Assessment of Plasma Sample Quality on Siemens Atellica COAG 360 System

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The assessment of sample quality is an essential requirement of a total quality management system in laboratory medicine,[1] as well as for routine and specialized coagulation testing.[2] Sample inspection, aimed to identify common sources of interference (i.e., hemolysis, turbidity, and icterus), has been performed for decades by means of visual assessment. Many limitations have been highlighted in this strategy, including high inaccuracy and large interobserver variability, along with challenges related to systematic recording of interference data either in the laboratory information system or in laboratory reports.[3] The new generation of analyzers for clinical chemistry, immunochemistry, and hemostasis testing is increasingly being equipped with the so-called serum or plasma indices, which have improved the ability to estimate the interference of hemolysis (H-index), icterus (I-index), and turbidity (L-index) by using multichromatic wavelength readings and formulas with adjustments for compensating potential spectral overlaps.[4] The widespread implementation of this approach may considerably increase quality and safety of laboratory testing, since routine use of reagent-specific thresholds of interference based on validated clinical acceptability criteria will enable suppressing data obtained analyzing potentially unsuitable specimens. Therefore, this study was planned to validate the plasma indices on the novel Siemens Atellica COAG 360 System (Siemens Healthcare Diagnostics Products GmbH).

The Atellica COAG 360 System is an analyzer consolidating five different analytical technologies (clotting, either optical or opto-mechanical, chromogenic, immunologic, high-sensitivity luminescence-based [LOCI], and platelet aggregation testing) on a single testing platform. Plasma quality assessment is performed by quantification of plasma indices ("HIL") on a 4-channel photometer using simultaneous multiple wavelengths scanning (from 365 to 645 nm). Measurements are performed in triplicate for each index, preceded by a blank measurement with purified water. The total sample volume used for HIL assessment is 50 µL and the measurement is completed within 16 seconds.

Our local validation of plasma indices on Atellica COAG 360 entailed the assessment of limit of blank (LOB), limit of detection (LOD), linearity, imprecision (within run, between-run, and total), result comparison with standard reference techniques, as well assessment of interference limits for five tests with nine different reagents and optical clotting methodology (►**Table 1**). The entire evaluation was based on routine sodium citrate plasma samples collected in primary evacuated blood tubes (Vacutest Kima, Kima). The study was approved by the local Institutional Review Board (University Hospital of Verona, Verona, Italy; SOPAV2, protocol number: 971CESC).

The calculation of both LOB and LOD was performed as earlier recommended:[5] [LOB] = [mean value] + 1.645 × [standard deviation, SD)] of 20 replicates of sample buffer; [LOD] = [LOB] + 1.645 × [SD] of 20 replicates of a routine citrate plasma sample with the lowest measurable value of each index (i.e., 1 for all indices). The linearity was tested using serial dilutions at fixed ratios (1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3, 8:2; 9:1) of a pool of 20 routine citrate plasma

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Table 1 Reagent specifications, allowable total error and interference limits of the tests used in this study

Test	Name	Reagent specifications	ATE	Interference limit		
				H-index	I-index	L-index
PT	Innovin	Recombinant human tissue factor and synthetic phospholipids	±5.3%	>8	>4	NI6
PT	Thromborel	Human placental thromboplastin	±5.3%	>5	NI5	>3
APTT	Actin FS	Ellagic acid and purified soy phosphatides	±4.5%	>3	>4	NI6
APTT	Actin FSL	Ellagic acid and purified soy and rabbit brain phosphatides	±4.5%	>5	>5	>4
APTT	Actin	Ellagic acid and liquid rabbit brain cephalin	±4.5%	>5	>3	NI6
APTT	Pathromptin LS	Silicon dioxide and vegetable phospholipids	±4.5%	>8	>5	NI6
Fibrinogen	Fibrinogen VR	Thrombin reagent (Clauss method)	±13.6%	NI9	>2	NI6
Antithrombin	Innovance AT	Chromogenic anti-activated factor X (FXa) assay	±8.3%	NI9	>4	>5
D-dimer	Innovance DD	Particle-enhanced immunoturbidimetric assay	±28%	NI9	>3	NI6

Abbreviations: APTT, activated partial thromboplastin time; ATE, allowable total error; NI5, no interference up to plasma index of 5; NI6, no interference up to plasma index of 6; NI9, no interference up to plasma index of 9; PT, prothrombin time.

samples displaying a high value of each index (H-index, 8; I-index, 6; L-index, 8) diluted with another pool of 20 routine plasma samples displaying a low value of each index (i.e., 1 for all plasma indices). All these dilutions were measured in duplicate and the linearity was finally assessed as Pearson's correlation coefficient.

The intraassay, interassay, and total imprecision was calculated using three different pools of 20 citrate plasmas. The pools were specifically selected to obtain low (i.e., 1 for all indices), intermediate (i.e., between 3 and 4), and high (i.e., between 5 and 8) values for each respective plasma index. The imprecision was measured in 20 consecutive runs for the intra-assay study and 10 runs in 10 working days for the interassay study, while total imprecision was estimated using the formula of Krouwer and Rabinowitz.[6] Results were finally reported as coefficient of variation (CV%). The interference limit was finally defined as the value of each plasma index above which the test result displayed a bias greater than the desirable specification for allowable total error, as summarized in ►**Table 1**.[7] This estimation was performed by serially diluting pools of 20 routine plasma citrate samples displaying high plasma indices values (9 for H-index, 5 for I-index, and 6 for L-index, respectively) with pools of 20 routine plasma citrate samples displaying the lowest measurable value of each index (i.e., 1 for all plasma indices). Method comparison was performed using 118 routine citrate plasma samples referred to the local laboratory for routine hemostasis testing. Plasma hemoglobin (i.e., reflecting hemolysis) was estimated as H-index on Roche Cobas c501 (Roche Diagnostics AG), as previously described.[8] The performance of this technique was proven to be optimally correlated with the reference cyanmethemoglobin assay.[8] The concentration of triglycerides (i.e., reflecting sample turbidity) and total bilirubin (i.e., mirroring icterus) in plasma was also measured on Roche Cobas c501, with the respective reference techniques. The statistical analysis was performed using Analyze-it (Analyze-it Software Ltd.).

The LOB and LOD was 1 for all plasma indices on Atellica COAG 360. The intra-assay imprecision was 0% for all indices, the interassay imprecision was 0% for both H-index and I-index, while it was comprised between 0 and 9.7% for L-index. The total imprecision was therefore 0% for both H-index and I-index, whereas it was between 0 and 9.7% for L-index (0% for L-index of 1, 9.7% for L-index of 3, and 8.3% for L-index of 8). The linearity was 0.993 for H-index, 0.980 for I-index, and 0.978 for L-index, respectively. According to linearity studies, we estimated that each increment of 1 index unit roughly corresponded to 0.28 g/L of hemoglobin for H-index, 56 µmol/L of total bilirubin for I-index, and 3.4 mmol/L of triglycerides for L-index, respectively. These values closely matched those provided by the manufacturer for hemoglobin (0.37 versus 0.28 g/L) and bilirubin (74 versus 55 µmol/L), whereas a larger difference was found for triglycerides (1.4 versus 3.4 mmol/L).

The limit of interference for all reagents tested is shown in ►**Table 1**. Briefly, actin FS was the most hemolysis-sensitive reagent, with clinically significant variation of values observed with H-index > 3. The other tests displayed intermediate sensitivity to hemolysis, while fibrinogen, antithrombin, and D-dimer were hemolysis-insensitive up to H-index of 9. Regarding the I-index, all reagents except Thromborel were found to be variably sensitive to bilirubin, with fibrinogen values already showing clinically significant bias with I-index > 2 (►**Table 1**). Thromborel was the most turbidity-sensitive reagent, since clinically significant variation of values was observed with L-index > 3. Actin FSL and antithrombin displayed a clinically significant bias with L-index > 4 and > 5, respectively, while the other tests were almost lipemia-insensitive up to L-index of 6. Regarding the comparison of Atellica COAG 360 plasma indices with hemoglobin, total bilirubin and triglycerides values obtained on Roche Cobas c501, the

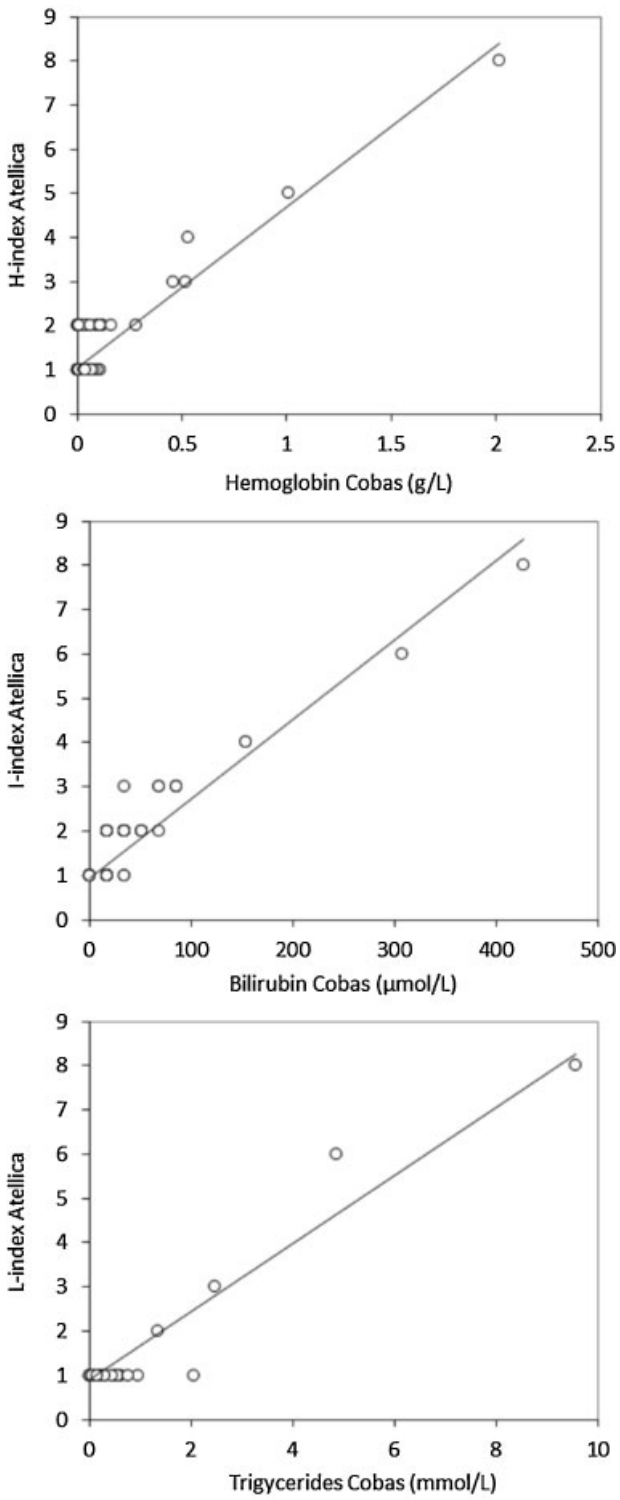

Fig. 1 Comparison of plasma indices measured by Atellica COAG 360 with hemoglobin, total bilirubin, and triglycerides values obtained on Cobas c501.

Pearson's correlations were 0.909 (95% confidence interval [95% CI], 0.871–0.936) for H-index, 0.922 (95% CI, 0.889–0.945) for I-index, and 0.964 (95% CI, 0.948–0.975) for L-index, respectively (all $p < 0.001$) (►**Fig. 1**). As specifically regards the H-index, Atellica COAG 360 displayed 1.00 sensitivity and 0.85 specificity compared with Cobas c501 at the upper limit of plasma hemoglobin concentration found in healthy subjects (i.e., 0.25 g/L).[9]

Taken together, the results of our study confirm optimal performance of plasma indices on Atellica COAG 360. The high interassay imprecision of L-index, mainly attributable to high variability encountered in frozen plasmas displaying L-index > 3, may be considered virtually insignificant in routine practice, since the threshold for turbidity interference was found to be > 3 for all reagents tested (►**Table 1**). We herein conclude that systematic assessment of sample quality through the use of plasma indices on Atellica COAG 360 is a reliable and viable option for enhancing the quality in hemostasis testing. As for other coagulation analyzers,[10] this study has also allowed estimation of some reagent-specific interference thresholds (►**Table 1**), which may be applied in routine practice to permit suppressing test results plagued by clinically unacceptable bias.

Conflict of Interest
None.

References

1 Sciacovelli L, Panteghini M, Lippi G, et al. Defining a roadmap for harmonizing quality indicators in laboratory medicine: a consensus statement on behalf of the IFCC Working Group "Laboratory Error and Patient Safety" and EFLM Task and Finish Group "Performance specifications for the extra-analytical phases". Clin Chem Lab Med 2017;55(10):1478–1488
2 Lippi G, Favaloro EJ. Preanalytical issues in hemostasis and thrombosis testing. Methods Mol Biol 2017;1646:29–42
3 Lippi G, Plebani M, Favaloro EJ. Technological advances in the hemostasis laboratory. Semin Thromb Hemost 2014;40(02): 178–185
4 Lippi G. Systematic assessment of the hemolysis index: pros and cons. Adv Clin Chem 2015;71:157–170
5 Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 2008;29(Suppl 1):S49–S52
6 Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clin Chem 1984;30(02):290–292
7 Westgard QC. Desirable Biological Variation Database specifications. https://www.westgard.com/biodatabase1.htm. Accessed September 27, 2017
8 Lippi G, Luca Salvagno G, Blanckaert N, et al. Multicenter evaluation of the hemolysis index in automated clinical chemistry systems. Clin Chem Lab Med 2009;47(08):934–939
9 Lippi G, Giavarina D, Gelati M, Salvagno GL. Reference range of hemolysis index in serum and lithium-heparin plasma measured with two analytical platforms in a population of unselected outpatients. Clin Chim Acta 2014;429:143–146
10 Lippi G, Ippolito L, Favaloro EJ. Technical evaluation of the novel preanalytical module on instrumentation laboratory ACL TOP: advancing automation in hemostasis testing. J Lab Autom 2013; 18(05):382–390