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Traceability of immunosuppressant's mass concentration results obtained using different commercial calibrators

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ABSTRACT

Background: Due to the high inter-variability in immunosuppressants pharmacokinetics, therapeutic drug monitoring of these drugs is essential in order to minimize the risk of rejection after organ transplantation. Thus, results facilitated to clinicians by clinical laboratories for these drugs should be as reliable as possible. The knowledge of metrological traceability and performing compatibility studies can allow ensuring reliability of these results, mainly, when laboratories introduce substantial changes in their measurement procedures, e.g., when they change the calibration materials. The aim of this study was to investigate, as an example, the traceability of the calibrator's assigned values from two manufacturers (Recipe and Chromsystems) and to perform compatibility studies for sirolimus and tacrolimus.

Material and methods: Traceability was described according to ISO 17511. Compatibility studies, based on the CLSI EP029-A guideline, were performed verifying the trueness processing the ERM®-DA111a and ERM®-DA110a reference materials.

Results: Traceability studies reveled that sirolimus and tacrolimus results were traceable to SI, except for sirolimus if Chromsystems' calibrators was used. Using Recipe's calibrators, the absolute relative biases and their expanded uncertainties were 1.23% and ± 6.10% for sirolimus, and 1.41% and ± 3.02% for tacrolimus. Furthermore, when Chromsystems' calibrators were used, these values were 12.2% and ± 6.02% for sirolimus, and 2.64% and ± 2.94% for tacrolimus.

Conclusions: Results reported a lack of traceability and compatibility of sirolimus' results when Chromsystems' calibrators was used. In order to avoid it, laboratories should only use calibrators with assigned values traceable to highest reference materials or, alternatively, perform a compatibility study and apply a bias correction factor.

1. Introduction

Immunosuppressive drugs are administered to recipients of solid organ transplants. The high variability in the pharmacokinetics of these drugs makes therapeutic drug monitoring essential for individualizing the dose and thereby preventing toxicity and adverse events in transplant patients [1–3]. At present, dose adjustments are made in part based on clinical laboratory results. Thus, reliable —comparable and equivalent— results of mass concentration of immunosuppressants in blood (cIS) should be provided by laboratories to ensure the monitoring of immunosuppressive therapy. Application of concepts as metrological traceability and measurement compatibility may allow knowing if results are comparable, and if they refer to the same measurand and have the same degree of equivalence, respectively [4]. So, laboratories should know the traceability of their reported results and verify, performing compatibility studies, if these results continue to be equivalent when substantial changes in their measurement procedures are implemented, for example, a change of commercial calibrators.

Nowadays, cIS are measured by a variety of high-performance liquid chromatography (HPLC)- or HPLC coupled to tandem mass spectrometry (HPLC-MS/MS)- and immunoanalysis-based procedures, which are all independently calibrated, but without agreement to common higher-order certified reference materials (CRM) or reference measurement procedures listed in the Joint Committee for Traceability in

Abbreviations: cIS, mass concentration of immunosuppressants in blood; HPLC, high-performance liquid chromatography; HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; CRM, certified reference material; JCTLM, Joint Committee for Traceability in Laboratory Medicine; IVD, *in vitro* diagnostic; SRL, sirolimus; cSRL, mass concentration of SRL in blood; TAC, tacrolimus; cTAC, mass concentration of TAC in blood; IQC, internal quality control; ISO, International Organization for Standardization; CLSI, Clinical and Laboratory Standards Institute; EMA, European Medicines Agency

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Laboratory Medicine (JCTLM) database [5]. Moreover, as shown in Table 1, most *in vitro* diagnostic (IVD) manufacturers prepare their calibration materials in-house without any traceability to these metrological references, although they are requested to comply with European Regulation 2017/746 on *in vitro* medical diagnostics [6]. This means that, currently, cIS's results are not comparable and compatible between measurement procedures or laboratories, posing potential risks to patients undergoing therapeutic drug monitoring. In order to minimize this lack of traceability and equivalence of results, JCTLM together with some IVD manufacturers, some proficiency-testing-scheme providers, clinical laboratories specialists, and different organizations have developed CRM for some immunosuppressants-related quantities, specifically, for sirolimus and tacrolimus mass concentration in blood (cSRL, cTAC) but, unfortunately, not for mass concentrations of cyclosporin A and everolimus in blood.

At present, our laboratory is considering the possibility of changing the manufacturer of calibration materials for the measurement of cIS. Taking advantage of the fact that currently CRM for cSIR and cTAC are available, and in order to know how the change of calibrators could affect the reliability of cIS's results; we investigated, as an example, the traceability of the calibrator's assigned values from two manufacturers and performed compatibility studies using cSRL and cTAC.

2. Material and methods

2.1. Materials

CRM ERM®-DA111a for cSIR and ERM®-DA110a for cTAC were purchased from LGC Standards (Middlesex, UK). Assigned values with their expanded uncertainties were (9.73 ± 0.55; $k = 2$) µg/L and (7.82 ± 0.25; $k = 2.3$) µg/L, respectively.

Calibration materials used were *ClinCal® Whole Blood Calibrators for Immunosuppressants* (Ref: 9933; lot: 1366) from Recipe (Darmstadt, Germany), and *6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood* (Ref: 28039; lot: 3716) from Chromsystems (Grafëlfing, Germany).

Internal quality control (IQC) materials used were Liquicheck™ Whole Blood Immunosuppressant Controls (Ref: 12000404; lot: 26250) from Bio-Rad Laboratories (Hercules, CA, USA).

CRM were ready for use. Calibrators and IQC materials were reconstituted with HPLC-MS grade water. All materials were stored and used as recommended by manufacturers.

2.2. Measurement procedures

cSRL and cTAC were measured using procedures previously described [9] based on HPLC-MS/MS. The measurement system used was an Acquity® UPLC®-TQD® (Waters, Milford, MA, USA).

2.3. Metrological traceability

Metrological traceability chain and calibration hierarchy of cSIR and cTAC results were described according to the ISO 17511 [10] guideline. Information related to the definition of the measurands and their intended use, selection of metrological references, calibration hierarchy, among other characteristics, was considered. Information were obtained from certificates and statements facilitated by the calibration materials manufacturers (Recipe and Chromsystems).

2.4. Measurement compatibility studies

According to the CLSI EP029-A guideline [11], compatibility studies were performed for cSIR and cTAC verifying the trueness using the CRMs described. Steps followed were:

1. Calibration of measurement system using both calibrators (Recipe and Chromsystems).
2. Verifying calibration curves.

According to the EMA guideline [12], calculated concentrations of the calibrators should all be within ± 15% of the nominal value, except for the lower limit of quantification for which a ± 20% interval could be allowed. Also, IQC measured values must fulfill the IQC algorithms established in our laboratory based on the six-sigma metrics strategy: 1–2,5 s (3) for cSRL and 1–3 s (3) for cTAC.

3. CRMs were processed under intermediate conditions in 20 non-consecutive days for cSIR; and in 15 nonconsecutive days for cTAC, following CLSI EP05-A3 recommendations [13].
4. Estimation, for each group of mean values, the relative bias (δ_r).
5. Estimation, for each group of mean values, the relative uncertainty related to the bias (u_δ).
6. Verification whether each δ_r obtained was lower or higher than two-fold the u_δ (relative expanded uncertainty, $k = 2$). No significant bias exists if $|\delta_r| \leq 2 \cdot u_\delta$.

The δ_r and u_δ were calculated as follows:

$$\delta_r = \left(\frac{\overline{x} - \mu}{\mu}\right) \cdot 100$$

$$u_\delta = \sqrt{u_{\overline{x}}^2 + u_\mu^2} = \sqrt{\frac{MS_{btw}}{n} + u_\mu^2}$$

where $\overline{x}$ are the mean values obtained after processing each CRM under intermediate conditions; μ, the CRM assigned values; $u_{\overline{x}}$, the relative uncertainties related to the mean; MS_{btw}, the between-run mean square uncertainties obtained from one-way ANOVA; n, the number of CRM processed ($n = 80$); u_μ, the relative standard uncertainties associated with the assigned values.

3. Results

3.1. Metrological traceability of cSRL results

Measurand was defined as *the mass concentration (µg/L) of the sirolimus in human whole blood, measured according to a laboratory-developed measurement procedure using an Acquity® UPLC®-TQD® measurement system, for monitoring the status of transplant patients and for checking whether the administered doses of SRL are effective*. Furthermore, this measurand also can be described as [8]:

Blood—Sirolimus; mass concentration(HPLC-MS/MS)

According to Recipe's statements, cSIR results could be considered traceable to the derived SI unit of measurement (µg/L) because traceability can be materialized to a stated reference (the higher-order CRM ERM®-AC021a) that would embody this SI derived-unit. Traceability chain applied to cSIR patients' results was:

a) SI unit of measurement. µg/L.
b) Primary reference measurement procedure. Gravimetry combined with assessment of impurities by chemical measurement procedures [14].
c) Primary measurement standard. ERM®-AC021a (European Reference Materials, European Commission-Joint Research Center, Brussels, Belgium) with mass fraction 98.89% ± 0.64% ($k = 1.97$) [14].
d) Manufacturer's selected measurement procedures. Gravimetry and volumetry using appropriate equipment (analytical balance and volumetric material calibrate by an accredited laboratory).
e) Manufacturer's working standards. A standard solution of SRL from the CRM ERM®-AC021a and a hemolysed drug-free pooled-human whole blood solution (manufacturer's master standard). Also, 7 solutions of SRL at different concentrations prepared from the master

Table 1
Metrological traceability of available immunosuppressant's commercial calibrators.

Pharmacological Quantity	IVD Manufacturer	Calibration material	Measurement system/ principle or method	Manufacturer declared metrological traceability[a]		Traceability of pharmacological quantity results
				Reference material	Measurement procedures to prepare/assign calibration materials	
B—Cyclosporin A; mass c.	Abbott Laboratories	Architect Cyclosporine Calibrators	Architect systems/CMIA®	In-house standard prepared from USP cyclosporin A certified reference standard	Gravimetry and LC-MS/MS	Abbott's product calibration materials
	AB Sciex	Sciex IVD-MS™ Immunosuppressants Six Point Whole Blood Calibrator Set	Sciex API 3200MD™ CE-IVD/ LC-MS/MS	In-house standard prepared from powdered cyclosporin A material	Gravimetry and LC-MS/MS	AB Sciex's product calibration materials
	Chromsystems	6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered cyclosporin A material	Gravimetry and LC-MS/MS	Chromsystems' product calibration materials
	Recipe	ClinCal® Whole Blood Calibrators for Immunosuppressants	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from Cerilliant C-093 certified reference material	Gravimetry and LC-MS/MS	Recipe's product calibration materials
	Roche Diagnostics	Elecsys® Cyclosporine CalSet	Cobas e platforms, Modular E170/ECLIA	In-house standard prepared from powdered cyclosporin A material	Gravimetry	Roche's product calibration materials
	Siemens Healthineers	Advia Centaur® Calibrators	Advia Centaur Systems/CLIA	In-house standard prepared from USP cyclosporin A certified reference standard	Gravimetry	Siemens' product calibration materials
		Siemens Dimension® CSA/CSAE Cyclosporine Calibrator	Dimension Series/ACMIA®	In-house standard prepared from USP cyclosporin A certified reference standard	Gravimetry and LC-MS/MS	Siemens' product calibration materials
		Siemens Dimension® CSA/CSAE Calibrators	Dimension Vista/LOCI®	In-house standard prepared from USP cyclosporin A certified reference standard	Gravimetry and LC-MS/MS	Siemens' product calibration materials
		Syva® Emit® 2000 Cyclosporine Calibrators	V-Twin and Viva-E and various commercial platforms/EMIT®	In-house standard prepared from USP cyclosporin A certified reference standard	Gravimetry and LC-MS/MS	Siemens' product calibration materials
	ThemoFisher Scientific	CEDIA® Cyclosporine PLUS Kit Calibrators	Adaptable to various commercial platforms/ CEDIA®	In-house standard prepared from powdered cyclosporin A material	Gravimetry	ThermoFisher's product calibration materials
	Zyvak Technologies	Lyophilized Whole Blood Calibrator Levels 0–6	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered cyclosporin A material	Gravimetry and LC-MS/MS	Zyvak's product calibration materials
B—Everolimus; mass c.	AB Sciex	Sciex IVD-MS™ Immunosuppressants Six Point Whole Blood Calibrator Set	Sciex API 3200MD™ CE-IVD/ LC-MS/MS	In-house standard prepared from powdered everolimus material	Gravimetry and LC-MS/MS	AB Sciex's product calibration materials
	Chromsystems	6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered everolimus material	Gravimetry and LC-MS/MS	Chromsystems' product calibration materials
	Recipe	ClinCal® Whole Blood Calibrators for Immunosuppressants	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from Cerilliant E-068 certified reference material	Gravimetry and LC-MS/MS	Recipe's product calibration materials
	Roche Diagnostics	Elecsys® Everolimus CalSet	Cobas e platforms, Modular E170/ECLIA	In-house standard prepared from powdered everolimus material	Gravimetry	Roche's product calibration materials
	ThemoFisher Scientific	QMS® Everolimus Calibrators	Adaptable to various commercial platforms/QMS®	In-house standard prepared from powdered everolimus material	Gravimetry and LC-MS/MS	ThermoFisher's product calibration materials
	Waters	MassTrak® Calibrators	Acquity® UPLC®-TQD®/LC-MS/MS	In-house standard prepared from powdered everolimus material	Gravimetry and LC-MS/MS	Waters' product calibration materials
	Zyvak Technologies	Lyophilized Whole Blood Calibrator Levels 0–6	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered everolimus material	Gravimetry and LC-MS/MS	Zyvak's product calibration materials

(*continued on next page*)

Table 1 (*continued*)

Pharmacological Quantity	IVD Manufacturer	Calibration material	Measurement system/ principle or method	Manufacturer declared metrological traceability[a]		Traceability of pharmacological quantity results
				Reference material	Measurement procedures to prepare/assign calibration materials	
B—Sirolimus; mass c.	Abbott Laboratories	Architect Sirolimus Calibrators	Architect systems/CMIA®	In-house standard prepared from powdered sirolimus material (purity ≥92%)	Gravimetry and LC-MS/MS	Abbott's product calibration materials
	AB Sciex	Sciex IVD-MS™ Immunosuppressants Six Point Whole Blood Calibrator Set	Sciex API 3200MD™ CE-IVD/ LC-MS/MS	In-house standard prepared from powdered sirolimus material	Gravimetry and LC-MS/MS	AB Sciex's product calibration materials
	Chromsystems	6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered sirolimus material	Gravimetry and LC-MS/MS	Chromsystems' product calibration materials
	Recipe	ClinCal® Whole Blood Calibrators for Immunosuppressants	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from ERM®-AC021a certified reference material	Gravimetry and LC-MS/MS	Derived-unit of SI (μg/L)
	Roche Diagnostics	Elecsys® Sirolimus CalSet	Cobas e platforms, Modular E170/ECLIA	In-house standard prepared from powdered sirolimus material	Gravimetry	Roche's product calibration materials
	Siemens Healthineers	Siemens Dimension® SIRO Calibrator	Dimension Series/ACMIA®	In-house standard prepared from powdered sirolimus material	Gravimetry and HPLC	Siemens' product calibration materials
		Syva® Emit® 2000 Sirolimus Calibrators	V-Twin and Viva-E and various commercial platforms/EMIT®	In-house standard prepared from powdered sirolimus material	Gravimetry and HPLC	Siemens' product calibration materials
	Zyvak Technologies	Lyophilized Whole Blood Calibrator Levels 0–6	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered sirolimus material	Gravimetry and HPLC	Zyvak's product calibration materials
B—Tacrolimus; mass c.	Abbott Laboratories	Architect Tacrolimus Calibrators	Architect systems/CMIA®	In-house standard prepared from powdered tacrolimus material (purity ≥98%)	Gravimetry and LC-MS/MS	Abbott's product calibration materials
	AB Sciex	Sciex IVD-MS™ Immunosuppressants Six Point Whole Blood Calibrator Set	Sciex API 3200MD™ CE-IVD/ LC-MS/MS	In-house standard prepared from powdered tacrolimus material traceable to the ERM®-DA100a certified reference material	Gravimetry and LC-MS/MS	Derived-unit of SI (μg/L)
	Chromsystems	6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered tacrolimus material traceable to the ERM®-DA100a certified reference material	Gravimetry and LC-MS/MS	Derived-unit of SI (μg/L)
	Recipe	ClinCal® Whole Blood Calibrators for Immunosuppressants	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered tacrolimus material traceable to the ERM®-DA100a certified reference material	Gravimetry and LC-MS/MS	Derived-unit of SI (μg/L)
	Roche Diagnostics	Elecsys® Tacrolimus CalSet	Cobas e platforms, Modular E170/ECLIA	In-house standard prepared from powdered tacrolimus material	Gravimetry	Roche's product calibration materials
	Siemens Healthineers	Siemens Dimension® TAC Calibrator	Dimension Series/ACMIA®	In-house standard prepared from powdered tacrolimus material	Gravimetry and HPLC	Siemens' product calibration materials
		Syva® Emit® 2000 Tacrolimus Calibrators	V-Twin and Viva-E and various commercial platforms/EMIT®	In-house standard prepared from powdered tacrolimus material	Gravimetry and LC-MS	Siemens' product calibration materials
	ThemoFisher Scientific	QMS® Tacrolimus Calibrators	Adaptable to various commercial platforms/QMS®	In-house standard prepared from USP tacrolimus certified reference standard	Gravimetry and HPLC	ThermoFisher's product calibration materials
	Waters	MassTrak® Calibrators	Acquity® UPLC®-TQD®/LC-MS/MS	In-house standard prepared from powdered tacrolimus material traceable to the ERM®-DA100a certified reference material	Gravimetry and LC-MS/MS	Derived-unit of SI (μg/L)
	Zyvak Technologies	Lyophilized Whole Blood Calibrator Levels 0–6	Adaptable to various LC-MS/ MS commercial platforms	In-house standard prepared from powdered tacrolimus material	Gravimetry and LC-MS/MS	Zyvak's product calibration materials

IVD, *in vitro* diagnostic; CMIA, chemiluminiscence microparticle immunoassay; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; ECLIA, electrochemiluminescence immunoassay; CLIA, chemiluminescence immunoassay; ACMIA, affinity chrome-mediated immunoassay; LOCI, luminiscent oxygen channeling immunoassay; EMIT, enzyme-multiplied immunoassay technique; CEDIA; cloned enzyme donor immunoassay; HPLC, high-performance liquid chromatography; SI, International System of Units; LC-MS, liquid chromatography coupled to mass spectrometry.
Pharmacological quantities are described according to the IFCC and IUPAC recommendations [8]. B, blood; mass c., mass concentration.

[a] Declared metrological traceabilities were facilitated from calibration materials/reagent kits manufacturers or obtained from U.S. Food & Drug Administration (FDA) [7].

standard and the hemolysed solution.
f) Manufacturer's standing measurement system. Different commercial HPLC-MS/MS measurement systems using Recipe's reagent kits.
g) Manufacturer's product standards. *ClinCal® Whole Blood Calibrators for Immunosuppressants*. Seven working standard materials whose cSRL values were assigned using the Recipe's measurement systems based on HPLC-MS/MS.
h) End-user's routine measurement procedure. A measurement procedure described in an Acquity® UPLC®-TQD® measurement system [9] calibrated using Recipe's calibrators.
i) Routine patient samples. Patient blood samples mainly from cardiac, hepatic, renal or alo-transplanted patients receiving SRL treatment. Samples were collected by a single venipuncture into BD Vacutainer® EDTA-K_3 tube (Becton Dickinson, Franklin Lakes, NJ, USA).
j) Measurement result. Measurement value obtained for a routine patient sample accompanied by and measurement expanded uncertainty.

According to Chromsystems' statements, cSIR results only would be traceable to the manufacturer product calibrators because it does not use an international conventional reference procedure nor an international CRM. Traceability chain applied to cSIR patients' results was:

a) Manufacturer's selected measurement procedure. Gravimetry and volumetry using appropriate equipment (analytical balance and volumetric material calibrate by an accredited laboratory).
b) Manufacturer's working standards. A standard solution of SRL from powder sirolimus (name and purity assessment not provided by manufacturer) and a hemolysed drug-free pooled-human whole blood solution (manufacturer's master standard). Also, 7 solutions of SRL at different concentrations prepared from the master standard and the hemolysed solution.
c) Manufacturer's standing measurement system. Different commercial HPLC-MS/MS measurement systems using Chromsystems' reagent kits.
d) Manufacturer's product standards. *6PLUS1® Multilevel Calibrator Set-MassTox® Immunosuppressants in Whole Blood*. Seven working standard materials whose cSRL values were assigned using the Chromsystems' measurement systems based on HPLC-MS/MS.
e) End-user's routine measurement procedure. A measurement procedure described in an Acquity® UPLC®-TQD® measurement system [9], calibrated using Chromsystems' calibrators.
f) Routine patient samples. Patient blood samples mainly from cardiac, hepatic, renal or alo-transplanted patients receiving SRL treatment. Samples were collected by a single venipuncture into BD Vacutainer® EDTA-K_3 tube (Becton Dickinson, Franklin Lakes, NJ, USA).
g) Measurement result. Measurement value obtained for a routine patient sample accompanied by and measurement expanded uncertainty.

The metrological traceability chains and calibration hierarchies for cSIR in patients' results using Recipe's and Chromsystems' calibration materials are shown in Fig. 1.

3.2. Metrological traceability of cTAC results

Measurand was defined as *the mass concentration (μg/L) of the TAC in human whole blood, measured according to a laboratory-developed measurement procedure using an Acquity® UPLC®-TQD® measurement system, for monitoring the status of transplant patients and for checking whether the administered doses of TAC are effective*; and their description as [8]:

Blood—Tacrolimus; mass concentration(HPLC-MS/MS)

Taking into account Recipe's and Chromsystems' statements, results could be considered traceable to a SI derived-unit of measurement (μg/L). Traceability chain applied to cTAC patients' results using both calibration materials was:

a) SI unit of measurement. μg/L.
b) Primary reference measurement procedure. Gravimetry and volumetry combined with assessment of impurities by chemical measurement procedures [15].
c) Primary measurement standard. A solution standard of 50 mg/L prepared weighing an appropriate pure TAC powder with mass fraction 97.3% ± 1.3% (k = 1.97) and dissolving it in acetonitrile [15].
d) Secondary reference measurement procedure. Isotope dilution mass spectrometry measurement procedure [15].
e) Secondary measurement standard. ERM®-DA110a (European Reference Materials, European Commission-Joint Research Center, Brussels, Belgium) with mass concentration 7.82% ± 0.25% (k = 2.3). This material was prepared spiking blank pooled human blood (containing EDTA-K_3 as preservative) with 100 μL of the acetonitrile solution standard 50 mg/L [15].
f) Manufacturer's selected measurement procedures. 1) Gravimetry and volumetry using appropriate equipment (analytical balance and volumetric material calibrate by an accredited laboratory) to prepare the manufacturer's working standards; and 2) HPLC-MS/MS to establish the traceability of assigned values of manufacturer's working standards to ERM®-DA110a.
g) Manufacturer's working standards. Manufacturer's master standards solutions from pure powder TAC and a hemolysed drug-free pooled-human whole blood solution. Also, 7 solutions from each CRM traceable manufacturer's master standard and the hemolysed solution.
h) Manufacturer's standing measurement system. Different commercial HPLC-MS/MS measurement systems using manufacturers' reagent kits.
i) Manufacturer's product standards. Commercial calibration materials (working calibration materials) with cTAC values assigned using the manufacturers' measurement systems.
j) End-user's routine measurement procedure. A measurement procedure described in an Acquity® UPLC®-TQD® measurement system [9] calibrated using manufacturers' calibrators.
k) Routine patient samples. Patient blood samples mainly from cardiac, hepatic, renal or alo-transplanted patients receiving TAC treatment. Samples were collected by a single venipuncture into BD Vacutainer® EDTA-K_3 tube (Becton Dickinson, Franklin Lakes, NJ, USA).
l) Measurement result. Measurement value obtained for a routine patient sample accompanied by and measurement expanded uncertainty.

The metrological traceability chain and calibration hierarchy for cTAC in patients' results using Recipe's and Chromsystems' calibration materials is schematized in Fig. 2.

3.3. Measurement compatibility

As described in Materials and Methods, calibration curves achieved the EMA criteria and IQC values accomplished the algorithms established by our laboratory (data not showed).

Data obtained in compatibility studies are showed in Table 2. According to the results obtained,no significant bias exists (1.23% < 6.10%) for cSRL when Recipe's calibrators were used, *i.e.*, the results were compatibles. Conversely, using Chromsystems' calibration materials, results were not compatibles because a significant bias was observed (12.2% > 6.02%). Furthermore, for cTAC and using both calibration materials, biases could be ignored due that they were lower than their expanded uncertainties (1.41% < 3.02% and 2.64% < 2.94%), indicating that results were compatibles.

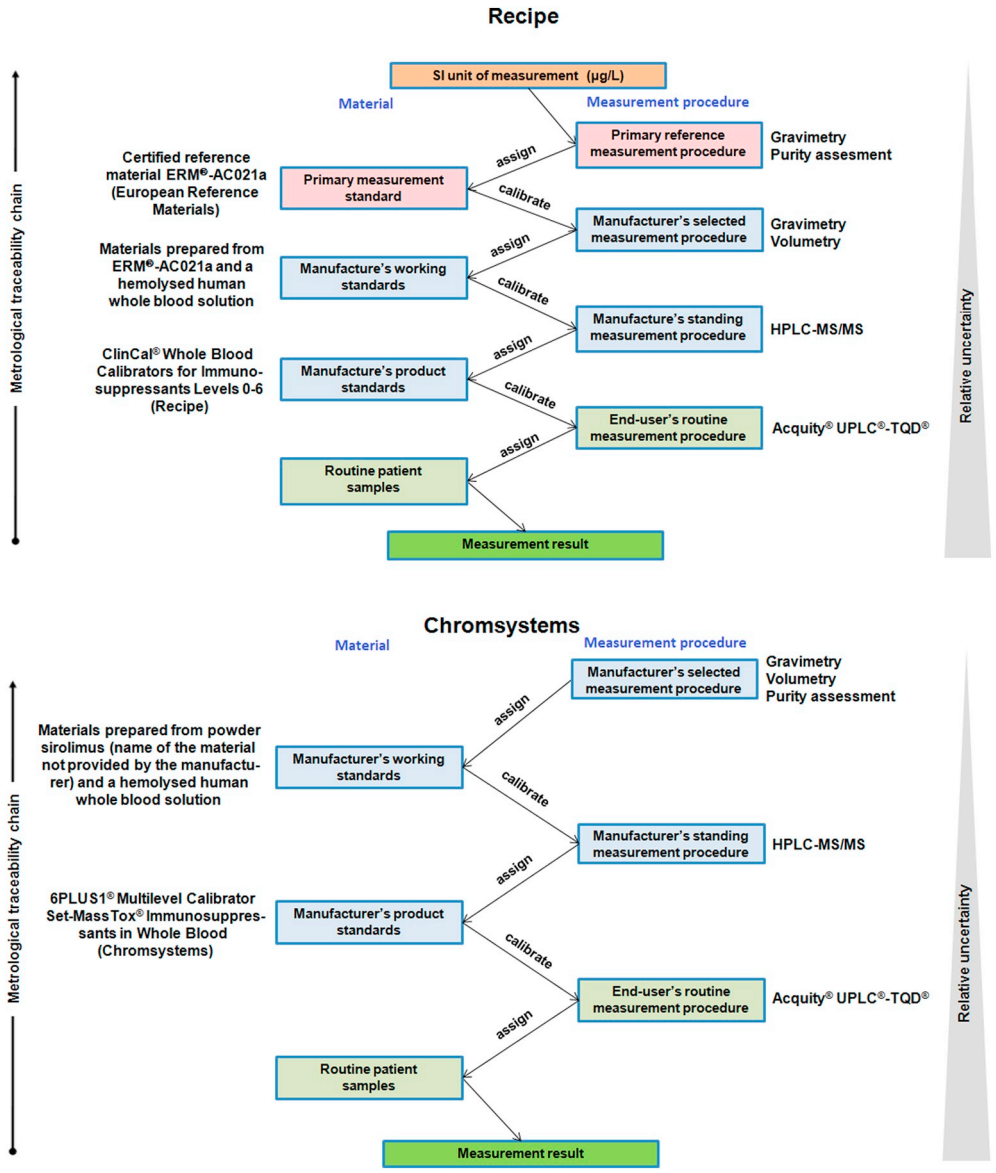

Fig. 1. Schemes of metrological traceability chain and calibration hierarchy for the mass concentration of sirolimus in blood results using Recipe's and Chromsystems' calibrators. The red, blue and greenboxes indicate that the responsibility for ensuringthe calibration hierarchy is metrology institutes and reference laboratories, manufacturers of end-user calibration materials and measuring procedures, and clinical laboratories, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Clinical laboratories measurement results obtained in the same laboratory over the time (time) and in different laboratories with similar or different measurement procedures (space) should be comparable (traceable) and compatible (equivalent). It is widely known that standardization ensure comparability and compatibility of measurement results [16,17] but, to achieve it, it is required that: 1) metrological international organizations produce high-order CRM and recommend reference measurement procedures that allows embodies measurement results into a SI units; 2) IVD manufacturers assign traceable values to calibration materials up to highest available metrological references, and develop measurement procedures as selective as possible based on international recommendations; and 3) clinical laboratories use measurement systems based on international recommended measurement procedures, use calibration materials with assigned values traceable to highest-order metrological references, use adequate IQC materials, as well as, participate periodically in external quality control schemes.

At present, several clinical laboratories measure immunosuppressants related-quantities using HPLC-, HPLC-MS/MS- and immunoanalysis-based measurement procedures. In addition to the individual metrological problems related with these procedures [18–20]; another additional problem lies in the use of commercial calibration materials (or in-house) without any traceability to the high-order metrological references listed in the JCTLM database although the fact that, currently exist these references for cSRL and cTAC on that list [6].

As an example, we investigated the traceability of the cSRL and cTAC calibration materials' assigned values and performed compatibility studies, in order to know how could affect the change of calibration materials in the reliability of cIS results. In the case of cSIR, the observed absolute relative bias (1.23%) using Recipe's calibration materials was covered by the expanded uncertainty interval of ± 6.10%, indicating a compatibility of results (non-significant bias exists). Further, even Recipe indicates that their calibrators values are traceable to ERM®-AC021a, cSIR results could be considered metrologically

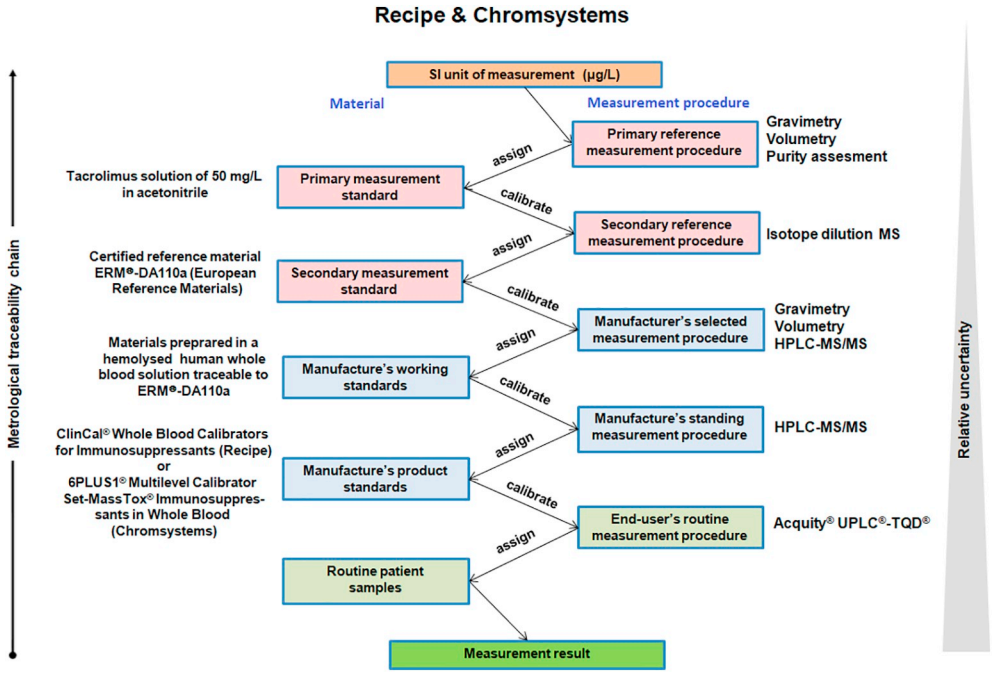

Fig. 2. Scheme of metrological traceability chain and calibration hierarchy for the mass concentration of tacolimus in blood results using Recipe's and Chromsystems' calibrators. The red, blue and greenboxes indicate that the responsibility for ensuringthe calibration hierarchy is metrology institutes and reference laboratories, manufacturers of end-user calibration materials and measuring procedures, and clinical laboratories, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Data obtained in the compatibility studies performed for mass concentrations of sirolimus and tacrolimus in blood, using Recipe's and Chromsystems' calibration materials.

	B—Sirolimus; mass c.(HPLC-MS/MS)[a]								B—Tacrolimus; mass c.(HPLC-MS/MS)[a]							
Manufacturer	n	$\bar{x}$ (µg/L)	$u_{\bar{x}}$ (%)	µ (µg/L)	u_{μ} (%)	δ_r (%)	MS_{btw} (µg^2/L^2)	u_{δ} (%)	n	$\bar{x}$ (µg/L)	$u_{\bar{x}}$ (%)	µ (µg/L)	u_{μ} (%)	δ_r (%)	MS_{btw} (µg^2/L^2)	u_{δ} (%)
Recipe	80	9.85	1.14	9.73	0.28	1.23	1.01	3.05	80	7.71	0.57	7.82	0.11	−1.41	0.15	1.51
Chromsystems	80	8.54	1.03	9.73	0.28	−12.2	0.62	3.01	80	8.03	0.47	7.82	0.11	2.64	0.11	1.47

HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; n, number of certified reference materials processed; $\bar{x}$, mean value obtained after processing certified reference material; μ, certified reference material assigned value; u_{μ}, relative standard uncertainty associated with the assigned values; δ_r, relative bias; MS_{btw}, between-run mean square uncertainties obtained from one-way ANOVA; u_{δ}, relative standard uncertainty related to the bias.
[a] Pharmacological quantities are described according to the IFCC and IUPAC recommendations [8]. B, blood; mass c., mass concentration.

traceable to ERM®-DA111a and, consequently, traceable to the SI. Using Chromsystems' calibration materials, results were not compatible (a significant bias exists) because the observed absolute relative bias (12.2%) was not included into the expanded uncertainty interval between ± 6.02%. Also, cSIR results only were traceable to the manufacturer product calibrators because Chromsystems does not use an international conventional reference procedure nor an international CRM. The lack of traceability of cSIR results using Recipe's and Chromsystems' calibration materials would indicate that there are not comparables, have not the same degree of equivalence (there are not compatibles) and, in consequence, they don't refer to the same measurand. Thus, if our laboratory would decide to use Chromsystems' calibration materials, to maintain the traceability and equivalence of cSIR results, we must apply a bias correction factor of $\mu/\bar{x} = 1.14$ and verify, periodically, the trueness' compliance. So, also standard uncertainty associated to the bias correction must be included in the calculation of measurement uncertainty together with other standard sources of uncertainty as those related with calibrator's-assigned values and the intermediate precision [11].

The observed absolute relative bias for cTAC (1.41% and 2.64%) using both calibration materials were considered negligible because they were included in their covered expanded uncertainties (± 3.02% and ± 2.94%, respectively) and, in consequence, the results were compatibles. Also, when both calibration materials were used, results were comparable because they were metrologically traceable to SI (they are traceable to the same high-order CRM ERM®-DA110a that embodies the derived-unit µg/L of the SI). Thereby, for cTAC results, no action would be required if we wanted to change the calibration materials, only we must verify periodically that bias remains insignificant among the time.

Based on the results obtained, we recommend to clinical laboratories for measurement of cSRL and cTAC only use calibrators with assigned values traceable to ERM®-DA111a and ERM®-DA110a, respectively. Conversely, if these calibrators cannot be used, they should perform compatibility studies using these CRM and to apply a bias correction factor (if this is necessary) to ensure the traceability and equivalence of their cSRL and cTAC results.

Regarding to other immunosuppressants, unfortunately, no CRM or reference measurement procedure exists for mass concentration of cyclosporin A and everolimus in blood. This fact is, of course, compromising the comparability and compatibility of their results over the time (if clinical laboratories implement substantial changes in their measurement procedures and they do not consider traceability information or do not perform compatibilities studies), and, mainly, over the space (between laboratories which use different measurement procedures and calibration materials); and, consequently, hinders their interpretation even more, considering that these results are usually compared with “universal” therapeutic intervals. For these reasons: 1) we would like request to metrological international institutes that, as far possible, produce metrological references or recommend reference procedures; 2) we claim to IVD manufactures a greater effort in applying different strategies described in international guidelines

[10,21–23] in order to establish the traceability of assigned values of their calibration materials; and 3) we recommend to clinical laboratories to perform compatibility studies using reference materials, *e.g.* IQC, if they introduce substantial changes in their measurement procedures until a high-order reference appears in JCTLM database.

5. Conclusions

In order to know how could affect the change of calibration materials in the reliability of cIS's results we investigated, as an example, the traceability of the calibrator's assigned values from two commercial calibration materials and performed different compatibility studies using highest CRM for cSRL and cTAC. Results reported a lack of comparability and equivalence of results only for cSIR. In order to avoid the lack of metrological traceability and measurement compatibility and obtain results as reliable as possible, clinical laboratories should only use calibrators with assigned values traceable to highest CRM for measurement of cSRL and cTAC. This would certainly be a first step towards standardization of cSRL and cTAC measurements, permitting to use common therapeutic interval for improving the results interpretation and, consequently, the patient's outcome.

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