted 28 April 2011
Available online 6 May 2011

Keywords:
Analytical interference
Hemolysis
Icterus
Lipemia
Roche Cobas

ABSTRACT

Background: Pre-analytical error accounts for major total laboratory errors. We assessed the impacts of hemolysis, icterus, and lipemia on laboratory tests on Roche Cobas 6000.
Methods: Various concentrations of hemoglobin, bilirubin, or Intralipid® were added into the plasma to simulate hemolytic, icteric, or lipemic samples. The analytes were then measured on Roche Cobas 6000 and the change of the analyte concentrations was determined.
Results: For most of the chemistry assays, our data were in a good agreement with Roche package inserts. However some assays had significant interference at lower index values while others were affected at higher index than the Roche package inserts indicated. In addition, we observed the positive interference by hemolysis on ALT, lipase, total protein, potassium, and iron. Negative interference was noted on calcium and CK. Most of the immunoassays were not affected by hemoglobin, bilirubin, and lipids although there were a few exceptions. Several therapeutic drugs were affected either positively or negatively by hemolysis, icterus, or lipemia to a certain extent.
Conclusions: We have demonstrated some test interferences which have not been reported previously on the Cobas 6000. The implementation of the cut-off indices on Cobas 6000 would provide more accurate test result reporting.

1. Introduction

Analytical interference caused by pre-analytical factors is a significant source of error in clinical laboratory measurements [1,2]. Analytical interference by hemolysis, bilirubin and lipids with laboratory assays is the most common concern in laboratory medicine. These altered results may lead to repeat tests, incorrect interpretation, wrong diagnosis, and potentially inappropriate intervention and unfavorable outcome for the patients [3–5]. Hemolysis, icterus, and lipemia commonly interfere with spectrophotometric methods with hemolysis being the most common [3,4,6,7]. This interference is mainly caused by components released from the red cells. Although direct spectral interference on chemistry analyzers has been minimized with bichromatic and kinetic analysis, the contents of red cells like potassium and lactate dehydrogenase falsely increase these constituents in plasma or serum. There are other constituents released from red cells that can interfere with test reactions. Finally, analytes can also get diluted in hemolysis [4]. Hemolysis can occur in vivo, but the major problem that clinical laboratories get is that it occurs during and after collection of specimens. Bilirubin can interfere with assays spectrally but also chemically in some reactions. Normally, bilirubin concentrations >35 mmol/l are clinically defined as hyperbilirubinemia, whereas icteric samples have bilirubin concentrations >100 mmol/l [3]. A lipemic sample is the result of high concentrations of chylomicrons and very-low density lipoprotein (VLDL). Lipemia may interfere in any assay that is based on the detection of light transmission or scattering or by volume displacement [8,9]. The presence of hemoglobin, bilirubin, and lipids in a specimen can cause a positive or negative interference in the measurement result of many analytes [10–12]. Depending on the magnitude of this interference, the results may lead to wrong interpretation and inappropriate intervention [5,13–15]. Interference on the Beckman system has been well investigated [8,16]. Nevertheless, the degree of interference varies with the methodology and individual instrumentation and comprehensive interference evaluation and published data are not available on Roche Cobas system (Indianapolis, IN).

2. Subjects and methods

To evaluate the impact of an interferent on analytes, the analyte was measured in a sample spiked with increasing concentrations of

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doi:10.1016/j.cca.2011.04.034

the interferent, and the relative deviation of the result from the non-spiked baseline value was then calculated.

2.1. Subjects

Pooled lithium heparinized plasma free of interferent was prepared as test sample. Hemolysate was prepared by washing packed red cells (0.9% saline and distilled water) and then freezing, and thawing. Various concentrations of hemolysate were added into the plasma to simulate different degrees of hemolysis. Icteric samples were obtained with the addition of unconjugated bilirubin (Sigma-Aldrich, St. Louis, MO) to specimens to achieve different concentrations of bilirubin. Because of poor solubility of unconjugated bilirubin, bilirubin was prepared in a stock solution in dimethyl sulfoxide [6,17]. The proportion of bilirubin in serum or plasma between conjugated and unconjugated is not a significant factor for interference [3]. Lipemic samples were prepared by adding Intralipid® (20%) (Baxter, Deerfield, IL). The analytes were then measured on Roche Cobas 6000 (c501 and e601) and the change of the analyte concentrations was compared with baseline samples.

2.2. Chemistry analyzer

The multi-channel Roche Cobas 6000 analyzer is a fully automated, computer-controlled system designed for the analysis of routine chemistry assays, immunoassays, and therapeutic drugs. The Cobas 6000 uses spectrophotometry to perform kinetic, end-point and non-linear reactions. To a certain extent, the system, similar to most modern analyzers, reduces spectral interference effects by application of two-reagent procedures and bichromatic spectrophotometry. The Cobas 6000 analyzer is able to detect hemolysis, icterus, and lipemia in samples and can generate quantitative index values for the major interfering substances of hemoglobin, bilirubin, and lipids expressed as H-index (hemolysis), I-index (icterus), and L-index (lipemia). The relationship of each HIL index with the SI unit or conventional unit is listed in Table 1.

2.3. Analytes investigated

The following tests were investigated: α1-antitrypsin (A1AT), acetaminophen, α-fetoprotein (AFP), albumin (Alb), alcohol, alkaline phosphatase (ALP), alanine aminotransferase (ALT), ammonia (Amm), aspartate aminotransferase (AST), β2-microglobulin (β2MI), β human chorionic gonadotropin (βHCG), complement C_3 (C_3), complement C_4 (C_4), calcium (CA), carbamazepine, carcinoembryonic antigen (CEA), ceruloplasmin (Cerulo), cholesterol (Chol), creatine kinase (CK), chloride (Cl), bicarbonate (CO_2), cortisol (Cort), creatinine (Creat), high-sensitivity C-reactive protein (hsCRP), direct bilirubin (D. Bili), digoxin, ferrous iron (Fe^{2+}), ferritin (FER), free triiodothyronine(FRT3), free thyroxine (FRT4), follicle-stimulating hormone (FSH), gentamicin, γ-glutamyltransferase (GGT), glucose, haptoglobin, high density lipoprotein cholesterol (HDL-C), homocysteine, immunoglobin A (IgA), immunoglobin G (IgG), immunoglobin M (IgM), potassium (K^+), lactate dehydrogenase (LD), luteinizing hormone (LH), lipase, magnesium (Mg^{2+}), myoglobin (Myo), sodium (Na^+), pre-albumin (PreALB), phenobarbital, phosphorus (Phos), prolactin (PRL), prostate-specific antigen (PSA), phenytoin, rheumatoid factor (RHF), salicylate, total bilirubin (T.Bili), total bilirubin in cord blood (TBICB), theophylline, tobramycin, total protein (TP), Troponin T (TnT), thyroid stimulating hormone (TSH), unsaturated iron binding capacity (UIBC), urea, uric acid, vancomycin, vitamin B12 (VB12), and valproic acid.

Table 1
Relationship between interferent concentration and the corresponding index.

	H = 1	I = 1	L = 1
Conventional units	1 mg/dl	1 mg/dl	1* (without units)
SI units	0.621 μmol/l	17.1 μmol/l	

* Lipemic index is determined by the turbidity qualitatively with no units.

Table 2
List of interference on chemistry assays by HIL.

Test	Hemolysis interference at index level	Icterous interference at index level	Lipemic interference at index level
Na^+	NS	NS	NS
K^+	Increased (H 200)	NS	NS
Cl^-	NS	NS	NS
CO_2	Decreased (H 130)	Decreased (I 14)	NS
A1AT	NS	NS	Increased (L 1000)
Alb	NS	NS	NS
Alcohol	Decreased(H200)	ND	ND
ALP	Decreased (H 235)	NS	NS
ALT	Increased (H 235)	NS	Decreased (L 150)
Amm	Increased (H 200)	Increased (I 1)	Decreased (L 50)
AST	Increased (H 40)	NS	Decreased (L 179)
β2MI	NS	NS	NS
CA^{2+}	NS	Decreased (I 7)	NS
Cerulo	NS	NS	Increased (L 50)
Chol	Increased (H 600)	NS	NS
CK	Increased (H 400)	Decreased (I 14)	Decreased (L 1200)
Creat	NS	Decreased (I 14)	NS
hsCRP	NS	ND	NS
D. Bili	Decreased (H 40)	NA	Increased (L 233)
Fe^{2+}	Increased (H 40)	ND	NS
GGT	Decreased (H 600)	Decreased (I 14)	Decreased (L 1200)
Glucose	NS	NS	NS
Haptoglobin	Decreased (H 118)	NS	Increased (L 1200)
HDL	NS	Decreased (I 25)	Decreased (L 1200)
Homocysteine	NS	NS	ND
LD	Increased (H 41)	NS	Decreased (L 1200)
Lipase	Increased (H 300)	NS	NS
Mg^{2+}	NS	NS	NS
PreALB	Increased (H 400)	NS	Increased (L 50)
Phos	Increased (H 200)	NS	NS
RHF	Decreased (H 200)	ND	NS
T.Bili	Increased (H 80)	NA	Increased (L 500)
TBICB	ND	ND	Increased (L50)
TP	Increased (H741)	Decreased(I 14)	NS
UIBC	Increased (H 50)	Decreased (I 3)	Decreased (L 180)
Urea	NS	NS	NS
Uric acid	NS	Decreased (I 25)	NS

The index value listed indicates that significant interference was observed at this level.
NA: not applicable, NS: not significant, ND: not determined.

2.4. Statistical analysis

Identify the measured hemolytic, icteric or lipemic index that corresponds to the point where the test analyte differs by >10% from the baseline. The percent change was calculated as: Interference% = 100 × (measured value − true value)/true value, where the measured value is the apparent analyte concentration in the presence of interferent, and the true value is the analyte concentration in the baseline without any addition of interferent. Significant interference was defined when the change of the analyte value exceeded 10% of the baseline value [13,17].

3. Results

3.1. Interferences of hemoglobin, bilirubin, and lipids on chemistry assays

The overall interferences of hemoglobin, bilirubin, and lipids on chemistry assays are listed in Table 2. For most of the chemistry assays, our data were in a good agreement with what Roche reagent package inserts indicated. However some assays were affected