



## Short Communication

## Random uncertainty of photometric determination of hemolysis index on the Abbott Architect c16000 platform



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## ABSTRACT

**Background:** Automatic photometric determination of the hemolysis index (HI) on serum and plasma samples is central to detect potential interferences of in vitro hemolysis on laboratory tests. When HI is above an established cut-off for interference, results may suffer from a significant bias and undermine clinical reliability of the test. Despite its undeniable importance for patient safety, the analytical performance of HI estimation is not usually checked in laboratories. Here we evaluated for the first time the random source of measurement uncertainty of HI determination on the two Abbott Architect c16000 platforms in use in our laboratory.

**Methods:** From January 2016 to September 2017, we collected data from daily photometric determination of HI on a fresh-frozen serum pool with a predetermined HI value of ~100 (corresponding to ~1 g/L of free hemoglobin). Monthly and cumulative CVs were calculated.

**Results:** During 21 months, 442 and 451 measurements were performed on the two platforms, respectively. Monthly CVs ranged from 0.7% to 2.7% on c16000-1 and from 0.8% to 2.5% on c16000-2, with a between-platform cumulative CV of 1.82% (corresponding to an expanded uncertainty of 3.64%). Mean HI values on the two platforms were just slightly biased (101.3 vs. 103.1, 1.76%), but, due to the high precision of measurements, this difference assumed statistical significance ( $p < 0.0001$ ).

**Conclusions:** Even though no quality specifications are available to date, our study shows that the HI measurement on Architect c16000 platform has nice reproducibility that could be considered in establishing the state of the art of the measurement.

## 1. Background

In clinical chemistry, an ‘interference’ is any clinically significant bias occurring in the measurement of an analyte caused by the presence in the sample of substances different from the measurand or by the physical/chemical properties of the sample itself [1]. Hemolysis, icterus and lipemia (HIL) represent the most frequent causes of interference [2]. Until not so long ago, identification of samples potentially interfered by HIL was carried out by laboratory professionals through the visual inspection of tubes. However, the human eye is not able to detect accurately and in a reproducible manner differences in sample coloring or turbidity. The visual inspection is highly subjective and difficult if not impossible to standardize, even with the help of color scales or specific operational instructions [3].

Among all the interferents, hemolysis is probably the most frequent cause of sample rejection and non-reported test results by clinical laboratories [4,5]. In hemolyzed samples, the hemoglobin (Hb) released

from erythrocytes causes significant errors on results of spectrophotometric assays that measure at wavelengths comparable to the absorbance spectrum of Hb itself (between 340 and 440 nm and between 540 and 580 nm, with a peak at 420 nm). Furthermore, free Hb's heme and iron can directly interact with reagent components or measurands themselves, potentially determining biased measurements. Hemolysis also causes the release in the sample of many other intracellular components present in the erythrocytes, such as ions (e.g., potassium and phosphate) and enzymes (e.g., lactate dehydrogenase and aspartate aminotransferase), which consequently suffer from positively biased results when measured in plasma. Finally, a negative bias is observable on some protein biomarkers, such as cardiac troponin T (cTnT) or insulin, which are catabolized by intracellular proteases released during hemolysis [6,7]. A quite recent study has shown that visual handling of hemolyzed samples increases the risk of reporting inaccurate results for cTnT, potassium and bilirubin, possibly affecting the clinical decision and patient safety [8].

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**Table 1**

Random uncertainty of the photometric measurement of hemolysis index (HI) on Abbott Architect c16000 platforms.

	No. of determinations	Mean HI	Monthly CVs <sup>a</sup>	Cumulative CV <sup>b</sup>	Relative expanded uncertainty <sup>c</sup>
Architect c16000-1	442	101.3	0.7% - 2.7%	1.59%	3.18%
Architect c16000-2	451	103.1	0.8% - 2.5%	1.63%	3.26%
Between platforms	893	101.7	1.2% - 2.7%	1.82%	3.64%

<sup>a</sup> Minimum and maximum values are reported.<sup>b</sup> Cumulative CV is calculated as the average of monthly CVs obtained in the study period.<sup>c</sup> By a coverage factor of 2.

To make up for these problems, during the last years the majority of analytical platforms has been provided with systems for the automatic photometric determination of HIL, which return more objective estimates and allow to automatically manage the potentially interfered results through laboratory software and middleware [9]. Nevertheless, the analytical performance of these measurements is not usually checked in clinical laboratories. In a multicenter evaluation, Fernandez et al. [10] showed a relatively good agreement between free Hb concentrations measured using the reference method and hemolysis index (HI) determined by some chemistry platforms, particularly those giving quantitative HI. On the other hand, no studies have so far considered the reproducibility of these measurements. Here, for the first time, we have estimated the uncertainty of HI measurement by the widely used Architect c16000 platform (Abbott Diagnostics) due to random error, which is often the largest contributor to the total uncertainty of measured quantity values on clinical samples [11].

## 2. Methods

### 2.1. HI determination

In our total laboratory automation (TLA) setting, two Architect c16000 platforms are used to automatically measure HIL indices on all plasma and serum samples on which chemistry and immunochemistry tests are requested. Both instruments are used and maintained by strictly following the manufacturer's instructions. In particular, the Architect c16000 analyzer measures HI through the dilution of samples with saline solution and the polychromatic photometric detection of the interferent. The mathematical calculation to determine HI uses a proprietary equation for the addition of absorbance measurements at four specific wavelength pairs (primary/secondary) covering the whole Hb absorbance spectrum, each one multiplied by a specific constant. The result is then corrected for the dilution factor [12].

### 2.2. Random uncertainty estimation

According to the 2007 Eurolab report, the random uncertainty of a measurement procedure should be quantified by calculating standard deviations obtained from replicate measurements. In particular, standard deviation should be obtained under within-laboratory intermediate reproducibility conditions in order to cover all relevant sources of variability, such as variations in instrumentation and measuring conditions, consumables and operators [13]. Materials used to estimate random uncertainty should be from a third-party source, independent from the company manufacturing the measuring system, should be commutable and should have a concentration level of the analyte relevant to the clinical application of the measurement [14].

Since commercial control materials for HIL indices with the characteristics previously described are lacking, for this study we prepared a serum pool with an HI value around 100 (~1 g/L of free Hb), manufactured with anonymized leftover samples from laboratory routine and stored in 250 µL aliquots at –20 °C. The 'target' HI value of ~100 was selected according to the threshold over which a clinically significant interference is expected when critical analytes such as cTnT and

potassium are determined [12]. From January 2016 to September 2017, HI was determined on both c16000 platforms using a freshly thawed aliquot every weekday. In the evaluated period, eight different batches of control material were prepared and employed. The transition from an old to a new batch always occurred at the beginning of the month. The random uncertainty was estimated by calculating for each instrument monthly CV and cumulative CV as the average of monthly CVs during the entire study period. Between-platform random uncertainty was also estimated since, in our TLA organization, patient samples are randomly sent to one of the two platforms.

## 3. Results

HI means and cumulative and monthly CVs (minimum and maximum values) are reported in Table 1. Monthly CVs ranged from 0.7% to 2.7% on c16000-1 and from 0.8% to 2.5% on c16000-2, with a between-platform cumulative CV of 1.82%. By applying a coverage factor of 2 (95% level of confidence), the relative expanded uncertainty of HI measurement on the Architect c16000 was 3.64%. Mean HI values on the two platforms were just slightly biased (101.3 vs. 103.1, 1.76% difference), but, due to the high precision of measurements, this difference assumed statistical significance (independent samples t-test,  $p < 0.0001$ ).

## 4. Discussion

As spectrophotometric reading produces HI results, they may be subject to technical drifts and failures like any other spectrophotometric laboratory measurement. The inability to give consistent measurements over time or the insurgence of a significant bias could have a negative impact on the correct acceptance/rejection of samples by the laboratory. Ultimately, any inaccuracy in the determination of HI could have negative impact on patient care [1,8]. Therefore, HI determination (and, more widely, all interference indices) should be considered like any other laboratory test, for which analytical performances should be controlled through appropriate quality control programs. The need to guarantee quality of the determination should rely on an appropriate definition of performance specifications defined by the same models used for other measurands [15].

With our study, we first demonstrated that it is possible for clinical laboratories to monitor the reproducibility of HI determination through an internal quality control program fulfilling the recommended characteristics for deriving random uncertainty [14]. From our results, we can conclude that the photometric method of the Architect c16000 platform shows an acceptable random uncertainty, which is consistent between different instruments. The obtained performance could be considered in establishing the state of the art of the measurement to be employed for the definition of quality specifications for HI measurement, by following the model 3 of the European Federation of Clinical Chemistry and Laboratory Medicine Strategic Conference [15].

From our results, we noted a slight, but statistically significant bias between the two c16000 platforms. At this stage, it is difficult to understand the impact, if any, of this bias on the clinical application of the HI measurement. Certainly, the organization of a proper External

Quality Assessment would be of pivotal importance in establishing the trueness of automatic HI determination by chemistry platforms.

One potential limitation of our study was that only one level for control material was employed, based on the HI threshold influencing the tests with major clinical impact (i.e., cTnT and potassium). The need of more than one level that spans the reportable range for the test for the appropriate estimate of measurement uncertainty has been previously underlined [16].

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