RANDOX

EDUCATIONAL GUIDE

Serum Indices

QUALITY CONTROL

Introduction

The release of the new version of ISO 15189 (2022), introduces increased focus on risk stratification and mitigation for patients and laboratory stakeholders and more emphasis on quality control to improve accuracy and validity of results. Interference caused by haemolysis, icterus and lipemia is responsible for many rejected results and incorrect diagnosis. It is therefore important to accurately capture the presence of interfering substances in samples before quantitative analysis is carried out.

HIL (Haemolysis, Icterus, Lipemia) interference can have detrimental effects on the determination of concentration of many analytes. It is therefore crucial to determine levels of HIL interference to improve laboratory efficiency and to reduce the frequency of erroneous results. HIL interference leads to poor test turnaround times and additional expenses as sample collection and analysis must be repeated in cases of haemolysis, icterus or lipemia in samples[6]. More serious consequences of poor HIL detection may be delayed or incorrect diagnosis of patient samples, leading to late or inappropriate treatment.

Historically, HIL levels were determined by a visual examination. However, this method is unreliable and subject to interpretation. The internationally recognised standard, C56-A Haemolysis, Icterus and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guidelines[7] advises that "visual inspection does not accurately capture the possible presence of interfering substances". Most clinical chemistry analysers on the market can detect HIL status and calculate an HIL alert index. The HIL alert index, or threshold level, can be defined as the minimum concentration at which HIL interferes with biochemical assays, yielding a bias of >10%[5]. C56-A states[7], "an automated HIL detection system offers an objective and consistent methodology for assessing sample quality."

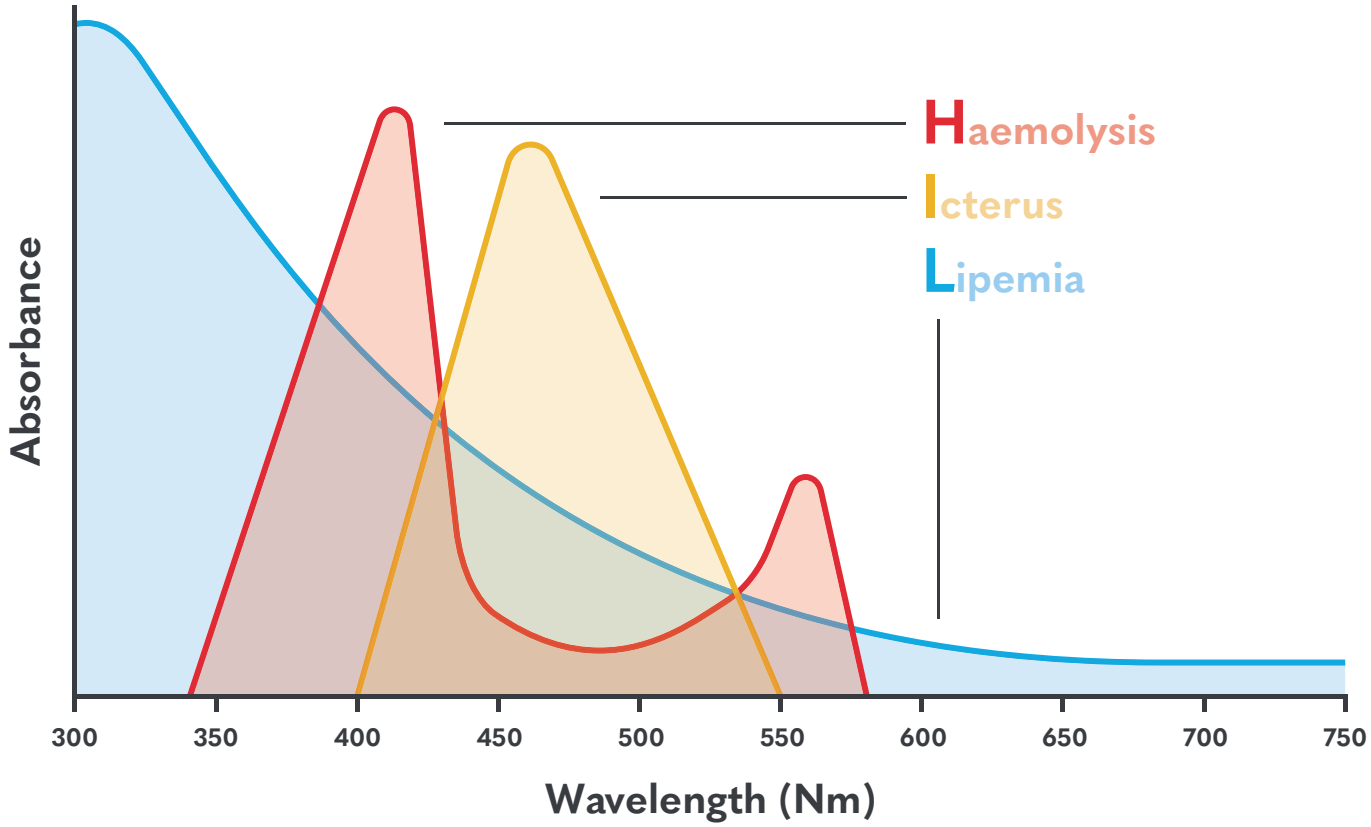

Figure 1. A graph displaying wavelengths at which interference caused by Haemolysis, Icterus and Lipemia are likely to be evident

Haemolysis

Haemolysis in patient samples is an extremely common form of pre-analytical error responsible for 40-70% of rejected laboratory samples[6] and is present in approximately 30% of samples in emergency departments[1]. Haemolysis is evident when red blood cells experience disruption of the membrane due to tangential stress, causing degradation of cellular integrity and the release of intracellular components. The release of these components, such as haemoglobin, potassium ions and aspartate aminotransferase, into serum or plasma can cause further degradation to analytes such as insulin and troponin T[1]. Common causes of haemolysis in samples are poor venepuncture techniques such as forceful blood extraction, poor sample storage such as repetitive freezing, inadequate sample transportation or incorrect sample preparation[3]. While *in vitro* haemolysis causes significant interference, *in vivo* haemolysis is not considered to be interference as the elevation of analytes may serve diagnostic purposes[7]. Haemoglobin concentrations above 200mg/dl are likely to display interference in samples through several mechanisms.

The simplest of these mechanisms is an increased concentration of analyte due to the release of intracellular components, through the lysis of the cell. This results in falsely high results in those analytes already present in high concentrations within the cell and falsely low results for analytes of low intracellular concentration[1]. As haemoglobin displays its greatest absorbance at around 415nm[1], haemolysis will cause interference in colorimetric assays measured between 340-440nm and 540-580nm[7] such as **iron, lipase, and albumin** (Figure 1). Furthermore, haemolysis can cause positive interference in **creatine kinase (CK) assays** through the release of adenylate cyclase[1].

Icterus

Icterus is caused by high bilirubin levels in a sample resulting in its yellowish pigmentation. This form of interference is most prevalent in neonatal departments with an incidence rate of over 30%. Icterus can be caused by hepatic necrosis, sepsis, or several other pathological conditions[6]. Colorimetric assays which measure absorbance between 400-550nm will be strongly affected by icterus with the greatest interference evident at 460nm (Figure 1). Examples of these assays include **phosphate assays** and the **Jaffee method of determining creatinine** concentration. In this assay, a creatinine picrate complex is formed, and the absorbance is measured at 500nm. The basic conditions in which this assay takes place cause the oxidation of bilirubin resulting in negative interference at 500nm and an erroneous result[1]. Assays for **cholesterol, triglycerides and uric acid** are subject to icterus interference, causing a negative bias, as bilirubin reacts readily with the antioxidant, hydrogen peroxide, an intermediate in the assays mentioned[1].

Lipemia

Lipemia is an aggregation of lipoproteins causing an effect on the turbidity of a sample and is seen in up to 2.5% of patient samples[4]. This is commonly caused by insufficient fasting prior to sample collection. This turbidity can be caused by a few mechanisms, most commonly the light scattering effect seen because of high concentrations of lipoproteins[1]. The primary type of lipoprotein responsible for this effect are chylomicrons. Chylomicrons are the largest form of lipoprotein and have the largest lipemic effect[2]. However, accumulations of large and intermediate VLDL can also induce interference. Whilst the most common cause of lipemia is diet, accumulation of lipoproteins can also be caused by high alcohol consumption, medications, and hereditary conditions[1].

The light scattering effect is the primary mechanism of lipemic interference and intensity is dependent on the size and quantity of particles suspended in the solution. Light scattering causes positive interference by reducing effective spectral linearity[7]. The effects from the light scattering effect can be seen between 300-700nm, however interference increases as wavelength decreases (figure 1). Therefore, assays, such as those which measure NAD(P)H concentration, at low wavelengths are at most risk from lipemic interference. In addition, high lipid concentration is a known factor in high rates of haemolysis. Other causes of lipemic interference are erythrocyte debris, platelets leukocytes, fibrin clots or contaminating particulate matter. Large concentrations of lipid particles may also cause negative interference on electrolyte quantification due to volume displacement[7]. Finally, hydrophobic analytes, reagents or reaction products may be absorbed by lipids particles, resulting in interference.

Normal

Haemolytic

Icteric

Lipemic

Figure 2. Illustration of Normal, Haemolytic, Icteric and Lipemic samples

Automated HIL detection vs Visual Assessment

C56-A states[7], "HIL indices should be measured on all samples which analytes are sensitive to haemolysis, icterus and lipemia/ turbidity." Due to the wide variety of wavelengths affected by these forms of interference, this statement encompasses a large variety of laboratory tests. Classical determination of HIL interference took the form of visual assessment. Haemolysed samples display a red colouration which is directly proportional to the concentration of haemoglobin and other interfering erythrocyte components. Similarly, icteric interference is characterised by a yellow pigmentation which increases proportionally to the concentration of conjugated and unconjugated bilirubin. Finally, the turbidity of samples increases proportionally to lipid concentration causing lipemic interference[7]. While previously considered acceptable, these methods are subject to user interpretation and lack harmonisation and uniformity across the industry.

Modern methods utilise onboard HIL detection methods in automated analysers to assess the level of HIL interference. These methods offer objective, semi-qualitative or qualitative analysis of interference providing a more accurate and consistent approach. The automation of these analyses aids laboratories in improving test turnaround times and enhances the reportability of patient results. Furthermore, the automation of this process increases laboratory throughput as the testing process does not have to be interrupted to carry out a visual assessment.

HIL Indices

To correctly analyse HIL interference, absorbance readings at different strategically selected wavelengths supplement the calculation of the interference indices. C56-A recommends laboratories consider several parameters when selecting an HIL interference analysis method[7]:

Interferant Test Concentration	The laboratory should consider the concentrations at which they test for HIL interferants. The concentrations should be clinically relevant and cover the entire range of possible interference. The index value should increase as interferant concentration increases.
Sample Volume	Neonatal, geriatric, and critical care patient samples are often supplied in very low volumes. Laboratories should consider the minimum sample volume required to determine an HIL index.
Wavelengths and Methods	Due to the large overlap in spectra of interferants, laboratories should consider the utility of wavelengths and methods selected.
Number of Indices	The number of indices provided by the HIL detection method should be considered. There is no recommendation for how many indices this should be, however laboratories should consider indices and related concentrations when choosing an HIL detection method.
Read Time	Laboratories should consider the test turnaround time for HIL detection and ensure it is practically applicable to their day-to-day activities.

Before results of any HIL detection method are used for patient samples, the specificity and sensitivity should be assessed at a minimum of two clinical decision concentrations. This evaluation should include the sensitivity of the icterus index to haemoglobin and lipids, the haemolysis index to bilirubin and lipids and the lipemic index to haemoglobin and bilirubin[7].
In the presence of HIL interference, laboratories are responsible for the handling of the associated results and samples. Under no circumstances should an HIL index be used to correct patient results. Generally, if a sample is deemed to be subject to one or more of these types of interference, the laboratory should reject the result and dispose of the sample correctly. However, in some cases, cut-off values can be defined. For example, haemolysis has a less significant effect on samples with high analyte concentration. In this case laboratories may wish to have a different procedure for handling these results than those which show haemolytic interference at low analyte concentration.

Verification and Quality Control of HIL Indices

C56-A states that laboratories should consider verification and quality control of expected performance to assess the following implications:

- HIL parameters, like all spectrophotometric measurements, are subject to drift and failure
- Failure to maintain consistent measurements may lead to changes in effective criteria for acceptance/rejection of specimens
- Inter-analyser variability can result in inconsistent acceptance/rejection criteria

To combat these implications, Randox Laboratories introduces the Serum indices Control and Serum Indices EQA.

Randox Serum Indices Control

The Randox Acusera Serum Indices (SI) control is designed to be used to monitor an IVD instrument's response in the detection of haemolyzed, icteric and lipemic (HIL) samples. This control can be utilised in laboratory interference testing to assist in improving error detection of pre-analytical errors affecting clinical chemistry testing. This control provides a full range of clinically relevant testing levels, including a negative (-) and three positives (+, ++ & +++).

ACUSERA

A third-party competitor product supplies only 1 level for each form of interference and a negative control, covering only a fraction of the range covered by the 4-level Randox Control. This competitor product is supplied in a liquid frozen format, increasing delivery costs. The Randox SI control is supplied lyophilised for enhanced shelf-life. Customers using this competitor product report that the control does not meet the stability claims of 14-day open stability. Customers report that significant variations are evident after as little as 1-3 days, increasing the waste produced by the laboratory.

Typical Values

	Haemolysis (H)		Icterus (I)		Lipemia (L)	
	Appearance scale	mg/dl	Appearance scale	mg/dl	Appearance scale	mg/dl
Level 1	-	<30	-	<2	-	<50
Level 2	+	30 - 100	+	2 - 4	+	50 - 100
Level 3	++	100 - 200	++	4 - 10	++	100 - 150
Level 4	+++	200 - 500	+++	10 - 20	+++	150 - 200

RIQAS Serum Indices External Quality Assessment

The RIQAS Serum Indices EQA programme is designed for the pre-analytical assessment of Haemolytic, Icteric and Lipemic (HIL) interferences. Available in a bi-monthly format with the option to report either quantitative or semi-quantitative results for the HIL parameters, this programme also provides an assessment on how these interferences impact on up to 25 routine chemistry parameters. This provides invaluable information on whether a correct judgement is being made to report results.

- Lyophilised for enhanced stability
- Human based serum ensuring commutable sample matrix
- Bi-monthly reporting
- HIL parameters include the option of quantitative or semi-quantitative reporting
- Interpretation of chemistry parameter results
- Submit results and view reports online via RIQAS.net

RIQAS

Conclusion

It is crucial laboratories test for haemolysis, icterus and lipemia to ensure the accuracy of their test processes are maintained. ISO 15189:2022 promotes the identification and control of non-conformities in the pre-analytical process, therefore, using Randox Serum Indices control and RIQAS Serum Indices EQA will help laboratories fulfil the requirements of the new edition of this standard.

Randox Serum Indices control displays improved consolidation, stability, and commutability to ensure laboratories are equipped to accurately determine pre-analytical interferences. Our Serum Indices control can be used with most major chemistry analysers including Roche, Abbot, Beckman, Ortho, and Siemens. When used in conjunction with Acusera 24.7, this control offers laboratories the ability to compare their HIL results with their peer group and identify potential failures in their pre-analytical process.

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If you would like further information please contact:

QC Marketing Team

T +44 (0) 28 9442 2413 | E acusera@randox.com | E marketing@randox.com

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