Practical Laboratory Medicine 25 (2021) e00232

Contents lists available at ScienceDirect

Practical Laboratory Medicine

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

# Optimization of hemolysis, icterus and lipemia interference thresholds for 35 clinical chemistry assays

Clement K.M. Ho [a,b,*], Claire Chen [c], Johnson W.S. Setoh [a], Willie W.T. Yap [c], Robert C.W. Hawkins [a,d]

[a] Department of Pathology and Laboratory Medicine, KK Women's & Children's Hospital, Singapore
[b] Duke-NUS Medical School, Singapore
[c] Abbott Laboratories, Singapore
[d] Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

ARTICLE INFO

*Keywords:*
Assay interference
Hemolysis
Icterus
Lipemia

ABSTRACT

*Objectives:* Interference of chemistry assays by hemolysis, icterus and lipemia (HIL) was investigated on the Abbott Alinity c system. We sought to empirically establish optimized HIL index thresholds for the purposes of reporting HIL interference in a hospital laboratory and advising clinicians on the interpretation of laboratory results in the presence of hemolysis, icterus or lipemia.

*Methods:* HIL index values measured by spectrophotometry were compared with concentrations of hemoglobin, bilirubin and Intralipid. HIL interference of 35 Abbott Alinity chemistry assays was subsequently investigated by pairwise comparison of test results in pooled serum or plasma with those in test preparations spiked with hemolysate, bilirubin or Intralipid. Data generated from the interference experiments were critically assessed according to assay-specific acceptance criteria adapted from multiple sources, and optimized thresholds for HIL indices were established.

*Results:* Correlations between HIL index values and their corresponding concentrations of hemoglobin, bilirubin and Intralipid were, in general, very good within the ranges of interferent concentrations tested. Hemolysis significantly affected 12 of 35 assays, whereas bilirubin and Intralipid interfered with four and three assays, respectively. Both the direction and magnitude of Intralipid interference with the direct bilirubin assay were dependent on the concentrations of the analyte.

*Conclusions:* HIL interference of the Abbott Alinity clinical chemistry assays investigated in this study was not uncommon. At present, there are no universally accepted criteria for defining significant assay interference for clinical practice. In establishing acceptance criteria for defining assay interference, each assay should be assessed according to both analytical criteria and clinical relevance.

## 1. Introduction

Blood specimens with hemolysis, icterus or lipemia (HIL) are often encountered in routine clinical laboratory practice. In our

* Corresponding author. Department of Pathology and Laboratory Medicine, KK Women's & Children's Hospital, 100 Bukit Timah Road, 229899, Singapore.
*E-mail address:* clement.ho@doctors.org.uk (C.K.M. Ho).

https://doi.org/10.1016/j.plabm.2021.e00232
Received 15 February 2021; Accepted 21 April 2021

experience, mild hemolysis is commonly found in blood specimens drawn from the pediatric populations, especially very young infants. A report on data collected from three Dutch hospitals showed that hemolysis, icterus and lipemia (corresponding to >50 mg/dL hemoglobin, >103 μmol/L bilirubin and >40 mg/dL Intralipid, respectively) were detectable in 1.06%, 0.54% and 0.82% of 124,567 specimens, respectively, by using automated spectrophotometry [1]. In the past, the extent of hemolysis, icterus and lipemia in plasma or serum specimens was visually assessed according to redness, yellowness and turbidity, and the procedures involved in visual estimation of hemolysis, icterus and lipemia were time consuming, highly subjective and not standardized. Visual assessment and management of hemolysis level, based on non-standardized subjective opinion, is discouraged and should be replaced with automated detection and management systems [2]. To date, major manufacturers of contemporary chemistry analyzers have developed instruments capable of more objective quantitation of HIL by spectrophotometric measurements [3].

Analytical processes and measurements may be perturbed by many interferents including hemoglobin, bilirubin or lipids through one or multiple mechanisms [4]. An interferent may originate from blood cells or intravenous fluid; for example, the release of potassium from hemolyzed blood cells can increase plasma and serum concentrations of potassium. An interferent may alter a chemical reaction by competing for reagents or binding to the catalytic site of an enzyme. An interferent may also have properties similar to those of the analyte or the enzymatic product of interest, such as color, fluorescence spectrum or co-elution in chromatography separation. Nonaqueous substances such as lipoproteins may affect analytical processes or measurement of analytes by the effect of light scattering (spectrophotometric interference), volume displacement or differential partitioning of analytes or reagents between the hydrophilic and hydrophobic phases of the sample [5].

To date, there are no universally-agreed allowable error goals for defining the acceptance criteria for assay interference. In considering which acceptance criteria to adopt for interference testing, both clinical relevance and analytical criteria are important [2, 4]. In this study, we compared multiple acceptance criteria for defining assay interference based on five separate sources including those HIL interference acceptance criteria recommended by the manufacturer, manufacturer's claimed maximum analytical imprecision of the assays, the individual assays' reference change values (RCVs), which are defined as the critical differences that must be exceeded between sequential results for a significant (or true) change to occur [6], and analytical performance/evaluation criteria from two separate external quality assessment programs, i.e. the College of American Pathologists (CAP) and the Royal College of Pathologists of Australasia (RCPA).

The aim of this study was two-fold. First, we sought to empirically investigate the nature and extent of HIL interference of routine clinical chemistry assays on the recently launched Abbott Alinity c system by using pooled samples of serum or plasma spiked with potential interfering substances. Second, through critical application of various criteria for defining assay interference to the data generated from the above HIL interference experiments, we aimed to establish in-house thresholds optimized for the reporting of HIL interference and interpretation of laboratory results in the presence of hemolysis, icterus or lipemia in a hospital laboratory with considerable workloads from pediatric patients.

## 2. Materials and methods

### 2.1. Study design

The effects of hemolysis, icterus and lipemia on 35 chemistry assays on the Abbott Alinity c system (Abbott Diagnostics, Abbot Park, IL, USA) were evaluated according to the CLSI guidelines EP07-A2 [4] and C56-A [5] wherever possible. Prior to the evaluation of interference, preliminary experiments were carried out to establish correlations between H index and hemoglobin concentrations, I index and bilirubin concentrations, as well as L index and Intralipid concentrations so that concentrations of hemoglobin, bilirubin and Intralipid could be estimated in subsequent interference evaluation by using the H, I and L index values. Analytes were assessed at or near the concentrations recommended by the CLSI guideline EP07-A2 [4] as much as practicable. All experiments were conducted in a routine clinical chemistry laboratory located in a tertiary, 830-bed hospital for women and children, with approximately half of the clinical chemistry test workload originating from pediatric patients. As only de-identified pools of residual serum and plasma samples were used in this study as part of the technical evaluation of new chemistry assays, institutional review board approval was not required for this study.

**Table 1**
Semiquantitative scale for HIL indices and corresponding concentrations of hemoglobin (H index), bilirubin (I index) and triglyceride (L index) on the Alinity c system, according to the manufacturer's specifications.

| | H index | I index | L index |
|---|---|---|---|
| Semiquantitative scale | Hemoglobin (mg/dL) | Bilirubin (μmol/L) | Triglyceride (mg/dL) |
| 0 | $H < 30$ | $I < 34.2$ | $L < 50$ |
| 1+ | $30 \leq H < 100$ | $34.2 \leq I < 68.4$ | $50 \leq L < 100$ |
| 2+ | $100 \leq H < 200$ | $68.4 \leq I < 171$ | $100 \leq L < 150$ |
| 3+ | $200 \leq H < 500$ | $171 \leq I < 342$ | $150 \leq L < 200$ |
| 4+ | $H \geq 500$ | $I \geq 342$ | $L \geq 200$ |

### 2.2. Chemistry assays and measurement of HIL indices

The Abbott Alinity c system is a fully automated clinical chemistry analyzer system for routine chemistry assays using photometric and potentiometric detection technologies. It measures HIL indices automatically by spectrophotometry and its HIL protocol employs a saline reagent which does not interfere with the measurement of HIL indices. Absorbance readings are taken at 4 wavelength pairs of 500/524, 572/604, 628/660 and 524/804 nm to calculate HIL index values, which approximately correspond to concentrations of the interferents hemoglobin, bilirubin and triglycerides; the HIL index values are then converted to semiquantitative categories ranging from 0, 1+, 2+, 3+ to 4+ according to the manufacturer's HIL protocol (Table 1). The 35 analytes were measured by Abbott Alinity chemistry assays in residual plasma (lactate assay) or serum (all other assays) pools according to the manufacturer's instructions for use.

Hemolysate stock solution was prepared by lysis of washed erythrocytes in residual samples, using the freezing and thawing method [7] followed by dilution with de-ionized water to a final hemoglobin concentration of 12,000 mg/dL. Bilirubin stock solution was prepared by dissolving unconjugated bilirubin consisting of three different α-isomers III, IX and XII (Sigma-Aldrich, St Louis, MO, USA) in 0.1 mol/L NaOH to a total bilirubin concentration of 8,000 μmol/L. Intralipid emulsion 20% (Fresenius Kabi, Uppsala, Sweden), main

**Table 2**
Concentrations of analytes tested and acceptance criteria for assay interference adopted in this study. CAP, College of American Pathologists; CVa, analytical coefficient of variation; RCPA, Royal College of Pathologists of Australasia.

| Assay | Unit | Concentrations tested | | Acceptance criteria for assay interference | Source of criteria |
|---|---|---|---|---|---|
| | | Level 1 | Level 2 | | |
| Alanine aminotransferase | U/L | 21.5–30.1 | 141.9–176.4 | 10.0% | Manufacturer |
| Albumin, bromocresol purple | g/L | 37.3–42.9 | 44.9–46.0 | 2.0 g/L if ≤ 33.0 g/L or 6% if > 33.0 g/L | RCPA |
| Alkaline phosphatase | U/L | 67.3–73.4 | 193.7–248.0 | 10.0% | Manufacturer |
| Amylase | U/L | 42.3–71.4 | Not available | 10.0% | Manufacturer |
| Aspartate aminotransferase (AST) | U/L | 19.2–21.9 | 107.4–265.8 | 10.0% | Manufacturer |
| Bile acids, total | μmol/L | 3.3–4.4 | Not available | 0.6 μmol/L if < 6.0 μmol/L or 10% if ≥ 6.0 μmol/L | Manufacturer |
| Bilirubin, total | μmol/L | 7.3–7.7 | 41.4–48.8 | 10.0% | Manufacturer |
| Bilirubin, direct | μmol/L | 3.1–4.6 | 23.4–27.3 | 10% or 1.7 μmol/L, whichever is greater | Manufacturer |
| C reactive protein (CRP) | mg/L | 2.24–4.54 | 73.0–140.0 | 5% or 0.5 mg/L, whichever is greater | Manufacturer |
| Calcium | mmol/L | 2.25–2.35 | Not available | 0.10 mmol/L if ≤ 2.50 mmol/L; or 4% if > 2.50 mmol/L | RCPA |
| Carbon dioxide | mmol/L | 20.3–22.7 | 24.8–27.9 | 4.3% | Manufacturer |
| Chloride | mmol/L | 99.2–100.7 | 107.1–108.3 | 3 mmol/L if ≤ 100 mmol/L or 3% > 100 mmol/L | RCPA |
| Cholesterol, HDL | mmol/L | 0.8–1.0 | 2.3–2.6 | 5.0% | Manufacturer |
| Cholesterol, total | mmol/L | 3.6–4.6 | 5.3–5.8 | 0.3 mmol/L if ≤ 5.0 mmol/L or 6% if > 5.0 mmol/L | RCPA |
| Complement C3 | g/L | 1.26–1.43 | Not available | 9.8% | 2.5 x CVa |
| Complement C4 | g/L | 0.27–0.32 | Not available | 10.0% | Manufacturer |
| Creatine kinase | U/L | 90.5–130.5 | 300.4–318.8 | 10.0% | Manufacturer |
| Creatinine, enzymatic | μmol/L | 55.3–57.6 | 161.7–218.6 | 8 μmol/L if ≤ 100 μmol/L or 8% if > 100 μmol/L | RCPA |
| Gamma-glutamyl transferase (GGT) | U/L | 36.3–40.1 | Not available | 10.0% | Manufacturer |
| Glucose | mmol/L | 2.3–2.5 | 10.8–15.6 | 6.0% | Manufacturer |
| Immunoglobulin A | g/L | 1.1–1.7 | Not available | 10.0% | Manufacturer |
| Immunoglobulin G | g/L | 11.7–12.3 | Not available | 8.5% | 2.5 x CVa |
| Immunoglobulin M | g/L | 0.8–1.2 | Not available | 10.0% | Manufacturer |
| Iron | μmol/L | 9.9–12.4 | Not available | 10% or 3.1 μmol/L, whichever is greater | Manufacturer |
| Lactate dehydrogenase (LDH) | U/L | 152.6–174.4 | 220.0–415.6 | 20 U/L if ≤ 250 U/L or 8% if > 250 U/L | RCPA |
| Lactic acid | mmol/L | 1.00–1.04 | 1.55–2.30 | 10% or 0.1 mmol/L, whichever is greater | Manufacturer |
| Magnesium | mmol/L | 0.80–0.85 | Not available | 7.5% | Manufacturer |
| Phosphorus | mmol/L | 1.3–1.5 | 1.7 | 0.06 mmol/L if ≤ 0.75 mmol/L or 8% if > 0.75 mmol/L | RCPA |
| Potassium | mmol/L | 4.3–4.7 | 5.2–5.8 | 0.2 mmol/L if ≤ 4.0 mmol/L or 5% if > 4.0 mmol/L | RCPA |
| Protein, total | g/L | 67.5–72.2 | 76.3–84.5 | 3 g/L if ≤ 60 g/L or 5% if > 60 g/L | RCPA |
| Sodium | mmol/L | 129.9–141.0 | 141.2–144.9 | 2.0% | Manufacturer |
| Transferrin | g/L | 2.6–3.0 | 3.4–4.2 | 0.2 g/L if ≤ 2.5 g/L or 8% if > 2.5 g/L | RCPA |
| Triglycerides | mmol/L | 1.1–1.8 | 3.0–6.0 | 10.0% | Manufacturer |
| Urea | mmol/L | 3.0–4.2 | 11.1–14.7 | 0.7 mmol/L or 9%, whichever is greater | CAP |
| Uric acid | μmol/L | 270.2–361.2 | 424.8–491.6 | 7.0% | Manufacturer |

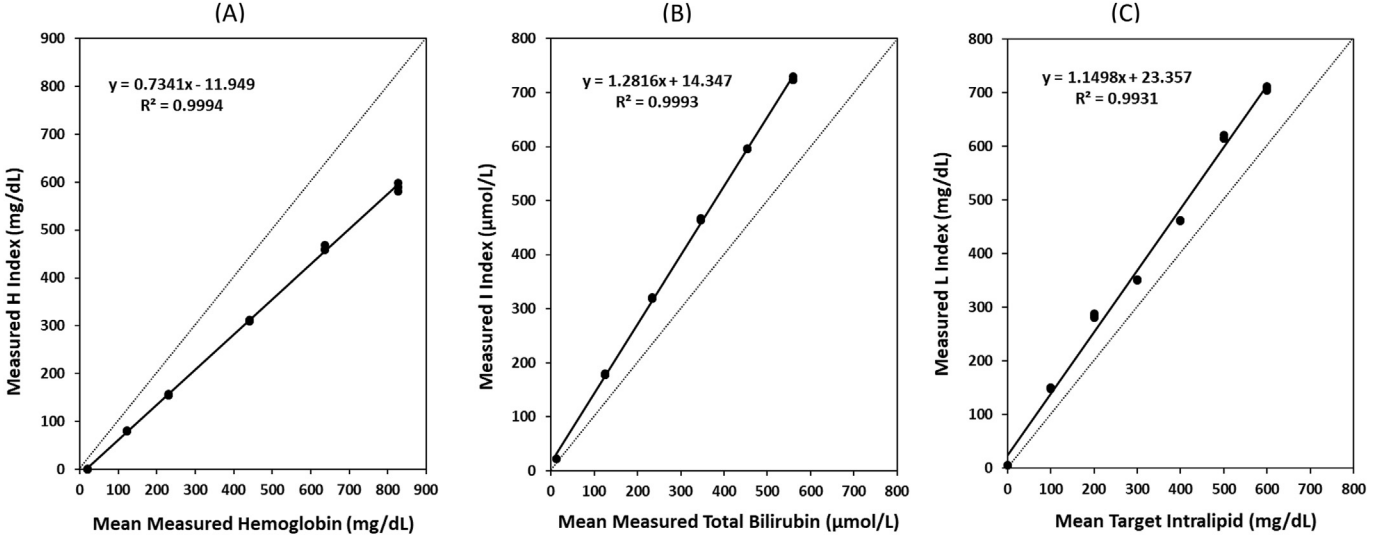


**Fig. 1.** Relationship between measured HIL index values on the Alinity c system and (A) measured concentrations of hemoglobin, (B) measured concentrations of total bilirubin and (C) calculated concentrations of Intralipid.

ingredients of which included soybean oil (20%, w/v) and glycerol (2.2%, w/v), was diluted with deionized water to give the Intralipid stock solution (10,000 mg/dL) for evaluating the potential effects of Intralipid. As Intralipid emulsion contained 2.2% glycerol, which could potentially interfere with some chemistry assays, various amounts of glycerol were added to those test samples for the evaluation of lipemia interference to keep the final glycerol concentrations in each preparation constant.

In the preliminary experiments, serum samples without visible hemolysis, lipemia and icterus were pooled together to generate a base serum pool. Aliquots of the base serum pool were spiked with increasing amounts of hemolysate, bilirubin or Intralipid stock solutions to prepare hemolysis serial samples up to a target hemoglobin concentration of 800 mg/dL, icterus serial samples up to a target total bilirubin concentration of 500 μmol/L, and lipemia serial samples up to a target Intralipid concentration of 600 mg/dL, respectively. Concentrations of hemoglobin and total bilirubin in both control and spiked samples were measured in triplicate on a Sysmex XN3000 analyzer (Sysmex, Kobe, Japan) and the Alinity c system, respectively. The HIL index values of the above serial samples were measured according to the manufacturer's HIL protocol. Statistical correlation between the HIL indices and hemoglobin, bilirubin and target Intralipid concentrations were then determined by linear least squares regression analysis.

In the subsequent evaluation of HIL interference, 35 assays were assessed at two different levels of the analytes wherever possible (Table 2). Visually non-hemolytic, non-icteric and non-lipemic control pools of plasma (for lactic acid assay) and serum (for the other assays) containing the desired concentrations of the analytes were spiked with increasing amounts of hemolysate, bilirubin or Intralipid stock solutions, respectively, to produce test preparations with H, I, or L index values ranging from 1+ to 4+. To minimize any potential matrix effect, the volumes of the stock solutions added to the control plasma or serum pools were limited to no more than 5% of the latter. Various volumes of 1.1% glycerol in water were added to the base pool in the Intralipid interference experiments in order to account for the glycerol present in Intralipid. Concentrations of hemoglobin, bilirubin and Intralipid in the test preparations were estimated by using the data obtained from the preliminary experiments described above. Concentrations of analytes in the test preparations and their corresponding controls were measured in quintuplicate according to the manufacturer's instructions. Mean apparent concentrations of the analytes in test preparations containing potential interferents (hemolysate, bilirubin or Intralipid) were then compared with mean concentrations of the analytes in control samples to calculate the biases due to assay interference.

### 2.3. Criteria for determining significant assay interference

The acceptance criteria used for defining significant assay interference were based on the lowest values of the following five criteria (Supplemental Table 1). First, the manufacturer's recommended acceptance criteria for assay interference were extracted from assay kit instructions. Second, values equivalent to 2.5 times the analytical coefficients of variation ($CV_a$) were calculated for the 35 assays, based on the manufacturer's claims for maximum assay imprecision. Five test replicates were required to detect a difference of 2.5 standard deviations from the mean in control samples for a two-sided statistical test with 5% probability of type I error and 90% power [4]. Third, reference change value (RCV), usually interpreted as the minimum (critical) difference that must be exceeded for a change in consecutive results in the same individual to become clinically significant [6], was calculated for each assay by using the following equation: $RCV = 2.77 \times (CV_a^2 + CV_i^2)^{1/2}$, where $CV_a$ is the analytical coefficient of variation (imprecision) and $CV_i$ is within-subject biological variation, based on a two-tailed test at 95% probability [2,6]. $CV_i$ values for the analytes were taken from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation database [8] or from the Desirable Biological Variation database (2014) [9] when EFLM biological variation data were not available. Lastly, analytical performance/evaluation criteria from two separate external quality assessment programs (the College of American Pathologists and the Royal College of Pathologists of Australasia) were also assessed.

Table 2 summarizes the acceptance criteria for defining significant assay interference adopted and the concentrations of analytes tested in this study. All statistical analyses were performed using the Analyse-it software version 3 (method validation edition, Analyse-it Software, Leeds, UK).

## 3. Results

### 3.1. Relationship between HIL indices and concentrations of hemoglobin, bilirubin and Intralipid

Data from preliminary experiments showed that correlations between HIL index values and their corresponding concentrations of hemoglobin, bilirubin and Intralipid were very good within the concentrations ranges of the three interferents tested (correlation coefficients $\geq$0.99; Fig. 1). Linear least squares regression analyses demonstrated essentially linear relationships between the HIL index values and their corresponding hemoglobin, bilirubin and Intralipid concentrations, allowing HIL index readings to be used for the estimation of hemoglobin, bilirubin and Intralipid concentrations in plasma or serum samples in subsequent assay interference experiments.

### 3.2. HIL interference of chemistry assays

Acceptance criteria for significant assay interference adopted in this study are summarized in Table 2.

In the hemolysis interference evaluation, it was observed that 12 assays were significantly affected. The addition of hemolysate resulted in consistent and dose-dependent trends towards overestimation of AST, chloride, iron, LDH, lactic acid, phosphorus, potassium, total cholesterol, total protein and triglycerides, whereas apparent concentrations of creatinine (enzymatic method) and direct bilirubin were substantially decreased when compared with controls without hemolysate. Dose-response curves generated from the

hemolysis interference evaluation are shown in Fig. 2A & Fig. 2B. Among the assays affected by hemolysate, significant assay interference was not always observed at both high and low concentrations of the analytes tested; for example, significant interference by hemolysate was detected only at high concentrations of total cholesterol (mean of controls, 5.71 mmol/L) and only at low concentrations of triglycerides (mean of controls, 1.75 mmol/L).

Bilirubin interfered with four assays including total cholesterol, lactic acid, total protein and triglycerides (Fig. 2C). Dose-dependent, negative interference of the total cholesterol and lactic acid assays was observed at both high and low concentrations of total cholesterol and lactic acid tested. In comparison, the total protein and triglycerides assays were significantly affected only at low concentrations of total protein (mean of controls, 72.2 g/L) and triglycerides (mean of controls, 1.75 mmol/L).

Three assays including direct bilirubin, iron and total protein were significantly interfered by the addition of Intralipid (Fig. 2D). In samples spiked with Intralipid, apparent concentrations of iron and total protein were falsely increased when L index values reached 4+. Differential interference of the direct bilirubin assay by Intralipid in opposite directions was observed at different concentrations of

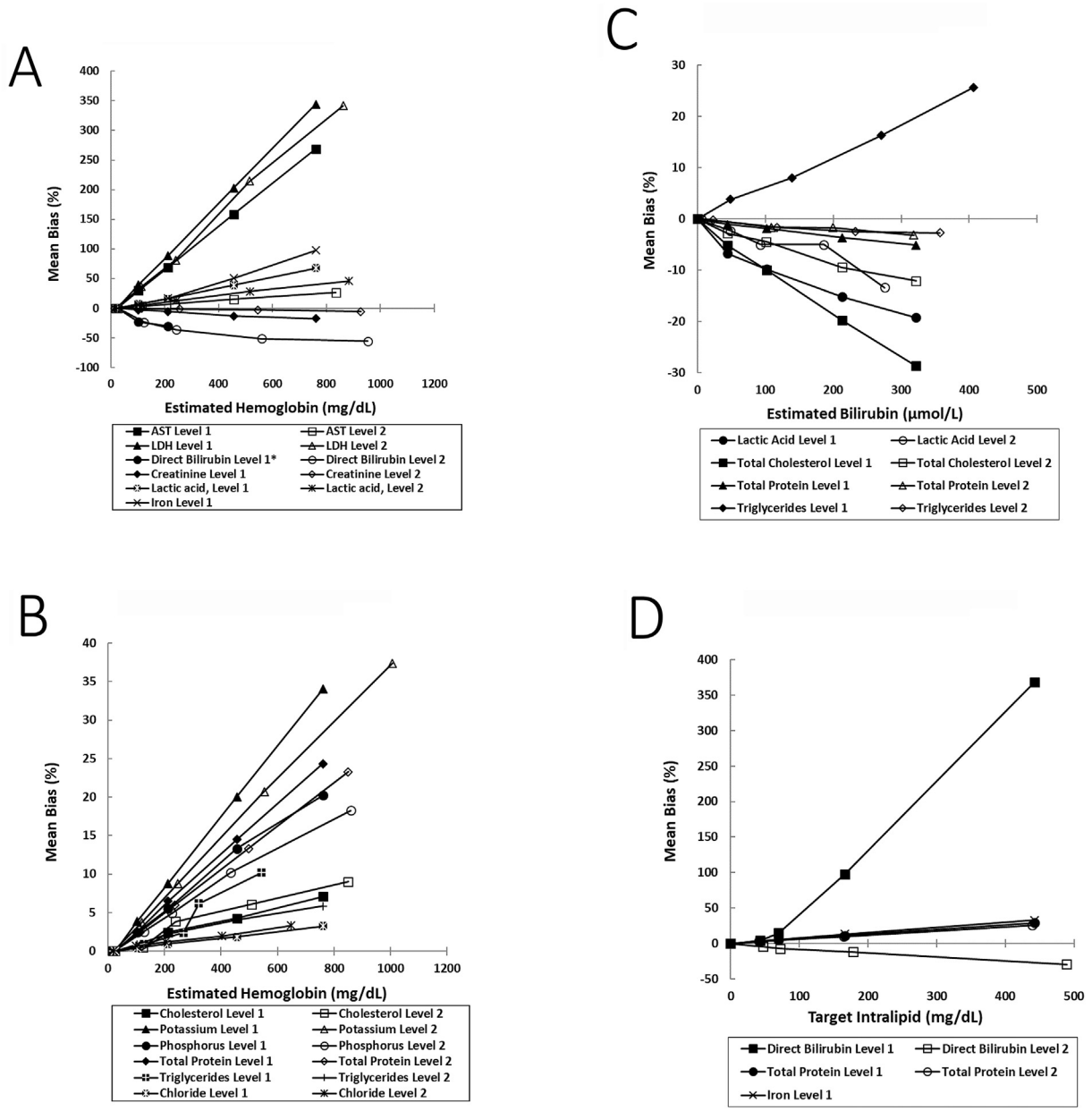


**Fig. 2.** Effects of spiking with (A & B) hemolysate, (C) bilirubin and (D) Intralipid on apparent concentrations of analytes. *Apparent direct bilirubin results were below the lower limit of the assay's measurement range and thus not reportable when estimated hemoglobin concentration was more than 212 mg/dL (Fig. 2A).

direct bilirubin tested (Fig. 2D). In a serum pool with a relatively low direct bilirubin concentration (mean ± SD, 4.6 ± 0.1 μmol/L), Intralipid spiking to approximately 442 mg/dL (L index 4+) elevated the apparent direct bilirubin concentrations (mean ± SD, 21.4 ± 0.5 μmol/L) by more than threefold (black squares, Fig. 2D). By contrast, similar Intralipid spiking (approximately 489 mg/dL; L index 4+) of a serum pool containing a relatively high concentration of direct bilirubin (mean ± SD, 27.3 ± 0.2 μmol/L) decreased the mean apparent direct bilirubin concentration by 30% to 19.3 (SD, ± 0.3) μmol/L (white squares, Fig. 2D).

HIL interference data generated from the above experiments were assessed according to the acceptance criteria listed in Table 2, and were used to establish optimized thresholds for HIL indices in our laboratory. Specimens with HIL index values above those optimized in-house thresholds are flagged for special attention by laboratory technologists, and comments are appended to those results which may have been affected by HIL interference (Table 3).

## 4. Discussion

The above results demonstrated that interference of the Alinity c system chemistry assays by hemolysis, icterus or lipemia was not uncommon. Hemolysis, in particular, was shown to interfere with 12 of the 35 investigated assays by using the criteria adopted in this study. It is well recognized that the release of intracellular components including hemoglobin from erythrocytes associated with hemolysis can lead to increased plasma and serum concentrations of a number of analytes which are present in high concentrations within cells, such as AST, LDH and potassium [3]. Moreover, hemoglobin can interfere with assay reactions, for example in the degradation of bilirubin through a probable oxidative reaction involving hydrogen peroxide (formed in the oxidation of hemoglobin) and acid hematin acting as a pseudoperoxidase [10]. In this study, hemolysate interference with the direct bilirubin assay (−30.2% to −36.2% at H index of 2+) was more marked than that with the total bilirubin assay (−1.7% to −3.1% at H index of 2+). A speculative explanation for the latter difference is that an additional reagent component included in the total bilirubin assay, but not in the direct bilirubin assay, can counteract the pseudoperoxidase activity of hemoglobin.

It is remarkable that both the direction and magnitude of Intralipid interference with the direct bilirubin assay were dependent on the concentrations of the analyte tested (Fig. 2D). The reason for the differential, dose-dependent Intralipid interference of the direct bilirubin assay in opposite directions observed at high and low concentrations of the analyte is unclear. It is possible that Intralipid-induced lipemia can have multiple effects such as light-scattering (turbidity), volume displacement, adsorption of lipophilic reagents or reaction products by lipids [3] and oxidation caused by peroxides in Intralipid [11]. At a certain concentration of an analyte, one effect exerted by Intralipid may be more dominant than others, leading to opposite directions of interference at different concentrations of the analyte.

Lipemia induced by Intralipid spiking had little effect on the measurement of serum electrolytes, including sodium, potassium and chloride, by indirect ion selective electrode methods in this study; mean apparent concentrations of sodium, potassium and chloride in all test serum pools were less than 1% different from those in the corresponding control serum pools (data not shown). However, the maximum target Intralipid concentrations in test preparations in this study were in general less than 500 mg/dL and the volume of Intralipid stock added to serum pools was limited to 5% of the final total volume. In comparison, some hyperlipidemic patients can have serum concentrations of triglycerides in excess of 1000 mg/dL. It is conceivable that higher concentrations of triglycerides or lipoproteins not investigated in this study may interfere with the above chemistry assays.

At present, there are no universally-accepted criteria for defining significant assay interference. In establishing acceptance criteria for interference testing, both clinical significance and analytical criteria should be considered [4]. Multiple quality goals have been

**Table 3**
Optimized thresholds for reporting HIL indices and comments for results in specimens with indices above the optimized thresholds.

| Analyte | Optimized thresholds | | | Comments for results in specimens with indices above the optimized thresholds |
|---|---|---|---|---|
| | H index | I index | L index | |
| Aspartate aminotransferase (AST) | 0 | 4+ | 4+ | Hemolysis index 1+ to 4+: may lead to falsely increased result |
| Bilirubin, direct | 0 | 4+ | 4+ | Hemolysis index 1+ to 4+: may lead to falsely decreased result |
| Chloride | 3+ | 4+ | 4+ | Hemolysis index 4+: may lead to falsely increased result |
| Cholesterol, total | 2+ | 1+ | 4+ | Hemolysis index 3+ to 4+: may lead to falsely increased result<br>Icterus index 2+ to 4+: may lead to falsely decreased result |
| Creatinine, enzymatic | 3+ | 4+ | 4+ | Hemolysis index 4+: may lead to falsely decreased result |
| Iron | 2+ | 4+ | 3+ | Hemolysis index 3+ to 4+: may lead to falsely increased result<br>Lipemia index 4+: may lead to falsely increased result |
| Lactate dehydrogenase (LDH) | 0 | 4+ | 4+ | Hemolysis index 1+ to 4+: may lead to falsely increased result |
| Lactic acid | 1+ | 2+ | 4+ | Hemolysis index 2+ to 4+: may lead to falsely increased result<br>Icterus index 3+ to 4+: may lead to falsely decreased result |
| Phosphorus | 2+ | 4+ | 4+ | Hemolysis index 3+ to 4+: may lead to falsely increased result |
| Potassium | 1+ | 4+ | 4+ | Hemolysis index 2+ to 4+: may lead to falsely increased result |
| Protein, total | 1+ | 3+ | 2+ | Hemolysis index 2+ to 4+: may lead to falsely increased result<br>Icterus index 4+: may lead to falsely decreased result<br>Lipemia index 3+ to 4+: may lead to falsely increased result |
| Triglycerides | 3+ | 3+ | 4+ | Hemolysis index 4+: may lead to falsely increased result<br>Icterus index 4+: may lead to falsely increased result |
| Other analytes | 4+ | 4+ | 4+ | Nil |

proposed including those based on outcome studies, biological variability of the measurand of interest, and state-of-the-art technology [4]. Manufacturers of chemistry assays often use ±10% as the default criterion of acceptability for HIL interference in their assay performance claims [3]. Although a deviation of less than 10% from the initial (control) value is commonly accepted as a tolerable analytical threshold, for some analytes such as sodium and chloride this criterion is considered too high for clinical purposes [1]. The second criterion we used in this study for determining the presence of assay interference was based only on the manufacturer's claimed analytical performance (imprecision) of the assays. In order to detect an interference effect yielding apparent results 2.5 x $CV_a$ deviant from control samples, a minimum of five replicates of both control and test samples were analyzed in order to achieve no more than 5% probability of type I error and 10% probability of type II error [4]. Among the five criteria for defining assay interference considered in this study, the 2.5 x $CV_a$ criterion was the smallest for and thus applied to two assays including complement C3 and immunoglobulin G (Table 2). The third criterion for defining assay interference considered in this study was the reference change value (RCV), a statistical concept that takes into account both within-subject biological variation and analytical imprecision [12]. Although measurement of analytes in samples before and after spiking with hemolysate, bilirubin or Intralipid did not involve any biological variation, clinicians and laboratorians often use RCV as a general guide for establishing clinical significance when two laboratory results are apparently different [2,6]. In none of the 35 assays investigated was RCV the smallest value among the five criteria considered for defining assay interference (Supplemental Table 1).

Despite the fact that HIL testing is readily available on chemistry analyzers from major manufacturers, there have been few published studies directly comparing HIL index results from different platforms [3]. Moreover, there are no standardized systems for reporting HIL indices among the major manufacturers of automated chemistry analyzers. HIL index values on the Alinity c system are measured by spectrophotometry and converted to a semiquantitative scale ranging from 0, 1+, 2+, 3+ to 4+. One of the advantages of a semi-quantitative scale for reporting HIL indices is its relative simplicity. In clinical practice, requesting doctors do not usually need to know the exact degree of hemolysis, icterus or sample turbidity induced by lipemia; instead, they sometimes rely on the laboratory to alert them when there is clinically significant hemolysis, icterus or lipemia that may affect their patients' laboratory results. In our hospital, a medical laboratory technologist is available 24/7, throughout the year, to advise requesting clinicians on the interpretation of the effects of HIL on test results. In-house data on HIL interference, including the direction and extent of interference, are readily accessible to our medical laboratory technologists in the clinical chemistry laboratory. At the time of writing, the maximally reportable L index on the Alinity c system is 4+, which approximately corresponds to concentrations of triglycerides >200 mg/dL. A disadvantage of the existing semiquantitative scale for L index is that there is no reporting mechanism to differentiate lipemic samples with severe hypertriglyceridemia in the range of 500–1000 mg/dL from those with moderate hypertriglyceridemia (200–500 mg/dL).

To date, there is no widely-accepted consensus on how to use the HIL index values to report and interpret laboratory results potentially affected by HIL interference. In a recent survey conducted by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) on the handling of preanalytical samples, about 70% of the participating laboratories rejected only some affected tests along with an appropriate comment, whereas about 20% of the participants released all test results with general information on hemolysis, icterus and lipemia [13]. It is remarkable that 14% of the participating laboratories declared to monitor neither preanalytical errors in general nor hemolysis, icterus and lipemia in particular [13]. In the same survey, for the definition of hemolytic samples, 54% of 1160 laboratories used parameter-specific cut-offs provided by the manufacturer, whereas only 14% of the responders used parameter-specific cut-offs derived in-house [13]. In our laboratory, we have configured our laboratory information system in such a way that specimens with HIL index values above their corresponding optimized thresholds (Table 3) are flagged for the attention of laboratory technologists and requesting clinicians. The corresponding comments, as detailed in Table 3, are also appended to those results for clinicians to decide if they would request a repeat test or interpret the results with the knowledge of potential assay interference by hemolysis, icterus or lipemia. Blood collection from younger pediatric patients can often be challenging and repeat collection of blood is usually avoided unless absolutely necessary. Our locally agreed policy dictates that test results are not suppressed even in the presence of severe hemolysis, icterus or lipemia.

This study has several limitations. The bilirubin mixture used in the preparation of stock solution consisted of unconjugated bilirubin isomers only. In clinical practice, patients' specimens can consist of mixtures of conjugated and unconjugated bilirubin in varying proportions. Conjugated and unconjugated bilirubin have been shown to interfere with triglycerides assays in opposite directions though this differential interference was uncommon [14]. In the present study, lipemia was investigated by spiking with Intralipid, which is a fat emulsion for intravenous nutrition and typically contains soybean oil, glycerol and other ingredients. Samples with added Intralipid do not adequately mimic lipemia found in patients with hyperlipidemia (except those patients on parenteral nutrition); the particles in Intralipid range in size from 200 to 600 nm, and misses the range of large particles of very low-density lipoprotein (VLDL) as well as the lower and upper ranges of chylomicron particles [5]. In a study which directly compared interference from native lipemic serum with interference induced by supplementation with Intralipid, the observed correlation between triglyceride concentrations and turbidity was relatively poor ($r < 0.90$) in both the Intralipid-supplemented samples and native patient serum samples with lipemia [15]. The same study reported that in some assays, lipemic interference in patient samples was not observed in Intralipid-supplemented samples with comparable or higher L index and triglycerides values [15]. Taken together, the use of samples supplemented with Intralipid does not provide an experimental method universally applicable to estimating assay interference present in lipemic patient samples. Another less commonly used method for investigating lipemia interference involves the measurement of the analytes of interest before and after ultracentrifugation or high-speed centrifugation of lipemic serum or plasma samples. However, the latter method of lipemia interference testing depends on the availability of serum or plasma samples containing various concentrations of lipoproteins, and thus dose-response effects of increasing lipemia on assays cannot usually be systematically investigated. Lastly, the effects of combinations of two or more HIL interferents on the above Alinity chemistry assays have not been explored in this study.

In conclusion, HIL interference of the 35 Abbott Alinity clinical chemistry assays investigated in this study was not uncommon. The

automated HIL protocol on the Alinity c system provides a readily-available method to assess the presence of hemolysis, icterus and lipemia that might potentially affect the results generated using the 35 clinical chemistry assays. Clinical laboratories should establish acceptance criteria for the thresholds of HIL interference according to both analytical and clinical relevance.

## Research funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Author contributions

All authors have contributed to the intellectual content and accepted responsibility for the entire content of this manuscript.

## Disclosures

C. Chen and W.W.T. Yap are employees of Abbott Laboratories. The other authors have no conflict of interest to declare.

## Declaration of competing interest

C. Chen and W.W.T. Yap are employees of Abbott Laboratories. The other authors have no conflict of interest to declare.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2021.e00232.

## References

[1] H.J. Vermeer, E. Thomassen, N. de Jonge, Automated processing of serum indices used for interference detection by the laboratory information system, Clin. Chem. 51 (1) (2005) 244–247.
[2] G. Lippi, J. Cadamuro, A. von Meyer, A.M. Simundic, European Federation of Clinical C, Laboratory Medicine Working Group for Preanalytical P, Practical recommendations for managing hemolyzed samples in clinical chemistry testing, Clin. Chem. Lab. Med. 56 (5) (2018) 718–727.
[3] C.J. Farrell, A.C. Carter, Serum indices: managing assay interference, Ann. Clin. Biochem. 53 (Pt 5) (2016) 527–538.
[4] CLSI, in: Interference Testing in Clinical Chemistry. CLSI Guideline EP07, third ed., Clinical and Laboratory Standards Institute, Wayne, PA, 2018.
[5] CLSI, Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis. CLSI Document C56-A, Clinical and Laboratory Standards Institute, Wayne, PA, 2012.
[6] F. Omar, G.F. van der Watt, T.S. Pillay, Reference change values: how useful are they? J. Clin. Pathol. 61 (4) (2008) 426–427.
[7] Meites S. Letter, Reproducibly simulating hemolysis, for evaluating its interference with chemical methods, Clin. Chem. 19 (11) (1973) 1319.
[8] EFLM, Biological variation: information site for laboratory medicine [accessed on 1 October 2019]: European Federation of Clinical Chemistry and Laboratory Medicine; [Available from: http://biologicalvariation.com/news.html.
[9] C. Ricos, V. Alvarez, F. Cava, J.V. Garcia-Lario, A. Hernandez, C.V. Jimenez, et al., Current databases on biological variation: pros, cons and progress, Scand. J. Clin. Lab. Invest. 59 (7) (1999) 491–500.
[10] B.C. Shull, H. Lees, P.K. Li, Mechanism of interference by hemoglobin in the determination of total bilirubin. I. Method of Malloy-Evelyn, Clin. Chem. 26 (1) (1980) 22–25.
[11] K.M. Silvers, B.A. Darlow, C.C. Winterbourn, Lipid peroxide and hydrogen peroxide formation in parenteral nutrition solutions containing multivitamins, J. Parenter. Enteral Nutr. 25 (1) (2001) 14–17.
[12] E.K. Harris, T. Yasaka, On the calculation of a "reference change" for comparing two consecutive measurements, Clin. Chem. 29 (1) (1983) 25–30.
[13] J. Cadamuro, G. Lippi, A. von Meyer, M. Ibarz, E. van Dongen, Lases, et al., European survey on preanalytical sample handling - Part 2: practices of European laboratories on monitoring and processing haemolytic, icteric and lipemic samples. On behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE), Biochem. Med. 29 (2) (2019), 020705.
[14] M. Uldall, M. Loikkanen, E. Olafsdottir, U. Ornemark, G. Nordin, H. Steensland, Nordic Interference Study (March 2002): Effects of Hemoglobin and Bilirubin on Some Common Serum Analysis, 2003.
[15] J.A. Bornhorst, R.F. Roberts, W.L. Roberts, Assay-specific differences in lipemic interference in native and intralipid-supplemented samples, Clin. Chem. 50 (11) (2004) 2197–2201.