Effects of serum indices interference on hormonal results from the Abbott Architect i2000 immunoassay analyser

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Accepted:25 September 2015

ABSTRACT

The routine chemical assays are affected by sample haemolysis, icterus and lipaemia, collectively known as serum indices; however, little attention has been given to the consequences of these conditions on hormonal assays (immunoassays). In this study, we assess the impact of interferences from exogenous serum indices on various endocrine assays performed on the Abbott Architect i2000 system. The pool of 20 serum samples was derived from a hospitalised population. The diluted serum samples were spiked with red cell haemolysate, Intralipid and bilirubin. The interferences were studied at baseline; 12.5%, 25%, 50%, 75% and 100% of 5.0 g/L haemoglobin; 1% of 20% Intralipid; and 0.342 mmol/L of bilirubin according to the EP7-A2 guideline (Interference Testing in Clinical Chemistry; CLSI, USA). Aliquots were analysed in duplicate and/or triplicate for various hormones on the Abbott Architect i2000 immunoassay analyser. Serum ferritin (r^2=0.84; P=0.074) and TSH (r^2=0.81; P=0.52) levels showed a direct relationship with haemolysis and therefore overestimated because of the effects of haemolysis. The vitamin B_{12} level progressively decreased as the amount of haemolysis increased (r^2=-0.76; P=0.136). There was a significant decrease in progesterone concentration owing to lipaemia (r^2=-0.983; P=0.003). For icteric interferences, a strong inverse correlation was observed for folic acid and was shown to be statistically significant (r^2=–0.94; P=0.017). Assays for ferritin, TSH, vitamin B_{12}, folic acid and progesterone showed various degrees of interference because of the variability in serum indices.

KEY WORDS: Hemolysis.
Icturus.
Immunoassay.
Indices.
Interferences.
Lipemia.

Introduction

Laboratory assays are subject to interference from many endogenous and exogenous sources. Extensive studies have explored the effect of serum indices interference on routine chemistry assays; however, few studies have focused on their effects on hormone immunoassays. Moreover, investigating the interferences by heterophilic antibodies in immunoassays has been the objective of many studies.[1] These interferences may result in serious errors in laboratory values and affect patient health plans and outcomes. Laboratory professionals have attempted to establish, introduce and apply many schemes and approaches to identify erroneous results arising from these interferences, but these approaches do not identify erroneous results arising from aberrant samples.

Substances that alter the measurable analyte concentration or alter antibody binding can potentially result in immunoassay interference, which may then lead to the misinterpretation of patient results by the laboratory and the incorrect course of treatment prescribed by the physician. Therefore, healthcare providers should pay close attention to the limitations of these assays. It is important to recognise the potential for interference in immunoassays and to set precautionary measures to identify them whenever possible. Detecting the presence of interference may require pre-treatment of the sample before the actual analysis.

One source of interference in immunoassays is haemolysis, which results in the release of red blood cell constituents into the serum, and may consequently influence antibody-antigen binding.[2] Ryder *et al*. noted that the interference resulting from haemoglobin, triglycerides and bilirubin in automated immunoassay techniques has not been examined systematically.[3]

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Therefore, the aim of our study is to establish a means of detecting, testing and reporting incidents of suspected interferences. As a result, additional comments will be attached to suspected results to inform the physician of the interference affecting the targeted values. It is our responsibility as laboratory specialists to maintain ongoing rapport between the laboratory and the physician, in order to raise the awareness of incorrect results arising from interference effects.

Materials and methods

This study followed the protocol EP7-A2, as well as the guidelines from the Clinical and Laboratory Standards Institute (CLSI).[4] A haemolysate solution was prepared by mechanically rupturing red blood cells and diluting the solution in the high pool (the preparation of which is described below) to a final haemoglobin concentration of 5 g/L. A series of sample dilutions of 0%, 12.5%, 25%, 50%, 75% and 100% were then prepared. Extra samples of the 12.5% dilution were used to improve the bias estimates at low interference levels. The 0% sample contained an equivalent volume of saline instead of the interferant solution.

The procedure was repeated to prepare the lipaemia solutions, in which a commercial fat emulsion preparation (20% Intralipid; Cutler Laboratories, Berkeley, CA, USA) was used and diluted in the high pool to a concentration of 1% (9.5 volumes serum + 0.5 volumes Intralipid).

For the icteric study, a stock solution of bilirubin (Sigma B4126, USA) was prepared by dissolving 20 mg bilirubin in 2 mL 0.1 mol/L NaOH, This solution provided a 17.1 mmol/L preparation. A volume of 0.1 mL stock solution was dissolved in a 5 mL pool of serum to produce a high pool of 342 μmol/L.

When the volumes were sufficient in size, the samples were tested in triplicate; otherwise the samples were tested in duplicate. The assays were performed quickly after receiving the samples, especially in the case of bilirubin, which is a light-sensitive substance. The assays were performed randomly to avoid bias, across all runs, testing for systematic effects arising from interference.

The Architect i2000 system (Abbott Laboratories, USA) uses chemiluminescent microparticle immunoassay (CMIA) technology. The microparticles are coated with capture molecules and then incubated with the analyte to form immune complexes. After separation of the unbound microparticles, the luminescent emission is measured. The hormone assays performed by this analyser included tests for cortisol, follicle-stimulating hormone (FSH), carcinoembryonic antigen (CEA), α-fetoprotein (AFP), ferritin, parathyroid hormone (PTH), free thyroxin (FT4), vitamin B_{12}, folic acid, prolactin, progesterone, luteinising hormone (LH), and β-human chorionic gonadotrophin (βhCG).

The average concentrations measured for the low pool (baseline) were subtracted from the sample results, and the respective differences were calculated. The evaluation was performed using the guidelines of the College of American Pathologists (CAP) for half of the total allowable error (TAE).[5] Any difference greater than half the goal of the TAE was considered to be above the allowable interference level and was deemed clinically significant. The calculated allowable interferences used in this study were found to vary from a minimum of 7.5% up to a maximum of 15%.

Results

Table 1 summarises the combined results of our interference studies and lists the P and r^2 values for various hormones versus interferences from haemolysis, icturus and lipaemia. There was a strong direct correlation for TSH and ferritin with the haemolysed samples. The measured levels of vitamin B_{12} were found to decrease progressively as the amount of haemolysis increased; therefore, we found a negative correlation between the two variables. However, these trends did not exceed the maximum allowable error for interference, which was calculated to be 15%. Cortisol and PTH showed a moderate to positive correlation for haemolysis, however, CEA showed a negative moderate correlation. Other hormone tests showed week correlation with haemolysis interference.

There was a significant decrease in progesterone concentration due to lipaemia, and the levels of progesterone exceeded the error limits. In addition, the levels of prolactin and vitamin B_{12} were found to correlate negatively with lipaemia, in contrast to folic acid and βhCG

Table 1. Combined results of interference studies.

Test	Haemolysis (r^2)	Lipaemia (r^2)	Icterus (r^2)
Cortisol	0.53 (P=0.28)	NA	NA
FSH	0.19 (P=0.72)	NA	–0.39 (P=0.52)
TSH	0.81 (P=0.52)	NA	NA
CEA	–0.62 (P=0.19)	NA	NA
AFP	–0.16 (P=0.76)	NA	NA
Ferritin	0.84 (P=0.074)	NA	NA
PTH	0.59 (P=0.299)	NA	NA
FT4	0.23 (P=0.71)	NA	NA
Vitamin B_{12}	–0.76 (P=0.136)	–0.81 (P=0.096)	NA
Folic acid	NA	0.60 (P=0.281)	–0.94 (P=0.017)
Prolactin	NA	–0.72 (P=0.169)	0.24 (P=0.698)
Progesterone	NA	–0.983 (P=0.003)	NA
LH	NA	0.073 (P=0.907)	NA
βhCG	NA	–0.43 (P=0.473)	–0.40 (P=0.51)

NA: not available.

Table 2. Comparison of various interferences studies in immunoassays.

Study	Test	Interference		
		Haemolysis	Lipaemia	Icterus
Lucena *et al*.[9]	Cortisol/Free T4	Yes/Yes	Yes/Yes	Yes/NA
Parra *et al*.[10]	Cortisol/Free T4	Yes/Yes	Yes/Yes	NA/NA
Armbrruster *et al*.[11]	Ferritin/Free T4	Yes (–ve)/Yes (–ve)	Yes (+ve)/Yes (+ve)	Yes (+ve)/Yes (+ve)
Current study	Cortisol	Yes (+ve)	NA	NA
	TSH	Yes (+ve)	NA	NA
	Ferritin	Yes (+ve)	NA	NA
	Vitamin B_{12}	Yes (–ve)	Yes (–ve)	NA
	Folate	NA	Yes (+ve)	Yes (–ve)
	Prolactin	NA	Yes (–ve)	NA
	Progesterone	NA	Yes (–ve)	NA
	βhCG	NA	Yes (–ve)	Yes (–ve)

NA: not available. +ve and –ve denote either positive or negative interference.

which were found to correlate positively with lipaemia. For icteric interferences, a strong inverse correlation was observed for folic acid, which was statistically significant.

Table 2 shows the comparison of various interference studies in immunoassays. All positive (+ve) and/or negative (–ve) interferences are shown.

Discussion

In this study, we evaluated the effect of interferences from serum indices on the Abbott Architect i2000 immunoassay analyser. We found that few hormone assays were affected by these interferences. The tests that were most affected by haemolysis interference were those for TSH, ferritin and vitamin B_{12}. The vitamin B_{12} assay showed a strong negative interference in the presence of haemolysis. This interference could result in clinically misleading results and thus a misdiagnosis of pathological vitamin B_{12} deficiency, which may lead to inappropriate remedies. Abbott Technologies reported that there was less than 10% potential interference from haemoglobin for the vitamin B_{12} assay; however, it was not indicated whether the interference was negative or positive.[6] Abbott used a cut-off value of 400 mg/dL haemoglobin as the endogenous marker for haemolysis, which was lower than the cut-off that we used in our study (500 mg/dL). Abbott stated that for optimal results, serum and plasma specimens should be free of fibrin, red blood cells and other particulate matter.[6] Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapies, may exhibit increased clotting times. They also reported that specimens containing some specific proteins, such as transcobalamin I/III or haptocorrin, at high concentrations, may depress the results of the vitamin B_{12} assay from the Architect i2000 analyser.[6] Red blood cells may contain these specific proteins and may therefore interfere with the vitamin B_{12} assay by producing negative effects. In addition to negative interference by haemolysis, there were strong inverse correlations between the vitamin B_{12}, progesterone and prolactin assays versus lipaemic interferences. However, only the progesterone assay demonstrated statistically significance values. In our study, lipaemia interference appears to be the most common interference in the immunoassay because the solubility of the antigen is measured in a lipid environment and is therefore no longer available to bind antibody.[6] The mechanism could also be defined by the physical masking of the antibody by lipids, thereby preventing antigen-antibody binding.[6] In their study comparing the Roche Elecsys testosterone II electro-chemiluminometric assay with liquid chromatography-mass spectroscopy (LC/MSMS), Owen *et al.* reported negative interference resulting from lipaemia at concentrations >22.5 g/L.[7] Jones stated that lipaemia can interfere with some immunoassays, especially those incorporating nephelometry and turbidimetry.[8]

For icteric interferences, we observed a significant inverse correlation for the folic acid assay and to less extent to βHCG assay. In general, interference from icterus in immunoassays has not been proved, because the wavelengths employed in chemiluminescent technology were separated from the absorbance maxima of bilirubin.[8] However, other studies have found that icteric samples may interfere with analytical methods for immunoassays.[9,10] For instance, Lucena *et al.* reported the effects of haemolysis, lipaemia and bilirubinaemia on an enzyme-linked immunosorbent assay for cortisol and free thyroxine in mammalian serum samples.[9] They indicated that haemolysis significantly interfered with the accuracy of FT4 determination (P=0.039), independent of the haemoglobin concentrations. They concluded that haemolysis should be avoided in FT4 testing using the competitive enzyme immunoassay. They also found that lipaemia significantly interfered with cortisol determination (P=0.0015) but not with F4 determination (P=0.41). They have also reported that the addition of bilirubin significantly interfered with cortisol testing (P=0), but, as with lipaemia, the magnitude of the differences was not of clinical significance. In another study, Parra *et al.* determined that haemolysis and lipaemia significantly interfered with cortisol and FT4 measurements, whereas bilirubinaemia did not affect the results in either mammalian or human serum as determined by time-resolved fluoroimmunoassays (TR-FIAs).[10]

In the evaluation study of FT4 and ferritin by enhanced luminescence immunoenzymometric assays (LIA), Armbruster *et al*. found that lipaemia and icterus samples produced positive interferences with FT4.[11] Similar effects of lipaemia were observed for ferritin; however, haemoglobin caused negative interference.[11] Our study showed that haemolysis, but not lipaemia, affects the ferritin immunoassay in positive interference. The differences between these studies can be attributed to differences in the nature and design of the immunoassays used in each study.

Other studies stated that immunoassays were generally unaffected by sample haemolysis and icterus, unlike other analytes that were measured.[12–14] Dimeski reported that there has not been any documented evidence of interference by icterus and lipaemia that were confined to immunonephelometric and immunoturbidimetric assays.[13] However, other types of immunoassay, such as ELISA and chemiluminometric assays, were not mentioned. Dimeski also stated that grossly lipaemic samples should be cleared for all assays to minimise volume displacement errors. It was further added that the instances of haemolysis interference were rare, except for the troponin assay, which is a commonly performed test that is frequently affected by haemolysis. Ji and Meng found that the majority of immunoassays were not affected by the presence of haemoglobin, bilirubin and lipids, although there were a few exceptions.[14] They used the Roche cobas 6000 system, that was a different immunoassay compared to the one we used in our study, which may explain this discrepancy, as every immunoassay has its own antibodies and unique structural design.

Many studies and reports have suggested various ways and approaches to eliminate or reduce endogenous and exogenous interferences and therefore improve the quality of the laboratory services.[13,15,16] For instance, Livesey and Dolam reported that haemolysis affects the measurement of plasma adrenocorticotrophic hormone (ACTH) by the immunoassay method.[17]

An editorial letter by Kricka stated that laboratories should guard against false-positive and false-negative immunoassay results from antibody or serum indices interferences by establishing a continuous dialogue between the physician and the clinical laboratory.[18] It was recommended that additional testing be arranged to confirm the test result (i.e., a repeat analysis on the specimen, a retest of a second specimen, or arranging for analysis by another method), and in case of antibody interferences, dilution or blocking studies can be performed to confirm the presence of an interferant. It was also suggested that patient education programmes increase patient knowledge and awareness about the limitations of immunoassay tests. Finally, it was emphasised that the manufacturers of immunoassays agree on guidelines for characterising the effects of interfering substances, such as that from CLSI.

Another possible approach to overcome these interferences in analytical methods is to automatically check the integrity of the samples against the serum indices. Glick *et al*.[19–21] have shown that technical disclaimers were generally not useful in the laboratory, because experienced technologists cannot accurately distinguish between various concentrations of lipaemia, haemolysis or icterus, even when provided with a visual reference specimen. The avoidance of inaccurate results resulting from interferences may necessitate the development of accurate electronic methods to identify and reject samples with these interferants, or, preferably, the development of instrument and reagent combinations that are not susceptible to these interferants. The authors concluded that there was little agreement between the actual concentration of each interferant and the assigned grade of turbidity, haemolysis or icterus, confirming the unreliability of human estimates of these potentially interfering substances.

We conclude that laboratories should be aware of the potential for interference in all immunoassays and that experimental artefacts may cause the misinterpretation of patient results and subsequently an incorrect diagnosis, leading to unnecessary treatments. □

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