

C56-A

Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline

This document provides background information on mechanisms of hemolysis, icterus, lipemia/turbidity (HIL) interference; intended usefulness of HIL indices; establishment of HIL alert indices; availability of automated HIL detection systems; and interpretation, strengths, limitations, and verification of HIL indices in the clinical laboratory.

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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

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Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline

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Merle B. Smith, MBA, MS, MT(ASCP)
Yung W. Chan, MT(ASCP)
Alberto Dolci, MD
Mark D. Kellogg, PhD
Christopher R. McCudden, PhD, DABCC, NRCC
Michelle McLean, MS, MT(ASCP)
James J. Miller, PhD, DABCC, FACB
Jack Zakowski, PhD, FACB

Abstract

Clinical and Laboratory Standards Institute document C56-A—*Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline* focuses on the intended usefulness and challenging issues of hemolysis, icterus, and lipemia/turbidity (HIL) indices as estimates of interference that may impact the validity and clinical utility of reportable patient results. C56 is closely aligned with CLSI document EP07¹; thus, the basic concepts for interference testing are briefly discussed in C56 to gain an understanding of the process by which HIL alert indices are established. Automated HIL systems currently available from various manufacturers are also described. This document enhances the continuous education of health care personnel by explaining the mechanisms of HIL interference, which in some cases include the noncorrelation of visual and (semi)quantitative HIL indices, the strengths and limitations of HIL measurements, and the verification of HIL indices in the clinical laboratory.

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SAMPLE

Foreword

One of the requirements for a clinical laboratory is that common interferences related to sample integrity such as hemolysis, icterus, and lipemia/turbidity (HIL) be evaluated with each reagent system. It has been a long-standing practice for clinical laboratory personnel to visually inspect the specimens for sample quality; however, visual inspection does not accurately capture the possible presence of an interfering substance or the combinations of interfering substances that may be present in the sample.

Because of limited resources and budgetary constraints, the clinical laboratory relies on the manufacturer to document HIL estimates and interference claims in the product labeling. However, it is important for the clinical laboratory personnel to verify the intended usefulness, strengths, and limitations of these estimates in their institutions.

An automated HIL detection system offers an objective and consistent methodology for assessing sample quality. HIL indices are calculations based on absorbance measurements that provide (semi)quantitative estimates of hemolysis, icterus, and lipemia/turbidity.

The document development committee, with the cooperation and support of *in vitro* diagnostic manufacturers, has reviewed a number of automated HIL systems currently available in the field.

There are few published guidelines concerning HIL measurements. As such, questions often arise about HIL measurement, calibration, QC, traceability, and identification and performance characteristics. This guideline was created to help address these questions, and under the CLSI consensus process included the international cooperation and collaboration of manufacturers, laboratory users, and government agencies. C56 is closely aligned with the recommendations in CLSI document EP07¹ for sample preparation and substances to use for HIL testing.

Several factors should be considered when examining the influence of lipemia on analytical methods. The heterogeneous nature of lipemia creates difficulties in simulating samples. Both very low-density lipoprotein (VLDL) and chylomicrons effectively scatter light, causing turbidity. VLDL exists in three size classes: small (27–35 nm), intermediate (35–60 nm), and large (60–200 nm). Chylomicrons represent a group of particles ranging in size from 70 to 1000 nm and varying greatly in size distribution and number among individuals. Because of the heterogeneity in particle size of VLDL and chylomicrons, a direct measure of triglycerides would not show good correlation with light scattering, visual lipemia, or the lipemic index.²

The examples used in C56 to demonstrate lipemia/turbidity testing and establish lipemia/turbidity indices use Intralipid[®] (or the equivalent) to simulate lipemia/turbidity due to lack of standard lipoprotein preparations. It has been used by reagent manufacturers to assess lipemia/turbidity interference because, unlike bilirubin or hemoglobin, there is no simple chemical substance that can be used to mimic the physical and chemical interfering properties of lipemic/turbid samples.²

Intralipid[®] is a registered trademark of Fresenius Kabi AG, Bad Homburg, Germany. Intralipid[®] is a synthetic, sterile nonpyrogenic fat emulsion for intravenous administration that can be added to serum or plasma to simulate lipemic samples. Samples with added Intralipid[®] (or the equivalent) do not perfectly mimic lipemic samples. Intralipid[®] (or the equivalent) is different from VLDL and chylomicrons. The particles in Intralipid[®] (or the equivalent) range in size from 200 to 600 nm with a mean of \approx 345 nm. Thus, Intralipid[®] (or the equivalent) completely misses the range of values for large VLDL and misses the lower and upper ranges for chylomicrons.

The committee also reviewed a typical path of workflow in the clinical laboratory during the preexamination (sample collection and assessment of sample quality using HIL indices), examination

(HIL detection), and postexamination (managing analyte results with interference flags) phases in which HIL is involved. This is summarized in Appendix A.

Note that the trade name Intralipid[®] is included throughout this document. It is Clinical and Laboratory Standards Institute's policy to avoid using a trade name unless the product identified is the only one available, or it serves solely as an illustrative example of the procedure, practice, or material described. In this case, the document development committee and consensus committee believe the trade name is an important descriptive adjunct to the document. In such cases, it is acceptable to use the product's trade name, as long as the words "or the equivalent" are added to the references. The examples used in C56 to demonstrate lipemia/turbidity testing and establish lipemia/turbidity indices use Intralipid[®] to simulate lipemia due to lack of standard lipoprotein preparations. It should be understood that information on this product in this guideline also applies to any equivalent products. Please include in your comments any information that relates to this aspect of C56.

Key Words

Alert indices, bias, hemolysis, icterus, lipemia/turbidity indices

Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline

1 Scope

Hemolysis, icterus, and lipemia/turbidity (HIL) indices are often measured on serum and plasma, to assess sample quality. This document offers consensus guidelines for the use of automated HIL indices by laboratories, as an aid to annotating potentially affected results as well as the rejection of a specimen or result.

This guideline is intended for use by:

- Manufacturers responsible for establishing HIL indices and alert indices (cutoff values) for use in the automated HIL detection systems in their clinical laboratory instruments
- Laboratory directors, managers, supervisors and medical technologists for establishing or evaluating HIL indices and making judgments about the acceptability of specimens and test results

C56 provides recommendations for:

- Establishing HIL indices to assess sample quality
- Estimating interference effects of hemoglobin, bilirubin, and turbidity to generate alert indices and gray zones
- Reporting (by manufacturers) interference effects of HIL in the reagent instructions for use (IFU)
- Establishing error flags for HIL interference
- Verifying HIL indices in the clinical laboratory
- Managing potential process outcomes of HIL measurements (reporting or rejecting specimens/results due to HIL interference)

This document provides a comprehensive review of the currently available automated HIL detection systems in clinical analyzers. C56 aims to enhance understanding of the mechanisms and interpretations of HIL indices and alert levels in the clinical laboratory. Procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results are not discussed in detail in C56, as these are described in CLSI document EP07.¹

2 Introduction

Prevention of medical errors is a goal of health care. The issue of medical errors that may arise from preexamination variables has received a great deal of attention.³ It has long been recognized that hemolysis, icterus, and lipemia/turbidity in patient specimens may interfere with accurate measurement of analytes (see Table 1).⁴ These preexamination variables can be attributable to *in vitro* processes, resulting from incorrect sampling procedures, transport, or storage of specimens, causing hemolyzed samples; *in vivo* physicochemical mechanisms such as the formation of chylomicrons and very low-density lipoprotein (VLDL) after food intake, causing sample lipemia/turbidity; and the presence of free

(unconjugated) and direct (conjugated) bilirubins in icteric samples. Employment of HIL indices does not solve the problem of preexamination errors; therefore, laboratories should continue to improve preexamination processes.

Ryder studied serum from outpatients and found 9.7% of specimens received contained at least one visible interferent. Of these, 76% were lipemic/turbid (probably due to nonfasting state), 16.5% were hemolyzed, and 5.5% were icteric.⁵

In a study conducted by Glick in an acute care hospital, the frequency was determined with which lipemia/turbidity, hemolysis, or icterus was encountered in serum samples. Thirty-two percent of all samples were found to have more than trace concentrations of an interferent. Of these, approximately 63% were icteric, 29% hemolyzed, and 8% lipemic/turbid.⁶

Incidence and relative frequencies of hemolysis, icterus, and lipemia/turbidity will vary depending on patient population (eg, neonatal or total parenteral nutrition patients); site practices; testing location (eg, emergency department); acquisition; and processing, storage, or transport problems on outpatient samples. More recent studies have verified that preexamination errors are the most common errors within the total testing process, and hemolysis is recognized as one of the most prevalent preexamination errors, and surely the most prevalent interference in clinical laboratory testing.⁷ Visual detection of hemolysis is arbitrary and, therefore, mostly unreliable, because it may over- or underestimate the actual severity of hemolyzed specimens.⁸

Inspection of individual specimens by laboratory technologists has been the system for detection and reporting of HIL interference for the past 30 years. However, Glick found that visual interpretation of hemolysis, lipemia/turbidity, and icterus showed very little agreement regarding the actual concentration of interferent. Even when comparison samples were used, visual grading was still problematic. He noted that because of this inconsistency, an unbiased method is recommended to accurately quantitate the level of interference.⁶

Initial visual observation of samples upon receipt in the laboratory for processing should not be totally abandoned. Detection of incorrect sample tubes and grossly hemolyzed or lipemic specimens by initial observation can initiate recollection of unacceptable specimens and reduce overall turnaround time (TAT).

The use of automated HIL indices overcomes the inherent limitations of visual estimation that have been used in the clinical laboratory for decades. This is particularly applicable in the highly automated laboratory where visual inspection is difficult due to the high volume of samples and the required speed for evaluating sample quality.

The addition of automation to the specimen inspection process can improve HIL detection by introducing harmonization and uniformity, improving quality and efficiency of laboratory processes, and, most importantly, enhancing the accuracy of reportable patient test results.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

C56-A addresses the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X EP07					

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

C56-A addresses the clinical laboratory path of workflow processes indicated by an “X.”

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
			X		X		X	

Related CLSI Reference Materials*

- EP07-A2** **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.
- M29-A3** **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

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950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: 610.688.0100 Toll Free (US): 877.447.1888 F: 610.688.0700

E: customerservice@clsi.org www.clsi.org

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