Letter to the Editor

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Derivation of performance specifications for uncertainty of serum C-reactive protein measurement according to the Milan model 3 (state of the art)

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To the Editor,

The definition and fulfillment of suitable analytical performance specifications (APS) for the measurement uncertainty (MU) is essential to ensure that laboratory measurements are clinically usable [1, 2]. There is now a global consensus that the derivation of APS for different measurands should be carried out by using the models established in 2014 by the Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) held in Milan, Italy [3]. In particular, model 1, based on the effect of analytical performance on clinical outcome, applies for measurands that have a central role in diagnosis and monitoring of a specific disease; model 2, based on the components of biological variation (BV) of the measurand, should be used for measurands under strict metabolic control; and model 3, based on the state of the art of the measurement (defined as the highest level of analytical performance technically achievable) should be used for measurands that cannot be included in the first two models [4].

C-reactive protein (CRP) is the most sensitive of the acute phase proteins and its concentrations in serum increase rapidly during inflammatory processes [5]. However, an elevation of CRP is not diagnostic of any one specific disease as it occurs in many diseases involving tissue damage or inflammation. Besides not having a central and well-defined role in the decision making of a specific disease, CRP is a biologically challenging analyte. We previously highlighted the difficulties in deriving reliable CRP BV data [6, 7]. The meta-analysed BV data made recently available on the EFLM database (https://biologicalvariation.eu/) are 33.5% for intraindividual (CV_I) and 87.7% for inter-individual variability (CV_G), respectively. As Franzini pointed out, the CV describing the distribution of a set of *positive* distributed values, as laboratory results are, cannot exceed 33.3% [8]. Higher CV_I and CV_G values probably represent incorrect estimates due either to a transitory illness of some subjects in the evaluated sample group or, more generally, to non-normal distribution of the CRP data in apparently healthy individuals.

Considering that neither of the first two EFLM models seems to be suitable for CRP, the model three constitutes the best option to derive APS for this measurand. According to the definition of the "state of the art" by the EFLM Strategic Conference, to derive APS for MU by using this model it is necessary to identify the highest quality of performance that is currently achievable [3]. With this aim, we compared experimentally estimated standard MU of four widely used commercial measuring systems for CRP measurement available in our laboratory: Alinity c and Architect c16000 (Abbott Diagnostics), AU680 (Beckman Coulter) and Cobas c501 (Roche Diagnostics), all 'Conformité Européenne'/CE marked. Table 1 summarizes the characteristics of the evaluated measuring systems.

According to recently published documents [1, 9], MU associated with laboratory results should combine two uncertainty components: the MU of calibrator value (u_{cal}) and the MU accounting for random sources (u_{Rw}). The former should in turn combine the MU of the higher-order reference selected by the *in vitro* diagnostics (IVD) manufacturer for implementing traceability with the MU deriving from the

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Table 1: Characteristics of measuring systems for C-reactive protein measurement included in the study.

Manufacturer	Platform	Commercial name and assay principle	Manufacturer's instruction for use version	Calibrator name	Stated traceability	Certified value of reference material ± standard uncertainty
Abbott Diagnostics	Alinity c	CRP Vario Wide Range Immunoturbidimetric method (art. 07P56)	G71182/R01, May 2017	CRP Vario Wide Range Calibrator kit	ERM-DA 472/IFCC	41.8 ± 1.25 mg/L
Abbott Diagnostics	Architect c16000	CRP Vario Wide Range Immunoturbidimetric method (art. 6K26)	306731/R04, August 2015	CRP Calibrator Set	ERM-DA 472/IFCC	41.8 ± 1.25 mg/L
Beckman Coulter	AU680	CRP Latex Immunoturbidimetry (art. OSR6199)	BLOSR6x99.04, July 2013	CRP Latex Calibrator Normal Set	ERM-DA 474/IFCC	41.2 ± 1.25 mg/L
Roche Diagnostics	Cobas c501	CRPL3 Particle Enhanced Immunoturbidimetric Gen. 3 (art. 04956842)	V 10.0, December 2018	C.f.a.s. Proteins	CRM 470	39.2 ± 0.95 mg/L

process for assignment of calibrator values. u_{Rw} gives information about the stability of the measuring system over time and its variability when employed by an individual laboratory. The recommended time span for this evaluation is 6 months, as it allows covering all the significant sources of u_{Rw} [9]. We previously described in detail how correctly estimating u_{Rw} through the internal quality control (IQC) information [10]. In this study, for each measuring system, we collected the information about the metrological traceability and MU of commercial calibrators at CRP concentration of 10 mg/L, the 99th percentile limit of reference value distribution, with the exception of Roche C.f.a.s. calibrator for which only one level is available at 79.9 mg/L concentration. Then, we calculated the u_{cal} by combining the standard MU of employed reference material, available on the corresponding certificate of analysis, with the standard MU of commercial calibrator as provided by the manufacturer. The u_{Rw} was experimentally estimated as intermediate reproducibility from 6-month consecutive measurement data of a serum pool with a selected CRP concentration near to 10 mg/L, randomly analysed daily during the ordinary laboratory activity. This material had the characteristics previously recommended for correctly deriving the MU of measuring systems due to random effects [1, 10]. In daily practice, the system alignment to the manufacturer's specifications was checked by measuring, three times per day, i. e. every 8 h, the control materials offered by each manufacturer as part of their CE-marked measuring system and by verifying that results were within the range declared by the manufacturer. Finally, the standard MU associated to clinical samples were calculated as $\sqrt{(u_{cal}^2 + u_{Rw}^2)}$ and the system with the best analytical performance identified (Table 2).

Although it is theoretically possible that there is some option on the in vitro diagnostics market performing better, our data show that the Architect c16000 performance in terms of MU on clinical samples (3.76%) may represent the

Table 2: Estimated combined standard measurement uncertainty of evaluated measuring systems for C-reactive protein (CRP) measurement.

Measuring system	Intermediate reproducibility data			Combined standard uncertainty of calibrator value (u_{cal})[a], %	Combined standard uncertainty on clinical samples[b], %
	Number of measurements in the 6-month period	Mean CRP, mg/L	Standard uncertainty accounting for random sources (u_{Rw}), %		
Alinity c	111	10.9	4.03	3.15	5.12
Architect c16000	147	10.2	2.06	3.15	3.76
AU680	184	10.5	2.41	3.16	3.97
Cobas c501	113	10.2	2.48	4.50	5.14

[a]Calculated by combining the standard MU of the employed reference material with the standard MU of commercial calibrator as declared by manufacturers. [b]Estimated as $\sqrt{(u_{cal}^2 + u_{Rw}^2)}$.

state of the art of the CRP measurement to be employed for the definition of APS for MU according to the model three of the EFLM Strategic Conference. If we consider this APS as desirable, we can also modulate the quality level to minimum goal (3.76% + ½ 3.76% = 5.64%), as previously described [10]. From data in Table 2, all the evaluated systems fulfilled this minimum quality goal for CRP MU. It is however expected that involved manufacturers work for improving the quality of assay performance to move towards the desirable quality goal in the next future. In this context, it is noteworthy the worsening of the analytical performance of CRP measurement between successive generations of platforms when Abbott Diagnostics products are considered. We recommend that manufacturers, before releasing any kind of technological upgrade, should pay major attention to assuring an equivalent, if not better, analytical quality of provided measurements.

In this brief communication, we gave an example of the concepts previously mentioned for deriving APS for MU using the Milan model 3 [10]. The major components of the process are as follows: 1) assess combined standard MU for the selected measurand, based on u_{cal} and random effects for commonly used measuring systems; 2) for random effects assessment, use material(s) other than those used to control the assay alignment and randomly spaced throughout the day; 3) identify the combined standard MU from the best performing system as the 'desirable' APS; and 4) establish the 'minimum' APS as being 50% greater than the desirable one. One potential limitation of our study was that only one level for pool material was employed, based on the CRP threshold having the major clinical impact (i. e., the upper reference limit). The need of more than one level that spans the reportable range for the test for the optimal estimate of MU has been previously underlined [1, 2]. Another study limitation is the use of only one version of each method, including the determined best system which is used to set the APS. This could not allow optimal capture of the variation in performance that would be seen in various laboratories.

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