Opinion Paper

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Practical recommendations for managing hemolyzed samples in clinical chemistry testing

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Abstract: We suggest here a pragmatic approach for managing results of clinical chemistry testing in hemolyzed samples collected from adults/older children, attempting to balance the need to produce quality laboratory data with clinical urgency of releasing test results. Automatic measurement of the hemolysis index (H-index) in serum or plasma is highly advisable, whilst low-quality assessment of this test remains less good than a visual inspection. Regarding its practical use, when the H-index value does not generate an analytically significant bias, results can be released, whilst when the value is associated with analyte variation in a range between analytically and clinically significant bias (i.e. variation does not exceed the reference change value [RCV]), results of hemolysis-sensitive tests can be released in association with a comment describing the direction in which data are potentially altered, suggesting the need to collect another sample. When the H-index is associated with analyte variation exceeding clinically significant bias (i.e. variation exceeds the RCV), results of hemolysis-sensitive tests should be suppressed and replaced with a comment that biased results cannot be released because the sample is preanalytically compromised and advising the recollection of another sample. If H-index values reach an even higher critical cut-off (i.e. H-index corresponding to a cell-free hemoglobin concentration ≥10 g/L), all laboratory data may be unreliable and should hence be suppressed and replaced with a comment that all data cannot be released because the sample is grossly hemolyzed, also suggesting the recollection of another sample. Due to inaccuracy and imprecision, the use of corrective formulas for adjusting data of hemolysis-sensitive tests is discouraged.

Keywords: hemolysis; interference; quality; safety.

Introduction

The preanalytical phase is an essential part of the total testing process along with both the analytical and postanalytical phases [1]. Solid evidence has been provided that the vast majority of diagnostic errors emerge from the many and still largely manually-intensive activities related to collection, handling and transportation of biological samples [2]. Among the various preanalytical problems, poor sample quality remains central [3]. More specifically, spuriously hemolyzed specimens are still the prevailing sources of unsuitable samples in clinical laboratories worldwide, with a frequency that is 5- to 10-fold higher than that of other conditions needing suppression of test results [4]. This is mainly due to the fact that breakdown of red blood cells (RBCs) and subsequent release of hemoglobin and other intracellular components in serum or plasma cause a kaleidoscope of biological, chemical and spectrophotometric interferences, thus finally often making test results unreliable [5].

Understandably, the practice of test results suppression in unsuitable samples is associated with many clinical (i.e. delayed diagnosis), economic (i.e. incremental costs needed for recollecting blood samples) and organizational (i.e. litigation with clinicians) consequences [6, 7]. In the past, several proposals on how to manage unsuitable specimens have been published [8, 9]. Nevertheless,

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an agreed harmonized strategy for managing unsuitable samples across different laboratories worldwide, is still lacking. The previously mentioned evidence, combined with the dramatic lack of harmonized strategies, have catalyzed a passionate debate about the most appropriate and reliable strategy for dealing with preanalytically altered laboratory test results, especially of those obtained in spuriously hemolyzed samples [10–13].

Bilirubin and lipids are also long since known as interfering substances, by altering the color or turbidity of the sample [14, 15], and causing interference due to spectrophotometric mechanisms, volume depletion effects, partitioning of the sample and through physicochemical mechanisms (lipemia), or through spectrophotometric mechanism and chemical interference of bilirubin (icterus) [5]. Although hemolysis mostly occurs during blood sampling and transportation, lipemia and icterus are associated with patient status and characteristics, so that they cannot be simply solved by repeated blood sampling. Moreover, due to different mechanisms through which interference occurs, hemolysis, icterus and lipemia cannot be looked at and managed as being the same phenomena. Interference from lipemia and icterus will thus not be discussed in this article, and their practical management in the laboratory will be analyzed in another specific document.

Therefore, the aim of this article is to provide recommendations for managing the results of clinical chemistry testing obtained in hemolyzed samples collected from adults/older children. Our proposal is a pragmatic approach, tentatively balancing the need to preserve quality of testing with the clinical urgency of releasing test results, especially when they are for patients with critical or life-threatening disorders. Moreover, the discussion on practical management of unsuitable samples in other areas of diagnostic testing (i.e. immunochemistry, coagulation, hematology, molecular biology and point-of-care testing) is outside of the scope of this article because the quality of published evidence about interference is still low beyond clinical chemistry. Moreover, type of samples, analytical techniques and clinical implications are considerably different.

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

1. Systematic assessment of the serum indices (H-index)

Recommendations:

1.1 Visual assessment and management of hemolysis level, based on individual, non-standardized subjective opinion, is strongly discouraged and should be replaced with automated detection and management systems.

1.2 Systematic automated measurement of the H-index in all serum and plasma samples and implementation of automated algorithm-based decision making rules is a strongly recommended approach for managing unsuitable specimens.

1.3 In facilities where systematic automated measurement is unavailable, visual assessment of hemolysis should be done by comparing coloration of the sample with a color chart (e.g. a photo with serum or plasma sample containing different cell-free hemoglobin values).

1.4 Laboratories where systematic automated measurement is unavailable are urged to implement automated H-index measurement for patient safety reasons.

The assessment of sample quality before testing is a mainstay of either routine or urgent laboratory diagnostics, as also endorsed by international organizations such as the International Organization for Standardization (ISO) 15189:2012 [16–18] and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [19, 20]. Although consensus has been reached that the importance of this activity is unquestionable, sample quality has for a long time been assessed by visual inspection of serum or plasma, with the aim of identifying hemolysis, turbidity or icterus. Nevertheless, several lines of evidence now attest that visual sample inspection has too many drawbacks (i.e. inaccuracy, imprecision, highly dependent upon the operator) [21], and may also jeopardize patient safety [22]. It has also been shown that not only visual detection, but also the decision on whether or not to report the test results from hemolyzed samples, may vary substantially between individuals when based on individual judgment [23–25]. To minimize errors associated with such practice, visual assessment of hemolysis level and management of hemolyzed samples based on individual, non-standardized subjective opinion is thus strongly discouraged, should no longer be performed and shall be replaced with automated detection and management systems [26]. In fact, automatic assessment of serum or plasma indices in preanalytical and clinical chemistry platforms not only provides a much

more precise, accurate and reproducible estimation of potential interference, but is also easy and inexpensive. Moreover, automated systems do not affect the turnaround time and are the only means for identification of unsuitable samples and direct data transfer to the laboratory information system (LIS) for laboratories using total laboratory automation [27]. Therefore, systematic automated measurement of the H-index in all serum and plasma samples and implementation of automated algorithm-based decision making rules have meaningless impact on laboratory budgets and are strongly recommended for managing unsuitable specimens. In facilities where the H-index is unavailable, we still support visual assessment of hemolysis by comparing sample hue with a color chart in order to maintain some sort of standardization. However, we urge these laboratories to implement automated H-index measurement for patient safety reasons.

2. Defining H-index cut-offs for flagging, alarming or suppressing tests results

Recommendations:

2.1 Test-specific cut-offs of the H-index above which an analytically significant bias may occur can be either adopted from manufacturers' assay sheets (when available), or can be locally calculated.

2.2 Analytically significant bias (i.e. analytically acceptable imprecision) for each test should be derived from available data in accordance with the consensus recommendations of the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), and its proposed hierarchy for establishing analytical specifications.

2.3 If quality specifications can be estimated from biological variability data, those may be either obtained from biological variation databases or from the available literature.

2.4 Clinically significant bias should be expressed as a reference change value (RCV).

Most manufacturers include in their assay sheets a specific documentation about the potential interference of cell-free hemoglobin on clinical chemistry tests, also providing instrument-specific cut-offs above which tests results are thought to be analytically biased. Although these thresholds have not been calculated according to the concept of "clinically significant" bias, they are still valid for establishing when the analytical quality specifications are no longer met.

When test-specific cut-offs of the H-index above which an analytically significant bias may occur cannot be adopted from manufacturers' assay sheets because they are either not available or are not applicable, a laboratory shall define its own cut-off for the analytically significant bias for each test. Specifications for significant analytical bias (i.e. analytically acceptable imprecision) should be derived from available data in accordance with the consensus recommendations of the 1st Strategic Conference of the EFLM, and its proposed hierarchy for establishing analytical specifications, which entails outcome studies, biological variation and state of the art [28, 29]. If quality specifications can be estimated from biological variability data, these may be either obtained from reliable sources, such as in the database developed by Carmen Ricos and her coworkers (and hosted on Westgard's website) or from the European Biological Variation Study (EuBIVAS) database, as well as from other scientific publications [30–32].

We recommend that clinically significant bias is expressed as RCV. The RCV which is also known as critical difference, is an objective tool for assessing the significance of differences in serial laboratory data obtained from the same subject. Originally proposed by Harris and Yasaka [33] in 1983, the RCV was then largely endorsed by Fraser [34] in the following years. The RCV is based on a specific formula, incorporating both within-subject biological variation (CV_I) and analytical variation (CV_A). Terms and symbols used to define the components of biological variation and other related aspects were recently proposed by Simundic et al. and endorsed by the EFLM Working Group for Biological Variation (WG-BV) [35, 36], as shown in Figure 1A.

A

$$RCV = \sqrt{2} * 1.96 * \sqrt{(CV_I)^{\wedge}2 + (Cv_A)^{\wedge}2}$$

RCV, Reference Change Value
CV_I, within-subject biological variation
CV_A, Analytical imprecision

B

$$CV_A = \frac{CV_A\ QC\ Level_1 + CV_A\ QC\ level_2 + \ldots\ CV_A\ QC\ Level_n}{n}$$

QC, Quality Control material

Figure 1: Calculation formula of the reference change value (A) and the analytical imprecision (B) of laboratory tests.

The RCV has become commonplace and almost irreplaceable in laboratory medicine, when establishing whether or not a certain variation of a measurable analyte can be considered "significantly" different from either the homeostatic point or from a previous value [37]. The application of RCV is a straightforward concept for identifying H-index cut-offs above which hemolysis-sensitive tests are plagued by "clinically significant" bias. Notably, this approach was developed and validated more than 10 years ago by Vermeer et al. [38], and has since been employed in many other subsequent investigations [39–42].

Importantly, whilst CV_I is conventionally considered to be rather constant in healthy individuals, the CV_A can vary widely using different analytical techniques, methods and reagents [34]. This is why each laboratory should preferably calculate and use its own RCV, on a local basis, with the same instrumentation and reagents used for measuring the H-index. Data collected with internal quality controls (IQA) or external quality assessment (EQA) programs can be reliable sources for this information. In these cases, as multiple levels of quality control materials are conventionally measured, the value of CV_A used for the RCV can be calculated as the mean CV_A obtained from the different levels of quality control materials tested (Figure 1B).

For laboratories seeking to perform local experiments for defining interference cut-offs, the whole procedure must be carried out with accurate procedures (e.g. calculating the bias in serum or plasma samples containing scalar amounts of cell-free hemoglobin), and results should then be stored until there is a change in reagents or instrumentation. The advantages and limitations of the various techniques for preparing hemolyzed specimens and performing interference studies have been previously discussed elsewhere [43, 44].

3. Reporting flagged or alarming tests results

Recommendations:

3.1 Test results measured in samples where H-index values are associated with a bias ranging between analytically and clinically significant cut-offs should be reported to the requesting clinicians.

3.2 If test result is reported from a hemolyzed sample (irrespective of being within the reference range or not), the laboratory report should be accompanied by a comment stating: "Value possibly decreased/increased by hemolysis. Consider recollecting another sample".

3.3 If test result is reported from a hemolyzed sample (irrespective of being within reference range or not), a comment can be either placed immediately below the numerical value, or added as a note at the end of the laboratory report.

3.4 Comments should always provide a clear indication of the direction in which the test result is potentially biased (i.e. increased or decreased).

Test results measured in samples where H-index values are associated with a bias ranging between analytically and clinically significant cut-offs should be reported to the requesting clinicians, as (i) this result will not likely trigger inappropriate care, because such a variation is still clinically acceptable and (ii) timely communication of test results in which bias is not clinically relevant, may often be necessary for establishing the most rapid and appropriate therapeutic measures, especially in patients with critical or life-threatening conditions. Potassium is the most paradigmatic example. Failure to communicate hyper- or hypokalemia measured in mildly hemolyzed samples is probably worse than suppressing a non-clinically biased data, as this can lead to delayed treatment of a potentially serious disorder.

Therefore, whenever H-index values of the specimen are associated with a bias ranging between the analytically and clinically significant cut-offs, test results can be added to the laboratory report accompanied by a specific comment like "Value possibly decreased/increased by hemolysis. Consider recollecting another sample". The comment can be either placed immediately below the numerical value, or added as a note at the end of the laboratory report. Both solutions seem suitable. In those cases where comments on laboratory reports tend to be ignored, missed or overlooked, it might be safer to replace test results with a specific sentence (e.g. "see comment") and providing the value within the comment section along with information on the hemolysis level. The appropriate way of displaying test results and interference information has to be decided individually for each health care setting based on patient safety risk analysis. Moreover, it seems advisable to always describe the direction in which the test result is potentially biased (i.e. increased or decreased), as this will help clinicians to more appropriately interpret the data (Figure 2).

4. Suppressing hemolysis-sensitive test results

Recommendations:

4.1 Results of hemolysis-sensitive tests measured in samples when H-index values are associated with a bias exceeding the clinically significant cut-offs

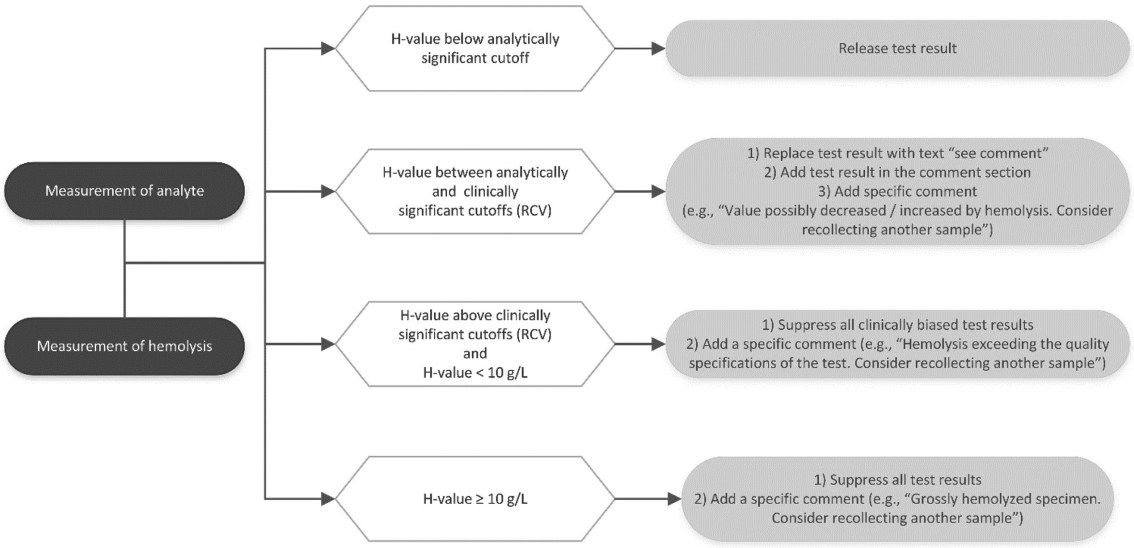

Figure 2: Summary of practical recommendations for managing hemolyzed specimens for clinical chemistry testing.

calculated according to the test-specific RCV should be suppressed.

4.2 Instead of a result, a comment should be added to the test report, stating: "Hemolysis exceeding quality specifications of the test. Consider recollecting another sample".

4.3 Test results measured on samples, for which H-index values are not associated with a bias exceeding the clinically significant cut-offs, should be reported to the requesting clinician (as already defined under 3.1–3.4).

Whenever the H-index values of the specimen are associated with a bias exceeding the clinically significant cut-offs calculated according to the test-specific RCV, the results of hemolysis-sensitive tests should be suppressed and replaced by a comment like "Hemolysis exceeding quality specifications of the test. Consider recollecting another sample" (Figure 2).

5. Suppressing all tests results

Recommendations:

5.1 In samples with >10 g/L of cell-free hemoglobin, all results of clinical chemistry testing should be suppressed and another sample should be requested.

5.2 If test results from a grossly hemolyzed sample (>10 g/L of cell-free hemoglobin) are suppressed, the laboratory report should be accompanied by a comment stating: "Hemolyzed specimen. Consider recollecting another sample".

The vast majority of studies have tested hemolysis interference up to 10 g/L of cell-free hemoglobin and no definitive evidence is available about the possible bias of clinical chemistry analytes above this threshold. Moreover, serum or plasma samples with such a high degree of hemolysis are not commonly observed in clinical laboratories [45]. Therefore, due to the lack of precise knowledge about the analytical and clinical consequences of bias in samples with higher degrees of hemolysis, it seems appropriate and more prudent to suggest that all results of clinical chemistry testing should be suppressed in these samples and another sample should be requested, using a comment like "Hemolyzed specimen. Consider recollecting another sample" (Figure 2). Notably, the injury of all blood cells and endothelia occurred during sample collection in serum or plasma grossly hemolyzed is probably so high that the vast majority of tests results will be thoroughly unreliable [46]. An overview of analytical and clinical significant cut-offs including the decisions on whether or not to release the test result with/without a comment are depictured in Figure 3.

6. Correcting data for the H-index

Recommendation:

6.1 Using corrective formulas for adjusting test results of hemolysis-sensitive tests from the serum or plasma concentration of cell-free hemoglobin is inaccurate and misleading, and is strongly discouraged.

Another approach for managing test results in hemolyzed samples has been proposed, entailing either correction of

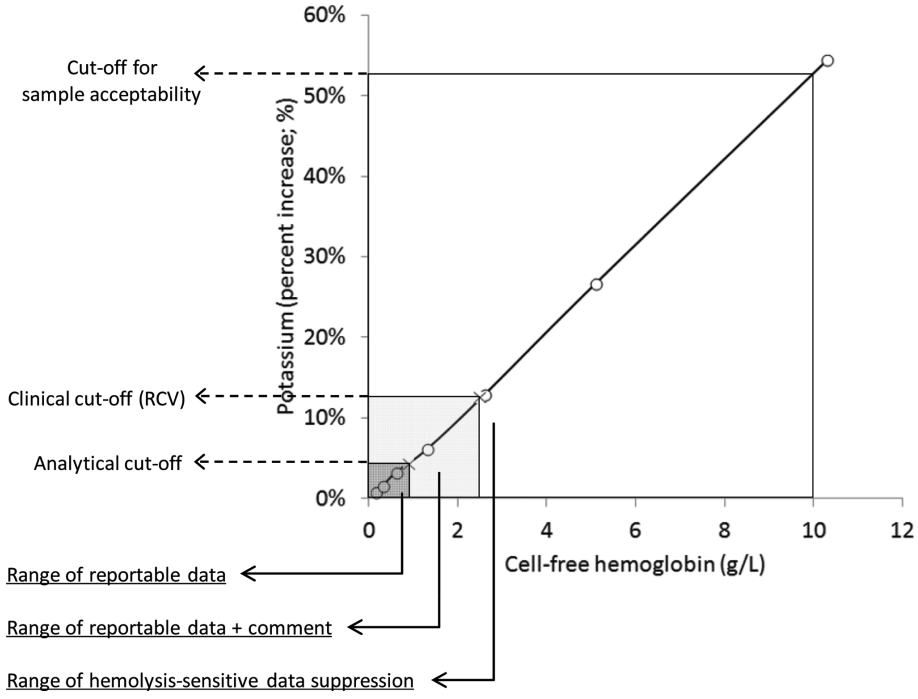

Figure 3: Interferogram, showing analytical and clinical significant decision limits.
RCV, reference change value.

data of hemolysis-sensitive tests for the H-index or including comments about the possible bias estimated from the serum or plasma concentration of cell-free hemoglobin [47–49]. Nevertheless, independent studies showed that this practice should be highly discouraged as spurious hemolysis is not homogeneous and sufficiently predictable in different subjects (i.e. the biological source of interference has an extremely wide inter-individual variation) [50, 51], and is also strongly dependent on some biological characteristics of RBCs (i.e. volume, size heterogeneity, etc.) and mechanism of interference [52]. Therefore, using corrective formulas for adjusting test results of hemolysis-sensitive tests from serum or plasma concentration of cell-free hemoglobin is inaccurate and misleading, and we advise against routinely using this strategy.

7. Including H-index data in the laboratory report

Recommendation:

7.1 Degree of hemolysis should be converted from the instrument-specific arbitrary units into g/L of cell-free hemoglobin to improve harmonization.

7.2 Degree of hemolysis (expressed as g/L of cell-free hemoglobin) should be provided in the laboratory test report.

7.3 Clinical laboratories should use control materials specific for the H-indices for continuously monitoring the analytical performance of the H-index.

The new generation of clinical chemistry analyzers can now measure the H-index with a degree of accuracy and precision that is comparable to that of many clinical chemistry tests. Moreover, reliable evidence suggests that the H-index is highly correlated with the serum or plasma concentration of cell-free hemoglobin measured with the reference cyanmethemoglobin technique [53, 54]. Therefore, inclusion of H-index data in the laboratory report, preceded by translation of instrument-specific arbitrary units into g/L of hemoglobin, seems advisable for many reasons, such as (i) the precision and accuracy of this measure is satisfactory, so that it can be safely reported to clinicians; (ii) the provision of an objective measure of sample quality will provide an objective basis for supporting the decision to suppress results of hemolysis-sensitive tests; (iii) spurious hemolysis is also an index of phlebotomy quality [55], so that systematic reporting H-index data may help improving phlebotomy practices, highlighting those healthcare settings where sample hemolysis is higher compared to others; (iv) the use of H-index will ease participating to quality programs based on quality indicators such as that of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working

Group "Laboratory Error and Patient Safety" [56, 57]; (v) in rare cases of patients with *in vivo* hemolysis (i.e. hemolytic anemia), providing an estimation of cell-free hemoglobin in serum or plasma may be valuable information for clinicians.

Although the availability of quality control materials specific for the H-indices remains limited, many diagnostic companies have these products in their pipeline. Once these quality control materials become widely available in the diagnostic market, clinical laboratories should use them for continuously monitoring the analytical performance of the H-index likewise in the routine practice of clinical chemistry testing. In the meanwhile, the same procedures applied for other quality controls can be applied to monitoring the analytical performance of this test. Therefore, routine serum or plasma samples with different levels of cell-free hemoglobin values may be aliquoted and stored, to be used as IQAs, whilst failure of quality control measures should prevent using the test for undertaking clinically significant decisions.

Conclusions

Sample quality is still considered a cornerstone of total quality in laboratory diagnostics and its assessment should be seen as an almost unavoidable routine activity. Yet, some areas of uncertainty remain. For example, the CV_I calculated in critical patients is very different from that estimated in ostensibly healthy subjects or in outpatients [34]. Therefore, too narrow limits of the RCV will lead to not releasing clinically useful data in patients with life-threatening conditions (e.g. results of coagulation testing in patients with liver disease) [58]. This

Table 1: Practical recommendations for managing test results in hemolyzed specimens.

1. Sample quality (i.e. presence of hemolysis) should always be checked before testing
2. Presence and degree of hemolysis should be preferably checked with automatic assessment of the H-index
3. When the H-index is unavailable, visual assessment of hemolysis by comparing sample hue with a color chart is advisable
4. Results of the H-index should always be transferred (and stored) into the laboratory information system (LIS) and consideration can be made to include them in the laboratory report
5. Results of the H-index shall always be converted into the corresponding hemoglobin concentration (i.e. in g/L)
6. Standard operating procedures (SOPs) should be defined for standardized management of test results in hemolyzed specimens (see Figure 2)
7. Quality control materials, both internal and external, should be used for continuously monitoring the analytical performance of the H-index

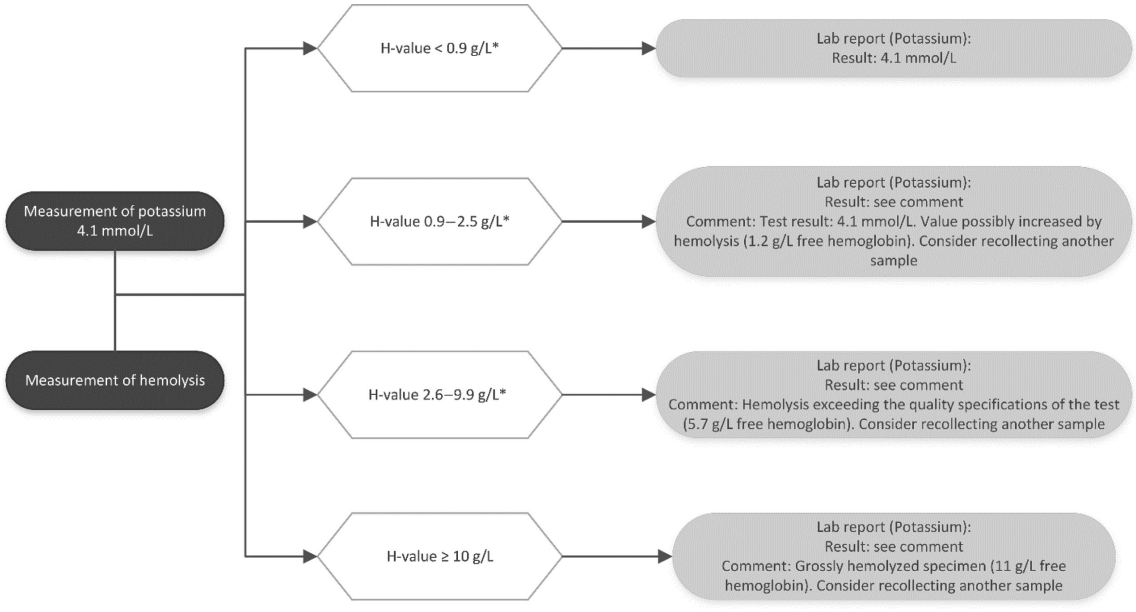

Figure 4: Application of the suggested strategy for managing potassium results obtained in hemolyzed samples.
*Cut-off values are fictional and have to be calculated for each individual setting of instrument and assay in use.

important issue is not actually covered by the available data on CV_I, but will probably be targeted by the "Biological variation database" prepared and maintained by the Task and Finish Group Biological Variation Database (TFG-BVD) and endorsed by the EFLM. The database will be hosted on the EFLM website, and will contain essential information about the biological variation and derived performance specifications for different measurands along with the evidence that supports it [59]. Then, provision of the H-index by different manufacturers is still plagued by poor harmonization, especially that referring to measurement procedure, variable definition of thresholds of interference after which test results may be "analytically" biased, and lack of information describing how interference cut-offs have been calculated.

The development and use of the H-index has introduced a paradigm shift in laboratory practice. The many advantages compared to visual inspection make its use highly advisable in all clinical laboratories. Irrespective of this virtually undisputable evidence, there is still fierce debate about the most effective and clinically safe strategy for dealing with sample hemolysis. In the authors' expectations, the pragmatic approach suggested in this document (Figure 1; Table 1) should provide an acceptable compromise between quality and clinical needs. Our approach is in favor of reporting hemolysis-sensitive test results in cases when the bias is clinically acceptable, so providing fundamental clinical information to clinicians. On the other hand, in cases when the predicted bias is clinically significant and may thus jeopardize patient safety, test results suppression seems the most reliable approach. A useful example of this strategy, regarding practical management of potassium test results in a hemolyzed sample, is shown in Figure 4 [60]. Notably, this approach can be easily incorporated into automatic algorithms, thus saving operator time, preventing the risk of entering erroneous data in the LIS and promoting harmonization in the management of clinical chemistry testing performed using potentially unsuitable specimens.

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