Raúl Rigo-Bonnin*, Francesca Canalias, Cristina El Haj, María Cristina González-Hernando, Noelia Díaz-Troyano, Laura Soldevila, Eva Benavent and Oscar Murillo

Measurement uncertainty of β-lactam antibiotics results: estimation and clinical impact on therapeutic drug monitoring

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Abstract

Background: Despite that measurement uncertainty data should facilitate an appropriate interpretation of measured values, there are actually few reported by clinical laboratories. We aimed to estimate the measurement uncertainty of some β-lactam antibiotics (β-LA), and to evaluate the impact of reporting the measurement uncertainty on clinicians' decisions while guiding antibiotic therapy.

Methods: Measurement uncertainty of β-LA (aztreonam [ATM], cefepime [FEP], ceftazidime [CAZ], and piperacillin [PIP]) values, obtained by an UHPLC-MS/MS based-method, was estimated using the *top-down* approach called the *single laboratory validation approach* (EUROLAB guidelines). Main uncertainty sources considered were related to calibrators' assigned values, the intermediate precision, and the bias. As part of an institutional program, patients with osteoarticular infections are treated with β-LA in continuous infusion and monitored to assure values at least 4 times over the minimal inhibitory concentration (4×MIC). We retrospectively evaluated the impact of two scenarios of laboratory reports on clinicians' expected decisions while monitoring the treatment: reports containing only the β-LA values, or including the β-LA coverage intervals (β-LA values and their expanded measurement uncertainties).

Results: The relative expanded uncertainties for ATM, FEP, CAZ and PIP were lower than 26.7%, 26.4%, 28.8%, and 25.5%, respectively. Reporting the measurement uncertainty, we identified that clinicians may modify their decision especially in cases where 4×MIC values were within the β-LA coverage intervals.

Conclusions: This study provides a simple method to estimate the measurement uncertainty of β-LA values that can be easily applied in clinical laboratories. Further studies should confirm the potential impact of reporting measurement uncertainty on clinicians' decision-making while guiding antibiotic therapy.

Keywords: β-lactam antibiotics; clinical interpretation; measurement uncertainty; top-down approach; UHPLC-MS/MS.

***Corresponding author: Raúl Rigo-Bonnin,** Laboratori Clínic, IDIBELL, Hospital Universitari de Bellvitge, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain, Phone: +34932607543, Fax: +34932607546, E-mail: raulr@bellvitgehospital.cat
Francesca Canalias: Laboratori de Referència d'Enzimologia Clínica, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra, Spain
Cristina El Haj, Laura Soldevila, Eva Benavent and Oscar Murillo: Servei de Malalties Infeccioses, IDIBELL, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
María Cristina González-Hernando and Noelia Díaz-Troyano: Laboratori Clínic, IDIBELL, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain

Introduction

Clinical laboratories obtain *measurement values* of biological quantities that may help in the diagnosis, treatment and monitoring of the human diseases. When a biological quantity is measured, random and systematic errors cause *measurement errors* in these values generating a doubt about the true values of the quantity. The estimation of *measurement uncertainty* enables knowing the magnitude of this doubt, as well as how reliable and accurate the laboratory measurement values really are. The *top-down* and the *bottom-up* approaches are mainly accepted amongst clinical guidelines in order to estimate measurement uncertainty [1–7], the latter being considered the more realistic and appropriate for clinical laboratories [1, 2, 4–7].

Currently, few laboratories report measurement uncertainty data. However, several studies have suggested that these data may help in the interpretation of measurement values and have an impact on clinicians' decisions, especially when they are compared with biological reference intervals, therapeutic intervals or clinical decision values (cut-off or critical values)

[1, 2, 8–11]. Therefore, clinical laboratories should report *measurement results*, which are expressed by a coverage interval that includes the measurement value and its measurement uncertainty [12].

There has been great interest recently on personalizing human therapies, such as the case of antimicrobials therapeutic drug monitoring (TDM). In the current worldwide era of infections caused by multidrug-resistant Gram-negative bacilli (MDR-GNB) and the limited pipeline of new antibiotics, clinicians deal with the need to optimize the antimicrobial therapy. In this line, β-lactam antibiotics (β-LA) can be administered in continuous infusion against difficult-to-treat infections (i.e. critically-ill patients, biofilm-related infection), in contrast with the traditional administration by intermittent boluses. When applying this strategy, the most relevant pharmacokinetic/pharmacodynamic indices of β-LA may be optimized; thus, TDM might assure a time the drug remains over the minimal inhibitory concentration (T > MIC) near 100% and the concentration of β-LA in plasma (cβ-LA) over 4 times the MIC (4 × MIC) [13–18]. In this specific setting of antimicrobials, few studies have approached the measurement uncertainty and have considered its impact on clinical practice [19–21].

In the present study we aimed to estimate the measurement uncertainty of different cβ-LA measurement values using a *top-down* approach called the *single laboratory validation approach*, and to evaluate the potential impact of reporting the measurement uncertainty on the clinicians' expected decisions to guide the TDM of β-LA administered in continuous infusion against biofilm-related osteoarticular infections.

Material and methods

Chemicals and reagents

LC-MS acetonitrile, LC-MS dimethyl sulfoxide (DMSO), LC-MS formic acid, LC-MS methanol and LC-MS water, were supplied by Merck KGaA (Darmstadt, Germany).

Certified reference materials of cefepime (FEP) (93.1%), ceftazidime (CAZ) (85.3%), and piperacillin (PIP) (94.4%), were purchased from European Pharmacopeia (European Directorate for the Quality of Medicines-Council of Europe, Strasburg, France). Reference material of aztreonam (ATM) (99.8%) was obtained from United States Pharmacopeia (Rockville, MD, USA).

Drug-free human plasma was obtained from the blood of patients who presented at Emergency Laboratory in our hospital. Blood was collected in a lithium-heparin tube (Vacuette, Kremsmünster, Austria) and centrifuged at 2000 *g* for 10 min at room temperature. The plasma obtained was pooled and stored at −80 °C until its use. An aliquot was separated to confirm the absence of any of the β-LA.

Measurement procedure, equipment and preparation of calibration and control materials

cβ-LA was measured using an UHPLC-MS/MS based-procedure previously developed and validated by our group [22]. The measurement system used was Acquity® UPLC®-TQD® (Waters, Milford, MA, USA).

The following equipment was used:

- ADA-120/L analytical balance from Adam Equipment (Bletchley, UK). Uncertainty indicated in the certificate of external calibration laboratory was (50.00 ± 0.53) mg ($k = 2$).
- Acura® 825 adjustable 100–1000 µL volume micropipette (pipette A) from Socorex Isba (Ecublens, Switzerland). Uncertainties indicated in the certificate of external calibration laboratory were: (1000.0 ± 2.6) µL ($k = 2$), (500.0 ± 1.8) µL ($k = 2$) and (100.0 ± 0.4) µL ($k = 2$).
- Nichipet® EX II adjustable 10–100 µL and 0.5–10 µL volumes micropipettes (pipettes B and C, respectively) from Nichiryo Co. Ltd. (Koshigaya-shi, Saitama, Japan). Uncertainties indicated in the certificate of external calibration laboratory were: (10.00 ± 0.18) µL ($k = 2$), (50.00 ± 0.22) µL ($k = 2$) and (100.0 ± 0.4) µL ($k = 2$) for pipette B; and (0.500 ± 0.02) µL ($k = 2$), (5.00 ± 0.08) µL ($k = 2$) and (10.00 ± 0.16) µL ($k = 2$) for pipette C.
- 20-mL BLAUBRAND® volumetric flask (Brand GMBH + CO KG, Wertheim, Germany). According to the manufacturer's data, the 20-mL volumetric flask accuracy was 0.04 mL.

Two stock solutions of β-LA from independent weighings were prepared at a concentration of 2.00 g/L. One stock solution was used for the preparation of plasma calibration materials, while the other one was used for plasma control materials. The stock solutions were prepared by weighing 40.08 mg of ATM, 42.96 mg of FEP, 46.89 mg of CAZ and 42.37 mg of PIP and dissolving these materials altogether in 20 mL water:metanol:DMSO (50:25:25, *v/v/v*). Eight working standards (5.00, 10.0, 50.0, 150, 450, 750, 1250 and 1750 mg/L) with a volume of 1-mL each one were prepared pipetting the corresponding volumes of stock solution in water. These solutions were stored light-protected for up to 6 months at (−75 ± 3) °C. One hundred microliters of calibration materials at 0.50, 1.00, 5.00, 15.0, 45.0, 75.0, 125 and 175 mg/L were prepared on the day of analysis diluting these working standards in human drug-free plasma in a ratio of 1:9. Working control materials were similarly prepared and stored, using a separate stock solution. Plasma control samples were ready-made at concentrations of 3.00, 30.0 and 120 mg/L.

Measurement uncertainty estimation

Measurement uncertainty estimation was performed following the CLSI [2] and EUROLAB [6, 7] guidelines. The steps followed were: (1) specification of the measurand and its intended use; (2) estimation of uncertainties associated with the assigned values of stock solution, working standard solutions and plasma calibration materials; (3) estimation of uncertainty associated with the intermediate precision of the measurement system; (4) estimation of the uncertainty associated with the bias; and (5) estimation of combined and expanded uncertainties.

Figure 1 shows a *cause and effect* diagram used to identify the main sources of measurement uncertainty.

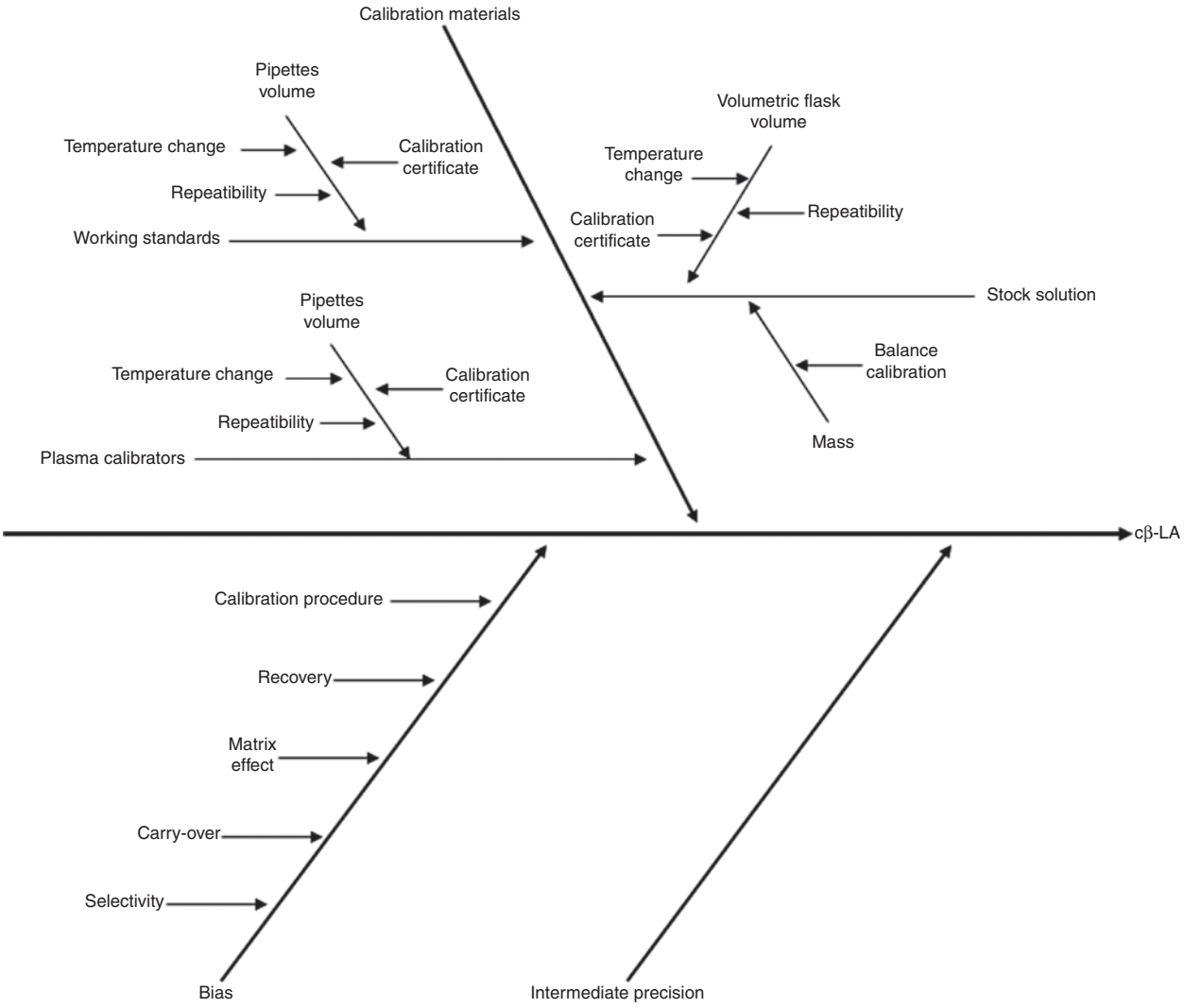

Figure 1: *Cause and effect* diagram of the most relevant measurement uncertainty sources of β-LA mass concentration in human plasma (cβ-LA) using the *single laboratory validation* approach.

Specification of the measurand: Specification of the measurands was based on the IUPAC and IFCC recommendations [23].

Uncertainty associated with the assigned value of stock solution: Uncertainties associated with the assigned value of stock solution (u_{stock}) were due to the reference materials purity (u_{purity}) and their mass weighted into the balance (u_{mass}), as well as the 20-mL volumetric flask (u_{flask}). Additionally, other uncertainty sources related to the volumetric flask volume variation due to room temperature fluctuations in the room temperature ($u_{temp,flask}$), and to the variation in filling the flask ($u_{filling,flask}$), were also considered.

The purity (p) of β-LA reference materials declared by manufacturers were ≥ p%. In order to estimate the u_{purity}, a type-B estimation using rectangular distribution was assumed to estimate it:

$$u_{purity} = \frac{100\% - (100 + p\%)/2}{\sqrt{3}}$$

The u_{mass} were calculated as:

$$u_{mass} = \frac{U_{balance}/2}{m} \cdot 100$$

where $U_{balance}$ is the expanded uncertainty obtained from the external calibration certificate of the analytical balance; and m, the reference material mass weighted into the balance.

According to the manufacturer's data, the 20-mL volumetric flask accuracy is 0.04 mL. To estimate the u_{flask}, a type-B estimation using a triangular distribution was used:

$$u_{flask} = \frac{0.04}{\sqrt{6} \cdot V_{flask}} \cdot 100$$

being V_{flask} the volume of the volumetric flask.

The $u_{temp,flask}$, assuming a type-B-rectangular distribution, was calculated using the following equation:

$$u_{temp,flask} = \frac{\alpha \cdot \Delta T}{\sqrt{3}} \cdot 100$$

where α is the coefficient of thermal expansion for water (2.14×10^{-4} °C^{-1}); and ΔT, the difference between the actual laboratory temperature and the temperature during the calibration of the volumetric flask ($\Delta T=5$ °C).

In order to obtain the $u_{\text{filling,flask}}$, a repeatability study was performed with a series of 10 fills with water and weigh experiments with volumetric flask. The standard deviation value obtained in the repeatability study was directly used as $u_{\text{filling,flask}}$.

Every uncertainty sources considered were combined to obtain the u_{stock}:

$$u_{\text{stock}}=\sqrt{u^2_{\text{purity}}+u^2_{\text{mass}}+u^2_{\text{flask}}+u^2_{\text{temp,flask}}+u^2_{\text{filling,flask}}}$$

Uncertainty associated with the assigned value of working standard solutions: Uncertainties related to the assigned values of each working standard solution (u_{ws}) included were, in addition to the u_{stock}, those associated with the pipetted stock solution volume ($u_{\text{pip,stock}}$), to the pipetted water volume ($u_{\text{pip,water}}$), and to the stability of the prepared working standards (u_{stab}). Uncertainty sources related to the pipetted volume variations due to the room temperature fluctuations ($u_{\text{temp,pip}}$) were also considered.

The $u_{\text{pip,stock}}$ and $u_{\text{pip,water}}$ were estimated using the following equation:

$$u_{\text{pip,stock}}\ \text{or}\ u_{\text{pip,water}}=\frac{U_{\text{pipette}_{\text{A,B,C}}}/2}{V_x}\cdot100$$

where $U_{\text{pipette}_{\text{A,B,C}}}$ is the expanded uncertainty obtained from the certificate of external calibration for the pipettes A, B or C; and V_x, the volume of stock solution or water pipetted using the pipettes A, B or C.

The $u_{\text{temp,pip}}$ were calculated as described above for volumetric flask:

$$u_{\text{temp,pip}}=\frac{\alpha\cdot\Delta T}{\sqrt{3}}\cdot100$$

where $\Delta T=2$.

Uncertainty related to the stability of working standard solutions (u_{stab}) was estimated as [24]:

$$u_{\text{stab}}=\frac{L_s}{\sqrt{18}}$$

where L_s is the maximum stability value obtained from European Medicine Agency criteria (15%) [25].

The standard uncertainty sources estimated were combined to obtain the u_{ws}:

$$u_{\text{ws}}=\sqrt{u^2_{\text{stock}}+u^2_{\text{pip,stock}}+u^2_{\text{pip,water}}+u^2_{\text{temp,pip,stock}}+u^2_{\text{temp,pip,water}}+u^2_{\text{stab}}}$$

Uncertainties associated with the assigned values of plasma calibration and control materials: Uncertainties associated with the assigned values of plasma calibration materials (u_{cal}) or plasma control materials (u_{ctrl}) considered, besides u_{stock} and u_{ws}, were due to the 10 µL-pipetted working standard solution volumes using pipette C ($u_{\text{pip C,ws}}$), to the 90 µL-pipetted drug-free plasma volumes using pipette B ($u_{\text{pip B,plasma}}$), and their respective uncertainties related to the pipetted volume variations due to the room temperature fluctuations ($u_{\text{temp,pip B}}$, $u_{\text{temp,pip C}}$).

The $u_{\text{pip C,ws}}$, $u_{\text{pip B,plasma}}$, $u_{\text{temp,pip B}}$ and $u_{\text{temp,pip C}}$ were estimated as previously described.

Finally, each u_{cal} or u_{ctrl} was calculated using the following equation:

$$u_{\text{cal}}\ \text{or}\ u_{\text{ctrl}}=\sqrt{u^2_{\text{stock}}+u^2_{\text{ws}}+u^2_{\text{pip C,ws}}+u^2_{\text{pip B,plasma}}+u^2_{\text{temp,pip B}}+u^2_{\text{temp,pip C}}}$$

Uncertainty associated with the intermediate precision: Estimation of the intermediate precision was performed using the three plasma control materials. Ninety-six values were collected over 12 months for each control material. The uncertainty associated with the intermediate imprecision measurement system (u_{p}) was calculated as:

$$u_{\text{p}}=\sqrt{\frac{\sum_{i=1}^{n}[(n_i-1)\cdot CV_i^2]}{\left(\sum_{i=1}^{n}n_i\right)-m}}$$

where n_i is the number of control material value for the month i; CV_i, the coefficient of variation obtained in the month i; and m, the number of months ($m=12$).

Uncertainty related to the bias: When a bias is estimated and found to be less than its expanded uncertainty (a metrological compatibility study), it is not necessary to correct the result for the bias. Despite this, when the main source of a bias cannot be estimated or eliminated, different procedures should be assessed for expanding the uncertainty interval to cover the bias [2].

In our case, the different possible sources of bias considered were: calibration procedure, recovery of extracted samples (REC), matrix effect (ME), carry-over (CO) and selectivity (SEL). The REC and the ME biases are corrected using internal standards, whereas the other sources remained uncorrected. A treatment of corrected and uncorrected biases was performed [26].

As there are no high-order reference materials listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database [27] to be used, the three plasma internal control materials were used as references to estimate the bias associated with the calibration procedure. Bias study was carried out as the intermediate precision study. The relative bias (δ_{r}) and its uncertainty (u_{δ}) were calculated as follows:

$$\delta_{\text{r}}=\frac{1}{m}\cdot\sum_{i=1}^{m}\left[\left(\frac{\bar{x}_i-\mu}{\mu}\right)\right]\cdot100$$

$$u_{\delta}=\sqrt{\delta^2_{\text{r}}+\left(\frac{u_{\text{p}}}{n}\right)^2+u^2_{\mu}}$$

where $\bar{x}_i$ is the mean obtained in our laboratory for the month i; μ, the reference value assigned by weighted or nominal value (3.00, 30.0 and 120 mg/L); n, the total number of control materials processed ($n=96$); and u_{μ}, the relative uncertainty associated with the assigned value of the plasma control material (u_{ctrl}).

To estimate the bias related to the REC, as well as the ME, the CO and the SEL, data from previously validated measurement procedure were considered [22]. Those biases were estimated using the following equations:

$$\delta_{\text{REC}}=\frac{1}{q}\cdot\sum_{j=1}^{q}(\text{REC}_j-\mu_{\text{REC}})$$

$$\delta_{ME} = \frac{1}{q} \cdot \sum_{j=1}^{q} (ME_j - \mu_{ME})$$

$$\delta_{CO} = CO - \mu_{CO}$$

$$\delta_{SEL} = \frac{1}{q} \cdot \sum_{I=1}^{q} (SEL_I - \mu_{SEL})$$

where q is the number of samples used to perform the REC, ME and SEL studies ($q=12$ for REC and ME; and $q=10$ for SEL); REC_j is the normalized REC in % (100 · REC sample/REC internal standard) value obtained for the sample j; μ_{REC}, the REC reference value assigned as 100% (indicating 100% of REC); ME_j is the normalized ME in % (100 · ME sample/ME internal standard) value obtained for the sample j; μ_{ME}, the ME reference value assigned as 100% (indicating that no ME exists); CO, the CO value in %; μ_{CO}, the CO reference value assigned as 0% (indicating that no CO exists); SEL_I, the selectivity value in % for the possible interference I; μ_{SEL}, the SEL reference value assigned as 0% (indicating that no interference exists).

The relative uncertainty associated with the REC (u_{REC}) and the ME (u_{ME}) were their respective coefficient of variation [22]. The relative uncertainties related to the CO (u_{CO}) and the SEL (u_{SEL}) were calculated as:

$$u_{CO} = \sqrt{\delta_{CO}^2 + u_{s-CO}^2}$$

$$u_{SEL} = \sqrt{\delta_{SEL}^2 + \sum_{l=1}^{q} (u_{s-SEL})_l^2}$$

where u_{s-CO} and u_{s-SEL} were estimated using a right-angled triangle distribution (type-B approach) as:

$$u_{s-CO} \text{ or } u_{s-SEL} = \sqrt{\frac{(b-a)^2}{18}}$$

where a and b are, respectively, the lower and upper limits of the interval; being $a=0\%$ in our case for CO and SEL; and b the CO value or the SEL value of each possible interference considered.

Every uncertainty bias sources were combined to obtain the uncertainty related to the whole bias (u_{bias}):

$$u_{bias} = \sqrt{u_\delta^2 + u_{REC}^2 + u_{ME}^2 + u_{CO}^2 + u_{SEL}^2}$$

Combined and expanded uncertainties: Once the individual contribution of uncertainty sources was estimated, a relatively combined standard uncertainty (u_c) was estimated according to the following equation:

$$u_c = \sqrt{u_{cal}^2 + u_p^2 + u_{bias}^2}$$

Finally, the relative expanded uncertainty (U) was achieved multiplying the u_c by a coverage factor of 2 [2].

Clinical interpretation of cβ-LA results

Since January 2015 in our hospital, β-LA are systematically administered in continuous infusion to treat patients with biofilm-related osteoarticular infections caused by GNB, and cβ-LA are measured weekly to monitor the treatment. This institutional program was approved by the Ethics Committee of our hospital and authorized by the Spanish Agency of Drugs and Sanitary Products (AEMPS).

Our protocol recommends starting the β-LA therapy with the intermittent bolus administration while awaiting definitive microbiological results from clinical samples; and then, β-LA administration is subsequently switched into continuous infusion. The choice of the β-LA for continuous infusion relies on the treating clinician, but is based on clinical issues, patients' characteristics and susceptibility testing profile of the isolates. The daily dosage of β-LA in continuous infusion is monitored to achieve target drug steady-state concentrations at least 4×MIC, as this concentration has been correlated with the maximum killing effect for β-LA [18, 28, 29]. For the particular situation of treatment of β-LA-non-susceptible isolates with high MIC values, continuous infusion administration of β-LA assures $T > MIC \approx 100\%$ and thus, may recover β-LA efficacy. In this situation, cβ-LA higher than 4xMIC were often difficult to achieve due to potential toxicity of β-LA.

In order to illustrate how the notification of measurement uncertainty in laboratory reports could facilitate the right interpretation of cβ-LA results for a possible and adequate clinical decision-making, we analyzed retrospectively some results of selected patients enrolled during a period from June 2015 to December 2016, evaluating two possible scenarios: (1) laboratory reports containing cβ-LA measurement values (Scenario 1); and (2) laboratory reports containing cβ-LA measurement results (coverage interval values which include measurement values and their respective expanded measurement uncertainties) (Scenario 2). Afterwards, for each patient, we evaluate the role of Scenario 1 and Scenario 2 in providing the most accurate information for clinicians to guide the treatment and to assure the targets mentioned.

Results

Measurement uncertainty

Measurands and their intended use were defined as *the mass concentration (mg/L) of (each) β-lactam antibiotic in human plasma measured according to a laboratory-developed measurement procedure using an Acquity® UPLC®-TQD® measurement system, in order to ensure its dose adjustment the achievement of pharmacodynamic targets associated with rapid bacterial killing and optimal clinical outcomes*.

Table 1 shows the different uncertainty sources related to the plasma calibrators-preparation (u_{stock}, u_{ws}) as well as the u_{cal}.

Table 2 indicates the different biases and their respective uncertainties. To most cβ-LA, the higher uncertainties were for those related to calibration procedure, whereas the uncertainties for REC and ME were approximately similar to each other. In the case of uncertainties related to CO and SEL, these were the ones with the most variable

Table 1: Uncertainty budget related to the plasma calibrators-assigned values for the measurement of β-LA mass concentration in plasma.

Quantity[a]	Calibrator value, mg/L	u_{stock}, %	u_{ws}, %	u_{cal}, %	U_{cal} ($k=2$), %	U_{cal} ($k=2$), mg/L
P–Aztreonam; mass c.(USP; UHPLC-MS/MS)	0.50	0.67	3.72	3.81	7.62	0.04
	1.00		3.78	3.87	7.74	0.08
	5.00		3.72	3.81	7.63	0.38
	15.0		3.71	3.80	7.6	1.1
	45.0		3.72	3.81	7.6	3.4
	75.0		3.70	3.80	7.6	5.7
	125		3.70	3.80	8	9
	175		3.70	3.79	8	13
P–Cefepime; mass c.(EP; UHPLC-MS/MS)	0.50	2.09	4.21	4.29	8.58	0.04
	1.00		4.27	4.35	8.69	0.09
	5.00		4.21	4.30	8.59	0.43
	15.0		4.20	4.28	8.6	1.3
	45.0		4.21	4.29	8.6	3.9
	75.0		4.20	4.28	8.6	6.4
	125		4.20	4.28	9	11
	175		4.20	4.28	9	15
P–Ceftazidime; mass c.(EP; UHPLC-MS/MS)	0.50	4.28	5.63	5.69	11.38	0.06
	1.00		5.67	5.73	11.47	0.11
	5.00		5.63	5.69	11.39	0.57
	15.0		5.62	5.68	11.4	1.7
	45.0		5.63	5.69	11.4	5.1
	75.0		5.62	5.68	11.4	8.5
	125		5.62	5.68	11	14
	175		5.62	5.68	11	20
P–Piperacillin; mass c.(EP; UHPLC-MS/MS)	0.50	1.74	4.05	4.13	8.26	0.04
	1.00		4.11	4.19	8.38	0.08
	5.00		4.05	4.14	8.27	0.41
	15.0		4.04	4.12	8.2	1.2
	45.0		4.05	4.13	8.3	3.7
	75.0		4.04	4.12	8.2	6.2
	125		4.04	4.12	8	10
	175		4.03	4.12	8	14

u_{stock} (%), relative uncertainty related with the assigned value of stock solution; u_{ws} (%), relative uncertainty associated with the assigned value of working standard solution; u_{cal} (%), relative uncertainty associated with the assigned value of the calibrator material; U_{cal} (%), relative expanded uncertainty related with the assigned value of the calibrator material; U_{cal} (mg/L), expanded uncertainty associated with the assigned value of the calibrator material in mg/L units. [a]Nomenclature and abbreviations are according to IFCC and IUPAC [23]: P, plasma; mass c., mass concentration. Other abbreviations: β-LA, β-lactam antibiotics; EP, European Pharmacopeia; USP, United States Pharmacopeia.

values. Figure 2 shows the bias sources with a main contribution to the uncertainty for cβ-LA.

Table 3 shows the measurement uncertainty budget containing the three main uncertainty sources (u_{cal}, u_p, u_{bias}), as well as the combined and expanded uncertainties. Expanded uncertainties at low values of cβ-LA were higher than at high values probably due to the heteroscedasticity proper to measurement systems based on UHPLC-MS/MS.

Clinical interpretation of cβ-LA results

Results of cβ-LA from selected patients treated with β-LA in continuous infusion are shown in Table 4. This table also gathers the potential impact of clinical laboratory reports on the clinicians' expected decision whether the measurement single value (Scenario 1) or the measurement coverage interval (Scenario 2) were informed by the laboratory. Two opposite situations were observed:

– Concordant situation. Patients with target values (4 × MIC) ≥ cβ-LA values (Scenario 1) and with 4 × MIC values ≥ the upper cβ-LA coverage interval values (Scenario 2) (see patients 1, 4, 7 and 10 from Table 4); or patients with 4 × MIC values < cβ-LA values (Scenario 1) and with 4 × MIC values < the lower cβ-LA coverage interval values (Scenario 2) (see patients 2, 5, 8 and 11 from Table 4). In these situations, both scenarios would provide similar information to clinicians (to maintain or to increase the β-LA dosage in

Table 2: Uncertainty budget related to the bias for the measurement of β-LA mass concentration in plasma.

Quantity[a]	QC mean, mg/L	δ_r, %	u_δ, %	δ_{REC}, %	u_{REC}, %	δ_{ME}, %	u_{ME}, %	δ_{CO}, %	u_{CO}, %	δ_{SEL}, %	u_{SEL}, %	u_{bias}, %
P–Aztreonam; mass	2.85	−5.00	5.90	−0.29	3.37	−0.72	3.12					7.60
c.(USP; UHPLC-MS/MS)	28.3	−5.67	6.52	3.31	2.06	6.71	3.49	1.30	1.30	0.43	0.43	7.79
	118	−2.08	3.77	6.39	1.57	12.8	2.96					5.23
P–Cefepime; mass	3.15	5.00	5.83	3.03	2.72	2.87	3.19					7.52
c.(EP; UHPLC-MS/MS)	30.9	3.00	4.29	6.73	3.01	6.81	3.69	0.60	0.60	2.14	2.14	6.79
	122	1.67	3.43	12.5	3.90	9.49	4.24					7.06
P–Ceftazidime; mass	3.11	3.67	4.50	0.79	3.82	0.89	2.56					8.75
c.(EP; UHPLC-MS/MS)	29.8	−0.67	2.78	5.05	4.31	1.94	3.76	1.50	1.50	5.73	5.74	8.70
	117	−2.25	3.45	12.9	3.77	5.28	1.22					7.92
P–Piperacillin; mass	3.05	1.67	2.90	−0.64	4.03	0.25	1.69					8.42
c.(EP; UHPLC-MS/MS)	30.3	1.00	2.67	3.5	1.69	2.22	3.44	4.20	4.21	5.06	5.07	8.07
	121	0.67	2.47	6.53	1.73	3.28	3.41					8.01

QC, quality control; δ_r (%), relative bias associated with the calibration procedure; u_δ (%), relative uncertainty related to the bias associated with the calibration procedure; δ_{REC}, bias related to the recovery of the extracted samples; u_{REC}, uncertainty associated with the bias related to the recovery of the extracted samples; δ_{ME}, bias associated with the matrix effect; u_{ME}, uncertainty related to the bias associated with the matrix effect; δ_{CO}, the bias associated with the carry-over; u_{CO}, uncertainty related to the bias associated with carry-over; δ_{SEL}, bias related to the selectivity; u_{SEL}, uncertainty associated with the bias related to the selectivity. [a]Nomenclature and abbreviations are according to IFCC and IUPAC [23]: P, plasma; mass c., mass concentration. Other abbreviations: β-LA, β-lactam antibiotics; EP, European Pharmacopeia; USP, United States Pharmacopeia.

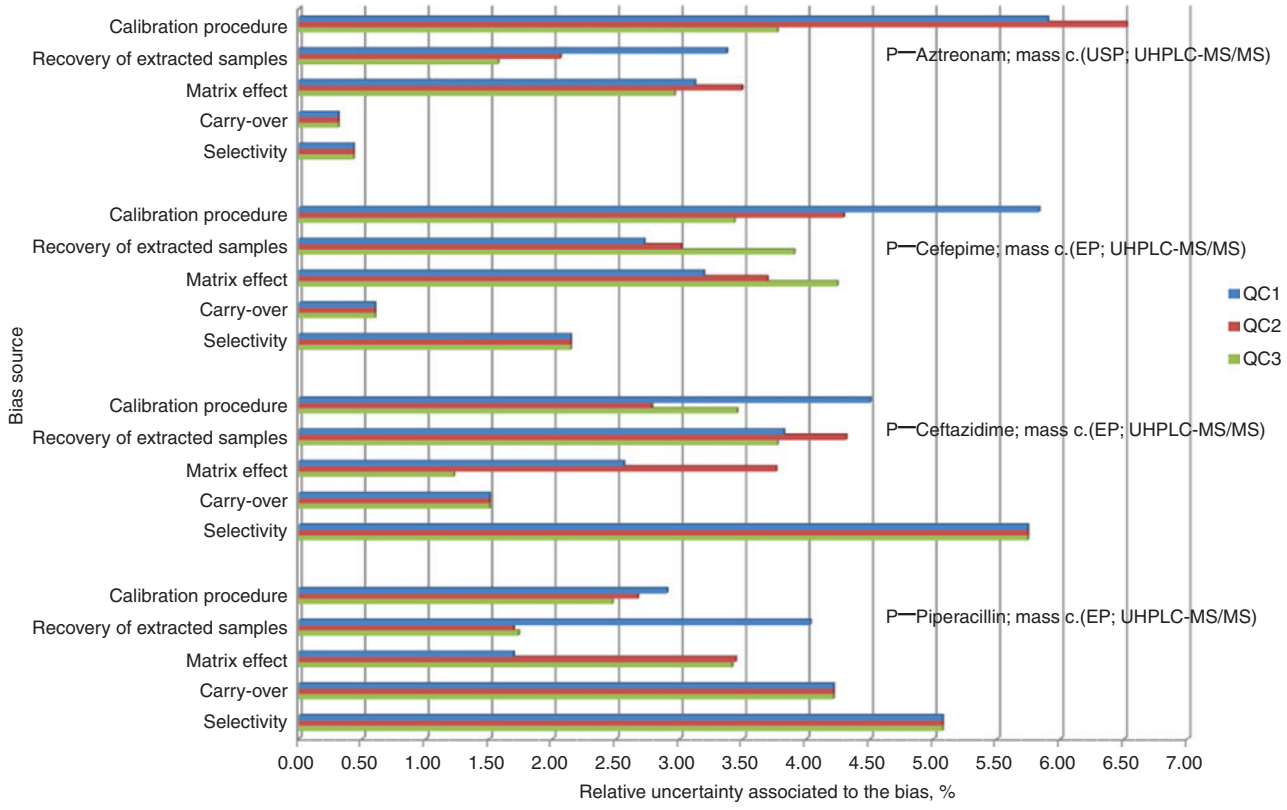

Figure 2: A block diagram about the relative uncertainties contributing to the bias uncertainty of different β-LA mass concentration in human plasma.

QC1, quality control material 1; QC2, quality control material 2; QC3, quality control material 3. Abbreviations according to IFCC and IUPAC [23]: P, plasma; mass c., mass concentration. Other abbreviations: β-LA, β-lactam antibiotics; EP, European Pharmacopeia; USP, United States Pharmacopeia.

Table 3: Measurement uncertainty budget for the measurement of β-LC mass concentration in plasma using the single laboratory validation approach.

Quantity[a]	QC mean, mg/L	u_{cal}, %	u_p, %	u_{bias}, %	u_c, %	U, %	U, mg/L
P–Aztreonam; mass	2.85		10.30	7.60	13.36	26.72	0.76
c.(USP; UHPLC-MS/MS)	28.3	3.81	7.10	7.79	11.21	22.4	6.4
	118		5.10	5.23	8.24	16	19
P–Cefepime; mass	3.15		9.99	7.52	13.22	26.43	0.83
c.(EP; UHPLC-MS/MS)	30.9	4.29	7.75	6.79	11.16	22.3	6.9
	122		5.00	7.06	9.66	19	24
P–Ceftazidime; mass	3.11		9.90	8.75	14.39	28.77	0.89
c.(EP; UHPLC-MS/MS)	29.8	5.69	6.60	8.70	12.3	24.6	7.3
	117		3.50	7.92	10	21	24.3
P–Piperacillin; mass	3.05		8.89	8.42	12.92	25.84	0.79
c.(EP; UHPLC-MS/MS)	30.3	4.13	6.20	8.07	10.99	22	6.7
	121		4.90	8.01	10.26	21	25

QC, quality control; u_{cal} (%), relative uncertainty associated with the assigned value of the calibrator material; u_p (%), relative uncertainty related to the intermediate precision; u_{bias} (%), relative uncertainty related to the bias; u_c (%), relative combined uncertainty; U (%), relative expanded uncertainty; U (mg/L), expanded uncertainty in mg/L units. [a]Nomenclature and abbreviations are according to IFCC and IUPAC [23]: P, plasma; mass.c., mass concentration. Other abbreviations: β-LA, β-lactam antibiotics; EP, European Pharmacopeia; USP, United States Pharmacopeia.

the first or the second example, respectively). Thus, the impact of reporting the measurement uncertainty by laboratory on the clinicians' expected decisions would be negligible.

– Discordant situation. Patients with target values (4 × MIC) < cβ-LA values (Scenario 1) and with 4 × MIC values within the cβ-LA coverage intervals (Scenario 2) (see patients 3, 6, 9 and 12 from Table 4). In this situation, results informed in the Scenario 1 would lead to maintain the β-LA dosage but in accordance to Scenario 2, clinicians should increase it. Thus, if the measurement uncertainty is not reported by the laboratory, clinicians could lead a wrong expected clinical decision.

Discussion

Results reported by clinical laboratories must be as accurate as possible, this suggesting also a role for reporting uncertainty data. When human infectious diseases are treated, a personalized therapy with antimicrobials would be desirable but it is not common that clinicians guide the therapy taking into account the measurement value and its uncertainty. Herein, we estimated the measurement uncertainty of cβ-LA values based on a *top-down* approach called a *single laboratory validation* approach, and evaluated the impact of applying this new information on the clinicians' decisions when treating biofilm-related osteoarticular infections with β-LA in continuous infusion.

The measurement uncertainty provides valuable information to interpret appropriately the results reported by the clinical laboratories. Unfortunately, the term uncertainty can easily be misunderstood, as it theoretically may represent a doubt about a measurement value. Taking into consideration that any measurement has a variation associated with it, measurement uncertainty indicates how reliable the value really is, providing information on the level of confidence of that same measurement.

According to the EUROLAB guidelines [6, 7], there are different approaches for the estimation of uncertainty: the *modeling* approach (so-called *bottom-up* approach by the GUM, EURACHEM and CLSI guidelines [2–4]), the *single laboratory validation* approach, the *proficiency testing* approach, and the *interlaboratory* approach (also known as *top-down* approach). Of all these *top-down* approaches, the first two are considered the most appropriate in clinical laboratories [4, 6, 7]. For the *single laboratory validation approach*, most of the uncertainty sources can often be assessed by a measurement procedure validation study. Estimates of intermediate precision and bias associated with calibration procedure can be obtained by organizing experimental works inside the laboratory. Combined with experimental investigation of other non-negligible individual sources of uncertainty, this approach provides essentially all of the data required for measurement uncertainty estimation. On the other hand, for the *proficiency testing* approach, measurement uncertainty

Table 4: Microorganisms and β-LA treatment from patients with biofilm-related osteoarticular infections and potential impact of clinical laboratory reports on the clinicians' expected decisions whether a measurement single value or a measurement value plus its measurement coverage interval is informed by the laboratory.

Patient	Microorganisms			Treatment		cβ-LA values		cβ-LA result	
	Microorganism[a]	MIC[b], mg/L	4xMIC, mg/L	β-LA	Dosage/ Frequency, g/h	cβ-LA single values, mg/L	Would it have been necessary to increase the dose?	cβ-LA value and its coverage interval, mg/L	Would it have been necessary to increase the dose?
1	*Pseudomonas aeruginosa*	16	64	ATM	5/24	42.6	Yes	42.6 [33.1–52.1]	Yes
2	*P. aeruginosa*	8	32	ATM	4/24	82.2	No	82.2 [69.0–95.4]	No
3	***P. aeruginosa***	**6**	**24**	**ATM**	**3/24**	**30.0**	**No**	**30.0 [23.3–36.7]**	**Yes**
4	*P. aeruginosa*	16	64	FEP	5/24	55.4	Yes	55.4 [43.0–67.8]	Yes
5	*Enterobacter cloacae*	2	8	FEP	4/24	31.3	No	31.3 [24.3–38.3]	No
6	***E. cloacae***	**4**	**16**	**FEP**	**3/24**	**20.3**	**No**	**20.3 [15.8–24.8]**	**Yes**
7	*P. aeruginosa*	32	128	CAZ	4/24	105	Yes	105 [83.0–127]	Yes
8	*P. aeruginosa*	2	8	CAZ	7/24	70.4	No	70.4 [55.6–85.2]	No
9	***P. aeruginosa***	**8**	**32**	**CAZ**	**6/24**	**40.2**	**No**	**40.2 [30.3–50.1]**	**Yes**
10	*P. aeruginosa*	16	64	PIP	12/24	33.0	Yes	33.0 [25.7–40.3]	Yes
11	*Acinetobacter baumannii*	8	32	PIP	10/24	52.4	No	52.4 [40.9–63.9]	No
12	***E. cloacae***	**8**	**32**	**PIP**	**12/24**	**40.2**	**No**	**40.2 [31.6–49.0]**	**Yes**

[a]The isolation of the microorganisms was carried out by microbiological conventional procedures. Identification was performed with the MALDI-TOF Biotyper® system (Bruker, Billerica, MA, USA). [b]MIC, minimum inhibitory concentration measured by E-test® method (bioMérieux, Marcy-l'Étoile, France). The MIC value corresponds to the specific β-lactam antibiotic administered in each patient. Abbreviations: MIC, minimal inhibitory concentration; β-LA, β-lactam antibiotic; ATM, aztreonam; β-LA, β-lactam antibiotics; FEP, cefepime; CAZ, ceftazidime; PIP, piperacillin; cβ-LA, mass concentration of β-lactam antibiotic in plasma. In **bold** are the discordant situations whenever clinical laboratory reports only a single cβ-LA value or a cβ-LA result (coverage interval values).

estimation can be performed as the *single laboratory validation* approach but calculating the bias related to calibration procedure with data from interlaboratory proficiency testing schemes, instead of those from internal quality control.

In this study, we described a detailed estimation of the uncertainty of cβ-LA values using the *single laboratory validation approach*. Estimate of intermediate precision and bias associated with calibration procedure were performed using long-term internal quality control data. We estimate this bias according to the EUROLAB guidelines formula [6, 7]. Despite this, other formulae and procedures could be used to estimate it, as that based on the root mean square error of bias (RMS) [6, 7, 30, 31].

Other important individual sources of uncertainty considered were those related to the preparation of calibration materials (to calibrator-assigned values), and to the bias associated with REC, ME, CO and SEL.

We found that the main contribution to the uncertainty measurement was from bias (except at low cβ-LA values, where the intermediate precision results were higher), followed by the intermediate precision and, finally, from calibrator's-assigned values. Once estimated and combined these three uncertainty sources, expanded uncertainties ranged between 16.5% and 26.7%, 19.3% and 26.4%, 20.7% and 28.8%, and 20.5% and 25.5% for plasma mass concentrations of ATM, FEP, CAZ and PIP, respectively.

For the estimation of measurement uncertainty, there are contradictory opinions about considering or not the uncertainty data related to different bias sources into the uncertainty budget. In accordance with others [8, 9], we think that when a measured value of a quantity is compared to a "universal" clinical decision value (4×MIC for the β-LA), the measurement uncertainty estimation should also include the biases and their respective uncertainties, although they have not exceeded their respective requirements. This fact may explain why the expanded uncertainties obtained in our study seem to be somewhat high if they are compared to uncertainty data published for other antibiotic related-quantities measured using HPLC-based procedures [19, 20].

Finally, we also showed the potential impact of reporting measurement uncertainty by clinical laboratories on the clinicians' expected decisions. On the basis of our results, we identified some situations where the

concentrations equivalent to 4×MIC values were within the cβ-LA coverage intervals and thus, they could only be observed if the measurement uncertainty is estimated. In accordance with these results, clinicians could modify the dosage of β-LA in order to ensure the effectiveness of antibiotic treatment.

A limitation in the clinical interpretation of cβ-LA values shown in this study (as it could also be applicable to other biological quantities) lies in the fact that we did not take into consideration other additional sources of variation (uncertainty sources) to which the cβ-LA values are subject, like those associated with the pre- and post-metrological phases, as well as to the within-subject pharmacological variability. If these sources of uncertainty would have been included in the uncertainty budget, the coverage intervals obtained would have been wider and, possibly, more valuable information could have been reported to clinicians. Another limitation is related with the fact that we have not considered that cut-off values used (4×MIC values in our case) also have an inherent measurement error and, consequently, a measurement uncertainty. Thus, we should compare the cβ-LA coverage intervals to the coverage interval from 4×MIC values rather than to a single 4×MIC value. Unfortunately, a more accurate interpretation of cβ-LA values cannot be performed as far as microbiology laboratories, diagnostic *in vitro* manufacturers or microbiological scientific societies report the measurement uncertainty of MIC's values.

Overall, we believe our results clearly show a potential impact on clinicians' expected decisions, especially when a measured value is close to the upper or the lower limit of the reference/therapeutic interval or close to the cut-off value.

In summary, this study shows how the measurement uncertainty can be estimated for different cβ-LA results obtained by UHPLC-MS/MS using a simple *top-down* approach that may be put into practice for others pharmacological quantities in order to improve the reliability of the results. Further studies should be performed to evaluate and to confirm the potential impact of reporting measurement uncertainty on clinicians' decisions while guiding antibiotic therapy.

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