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Heterogeneity of manufacturers' declarations for lipemia interference — An urgent call for standardization

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ABSTRACT

Introduction: Due to the budget limitations, laboratories mostly rely on the manufacturers' information about the influence of interfering substances on laboratory results. However, some manufacturers do not follow the recommended procedures for testing interferences (CLSI standard) and there is a great variability in the presentation of data regarding lipemia interference.
Materials and methods: We aimed to verify the manufacturers' specifications for lipemia interference for clinical chemistry reagents provided by Beckman Coulter, Roche and Siemens. Bias was determined using the Intralipid® simulated lipemic samples. Furthermore, we aimed to compare obtained data with the manufacturers' claims and desirable specification for imprecision derived from biological variation.
Results: i) Manufacturers' declarations were not confirmed for all three manufacturers; ii) the magnitude and direction of the effect of lipemia on laboratory results differ substantially between the three tested analytical systems; and iii) manufacturers are using arbitrary limits in declaring the expected effect of interference on laboratory results.
Conclusions: There is an urgent need to standardize the way manufacturers test and report their data on the lipemia interference. We propose that, instead of arbitrary limits, manufacturers use evidence based quality specifications for assessing the allowable biases. Moreover, laboratories should be aware of the possible lack of replicability of manufacturers' declarations.

1. Introduction

Analytical interferences like hemolysis, lipemia and icteria can be considerable sources of laboratory errors [1–3]. Interfering substances introduce variability into laboratory measurement. If the variability is significant, it can be mistaken for the clinically relevant change in the laboratory result and leads to a medical decision deleterious for the patient. It is, therefore, essential for the laboratory experts to recognize directions and magnitudes of interfering substances' effects for all measured analytes [4,5].

In the everyday practice, laboratories mostly rely on information provided by the manufacturers in the product documentation, because in most cases limited laboratory budget doesn't allow performing extensive interference studies. Also, for lipemia in particular, unlike for hemolysis or icteria, there is an additional problem in simulation of unsuitable samples. A standardized material is not available. A lipemic sample from a patient often can't be obtained in sufficient volumes, and accumulation of such samples for spiking study is impossible since the material loses its properties if frozen [6]. Currently the most accepted approach includes spiking of native serum samples with fat emulsions used for parenteral diet, e.g. Intralipid® [7]. Due to differences in the particle size and composition, Intralipid® simulated lipemia differs from (patho)physiological lipemia [8], however it is currently the best way to test lipemia interference.

According to the CLSI (Clinical and Laboratory Standards Institute) C56-A document, manufacturers of in vitro diagnostic analytical systems are obliged to declare the results of their interference studies by reporting the concentration of analyte, concentration of Intralipid® (or other products used) and percentage of bias observed. When there is no interference, the highest concentration of interference tested should be reported. When there is interference, manufacturers should report the lowest concentration that causes significant bias [9]. However, certain manufacturers do not follow the recommended procedures and there is a great variability in the presentation of data regarding lipemia

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; DSI, desirable specification for imprecision.

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interference. Also, manufacturer's data may differ when applied in a laboratory setting due to sample manipulation, combining interferences or deterioration of analytical equipment.

Hence, the aim of our study was to verify manufacturer's specifications for lipemia interference for clinical chemistry reagents provided by three different manufacturers (Beckman Coulter, Roche and Siemens) by determining bias in Intralipid® simulated lipemic samples and compare obtained data with the manufacturers' claims and desirable specification for imprecision (DSI) derived from biological variation.

2. Materials and methods

2.1. Study design

This multicenter study was performed in the period from November 2012 to March 2013. The research protocol was developed in one laboratory in Croatia (Lab 1: University Department of Chemistry, Medical School University Hospital Sestre Milosrdnice in Zagreb) and then replicated in two laboratories in Verona, Italy (Lab 2: Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona and Lab 3: Laboratory of Clinical Biochemistry and Hematology, Borgo Trento Hospital). All the participating laboratories are accredited: Lab 1 according to ISO 15189 standard; Lab 2 and Lab 3 according to Clinical Pathology Accreditation — CPA (UK) based on ISO 15189 standard and certificated according to ISO 9001:2008.

2.2. Samples

Samples used for this protocol were obtained from outpatients referred to the laboratory for blood testing. After laboratory testing was done, remaining portions of serum samples were used. No additional blood sampling was done for this research.

Blood sampling was done in the morning after an overnight fast using a 21 G × 1 1/2″ needle (Greiner Bio-One). Blood was collected into tubes with a serum clot activator without gel separator (ref. 454204; 4 mL, 13 × 75 mm; Vacuette, Grainer Bio-One, Kremsmünster, Austria). After the sampling, the tubes were mixed according to the manufacturer's recommendations and left in an upright position for 40 min allowing the coagulation process to complete. Samples were then centrifuged for 10 min on 2800 ×*g* [10]. Sera without any visible interferences of hemolysis, icteria or lipemia were used for preparing sample pools. Twenty serum pools (total volume of 20 mL each) were made in order to cover a wide concentration range for tested analytes. Sera were collected, mixed, immediately frozen at −20 °C for 48 h, thawed and afterwards filtered once using a filter paper. These samples were labeled as native samples (Intralipid® = 0).

In order to simulate lipemia, increasing amounts of Intralipid® solution (Fresenius Kabi AB, Uppsala, Sweden) were added to native samples. The final volume of all samples was 1 mL. According to the CLSI guideline C56-A (Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis) [9]; the maximum tested concentration of Intralipid® solution was 1000 mg/dL. Other dilutions were done to cover the most frequent concentration levels declared in the manufacturer's recommendations (100, 300, 500, 700, 800, 900 and 1000 mg/dL Intralipid®).

2.3. Methods

A total of 24 clinical chemistry analytes were measured on analyzers from three different manufacturers. In Lab 1 measurements were preformed on a Beckman Coulter AU 680 analyzer (Beckman Coulter, Tokyo, Japan). In Lab 2 one parameter was not determined (CK-MB) and all other measurements were done on a Cobas® 6000 <c501> analyzer (Roche Diagnostics GmbH, Penzberg, Germany). In Lab 3 measurements were done on a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics, Munich, Germany). Proprietary reagents and applications were used as recommended by the manufacturer for all Beckman Coulter and Roche reagents, while on the Siemens platform iron was determined using a reagent from another manufacturer (Sentinel Diagnostics, Milan, Italy). Only proprietary calibrators and controls were used. Each pool was analyzed separately. Single measurements were done in native and Intralipid® spiked samples. The list of tested parameters, methods, and manufacturer's claims regarding lipemia interference is presented in Table 1.

2.4. Data analysis

The mean concentration of the 20 measurements was calculated for native samples and all respective Intralipid® concentration levels. For each concentration level, bias against native sample was calculated as percentage difference according to the formula:

$$\text{Bias(x)}(\%) = (\text{conc[x]}-\text{conc[native]})/\text{conc[native]} \times 100\%;$$

where x corresponds to mg/dL of Intralipid® (100, 300, 500, 700, 800, 900 and 1000).

The results of the measurements on all three platforms for each analyte are presented separately. Concentrations of Intralipid® solution are plotted on x-axis and respective bias values on y-axis of interferograms.

The bias measured at the declared acceptable Intralipid® concentration level was compared to the declared bias. If measured bias was lower than the declared bias, specification was confirmed. Otherwise, the manufacturer's specification was not met.

DSI derived from biological variation (0.5 of within-subject biological variation) according to Dr. Carmen Ricos and colleagues [11] was used as our criteria of acceptance in lipemia interference testing. The highest Intralipid® concentration where measured bias was still lower than the DSI criteria was established as the acceptable Intralipid® concentration.

Data are collected and analyzed in MS Excel 2007 (Microsoft, Redmond, Washington).

3. Results

Results of measurement and calculated bias values for all Intralipid® concentration levels are presented in Table 2. Lipemia interferograms for the 24 clinical chemistry analytes are presented in the Supplementary data file. The results show some significant differences in magnitude and direction of lipemia interference. For direct bilirubin, Roche reagent shows strong positive bias in lipemic samples, while Beckman Coulter and Siemens reagents display negative bias. Also, for bilirubin reagent, Siemens displays strong positive, Roche negative and Beckman Coulter negligible bias. Interestingly, GGT and glucose were almost not affected by adding Intralipid® solution for Roche and Beckman Coulter reagents, while there was a strong bias, positive for glucose and negative for GGT in Siemens reagent. For magnesium and lipase, Beckman Coulter reagents display a significantly greater positive bias than the two other manufacturers.

Results of comparison of the values declared by the manufacturers and measured data are presented in Table 3. Out of the 24 tested clinical chemistry parameters for Beckman Coulter reagents, the manufacturer's declaration regarding lipemia was confirmed only for less than half of the tested parameters (11/24). For 4 parameters (creatinine, glucose, phosphates and albumin) declared bias was met at a higher Intralipid® concentration, overestimating the interfering effect of lipemia. For those parameters, a higher Intralipid® concentration than declared would still be acceptable. On the other hand, for 9 parameters (ALT, AST, CK, CK-MB, LD, AMY and ALP) influence of lipemia was seriously underestimated, meaning that the measured bias at declared Intralipid® concentration was higher than that reported by the manufacturer.