Practices for Identifying and Rejecting Hemolyzed Specimens Are Highly Variable in Clinical Laboratories

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• Context.—Hemolysis is an important clinical laboratory quality attribute that influences result reliability.

Objective.—To determine hemolysis identification and rejection practices occurring in clinical laboratories.

Design.—We used the College of American Pathologists Survey program to distribute a Q-Probes-type questionnaire about hemolysis practices to Chemistry Survey participants.

Results.—Of 3495 participants sent the questionnaire, 846 (24%) responded. In 71% of 772 laboratories, the hemolysis rate was less than 3.0%, whereas in 5%, it was 6.0% or greater. A visual scale, an instrument scale, and combination of visual and instrument scales were used to identify hemolysis in 48%, 11%, and 41% of laboratories, respectively. A picture of the hemolysis level was used as an aid to technologists’ visual interpretation of hemolysis levels in 40% of laboratories. In 7.0% of laboratories, all hemolyzed specimens were rejected; in 4% of laboratories, no hemolyzed specimens were rejected; and in 88% of laboratories, some specimens were rejected depending on hemolysis levels. Participants used 69 different terms to describe hemolysis scales, with 21 terms used in more than 10 laboratories. Slight and moderate were the terms used most commonly. Of 16 different cutoffs used to reject hemolyzed specimens, moderate was the most common, occurring in 30% of laboratories. For whole blood electrolyte measurements performed in 86 laboratories, 57% did not evaluate the presence of hemolysis, but for those that did, the most common practice in 21 laboratories (24%) was centrifuging and visually determining the presence of hemolysis in all specimens.

Conclusions.—Hemolysis practices vary widely. Standard assessment and consistent reporting are the first steps in reducing interlaboratory variability among results.

In this study, we sought to quantify the frequency of hemolyzed specimens, to identify the methods for detecting the presence and amount of hemolysis, to characterize the current responses of laboratory personnel to the appearance of hemolysis, and to suggest practice improvements.

MATERIALS AND METHODS

Members of the College of American Pathologists (CAP) Quality Practices Committee developed an online questionnaire, in the usual CAP Q-Probes format, which was sent to participants in the 2011 CAP C1, C3, C3X, CZ, CZX, CZ2, and CZ2X chemistry surveys. One reminder notice was sent to nonrespondents encouraging participants to complete their questionnaires and return them to the Q-Probes CAP committee staff, who then tabulated the results according to previously established Q-Probes procedures.7 The questionnaire contained 28 multiple-choice questions, with “other, please list” as a choice in 7 questions. If participants did not answer specific questions, they were excluded from the data only for the question(s) they did not answer.

RESULTS

Of the 3495 CAP customers enrolled in the Chemistry Surveys, 846 (24%) responded. Seen in Figure 1 are hemolysis rates for 772 laboratories. In 276 laboratories (36%), the overall hemolysis rate was less than 1%; in 267 laboratories (35%), it was 1% to 2.9%; and in 8 laboratories (1%), it was 15.0% or greater. In 71 laboratories (9%), personnel were unsure of the overall hemolysis rate.

A visual scale of evaluating hemolysis was used in 369 of 779 (48%) of the responding clinical laboratories, an instrument scale was used in 87 laboratories (11%), and both visual and an instrument scales were used by 323 (41%) of responders. As shown in the Table, when visually grading a specimen for hemolysis, a picture of the degree of hemolysis was used to classify each specimen in 271 of 680 laboratories (40%); however, the visual grading of specimens for hemolysis was included as part of the clinical laboratories’ competency assessment program in 120 of 678 laboratories (18%). In 64 of 756 laboratories (8%), a manual procedure, such as placing a drop of specimen on gauze, followed by visual inspection, was used to differentiate hemolysis from icterus in difficult cases. Of 748 participants, 269 (36%) forwarded automated hemolysis flags that accompany hemolyzed results from their major chemistry instrument to the patients’ medical record. For all analytes measured on their primary chemistry analyzers in 777 laboratories, 632 laboratories (81%) used the same hemolysis scales, 495 of 672 (74%) used the same hemolysis scales surveys.

<table>
<thead>
<tr>
<th>Procedures Used in Identification of Hemolysis</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Total, No.</td>
</tr>
<tr>
<td>When visually grading a specimen for hemolysis, is a picture of the degree of hemolysis used to classify each specimen?</td>
<td>680</td>
</tr>
<tr>
<td>Is the visual grading of specimens for hemolysis included as part of your laboratory’s competency assessment program?</td>
<td>678</td>
</tr>
<tr>
<td>Are any manual procedures used to identify the presence of hemolysis in difficult cases (ie, place a drop of specimen on gauze, inspect the color and differentiate between hemolysis and icterus [bilirubin])?</td>
<td>756</td>
</tr>
<tr>
<td>Is your laboratory able to forward automated hemolysis flags that accompany hemolyzed results recorded on your major chemistry instrument to the patients’ medical records?</td>
<td>748</td>
</tr>
<tr>
<td>Does your laboratory use automated result verification for the primary chemistry analyzer?</td>
<td>830</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry analyzer?</td>
<td>777</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry and immunoassay analyzers?</td>
<td>723</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry analyzer and your backup chemistry analyzer?</td>
<td>672</td>
</tr>
<tr>
<td>Does your laboratory systematically and regularly monitor the percentage of hemolyzed specimens as part of your laboratory’s performance improvement plan?</td>
<td>692</td>
</tr>
</tbody>
</table>
on their primary and secondary chemistry analyzers, and 503 of 723 (70%) used the same hemolysis scales on the primary chemistry analyzer and immunoassay analyzers. In a few laboratories (325 of 692; 47%), hemolysis was systematically and regularly monitored as part of the laboratory’s performance improvement plan.

In most laboratories (630 of 710; 88%), specimens were rejected depending on the level of hemolysis. However, in 50 clinical laboratories (7.0%), all hemolyzed specimens were rejected, and in 25 clinical laboratories (4%), no hemolyzed specimens were rejected.

Sixty-nine specific terms were used to describe hemolysis: 21 terms were used commonly with each term used in at least 10 participating laboratories to classify hemolysis. Slight, moderate, gross, and marked were used in 389, 389, 326, and 161 of 707 laboratories (55%, 55%, 46%, and 23%), respectively (Figure 2). Of the responders, 127 (18%) indicated they used all 3 modifiers, slight, moderate, and gross in their descriptive scales. The most commonly used numeric terms were 1, 2, 3, and 4 used in 195, 188, 187, and 170 laboratories (28%, 27%, 26%, and 24%), respectively. In the remaining 56 laboratories (8%), 48 additional words, numbers, or phrases described hemolysis.

Figure 3 provides a list and the percentages for 20 cutoff values used to describe specimens rejected for hemolysis in 597 laboratories. In those laboratories, 215 (36%) used a numeric level, and 334 (56%) used a descriptive word to describe the cutoff for specimen rejection. The most common cutoffs that resulted in rejection were moderate hemolysis, used in 182 laboratories (30%), followed by gross hemolysis, which was used in 66 laboratories (11%). The category other was used in 43 additional laboratories (7.2%), where a unique term or additional modifier(s) was added to one of the 20 other terms described in Figure 3. No laboratories used 6 for rejection, although one laboratory used “greater than 6.”

Figure 4 summarizes practices to detect hemolysis in whole blood specimens in 91 clinical laboratories that use whole blood analyzers not at the point-of-care. Approximately one-half of those using whole blood analyzers measured glucose, potassium, sodium, and chloride, whereas about one-third of those with whole blood systems measured calcium as well as other analytes. Of the 86 laboratories that used whole blood electrolyte analyzers, 48 (56%) did not determine whether specimens were hemolyzed. The most common method, used by 18 (21%) of these laboratories, was...
respondents to determine hemolysis, was to centrifuge all whole blood specimens after measurement and then visually determine whether hemolysis was present. In a few clinical laboratories (9 of 86; 10%), personnel stored and then visually inspected all specimens within an hour after measurement to determine whether hemolysis was present. In 5 (6%) of the clinical laboratories, only when potassium was elevated were specimens either centrifuged (3 or 3%) or stored for up to an hour and then visually inspected for hemolysis (2 or 2%). When hemolysis was detected in any of these circumstances, then a comment was added to the quantitative result indicating the specimen was hemolyzed.

**COMMENT**

The overall hemolysis rate in 772 laboratories varied greatly, ranging from less than 1% in 36% of laboratories (n = 276) to 15% or more in 1% of laboratories (n = 8). In more than 70% of laboratories (n = 543), the hemolysis rate was less than 3%, and 9% (n = 71) were unsure of their overall hemolysis rates. This contrasts with a 1997 Q-Probes report in which 0.2% of more than 10 million specimens in 453 clinical laboratories were rejected for hemolysis. A number of participants commented that the hemolysis rates were highest in specimens from their emergency departments; similar findings were described in a number of studies reported in the literature, with hemolysis rates of 3.4%, 12.8%, 12.9%, 4, and 19.8%. It is not surprising to us that in 9% of clinical laboratories (71 of 772), personnel did not know what their overall hemolysis rates were, as common practice is only to monitor and reduce the rates at specific patient locations such as emergency departments where rates are highest.

Some laboratories rejected all hemolyzed specimens. This policy potentially places certain patients at risk such as the case reported by Ismail and colleagues, who described a patient with renal failure and unrecognized intravascular hemolysis who died from the presumed arrhythmia of hyperkalemia because their clinical laboratory had a policy of not measuring potassium values when specimens were hemolyzed. Although the World Health Organization currently recommends that potassium results not be reported when specimens are hemolyzed, we strongly suggest that the laboratory community revisit this issue with consideration of reporting results qualified by noting the presence and degree of hemolysis and an assessment of the effect it may have on the test result, allowing clinicians responsible for the patient’s care to integrate this information into the differential diagnosis and subsequent treatment or to repeat the analysis on another specimen obtained by techniques minimizing in vitro hemolysis.

Most laboratories still used visual scales to grade hemolysis, despite most chemistry analyzers used in central hospital laboratories now measuring hemolysis. Visual estimations of hemolysis are unreliable, and automated methods are preferable. In addition, charts that are used for visual estimation of hemolysis fade, leading to erroneous interpretations. In most laboratories, the description of hemolysis cannot be sent automatically to the patient’s medical record, thereby underestimating the frequency of reporting because technologists working in fast-paced chemistry laboratories could easily forget to perform nonautomated tasks, such as adding a modifier to a patient’s medical record result. Because these hemolysis results cannot be sent automatically to the laboratory information system, these laboratories are probably prevented from using an automated verification system. There is a second consideration regarding nonautomated assessment of hemolysis. Although personnel performing laboratory tests in the United States are required to be checked for color blindness, only 120 of 678 laboratories (18%) evaluated clinical laboratory employees for competency in visually evaluating hemolysis.

Within laboratories, practices for identifying and reporting hemolysis also appeared to vary greatly. Fourteen percent of laboratories (105 of 777) did not use the same hemolysis scale on their primary and secondary chemistry analyzer, and in 23% of clinical laboratories (165 of 723), different hemolysis scales were used on the primary chemistry analyzer and the primary immunoassay analyzer. Different scales emanating from the same laboratory are bound to confuse clinicians. Other examples of variability in hemolysis procedures within the same laboratory included specific reporting procedures that changed according to the analyte measured.

Explanations of hemolysis in some textbooks suggest that quantitative measurements be used to describe hemolysis, and in the United States, these results frequently are reported in milligrams per decaliter. However, in the classic manuscript, Glick et al used 6 numeric, semiquantitative categories, each followed by a plus sign: 0, contains no or trace interferent and 1+, 2+, 3+, 4+, or 5+. Other workers used descriptive terms that approximately correspond with 7 reportable, numeric categories, such as none (0), none to slight (1), slight (2), slight to moderate (3), moderate (4), moderate to gross (5), and gross (6). Some publications relate descriptive terms to quantitative measurements, such as the use of a scale such as mild (1 g/L), moderate (2.5 g/L), and severe (5 g/L) by Hawkins or the use of slight (50 mg/dL), moderate (100 mg/dL), and gross (200 mg/dL) by Spencer and Roger. Our sampling confirmed the varied classification with 69 different terms, numbers, or combinations of numbers and terms used. Some newer major chemistry analyzers allow users to decide to implement a feature of a quantitative, continuous scale throughout the measurement range to describe hemolysis.

In our experience, we have found that laboratorians try to simplify nonautomated processes by having the same rule for all measurements performed on the same specimen type. Hence, it was not surprising to us that most laboratories
(74%; 495 of 672) used one hemolysis level to reject or accept specimens for all measurements. We found in approximately 30% of laboratories (182 of 597), specimens were rejected when hemolysis reached moderate levels, and 11% (66 of 597) when hemolysis levels were gross. For those laboratories using a numeric scale, the most common discrimination level for rejection or acceptance was 2, but that level was used only in 9% (54 of 597) of the laboratories.

Whole blood measurements have gained favor when speedy analyses are required, but because red blood cells are distributed throughout these specimens, it is impossible to detect whether some of the red color in whole blood specimens is from hemolysis. Hawkins35 studied the hemolysis frequency in whole blood gas analysis in his clinical laboratory and found that hemolysis was more than 5 times higher in whole blood used for blood gases compared with serum and that 42% of potassium results in these specimens were downgraded from normokalemic to hypokalemic or from hyperkalemic to normokalemic or hypokalemic. The Hawkins36 data provide support in critical whole blood potassium measurements for the need to determine whether hemolysis is present.

There is no quick and reliable way to determine whether hemolysis is present in whole blood specimens. In most laboratories (56%; 48 of 86) in our study using whole blood analyzers, laboratory personnel did not take additional steps to detect whether whole blood specimens were hemolyzed. Of the few who evaluated hemolysis in whole blood, the most common practice among 21% of responders was to centrifuge all whole blood specimens and then visually examine whether the supernatant was red. The second most common practice (10%) was to store the whole blood specimen for up to an hour allowing the red blood cells to settle and then visually noting whether hemolysis was present. When potassium was elevated, some laboratories identified hemolysis in whole blood specimens by storing the whole blood specimen upright for up to an hour and then visually examined the specimens for hemolysis (3%), or inspecting other specimens obtained at the same time for hemolysis (3%).

One obvious disadvantage of identifying hemolysis in whole blood specimens is the time required. Visual inspection required up to an hour before red blood cells fully settled or required 10 minutes when separation was hastened by centrifugation. Waiting for these procedures to occur before visual inspection defeats the main objective of rapid whole blood testing result reporting.

A second issue with identifying hemolysis in whole blood specimens is that visual estimation of the amount of hemolysis has been unreliable because experienced technologists cannot accurately distinguish between various concentrations of hemolysis.6,12,13 A third issue is whether or not hemolysis affects whole blood assays other than potassium results. Whole blood measurements that are commonly performed include sodium, calcium, magnesium, and bilirubin. Effect on these measurements in hemolysed whole blood would not always be detected if clinical laboratory procedures require evaluation of hemolysis only when potassium was elevated. A potential solution alerting physicians to hemolysis in a timely manner is to automatically quantitate hemolysis or free hemoglobin in a whole blood specimen at, or before the time of, measurement of the whole blood specimen. At this time, there are no US Food and Drug Administration–approved methods or instruments available that can make such a measurement.

Widespread use of multianalyte, whole blood point-of-care devices, such as the Abbott iSTAT (Abbott Laboratories, Abbott Park, Illinois) and the Alera epoc (Alera Inc, Waltham, Massachusetts) blood gas analyzers, as well as single-test whole blood devices, including glucose meters, are subject to hemolysis interferences, but based on their use at locations where no centrifuges are located, procedures used in clinical laboratories to identify hemolysis are not applicable. The only useful procedure is that if a result is unexpected and it could have been influenced by hemolysis, a serum specimen should be sent to the clinical laboratory for remeasurement.

Quantitative hemoglobin measurements are some of the most common and accurate measurements made in hospital clinical laboratories because hemoglobin routinely is measured by arterial blood gas instruments, complete blood cell count analyzers, co-oximeters, pulse oximeters, and many point-of-care devices. There is even a fourth edition of reference methods for automated hemoglobin measurements in human blood.39 Despite these resources, laboratory personnel continue to use inaccurate visual methods to estimate hemolysis and report hemolysis using poorly reproducible terms that confuse clinicians. Finally, most laboratories continue to accept or reject specimens for measurement of various analytes using unscientific principles.

We certainly agree with van Rheenen and de Moor39 that one place where visual estimates of hemoglobin may be the best that can be achieved is in resource-poor countries where no automated analyzers are available. In developed countries, where clinical laboratory instruments that quantify hemoglobin accurately are in the same clinical laboratory, it is time for laboratory personnel to standardize policies and procedures for measurement and reporting of hemolysis. These policies and procedures should include quantification of hemoglobin on a method-by-method basis.

Until standard assessment and reporting occur, variability among results from identical hemolysed patient specimens measured in different clinical laboratories will continue. Besides standard reporting and consistent assessment of interferences, we also recommend that instrument manufacturers consider developing whole blood analyzers that simultaneously measure hemolysis.

References


