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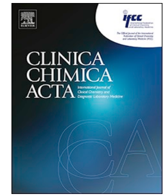

Effect of hemolysis, icterus, and lipemia on three acetaminophen assays: Potential medical consequences of false positive results

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ABSTRACT

Background: We evaluated the effect of hemolysis, icterus and lipemia on 3 acetaminophen assays: namely the Syva® EMIT®, the Microgenics DRI® assay, and the Roche assay on a Roche Cobas® c501 or an Integra 800 analyzer.

Methods: Discarded acetaminophen – free serum samples (blank pool) and patient serum with acetaminophen overdose were used to prepare samples. Three levels of acetaminophen (5, 10, and 30 μg/ml) were evaluated for interference: hemolysis (H index range: 0–1000), icterus (I index range: 0–40), and lipemia (L index range: 0–1000).

Results: Measurements showed that the EMIT® assay was not significantly affected by hemolysis or icterus at all 3concentrations evaluated, but was negatively affected by lipemia at all three levels at 1000 mg/dl intralipids. The DRI® assay was similarly affected by hemolysis and icterus, but lipemia (at 1000 mg/dl intralipids) only affected the 5 μg/ml level. The Roche acetaminophen assay was significantly affected by hemolysis at all three concentrations. It was significantly affected by icterus at 20 mg/dl bilirubin and > 5 μg/ml and at icterus levels of 30 and 40 mg/dl bilirubin at 10 and 30 μg/ml acetaminophen concentrations, respectively. However, the Roche assay was least affected by lipemia.

Conclusion: Hemolysis and icterus had insignificant interference on the Syva EMIT® and the DRI® assays for the analysis of acetaminophen, but significant interference effect on the Roche assay. On the other hand lipemia interfered less markedly with the Roche assay. The effect of hemolysis, icterus and lipemia should always be considered. Cautions are warranted when interpreting results for the potential false positive results in the presence of hemolysis and icterus at the concentrations evaluated in this study.

1. Introduction

Acetaminophen is one of the most common non-prescription analgesics used for the management of pain and fever in the U.S., with an estimated 25 billion doses sold yearly [1]. The prevalence of acetaminophen in multiple medications along with a very narrow therapeutic range (10–20 μg/ml) can lead to intentional or unintentional overdosing. An average of approximately 80,000 emergency department visits (data from 2006 to 2010) have been associated with acetaminophen overdose in the U.S. annually with roughly 70% of the visits being associated with self-directed violence [2–4].

The hepatotoxicity of acetaminophen, arising from the metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI), has been well documented [5,6]. Acetaminophen poisoning has been recognized as one of the most common causes for acute liver failure in the U.S. and U.K. [7,8]. Widespread usage combined with its hepatotoxicity has placed acetaminophen high on the list of suspected agents for patients with acute liver failure. Serum concentrations of acetaminophen have been routinely used to diagnose drug overdose, to predict the hepatotoxicity and to identify acetaminophen consumption in patients with acute liver failure.

The interference of bilirubin on acetaminophen assays has raised significant concerns for accurate measurement of acetaminophen [9–14]. This can be a significant concern when patients undergo hepatotoxicity in the event of acetaminophen overdose, as bilirubin levels can increase due to hepatic necrosis. Furthermore, false positive results

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of acetaminophen in patients with acute hepatitis and hyperbilirubinemia may be mistaken for acetaminophen overdose, leading to unnecessary treatment and may delay the recognition of the underlying causes, impeding timely and proper treatment [15].

Accurate measurement of serum acetaminophen levels, therefore, plays a vital role in patient management with acetaminophen overdose and determination of acetaminophen use in patients with acute liver failure. We suspected that an assay affected by icterus might also be affected by hemolysis and possibly by lipemia due to the intrinsic optics or spectroscopy nature of colorimetric assays.

2. Materials and methods

2.1. Assay principles and analysis parameters

Three assays were evaluated in this study: the Roche acetaminophen assay, the Syva® EMIT® assay (Siemens Healthcare Diagnostics Ltd.) and the DRI® (Microgenics Corp.) assay. These assays are based on the monitoring of different light-producing reactions. For example, the Roche assay is an enzymatic method, whereby aryl-acylamidase is employed to hydrolyze acetaminophen to *p*-aminophenol and acetate. The *p*-aminophenol is then converted to an indophenol which absorbs strongly at 629 nm. Hence, the change in absorbance due to indophenol formation is directly proportional to acetaminophen concentration in serum [19].

The EMIT® and DRI® assays are based on the homogenous enzyme immunoassay technique, in which endogenous acetaminophen competes with the glucose-6-phosphate dehydrogenase (G6PDH)-labeled acetaminophen for the antibody binding sites. The binding of antibodies with the G6PDH-labeled acetaminophen resulted in the loss of enzyme activity. Competitive binding frees and activates the enzyme to reduce NAD to NADH, which produces a change in the absorbance at 340 nm [20,21]. The Roche assay was performed on a Cobas® Integra 800 system using the manufacturer's defined parameters. The Syva® EMIT® and the Microgenics DRI® assays were evaluated on a Roche COBAS® c501 analyzer as open-channel assays with user-defined analysis parameters (Supplementary Information Table 1).

2.2. Serum samples and stock solutions

A total of 19 discarded de-identified serum samples from patients with acetaminophen overdose (with concentrations up to 360 μg/ml) obtained from the Beth Israel Deaconess Medical Center were used as the source of acetaminophen to prepare samples for this study. Samples from apparently healthy individuals without detectable levels of acetaminophen were from the Children's Hospital Boston and used as blanks and diluents.

Fresh human red blood cells obtained from an apparently healthy individual were subjected to multiple freeze-thaw cycles to prepare concentrated hemolysate. A hemolysate stock solution (10 times concentrated) of 10,000 mg/dl hemoglobin was prepared from the concentrated hemolysate. A series of stock solutions (10 times concentrated) at levels of 8000, 6000, 4000, 2000, 1500, 1000, and 500 mg/dl were prepared by mixing the 10,000 mg/dl solution proportionally with saline.

Unconjugated bilirubin was from Sigma-Aldrich and used to prepare samples for icteric interference. Stock solutions (10 times concentrated) at the concentrations of 400, 300, 200, 100, 50, and 25 mg/dl bilirubin were prepared in saline. Intralipid® (20%) was from the pharmacy department at the Children Hospital Boston. Stock solutions (10 times concentrated) were prepared at Intralipid concentrations of 10,000, 8000, 4000, and 2000 mg/dl.

2.3. Serum sample preparation for interference

Three levels of acetaminophen at levels of 5, 10, and 30 μg/ml were prepared by mixing serum samples with high levels of acetaminophen with blank serum samples.

The therapeutic range for acetaminophen varies and has been reported to be 10–20 μg/ml. These concentrations were chosen to represent the concentrations below, across and above the therapeutic range.

In addition, we chose acetaminophen concentrations at 5, 10, and 30 μ g/ml due to several other considerations. First, in patients with acute acetaminophen overdose, the initial clinical presentation might be mild and non-specific and the manifestations of hepatotoxicity might not appear until 24–48 h after the acetaminophen overdose [17,25,26] when bilirubin in patients with acute acetaminophen overdose could have a dramatic increase [15]. Furthermore, acetaminophen has a short half-life and is rapidly metabolized and absorbed. As indicated by the modified Rumack-Matthew Treatment nomogram, a commonly used approach to guide the decisions on overdose treatment, serum concentration from patients with serum acetaminophen concentrations of 150 μg/ml at 4 h after injection quickly dropped to 18.8 μg/ml at 16 h [16,17]. The concentration selected in this study reflected this range when expected hepatotoxicity and therefore potential bilirubin interference. We chose these levels to provide insights into the overall interference for acetaminophen analysis.

Samples with interferences were prepared by mixing the 3 levels of serum samples with 10 times concentrated interference stock solution at 9:1 ratio. The 9:1 ratio was used to minimize the matrix effect so that ≤10% of the final sample volume was from the concentrated interference stock solutions. A corresponding amount of saline was used to generate the blank reference samples.

Specifically, 180 μl of serum sample at the designated acetaminophen concentrations (5, 10, or 30 μg/ml) was mixed with 20 μl of the corresponding concentrated (10 times concentrated) interferent solutions. All the indices were determined on the Roche Integra 800 analyzer to characterize the interference. The calculated and measured interference levels are indicated in Table 1. These samples were analyzed by the 3 assays at the same time at Children's Hospital Boston with each sample measured once.

One limitation for this study was the use of unconjugated bilirubin. To evaluate the impact of endogenous bilirubin, 20 random patient samples without obvious acetaminophen history were collected. Their acetaminophen concentration and I index were measured to show the impact.

Table 1
Summary of calculated and measured H I L index levels.

	Calculated concentration (mg/dl)	Measured index
Hemolysis (H)	0	12
	50	94
	100	150
	150	206
	200	262
	400	496
	600	734
	800	975
	1000	1271
Icterus (I)	0	0
	2.5	5
	5	8
	10	16
	20	31
	30	39
	40	45
Lipemia (L)	0	20
	200	259
	400	461
	800	893
	1000	1110

Note 1. The Indices were determined on COBAS INTEGRA 800 Instrument. 2. Calculated H, I, L concentrations were based on hemoglobin, unconjugated Bilirubin, and Intralipid concentrations, respectively.

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