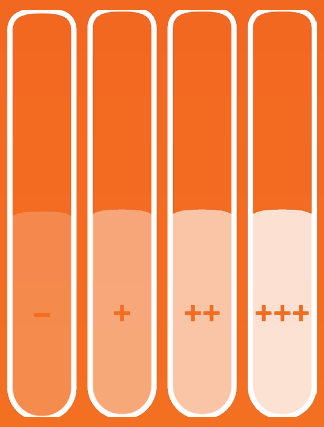
RANDOX

The Implications of Haemolytic, Icteric and Lipaemic Interference



QUALITY CONTROL

Interference caused by haemolysis, icterus and lipaemic (HIL) is a scourge on efficient laboratory test turnaround times (TAT) and timely diagnosis, with consequences for both the laboratory and the patient. Impacts on the laboratory may be financial or reputational, however, the repercussions for the patient may be more severe. Errors can occur at any stage of the analytical process, but those which begin in the pre-analytical phase are often avoidable. Herein, we explore the causes of HIL interference, their implications and the actions that can be taken to minimise their occurrence.

Forms of Interference

Haemolysis

Haemolysis describes the rupturing of red blood cells (RBCs) due to tangential stress and a loss of cellular integrity resulting in the release of intracellular components into the surrounding fluid. These components include haemoglobin, potassium ions and aspartate aminotransferase (AST). They are released into plasma or serum, often causing further degradation of analytes like insulin and troponin.

Haemolysis is the most common form of interference in emergency departments; it is present in approximately 30% of samples[1], accounting for between 40-70% of all rejected laboratory samples[2]. Most clinical chemistry analytes are affected by haemolysis, but the severity of interference will depend on both the levels of haemolysis and the assay methodology. Interference can occur through several mechanisms:

Spectrophotometric Interference

The intracellular components released through haemolysis can interfere with spectrophotometric assays at a range of wavelengths. Haemoglobin displays its greatest absorbance at 415nm but can affect assays measured between 340-440nm and 540-580nm. Examples of these assays include iron, lipase, and albumin[1].

Chemical Interference

The liberated RBC components can interfere with analyte quantification by interacting with components of the assay in direct or indirect methods. Direct methods include competition for substrate or with other assay components, and direct inhibition of assay reactions. For example, haemoglobin inhibits the diazonium colour formation in the Jendrassik-Grof assay for bilirubin[3]. Indirect methods include precipitation of the analyte, proteolysis, and the complex formation between RBC and assay components[3].

Liberated RBC components

Many of these analytes are more concentrated within RBCs than they are in serum and plasma. Analytes with RBC intracellular concentrations exceeding serum/plasma levels by a factor of 10, as seen with substances like lactate dehydrogenase, inorganic phosphate, potassium, and AST, can cause significant interference, even in cases of mild haemolysis[3].

Sample Dilution

In contrast, some analytes, found at much lower concentrations inside RBCs than in serum and plasma, can cause a negative bias when they are quantified using biochemical assays. These analytes include albumin, bilirubin, glucose, alkaline phosphatase, chloride, sodium and gamma-glutamyl transferase (GGT). However, it requires severe haemolysis to achieve a significant clinical bias through this mode of interference[3].

While *in vitro* haemolysis is a nuisance for the laboratorian, *in vivo* haemolysis may be indicative of several conditions. Accounting for around 3% of haemolytic samples, *in vivo* haemolysis may occur due to one or more of the following: Infection, immune-mediated mechanisms (autoimmune haemolytic anaemia), inherited RBC disorders, mechanical aid (prosthetic heart valves) toxins (drug overdose, chemotherapy, or venoms), burns, and more[3].

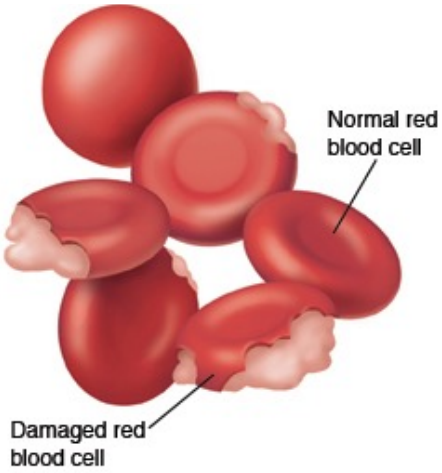


Figure 1. Healthy and ruptured red blood cells.[20]

Lipaemic

Lipaemic accounts for around 2.5% of rejected patient samples and is most common in outpatient samples[4]. Lipaemic is defined as an aggregation of lipoproteins which affects the turbidity of the sample. The primary method of interference is the light-scattering effect caused by high concentrations of lipoproteins[1]. This effect is dependent on the size and quantity of the interfering particle suspended in the sample. Larger lipoproteins, such as chylomicrons, have a larger lipaemic effect. This causes positive interference through the reduction of spectral linearity between 300-700nm[1]. Interference by light-scattering increases as the wavelength decreases; assays which measured NAD(P)H concentrations at low wavelengths are the most affected by lipaemic interference[1].

The accumulation of lipoproteins can also cause interference by blocking the antigen-antibody reactions commonly used in immunoassays[4]. Depending on the nature of these reactions, this interference can cause falsely high or low results. Other causes of lipaemic interference are erythrocyte debris, platelets leukocytes, fibrin clots or contaminating particulate matter. Large concentrations of lipid particles may also cause negative interference in electrolyte quantification due to volume displacement[4]. Finally, hydrophobic analytes, reagents or reaction products may be absorbed by lipid particles, resulting in interference[4].

The most common reason for lipaemic in samples is insufficient fasting before sample collection. However, there are some pathophysiological factors which can cause lipaemic. Conditions including multiple myeloma, diabetes mellitus, acute pancreatitis and kidney failure can result in lipaemic samples[4].

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%[2]. Icterus can be caused by hepatic necrosis, sepsis, or several other pathological conditions[2]. Colorimetric assays which measure absorbance between 400-550nm will be strongly affected by icterus with the greatest interference evident at 460nm[1]. Examples of these assays include phosphate assays and the Jaffe method of determining creatinine concentration. In this assay, a creatinine picrate complex is formed, and the absorbance 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[5]. Assays for cholesterol, triglycerides and uric acid are subject to icteric interference, causing a negative bias, as bilirubin reacts readily with the antioxidant, hydrogen peroxide, an intermediate in these assays[1].

Implications for Patients

The 2022 edition of ISO15189 places more emphasis on risk management and mitigating risk to the patient. However, the impact of rejected samples and delayed tests can have as serious an impact as an erroneous result. We've discussed why these forms of interference lead to sample rejections, but what are the consequences? Firstly, the need to repeat sample draws can lead to additional discomfort or distress for patients, particularly children. More importantly, the delay in testing, or the need to retest, leads to a delay in diagnosis which could adversely affect outcomes. Delays in result reporting are associated with 61% longer emergency department residency and 43% delay in receiving treatment[6].

For conditions like stroke and heart disease, every minute counts. Any delay in diagnosis can have ruinous effects on morbidity and mortality[7]. Many of the assays used to assess biomarkers related to heart problems, like creatine kinase, CKMB, and Troponin T, are largely affected by one or more of the types of interference discussed.

Furthermore, a study of over 5 million patients in England showed that waiting more than 5 hours in emergency care before hospital admission is linked to a heightened risk of death from any cause within the next 30 days[8]. Another example comes from a study which investigated the effect of extended cumulative clinical read time (CRT) on length of hospital stay[9]. This paper shows that for each day a diagnosis is delayed, the length of stay was prolonged by 13.2%. When the analysis was restricted to delay by abnormal results, each day of delay increased the likelihood of delayed discharge by 33.3%[9].

These examples do not directly investigate HIL interferences but discuss the effect of delays on patient outcomes. Therefore, it follows that delays in diagnosis due to rejected samples could result in similar outcomes. Delayed test results can cause additional stress on patients and may cause them to lose confidence in health services. Finally, extended stays in emergency departments mean fewer beds for other patients who require attention, feeding the vicious cycle of diagnosis and treatment delays.

Implications for the Laboratory

The consequences for the laboratory may not be as existentially threatening as they are for patients but may still have a more drastic impact than is initially apparent. There is the immediate cost of materials for resampling and retesting, but this is only the beginning.

A paper published in 2019 by Maul *et al*., investigated the savings associated with reducing the rate of haemolysed samples from 10% to 2%. After determining that the avoidable expenditure from haemolysed samples might be as high as 23% of the total cost of serum sample collections, an in-depth analysis was conducted. They concluded that an 8% reduction in haemolysed samples could result in a saving of up to $144,000 USD for similar hospitals[10]. A breakdown of the data can be seen in Figure 2.

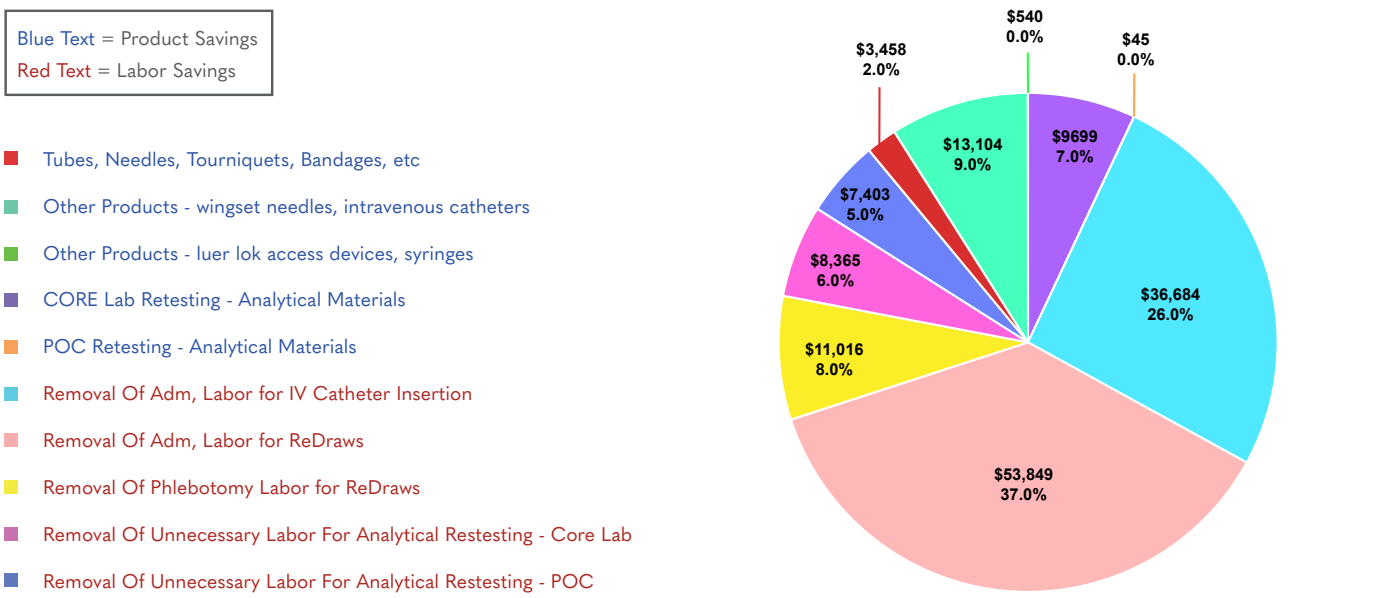


Figure 2. Pie chart illustrates the total potential annual savings with a 2% haemolysis rate[10]

Another source states that for US hospitals with 650 beds, haemolysis in samples may cost up to $1,199,122 USD annually[11]. A different approach showed that blood sample rejection was associated with an increased length of hospital stay. The authors estimate that the extended length of stay resulted in an additional fixed cost of £26,824 GBP[12]. Additional repercussions for the laboratory may involve negative impacts on reputation, stock management and laboratory throughput.

HIL Detection

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 lipaemic interference (Figure 3). Although data suggests that up to 37% of laboratories in Europe still use this method[13], visual determination of HIL interference is now strongly discouraged as it is subject to user interpretation and lacks standardisation 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. All analysers use the same principle for HIL indices - absorbance readings at different strategically selected wavelengths supplement the calculation of the interference indices. In the most straightforward method, 3 pairs of absorbance readings are taken. As there is a spectral overlap between HIL interferences,

Figure 3. Illustration of Normal, Haemolytic, Icteric and Lipaemic samples

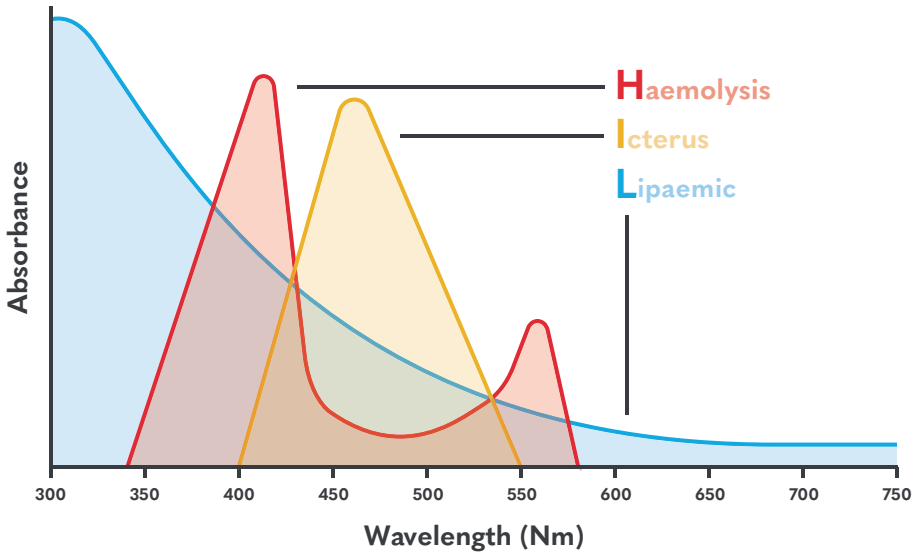


Figure 4. Absorbance spectra for haemolytic, lipaemic and icteric samples

(Figure 4), correction factors are calculated and applied to account for the multiple contributions to absorbance at some wavelengths[1]. Some analysers use more than 3 pairs of readings, however, the more readings that are included, the more complicated the calculation of the correction factors[1].

The automation of these analyses aids laboratories in improving test turnaround times and enhances the reportability of patient results. Furthermore, the automation of HIL detection increases laboratory throughput as the testing process does not have to be interrupted to carry out a visual assessment.

Guidance from the Clinical and Laboratory Standards Institute, C56-A, recommends laboratories consider several parameters when selecting a HIL interference analysis method which can be seen in Table 1 [14].

Table 1. CLSI recommendations regarding HIL interference testing

Interferant Test Concentration	The laboratory should consider the concentrations at which the 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 & 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 laborties 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 pratically applicable to their day-to-day activities.

Before the results of any HIL detection method are used to confirm or reject 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 lipaemic index to haemoglobin and bilirubin. In the presence of HIL interference, laboratories are responsible for the handling of the associated results and samples. Under no circumstances should a HIL index be used to correct patient results[14].

Minimising and Managing HIL Specimens

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.

Minimising Haemolysis

As previously mentioned, haemolysis may occur in samples taken from patients with increased RBC fragility as is seen in those with diabetes mellitus, cancer, and multiple sclerosis. RBC integrity may also be compromised in post-menopausal women and those undergoing chemotherapy or anticoagulant therapy.

The most common causes of *in vitro* haemolysis occur during phlebotomy, normally because of increased tangential stress or shear force. Aggressive blood drawing is a highly cited reason for haemolysis. Up to 80% of haemolytic samples have been associated with the use of syringes for sample collection[3]. When compared with other equipment like evacuated tubes, shear forces are much higher when using syringes which increases the likelihood of the RBC rupture. Similarly, a direct correlation is found between haemolysis rates and vacuum pressure of intravenous catheters for blood collection[3].

A paper by Lee *et al. (2023),* describes a novel technique involving sample collection without removing the catheter needle. When compared with the conventional method, haemolysis rates decreased by over 5%[15]. Furthermore, softer catheters are associated with a negative pressure increase when drawing blood, which may cause the collapse of the catheter and disruption of RBC membranes[15].

When using evacuated tubes, they should be filled. Additives such as clot accelerators, anticoagulants or anti-glycolytic agents are added at concentrations that presume a filled tube. Tubes not filled will contain a high relative concentration of additives increasing the chances of osmotic rupturing of RBCs[3].

After collecting the sample, it's essential to transport it at a consistent temperature. Avoiding extremes of heat or cold is crucial to prevent haemolysis. During sample preparation, appropriate centrifugation is important – excessive speeds or durations can cause shear damage and haemolysis.

Managing Lipaemic

As discussed, the most common cause of lipaemic samples is inadequate fasting before sample collection. While this can be difficult to avoid in emergency departments, outpatient clinics should provide patients with proper instructions about fasting to reduce the likelihood of lipaemic[4]. However, this is complicated by the differing recommendations around the world. For example, in Italy, it is recommended that patients fast for at least 8 hours before their sample draw, whereas in Australia, recommendations state fasting for 10-16 hours is required[4]. Furthermore, many countries, like the UK, only require patients to fast for tests directly affected by food consumption, like glucose or iron tests[16]. Normally, lipaemic can be removed from a clear specimen through several methods. Determining the appropriate method will depend on the test that is to be used. The first method is centrifugation. Ultracentrifugation is most effective at separating lipids. However, this expensive equipment is not available in many laboratories[14]. High-speed centrifugation (10,000 x g) has been shown to be almost as effective. However, centrifugation at lower forces is only able to separate the largest lipaemic culprits, chylomicrons[17]. Upon successful separation of the lipid layer, it can be removed, and analysis conducted on the infranatant. Centrifugation is not acceptable for the measurement of analytes that are distributed in the lipid layer such as hormones, drugs, and other hydrophobic substances – this will result in falsely low results[4].

Next, lipids can be extracted using polar solvents such as polyethylene glycol. Some laboratories use manual protocols, but commercial kits are available. Once added and centrifuged, lipids are bound to the non-toxic, non-ionic polymers and precipitate at the bottom, allowing for analysis of the clear supernatant[4]. This method

has been shown to be unsuitable for a variety of analytes such as GGT, CKMB, C-reactive protein[18], Troponin T[19].

Finally, sample dilution is acceptable for some analytes distributed in the lipid layer. An appropriate dilution factor is essential. A sample should only be diluted enough to eliminate the turbidity of the specimen and not overdiluted, ensuring the analyte concentration remains within the analytical capabilities of the assay[1,4].

Icterus

Icteric interference is more complicated. Unlike lipids, bilirubin is not easily removed from serum or plasma. One successful method uses potassium ferricyanide to oxidise bilirubin to biliverdin prior to initiating the Jaffe creatinine method[1]. Rate-blanking has also shown success; sodium hydroxide is added to the sample and the rate of colour change is measured and used as a correction factor. Alternatively, high-efficiency hydrogen peroxide acceptors or ferrocyanide may help minimise icteric interference in assays where hydrogen peroxide is an intermediate[1].

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

Acusera 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 haemolytic, icteric and lipaemic (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 (+, ++ & +++).

RIQAS Serum Indices EQA

The RIQAS Serum Indices EQA programme is designed for the pre-analytical assessment of Haemolytic, Icteric and Lipaemic (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.

Conclusions

HIL interference in laboratory testing has profound implications for both laboratories and patients. It leads to sample rejections, delayed diagnoses, and erroneous results. For patients, this means discomfort, stress, and potentially higher morbidity and mortality due to treatment delays. Laboratories face financial costs, reputation risks, and workflow disruptions.

Modern automated analysers offer objective HIL detection, improving turnaround times and result reporting. However, managing HIL specimens requires specific protocols.

Minimizing haemolysis involves addressing blood collection, transportation, and appropriate centrifugation. Lipaemic prevention includes patient fasting instructions and management through methods like centrifugation, lipid extraction, or sample dilution. Icteric interference, linked to bilirubin levels, requires assay-specific approaches.

While modern approaches help to improve the detection of these forms of interference, there is a need for greater standardisation of the methods used to calculate HIL indices and the procedures used to deal with them.

References

1. Farrell CJL, Carter AC. Serum indices: managing assay interference. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 2016;53(5):527-538. doi:10.1177/0004563216643557

2. Tian G, Wu Y, Jin X, *et al*. The incidence rate and influence factors of hemolysis, lipemia, icterus in fasting serum biochemistry specimens. PLoS One. 2022;17(1):e0262748. doi:10.1371/journal.pone.0262748

3. Simundic AM, Baird G, Cadamuro J, Costelloe SJ, Lippi G. Managing hemolyzed samples in clinical laboratories. Crit Rev Clin Lab Sci. 2020;57(1):1-21. doi:10.1080/10408363.2019.1664391

4. Nikolac N. Lipemia: causes, interference mechanisms, detection and management. Biochem Med (Zagreb). Published online 2014:57-67. doi:10.11613/BM.2014.008

5. Mainali S, Merrill AE, Krasowski MD. Frequency of icteric interference in clinical chemistry laboratory tests and causes of severe icterus. Pract Lab Med. 2021;27:e00259. doi:10.1016/j.plabm.2021.e00259

6. Dawande PP, Wankhade RS, Akhtar FI, Noman O. Turnaround Time: An Efficacy Measure for Medical Laboratories. Cureus. Published online September 6, 2022. doi:10.7759/cureus.28824

7. Kwok CS, Burke H, McDermott S, *et al*. Missed Opportunities in the Diagnosis of Heart Failure: Evaluation of Pathways to Determine Sources of Delay to Specialist Evaluation. Curr Heart Fail Rep. 2022;19(4):247-253. doi:10.1007/s11897-022-00551-4

8. Jones S, Moulton C, Swift S, *et al*. Association between delays to patient admission from the emergency department and all-cause 30-day mortality. Emergency Medicine Journal. 2022;39(3):168-173. doi:10.1136/emermed-2021-211572

9. Ong MS, Magrabi F, Coiera E. Delay in reviewing test results prolongs hospital length of stay: a retrospective cohort study. BMC Health Serv Res. 2018;18(1):369. doi:10.1186/s12913-018-3181-z

10. Maul P, Phelan M, Reineks E, Bontempo A, Green S. Quantifying the Economic Impact of Poor Quality (Hemolyzed) Blood Samples from the Emergency Department. J Health Care Finance. 2019;45(4). Accessed September 20, 2023. https://healthfinancejournal.com/~junland/index.php/johcf/article/view/179

11. Green SF. The cost of poor blood specimen quality and errors in preanalytical processes. Clin Biochem. 2013;46(13-14):1175-1179. doi:10.1016/j.clinbiochem.2013.06.001

12. Bodansky DMS, Lumley SE, Chakraborty R, *et al*. Potential cost savings by minimisation of blood sample delays on care decision making in urgent care services. Annals of Medicine & Surgery. 2017;20:37-40. doi:10.1016/j.amsu.2017.06.016

13. Cornes M, Simundic AM, de la Salle B, *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 (EF. Biochem Med (Zagreb). 2019;29(2):334-345. doi:10.11613/BM.2019.020705

14. Clinical and Laboratory Standards Institute. Hemolysis, Icterus and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline. CLSI Document C56-A. 2012;32.

15. Lee H, Lee H, Kim C, Shin H, Lee I, Kim Y. Hemolysis Control in the Emergency Department by Interventional Blood Sampling. J Pers Med. 2023;13(4):651. doi:10.3390/jpm13040651

16. NHS. Can I eat and drink before having a blood test? Operations, tests and procesures. Published February 25, 2021. Accessed September 21, 2023. https://www.nhs.uk/common-health-questions/operations-tests-and-procedures/can-i-eat-and-drink-before-having-a-blood-test/#:~:text=Examples%20of%20blood%20tests%20that,12%20hours%20before%20the%20testhttps://www.nhs.uk/common-health-questions/operations-tests-and-procedures/can-i-eat-and-drink-before-having-a-blood-test/

17. Dimeski G, Jones BW. Lipaemic samples: Effective process for lipid reduction using high speed centrifugation compared with ultracentrifugation. Biochem Med (Zagreb). Published online 2011:86-94. doi:10.11613/BM.2011.016

18. Vermeer HJ, Steen G, Naus AJM, Goevaerts B, Agricola PT, Schoenmakers CHH. Correction of patient results for Beckman Coulter LX-20 assays affected by interference due to hemoglobin, bilirubin or lipids: a practical approach. Clinical Chemical Laboratory Medicine. 2007;45(1). doi:10.1515/CCLM.2007.004

19. Saracevic A, Nikolac N, Simundic AM. The evaluation and comparison of consecutive high speed centrifugation and LipoClear® reagent for lipemia removal. Clin Biochem. 2014;47(4-5):309-314. doi:10.1016/j.clinbiochem.2014.01.001

20. Saint Luke's Hospital. When Your Child Has Hemolytic Anemia. Health Library. Published 2023. Accessed October 14, 2023.

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QUALITY CONTROL

MDSAP Certification Number:
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