EFLM WG-PRE recommendations for managing hemolyzed samples

Ana-Maria Šimundić

Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, CROATIA

Faculty of Pharmacy and Biochemistry, Zagreb University, Zagreb, Croatia
The outline of my talk

- How frequent is hemolysis?
- What is the effect of hemolysis?
- What is wrong with visual detection of the degree of hemolysis?
- Situation in Europe?
- How reliable are manufacturer’s declarations?

- What has EFLM WG-PRE done to help?
The frequency of hemolysis?

How big is the problem?
Out of all rejected samples in a chemistry lab, hemolysis accounts for:

a) 3%
b) 10%
c) 40%
d) 60%
e) 90%
Hemolysis is the most frequent laboratory error


<table>
<thead>
<tr>
<th>Reason for Rejection</th>
<th>No. of Specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen hemolyzed</td>
<td>22 531 (59.6)</td>
</tr>
<tr>
<td>Insufficient specimen quantity to perform test</td>
<td>4 313 (11.4)</td>
</tr>
<tr>
<td>Inadequately labeled (or unlabeled) container or inadequately filled-out requisition</td>
<td>2 549 (6.7)</td>
</tr>
<tr>
<td>Specimen lost/not received</td>
<td>1 516 (4.0)</td>
</tr>
<tr>
<td>Improper collection container</td>
<td>1 337 (3.5)</td>
</tr>
<tr>
<td>Specimen clotted</td>
<td>621 (1.6)</td>
</tr>
<tr>
<td>Excessive time delay prior to centrifugation</td>
<td>503 (1.3)</td>
</tr>
<tr>
<td>Laboratory accident</td>
<td>419 (1.1)</td>
</tr>
<tr>
<td>Incorrect storage temperature prior to testing</td>
<td>330 (0.9)</td>
</tr>
<tr>
<td>Inadequate separation of serum/plasma from clot/cells</td>
<td>230 (0.6)</td>
</tr>
<tr>
<td>Specimen damaged in transit</td>
<td>144 (0.4)</td>
</tr>
<tr>
<td>Insufficient specimen quantity for proper anticoagulant-to-blood ratio</td>
<td>102 (0.3)</td>
</tr>
<tr>
<td>Specimen hemolyzed and insufficient test quantity</td>
<td>103 (0.3)</td>
</tr>
<tr>
<td>Multiple reported reasons</td>
<td>53 (0.1)</td>
</tr>
<tr>
<td>Other</td>
<td>3 082 (8.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37 833 (100)</strong></td>
</tr>
</tbody>
</table>

* The reason for rejection was unknown for 130 specimens.
Hemolysis is the most frequent laboratory error. Simundic AM, Nikolac N, Vukasovic I, Vrkic N. The prevalence of preanalytical errors in a Croatian ISO 15189 accredited laboratory. CCLM 2010;48(7):1009-14.
Frequency depends on the collection facility

Hemolysis is more frequent in collections involving syringes and catheters.

Table 1. Causes of specimen hemolysis.

<table>
<thead>
<tr>
<th>Cause</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood drawn too vigorously through</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Needle into syringe</td>
<td>155</td>
<td>30.7</td>
</tr>
<tr>
<td>Butterfly needle into syringe</td>
<td>101</td>
<td>20</td>
</tr>
<tr>
<td>Intravenous catheter into syringe</td>
<td>83</td>
<td>16.5</td>
</tr>
<tr>
<td>Infusion access into syringe</td>
<td>58</td>
<td>11.5</td>
</tr>
<tr>
<td>Catheter partially obstructed</td>
<td>35</td>
<td>6.9</td>
</tr>
<tr>
<td>Blood forced into the tube</td>
<td>26</td>
<td>5.1</td>
</tr>
<tr>
<td>In vivo hemolysis</td>
<td>9</td>
<td>1.8</td>
</tr>
<tr>
<td>Extracorporeal circulation</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>Specimen frozen</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Errors in handling</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cause unknown</td>
<td>26</td>
<td>5.1</td>
</tr>
<tr>
<td>Total</td>
<td>505</td>
<td>100</td>
</tr>
</tbody>
</table>

Hemolysis is associated with the pressure in the blood collection tube.

Figure 2: Relationship between “negative pressure” ($P_v$) of blood collections tubes and mean free hemoglobin concentrations.

The effect of hemolysis?
Assay- and instrument-specific effect


Assay- and instrument-specific effect

Ortho Eci

Roche Elecsys

How do you detect the degree of hemolysis in your lab?

(For chemistry assays)

a) Visual check
b) Automated HIL indices
c) A combination of both
What is wrong with visual detection of hemolysis?
Visual inspection is highly unreliable and inconsistent

Comparison of visual vs. automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye?

Ana-Maria Simundic*, Nora Nikolac, Valentina Ivankovic, Dragica Ferenec-Ruzic, Bojana Magdic, Marina Kvaternik and Elizabeta Topic

University Department of Chemistry, School of Medicine, Faculty of Pharmacy and Biochemistry, Zagreb University, University Hospital “Sestre Milosrdnice”, Zagreb, Croatia

Introduction

Most errors occur in the preanalytical phase of clinical laboratory testing (1, 2). Successful monitoring and management of preanalytical sources of interferences is therefore crucial to the quality of laboratory diagnostic process and to the quality of patient care. Results from hemolyzed, icteric, and lipemic samples
Comparability between visual and automated detection is poor.

Comparability between assessors is low


CONCLUSIONS:
Visual inspection of lipemic, icteric and hemolyzed samples is highly unreliable and should be replaced by automated systems that report serum indices.
Ana Helena Luksic*, Nora Nikolac Gabaj, Marijana Miler, Lora Dukic, Ana Bakliza and Ana-Maria Simundic

Visual assessment of hemolysis affects patient safety

- retrospective study
- emergency chemistry laboratory (data collected in 2015)
- visual assessment + manual handling and management
- re-assesment for all samples received in the period of 1 week
1/3 of all samples incorrectly handled


Figure 2: Percentages of correctly and incorrectly released or suppressed tests results as well as total (n = 2518) correct and incorrect handling of test results from hemolyzed samples.
Risk analysis according to ISO 14971 standard: Application of Risk Management to Medical Devices

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TnT</td>
</tr>
<tr>
<td>04</td>
<td>AST, LDH</td>
<td></td>
<td>Bil (dir)</td>
<td>Total Bilirubin</td>
<td>K</td>
</tr>
<tr>
<td>03</td>
<td></td>
<td></td>
<td>Amil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>ALT</td>
<td></td>
<td>Ca, Cl</td>
<td></td>
<td>CRP, Urea, Na, Creat</td>
</tr>
<tr>
<td>01</td>
<td>ALP, GGT</td>
<td>P, Mg, PROT</td>
<td></td>
<td>Lakt, LIP, ALB, Ethanol, CK</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

Situation in Europe?
Part 1. How do European laboratories monitor the preanalytical phase and how data from this monitoring is currently used.

Part 2. Detection and management of hemolysis, lipemia and icterus

1,405 responses from 37 European countries.

- Primary care laboratory (18%)
- Hospital laboratory (38%)
- Laboratory that serves both in- and out-patients (40%)
HIL indices are monitored by 86% of the 1,347 respondents who stated to analyze blood samples.

How do you measure the degree of hemolysis, lipemia and icterus in your lab?

- Visual detection: 30%
- Automated HIL indices: 28%
- Both: 30%
- Automated HIL indices: 42%

High proportion of labs still perform visual HIL check.

Those who perform a visual check...

Do you use a color scale for visual hemolysis detection?
(n=434)

Yes 28.6%
No 71.4%

Majority of labs do not even use a color scale for a visual HIL check

We use different cut-offs to define hemolysis

We manage hemolyzed samples differently.

We use different cut-offs to manage hemolyzed samples.
Are manufacturer’s declarations reliable? Useful? Safe?
Interfering Substances
Results of studies\(^3\) show that the following substances interfere with this Total Bilirubin procedure. The criteria for no significant interference is recovery within [10\%] of the initial value.

Hemolysis: No significant interference up to 500 mg/dL Hemolysate

(5g/L)


- Bias is **not evidence based** (biological variation/clinical significance of the test)
- Most use **10\% bias** as allowable deviation (or 3SD)
- Instead of continuous data, just a single cut-off is provided
Harmonization in hemolysis detection and prevention. A working group of the Catalanian Health Institute (ICS) experience.
Manufacturer’s declarations often cannot be verified in the lab


**Table 5** Proposed hemolysis interference limit (median per group of analyzers), (A) versus limit recommended by the manufacturer (B).

<table>
<thead>
<tr>
<th>Test</th>
<th>Hemolysis interference limit, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Advia 2400 (Siemens)</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>ALT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>AST&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6</td>
</tr>
<tr>
<td>CK&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>COL</td>
<td>&gt;6.9</td>
</tr>
<tr>
<td>P</td>
<td>2.4</td>
</tr>
<tr>
<td>FAL</td>
<td>2.4</td>
</tr>
<tr>
<td>FE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>GLU&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>GGT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>K</td>
<td>0.6</td>
</tr>
<tr>
<td>LD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>PT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>TG</td>
<td>6.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Test with different analytical methods according to the analyzers. na, not available.
Heterogeneity of manufacturers' declarations for lipemia interference — An urgent call for standardization

Nora Nikolac a,⁎,1, Ana-Maria Simundic a, Manuela Miksa a, Gabriel Lima-Oliveira b,1, Gian Luca Salvagno b, Beatrice Caruso c, Gian Cesare Guidi b,c

a University Department of Chemistry, Medical School University Hospital Sestre Milosrdnice, Vinogradska 29, 10000 Zagreb, Croatia
b Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona, 37126 Verona, Italy
Laboratory of Clinical Biochemistry and Hematology, Borgo Trento Hospital, P. le Aristide Stefani 1, 37126 Verona, Italy

The aim: to verify the manufacturers' specifications for lipemia interference for clinical chemistry reagents provided by Beckman Coulter, Roche and Siemens.
The aim: to verify the manufacturers’ specifications for lipemia interference for clinical chemistry reagents provided by Beckman Coulter, Roche and Siemens.

### Table 3
Comparison of declared and measured data on lipemia interference.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beckman Coulter AU 680</th>
<th>Cobas® 6000 &lt;c501&gt;</th>
<th>Dimension Vista System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Potassium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Chlorides</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lipase</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Iron</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>ALT</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AST</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bilirubin, direct</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Urea</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Creatinine</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Glucose</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Phosphates</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Albumin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CK-MB</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CK</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>LD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AMY</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>ALP</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>GGT</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Magnesium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Calcium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Total proteins</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CRP</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

ALP — alkaline phosphatase; ALT — alanine transaminase; AMY — amylase; AST — aspartate transaminase; CK — creatine kinase; CK-MB — creatine kinase MB isoenzyme; CRP — C-reactive protein; GGT — gamma-glutamyltransferase; LD — lactate dehydrogenase.

(✓) — Measured data confirms declared data.

(✓*) — Measured data conditionally confirms declared data (we didn’t test exact declared Intralipid® concentrations).

(+) — Underestimation of bias, i.e. declared bias value is met at higher Intralipid® concentration.

(−) — Overestimation of bias, i.e. declared bias value is met at lower Intralipid® concentration.
Did you verify hemolysis cutoffs declared by the manufacturers? (n=624)

- Yes, all of them
- Yes, some of them
- No

Majority of the labs do not verify manufacturer’s declarations

To summarize...

- Hemolysis is the most common preanalytical error.
- It affects many analytes.
- Our practices are heterogeneous and not standardized.
- This creates the risk of reporting wrong results.

Opportunity for diagnostic errors (missed, delayed or wrong diagnosis).
We have to do something about it.

*Houston, we have a problem*
Standardize the way we detect and manage hemolyzed samples

Producing and systematically using the evidence based, patient oriented guidelines, rules, and specifications
What has EFLM WG-PRE done to help?
Opinion Paper

Giuseppe Lippi*, Janne Cadamuro, Alexander von Meyer and Ana-Maria Simundic, on behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE)

Practical recommendations for managing hemolyzed samples in clinical chemistry testing


AIM: To suggest a pragmatic approach for managing results of clinical chemistry testing in hemolyzed samples.
Our proposal outlined in the rest of this document, includes recommendations on:

1. how to systematically assess serum indices,
2. how to define hemolysis index (H-index) cut-offs for flagging, alarming or suppressing tests results
3. reporting flagged or alarming tests results
4. suppressing hemolysis-sensitive test results
5. suppressing all tests results
6. correcting data for the H-index
7. including H-index data in the laboratory report


AIM: To suggest a pragmatic approach for managing results of clinical chemistry testing in hemolyzed samples.
Figure 2: Summary of practical recommendations for managing hemolyzed specimens for clinical chemistry testing.
Are you using IQC for HIL indices in your lab?

a) Yes, I use commercial controls
b) Yes, I prepare my own pool
c) No
Local quality assurance of serum or plasma (HIL) indices

Giuseppe Lippi\textsuperscript{a,*}, Janne Cadamuro\textsuperscript{b}, Alexander von Meyer\textsuperscript{c}, Ana-Maria Simundic\textsuperscript{d}, on behalf of the European Federation of Clinical Chemistry, Laboratory Medicine (EFLM) Working Group, for Preanalytical Phase (WG-PRE)

\textsuperscript{a} Section of Clinical Biochemistry, University of Verona, Verona, Italy
\textsuperscript{b} Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria
\textsuperscript{c} Institute for Laboratory Medicine, Kliniken Nordoberpfalz AG, Klinikum St. Marien, Amberg, Weiden, Germany
\textsuperscript{d} Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia

**The aim:** to provide an expert opinion about management of internal quality control (IQC) assessment for HIL indices.
- we support the use of in-house prepared IQC materials
- we give you advice on how to prepare pools for IQC materials
- at least 2 levels for each interfering substance should be used
- IQC testing for HIL indices should be performed at least 2 times per day in routine and stat laboratories
- IQC testing should be systematically recorded
- IQC should be interpreted and acted upon in the same manner as with any other IQC result
RESEARCH ARTICLE

Internal quality assurance of HIL indices on Roche Cobas c702

Giuseppe Lippi¹, Janne Cadamuro², Elisa Danese¹*, Matteo Gelati¹,
Martina Montagnana¹, Alexander von Meyer³,⁴, Gian Luca Salvagno¹*, Anna-
Maria Simundic⁵,⁶

¹ Section of Clinical Biochemistry, University of Verona, Verona, Italy, ² Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria, ³ Institute of Laboratory Medicine, Klinikum Nordoberpfalz AG, Weiden, Germany, ⁴ Institute of Laboratory Medicine, Klinikum St. Marien, Amberg, Germany, ⁵ Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia

* These authors contributed equally to this work.
* elisa.danese@univr.it
Fig 3. Stability of in-house prepared internal quality control (IQC) materials for quality assurance of HIL (Hemolysis, H; Icterus, I; Lipaemia, L) indices on Cobas c702, with target values set on the first frozen-thawed aliquot, on day 1. The continuous line is set at the target value, whilst the dotted lines define the performance goals.

Call for more transparency in manufacturers declarations on serum indices: On behalf of the Working Group for Preanalytical Phase (WG-PRE), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

Alexander von Meyer\textsuperscript{a,*}, Janne Cadamuro\textsuperscript{b}, Giuseppe Lippi\textsuperscript{c}, Ana-Maria Simundic\textsuperscript{d}

\textsuperscript{a} Institute for Laboratory Medicine, Klinikum Nordoberpfalz AG and Klinikum St. Marien, Weiden and Amberg, Germany
\textsuperscript{b} Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria
\textsuperscript{c} Section of Clinical Biochemistry, University Hospital of Verona, Verona, Italy
\textsuperscript{d} Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia

Discussion

This document aims to propose some reliable solutions that may be adopted by manufacturers for increasing worldwide harmonization of serum indices.
1.3.1. Assessment of indices

Data obtained by the manufacturers during the process of validating the impact of serum indices on laboratory test results are necessary and of utmost importance for medical laboratories [21]. Manufacturers should therefore ideally provide the following information about assessment of serum indices:

a) Wavelengths used for measuring each serum index
b) Calculating formula
c) Sensitivity, linearity and range of measurement
d) Sample volume and type (e.g., serum or plasma)
e) Sample buffer for blank measurement and for sample dilution
f) Traceability of hemolysis and icteric indices to the concentration of free hemoglobin and total bilirubin, respectively
g) A possible correlation between lipemic index and triglycerides concentration should also be made available.
1.3.2. Reporting of the indices

a) Manufacturers should report all results of serum indices as continuous values.
b) All results should be transferable to the LIS on a separate channel.

All indices must be preferably reported as continuous, quantitative values. Semi-quantitative measurements (i.e., based on interval scales) may not be suitable for optimal interpretation of interference. All interval (ordinal) scales are not suitable for reliably monitoring sample quality, for taking decisions about releasing or suppressing a potentially biased test result, or for making comparison (e.g. EQA). All results of the indices should then be transferable into the LIS along with routine transmission of conventional test results, so that they could even be included in the laboratory report, according to local standard operating procedures (SOPs). The interface structure of a normal assay transmission should be used.
The outline of my talk

- How frequent is hemolysis?
- What is the effect of hemolysis?
- What is wrong with visual detection of the degree of hemolysis?
- Situation in Europe?
- How reliable are manufacturer’s declarations?

- What has EFLM WG-PRE done to help?
“Amid all of the pressing priorities, we must remember that the elimination of harm to our patients and workforce is our foremost moral and ethical obligation.”

Gary S. Kaplan, MD, FACMPE
Charles D. Stokes, RN, BSN, FACHE
Co-Chairs of the Leading a Culture of Safety Project

Arthur Ashe was the first black tennis player who was selected to the United States Davis Cup team and the only one ever to win the singles title at Wimbledon, the US Open, or the Australian Open.

"Start where you are. Use what you have. Do what you can."

– Arthur Ashe

https://due.com/blog/start-where-you-are-arthur-ashe/