

To Convert or Not to Convert? Official International Association of Therapeutic Drug Monitoring and Clinical Toxicology Guideline: Considerations and Recommendations for Converting Capillary Blood Microsampling Concentrations to Plasma Concentrations

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Background: Capillary blood microsampling enables the sampling of small blood volumes, making it suitable for vulnerable populations, remote collection, and repeated sampling. Owing to its minimal invasiveness, blood microsampling has emerged as an alternative to venipuncture in many fields. However, as capillary blood differs from venous blood in composition and analytes vary in their distribution between the blood cell and plasma fractions, plasma and capillary blood concentrations may differ. Because plasma is often the standard matrix for routine analyses, this discrepancy can compromise the clinicians' interpretation of capillary blood results. Hence, to ensure

comparability with results obtained through plasma-based assays and reference ranges, accurate conversion to plasma concentrations may be required, which may improve the reliability and validity of microsampling-based outcomes. Despite its relevance, guidance on whether conversion is appropriate or desired and on how to translate and validate the translation of capillary blood microsampling results to plasma concentrations is lacking.

Method: To address this gap, members of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) expert committees have prepared this guideline to discuss the “what, when, and how” of converting capillary to plasma concentrations and provide guidance on the decision of fit-for-purpose conversion. In addition, guidance to assess capillary blood results when the reference matrix is whole blood and to assess liquid capillary plasma results is provided. The included topics, as well as considerations and recommendations presented in this guideline, were based on a previously published dried blood spot-based IATDMCT guideline, literature review and expert opinions of the authors.

Results and conclusions: Key points include the importance of conducting a comprehensive clinical validation study to fully understand capillary blood microsampling results. In addition, the performance of the conversion method should be evaluated case by case, as it depends on both the microsampling-based method and analyte of interest. Furthermore, an independent set of paired capillary and venous samples should be used to validate an established conversion formula. Finally, a key point for future studies is to focus on the clinical impact of (converted) capillary blood concentrations in comparison with decisions based on results in the reference matrix to guarantee any microsampling-based outcome.

Key Words: microsampling, capillary blood concentrations, dried blood spots, conversion methodologies, guideline

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INTRODUCTION

Capillary blood microsampling, such as following a finger-prick, has emerged as a minimally invasive

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alternative to venous blood collection. Over the past decade, conventional sampling of dried blood spots (DBS) using filter paper has evolved to collect capillary blood using sophisticated alternatives.^{1–3} Unlike venipuncture, which requires dedicated clinical staff and is often burdensome, capillary blood can be collected in a remote (home-based) setting by the users themselves or other nonhealth care–trained individuals, without the need to travel to a doctor or hospital. Moreover, owing to the collection of small volumes (often <50 μ L), blood microsampling is particularly suitable for fragile populations, such as elderly and pediatrics, and also offers the potential for repeated sampling, such as in the context of pharmacokinetic (PK) studies and therapeutic drug monitoring (TDM).^{4–8}

Despite these benefits, major challenges arise when interpreting capillary blood concentrations. Capillary blood differs from venous blood in composition as it contains interstitial fluid as well as both arterial and venous blood, typically with a higher proportion of arterial blood, potentially affecting the capillary blood concentration of the envisaged analyte(s).^{4,9–11} Moreover, the arterial-to-venous blood ratio largely depends on factors such as the sampling site, whereas the interstitial fluid proportion is affected by the pressure applied around the puncture site to enhance blood flow.^{12,13} In addition, owing to the challenges historically associated with working with whole blood, most reference ranges and clinical cutoffs have been established in plasma or serum, except for a few specific compounds, such as tacrolimus. Therefore, plasma and serum are considered as standard matrices in clinical practice. As analyte distributions vary between the red blood cell (RBC) and plasma fractions, concentrations in capillary blood and venous plasma may differ, which may compromise the clinicians' interpretation of microsampling-based results.^{9,11} Theoretically, new microsampling-based reference ranges and cutoff values can be established if the values differ, which would be expensive and time-consuming. Moreover, as clinicians are familiar with existing reference ranges, introducing alternative whole blood-based ranges may cause confusion and wrong interpretations.¹⁴ Hence, capillary blood concentrations should be accurately converted to plasma or serum concentrations to ensure the validity and clinical utility of microsampling-based results, depending on the matrix that is routinely used. This is essential to allow comparison with results obtained through conventional plasma or serum methods and existing reference ranges.¹

Although a blood microsampling-based method can only be implemented in clinical practice once the capillary results are interchangeable with those obtained using the reference method, comprehensive guidance on how to appropriately convert capillary blood concentrations into venous plasma or serum concentrations is currently lacking. Furthermore, multiple conversion methods have been proposed even for a single analyte and application, underscoring the necessity for a streamlined approach to translate microsampling-based blood results into plasma or serum concentrations.^{15–20}

Therefore, this guideline aims to provide recommendations on how to convert capillary blood concentrations (C_{cap}) to estimated venous plasma concentrations (EC_{pl}) and correctly

evaluate the performance of the applied conversion approach. While these topics have already been touched upon in a previous guideline on the analytical and clinical validation of conventional DBS,¹ the presented guideline covers additional matrices and collection techniques, aiming to further promote the implementation of blood microsampling-based methods in clinical practice. Notably, this guideline mainly focuses on the conversion of (particularly dried) capillary blood to venous plasma results, but the provided recommendations are also applicable when the reference matrix is serum. Ultimately, as differences may exist between plasma- and serum-based results, as described for glucose and potassium,^{21,22} capillary blood results must be compared with the reference matrix in question. Furthermore, the recommendations are general and not microsampling device-specific. Consequently, they can be applied across a range of devices used to collect capillary blood microsamples, such as DBS cards, Capitainer, HemaXIS, Mitra volumetric absorptive microsampling (VAMS), and Tasso M20. In addition, the recommendations are applicable to various sampling purposes, including TDM, PK studies, and follow-up of (endogenous) compounds or biomarkers, such as the assessment of vitamin D status and/or sampling sites, including finger, arm, and ear pricks. For convenience, finger-prick sampling was used as a representative example throughout this guideline.

This guideline begins with an overview of how to properly conduct a clinical validation study (section “Clinical validation”). This is followed by an overview of the different available approaches to “translate” C_{cap} to EC_{pl} (section “Overview of the different approaches to convert capillary blood concentrations to venous plasma concentrations”). The next section provides an overview of the instances where conversion to EC_{pl} is considered unnecessary, such as when the reference matrix is whole blood instead of plasma or serum, or even when it is contraindicated (section “When is conversion to venous plasma-based results not recommended?”). Furthermore, guidance on the decision to adopt a fit-for-purpose conversion approach is provided (section “Considerations and recommendations to convert capillary blood concentrations to venous plasma concentrations”). This section encompasses recommendations for (dried) microsampling-based assays for endogenous compounds and biomarkers as well as exogenous or administered compounds. Finally, dried plasma sample use as an alternative to capillary blood microsampling is discussed (section “Dried plasma spots to eliminate the need for conversion?”).

CLINICAL VALIDATION

A newly developed and analytically validated microsampling-based method can be implemented as an alternative to the standard procedure in routine care only after it has been successfully validated in a clinical validation study, demonstrating that the results from capillary samples are clinically interchangeable with those obtained using a reference matrix, such as venous blood, plasma, or serum.¹ Given the importance of a well-designed clinical validation study, its practical aspects and points of focus are briefly discussed. Further details can be found in the International Association

of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) guideline by Capiou et al.¹

In a clinical validation study, paired venous whole blood and capillary samples are collected from at least 40 participants, using a single sample per participant, or 25 participants if multiple samples (ideally 2–3) per participant are collected.^{1,23} This minimum sample size threshold is based on the recommendations stated in the guideline for measurement procedure comparison provided by the Clinical and Laboratory Standards Institute (CLSI) and the guideline by Capiou et al.^{1,23} In brief, the required sample size is calculated as described by Linnet et al and primarily depends on the range ratio (maximum value divided by the minimum value of the concentration range) and coefficient of variation of the methods. Because the range ratio and coefficient of variation of most microsampling-based methods are above 25 and 5%, respectively, the number of samples required following Linnet calculation will be 36 or 45.²⁴ Therefore, 40 participants in case a single sample is collected per participant, or 25 participants when multiple samples per participant are collected, should be used as a rule of thumb in clinical validation studies. These samples should be equally distributed over the entire concentration range of interest and, in case of capillary samples, should be collected by the person intended to take the samples clinically, including the participants themselves, health care providers, or nonhealth care-trained persons. Moreover, venous and capillary blood should be collected nearly simultaneously and in a randomized order, with the venous collection occurring randomly before or after the capillary collection to ensure optimal comparison of the results obtained from the paired samples.^{1,25,26}

Analytical results obtained from different blood-based matrices are compared and statistically evaluated in a clinical validation study (Fig. 1).^{1,23} Statistical evaluations are described in section “Considerations and recommendations to convert capillary blood concentrations to venous plasma concentrations”; Bland–Altman comparison and Passing–Bablok regression analysis can be used for this purpose. For dried capillary blood microsampling (Fig. 1A), the potential differences between capillary and venous blood concentrations can be assessed by comparing the results obtained from dried microsamples collected after a finger-prick with those from microsamples prepared from venously collected blood. In addition, the venous blood sample can be used to generate plasma. Consequently, the results obtained from venous liquid whole blood and plasma can be compared and used to experimentally derive the blood-to-plasma (C_{bl}/C_{pl}) ratio of the analyte of interest. Furthermore, the results obtained from dried and liquid venous blood samples could be compared and should yield similar outcomes. A discrepancy observed at this point indicates an impact of the dried matrix approach in se (“methodological bias”),¹ which will also be present when analyzing dried capillary samples and may need to be accounted for at some point. Alternatively, if liquid capillary blood microsamples are collected (Fig. 1B), liquid capillary plasma or serum could be obtained from the anticoagulated or coagulated capillary blood, respectively. The results obtained from these samples can then be compared with those obtained from venous equivalents to assess potential capillary–venous differences.

This clearly implies that a comprehensive clinical validation covering all possible comparisons is of utmost importance to ensure a complete understanding of the results obtained from different matrices (Fig. 1). Depending on the matrix that is routinely used, the results obtained from liquid or dried finger-prick samples can be compared with those obtained from the standard matrix (venous blood, plasma, or serum). The presented guideline addresses these comparisons. If the results of capillary blood and the standard matrix are not interchangeable, whether a conversion factor or formula to translate C_{cap} can be established should be evaluated. Importantly, if this conversion is derived from experimental data, an additional independent sample set not used to derive the conversion factor or formula is required to validate the conversion approach.¹ This aspect has often been overlooked and is discussed in detail in section “Approaches based on experimental data”. Paired samples for independent test and validation sets can be collected from the same participants at different time points to avoid the need for a new cohort of participants.¹ Further specific recommendations for sample size and type of conversion factor or formula to be applied are provided in section “Considerations and recommendations to convert capillary blood concentrations to venous plasma concentrations”.

OVERVIEW OF THE DIFFERENT APPROACHES TO CONVERT CAPILLARY BLOOD CONCENTRATIONS TO VENOUS PLASMA CONCENTRATIONS

Table 1 summarizes an overview of the different methods used to convert C_{cap} to EC_{pl} , as outlined in the literature, along with examples of the analytes to which these approaches have been applied. This overview is based on a review of the literature on the conversion of C_{cap} to EC_{pl} for commonly known microsampling-based applications such as TDM and PK studies. As summarized in Table 1 and elaborated below, conversion methods can be categorized into approaches based on the hematocrit (Hct) and PK parameters, experimental data, or a combination of both. A summary of the advantages and disadvantages of different conversion approaches is given in Table 2.

Approaches Based on the Hct and PK Parameters

The C_{bl}/C_{pl} ratio of the compound is the first factor to be considered when calculating EC_{pl} from C_{cap} . This ratio depends on the distribution of the compound between the RBC and plasma fractions (Fig. 2) and can be expressed by the following equation under steady-state conditions (Equation 1)⁹:

$$\frac{C_{bl}}{C_{pl}} = (1-Hct) + Hct \times \rho \times f_u \quad (1)$$

where C_{bl} represents the total blood concentration, C_{pl} the total plasma concentration, ρ the red blood cell-to-unbound plasma concentration ratio, f_u the unbound fraction in plasma,

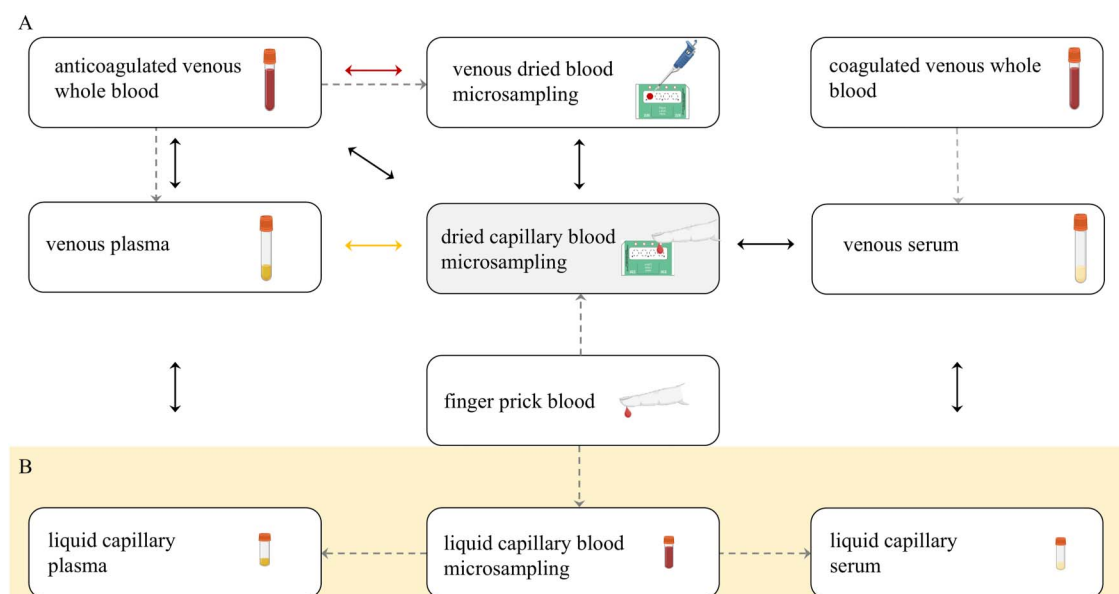


FIGURE 1. Schematic overview of the samples that can be collected during a clinical validation study, for (A) dried or (B, indicated by the yellow-shaded area) liquid capillary blood microsampling-based assays. The gray and bold black arrows indicate the samples that can be generated from individual sampling methods and compared, respectively. The yellow arrow shows the comparison between dried capillary blood and venous plasma samples (the main focus of this guideline). The red arrow indicates the essential comparison between dried and liquid venous blood samples, which should in essence give the same results. Figure inspired by Capiu et al.¹

and Hct the hematocrit.^{9,11} An overview of the factors commonly used in this guideline, along with their corresponding classifications, is summarized in Table 3.

Because capillary blood is at the transition from arterial to venous blood, it consists of a mixture of both and contains interstitial fluid.^{1,4,9–11} Consequently, C_{cap} might differ from C_{bl} , which is particularly important when there is a significant volume of interstitial fluid present in the capillary blood, known as “dilution” of the sample owing to excessive squeezing of the fingertip and should be avoided upon sampling.^{1,27} Moreover, owing to the PK delay between the two matrices, the differences between C_{cap} and C_{bl} may be significant during the distribution phase of a component.^{1,4,9–11,28} Equation 1 describes a compound’s distribution between the RBC and plasma fractions under steady-state conditions. Hence, assuming no significant difference between C_{cap} and C_{bl} at steady state, C_{bl} can be substituted with C_{cap} in Equation 1 (Equation 2), enabling the calculation of EC_{pl} based on C_{cap} using Equation 3:

$$\frac{C_{cap}}{C_{pl}} = (1 - Hct) + Hct \times \rho \times f_u \quad (2)$$

$$EC_{pl} = \frac{C_{cap}}{(1 - Hct) + Hct \times \rho \times f_u} \quad (3)$$

This relationship suggests that the Hct and the affinity of the compound for cellular and plasma fractions are the primary factors determining the C_{cap}/C_{pl} ratio. As the affinity of the compound for cellular and plasma fractions is

dependent on its physicochemical properties, it is essential to evaluate on a case-by-case basis which of the three parameters (ie, Hct, f_u , or ρ) has the most significant influence on the C_{cap}/C_{pl} ratio and should be considered when aiming at converting C_{cap} to EC_{pl} using this approach.^{9,11}

First, for compounds that are (almost) exclusively present in plasma, such as therapeutic proteins, or show high plasma protein binding, and hence low plasma f_u (Table 3), distribution in the RBCs can be neglected, and the RBC fraction can, in essence, be considered a “dilution” of the plasma fraction.²⁹ These compounds typically display low C_{bl}/C_{pl} and C_{cap}/C_{pl} ratios (Table 3). Because f_u and ρ can be considered negligible in this case, the Hct will be the most important parameter influencing the relationship between C_{cap} and C_{pl} .^{11,15,29} Therefore, Equation 3 can be simplified to Equation 4, which enables the calculation of EC_{pl} from C_{cap} based solely on the Hct. The mean or median Hct of the intended population or individual Hct of the sample can be used to calculate EC_{pl} . Suggestions for determining the individual Hct value from dried blood microsamples are provided in section “Endogenous compounds and biomarkers”.

$$EC_{pl} = \frac{C_{cap}}{1 - Hct} \quad (4)$$

Examples of analytes to which this simplified conversion method was applied are summarized in Table 1. For example, because iohexol is fully excluded from RBCs,^{30,31} Zwart et al³² converted DBS-based C_{cap} to EC_{pl} based on Equation 4 using both the mean Hct of the target population and individual Hct. The details of the calculations are

TABLE 1. Overview of Different Approaches to Convert Capillary Blood Microsampling Concentrations to Plasma Concentrations, Along With Some Examples Outlined in Literature (Nonlimiting)

Parameter(s)	Conversion Formula	Selected examples ^{ref}
Hct- and PK-Based Approach		
Hct	$EC_{pl} = \frac{C_{cap}}{(1-Hct)}$	Iohecol^{32,107} Vitamin D* 33,38,143,144 Androstenedione, 17-OH-progesterone, and testosterone¹⁴⁵ Mycophenolic acid^{19,68} Linezolid⁹⁶ Crenezumab and etrolizumab⁹⁷ Radotinib[†],¹¹⁴ dabrafenib⁴⁶ Vancomycin and creatinine[‡] ⁹⁵ Risperidone (and metabolite)¹¹⁷ Adalimumab¹⁴⁶
Hct, ρ , and f_u	$EC_{pl} = \frac{C_{cap}}{(1-Hct)+Hct \times \rho \times f_u}$	Nilotinib³⁹ Nilotinib, cabozantinib, dabrafenib, trametinib, and ruxolitinib²⁰
Hct and $K_{RBC/pl}$	$EC_{pl} = \frac{C_{cap}}{(1-Hct)+Hct \times K_{RBC/pl}}$	Mycophenolic acid⁶⁸ Vemurafenib⁴⁴ Palbociclib,⁴⁵ trametinib⁴⁶ Tamoxifen and endoxifen⁴³
Experimental approach		
$\frac{C_{chl}}{C_{pl}}$ ratio	$EC_{pl} = C_{cap} \times \frac{1}{\frac{C_{chl}}{C_{pl}}}$	Nirmatrelvir¹⁴⁷ Creatinine[§] ⁵¹ Imatinib and dasatinib⁵⁰
$\frac{C_{cap}}{C_{pl}}$ ratio	$EC_{pl} = C_{cap} \times \frac{1}{\frac{C_{cap}}{C_{pl}}}$	Iohecol³² Mycophenolic acid^{32,68} Linezolid¹⁴⁸ Adalimumab¹⁰⁴ Imatinib^{59,149} Nilotinib, cabozantinib, dabrafenib, trametinib, and ruxolitinib²⁰ Palbociclib, ribociclib, and letrozole⁴⁵ Paclitaxel⁵⁸ Docetaxel⁵² Lacosamide, lamotrigine, and levetiracetam¹⁸
Slope (and intercept) based on linear or (weighted) Deming or Passing–Bablok regression analysis	$EC_{pl} = \frac{C_{cap}}{\text{slope}}$ or $EC_{pl} = \frac{C_{cap} - \text{intercept}}{\text{slope}}$	Mycophenolic acid^{19,115} and metabolite⁴⁸ Vitamin D^{150,151} Creatinine^{19,26,69,95,115} Radotinib,¹¹⁴ anlotinib¹¹⁶ Nilotinib,³⁹ Nilotinib, cabozantinib, dabrafenib, trametinib, and ruxolitinib²⁰ Vemurafenib⁴⁴ Paclitaxel⁵⁸ Lacosamide, lamotrigine, and levetiracetam¹⁸ Venlafaxine, desvenlafaxine, quetiapine, norquetiapine, and mirtazapine¹⁵² Vancomycin⁹⁵ Amlodipine, enalaprilate, hydrochlorothiazide, losartan (and metabolite), nifedipine, perindopril (and metabolite), spironolactone (and metabolite), and valsartan¹¹⁸ Pipamperone¹¹⁷ 5-Fluorouracil⁷⁸

(continued on next page)

TABLE 1. (Continued) Overview of Different Approaches to Convert Capillary Blood Microsampling Concentrations to Plasma Concentrations, Along With Some Examples Outlined in Literature (Nonlimiting)

Parameter(s)	Conversion Formula	Selected examples ^{ref}
Combinatorial approach		
Hct and conversion factor	$EC_{pl} = \frac{C_{cap}}{(1-Hct)} \times \text{conversion factor}$	Iohexol ⁵¹ Imatinib ⁵⁹ Palbociclib, ribociclib, and letrozole ⁴⁵ Tamoxifen (and metabolites) and endoxifen ⁵⁷ Paclitaxel ⁵⁸ Docetaxel ⁵² Aripiprazole (and metabolite) ¹¹⁷

*Heughebaert and Ghesquière et al applied an additional Hct-based conversion factor to account for the Hct of DBS-based calibrators.

†Radotinib plasma concentrations were estimated based on the Hct using a second-degree polynomial function.

‡Vancomycin and creatinine plasma concentrations were estimated based on linear regression with the Hct as an independent variable.

§Plasma creatinine concentrations were estimated based on the time-dependent C_{bl}/C_{pl} ratio.

presented in Case Example 1. The mean Hct for conversion was also successfully applied by Jensen et al³³ to assess vitamin D levels in capillary DBS collected from healthy adults. However, when applying the same approach to DBS-based results obtained from neonates, a large bias between EC_{pl} and actual C_{pl} was found. Because the Hct range in a neonatal population is broader than that in a healthy adult population,³⁴ using the mean or median population-based Hct could be an oversimplification for populations with (more) heterogeneous Hct values. Notably, for all Case Examples, the values were obtained directly from the referenced study, where available. When specific data were not provided, values were selected arbitrarily. For example, the individual Hct levels in Case Example 1 were arbitrarily chosen to reflect a wide Hct range.

Wang et al³⁵ proposed an alternative approach for obtaining EC_{pl} for components that are (almost) exclusively present in the plasma fraction. Instead of converting DBS-based concentrations to EC_{pl} , vitamin D was quantified in fixed-size DBS subpunches using matrix-matched artificially prepared calibrators, the concentrations of which had already been translated to equivalent C_{pl} . Therefore, the need to convert DBS-based results was circumvented,

as plasma-based rather than blood-based vitamin D quantitative results were obtained upon DBS sample analysis. However, this simplified approach should be used with caution, as the difference in Hct between actual DBS samples and artificially prepared calibrators, which is most pronounced at the Hct extremes, can result in inaccurate quantification. This was also described by Wang et al:³⁵ the accuracy of samples with a Hct ranging from 0.30 to 0.70 L/L varied from 115.5% to 83.0%, respectively. Because vitamin D is mainly present in the plasma fraction and specifically for DBS samples, as the spreadability of blood onto filter paper is inversely dependent on the Hct, the plasma fraction being analyzed in a fixed-size DBS subpunch will differ when the Hct (strongly) differs from the Hct of the calibrators, resulting in inaccurate quantification of the sample of interest. Hence, for DBS-based approaches specifically using a fixed-size subpunch, an additional correction factor to account for the variation in the Hct of the sample compared with the value of the calibrators should be included unless only samples with a Hct close to that of the matrix-matched calibrators are analyzed. As proposed by Lote-Oke et al,³⁶ an additional correction factor was recently explored by Heughebaert et al using Equation 5:

TABLE 2. Summary of the Advantages and Limitations of the Different Conversion Approaches Discussed in Section “Overview of the different approaches to convert capillary blood concentrations to venous plasma concentrations”

Approach	Advantages	Limitations
Hct- and PK-based approach	No independent sample set required for validation	Compound distribution in different blood fractions and different parameters (Hct, ρ , and f_u) should be known
	If compound is solely present in the plasma fraction, conversion is solely based on the Hct	Impact of intra- and interindividual variability of parameters on performance conversion formula
Experimental approach	Inclusion of other clinical variables inherent to study population	Independent sample set required for validation (such as, more paired samples needed)
	Coverage of the entire concentration range of interest in case of conversion based on regression analysis	Conversion factors dependent on the population characteristics; hence, limited extrapolation to extended population
Combinatorial approach	Additional factor included for accurate conversion	Independent sample set required for validation (such as, more participants needed)
		Additional factor cannot cope with issues related to the (im)precision

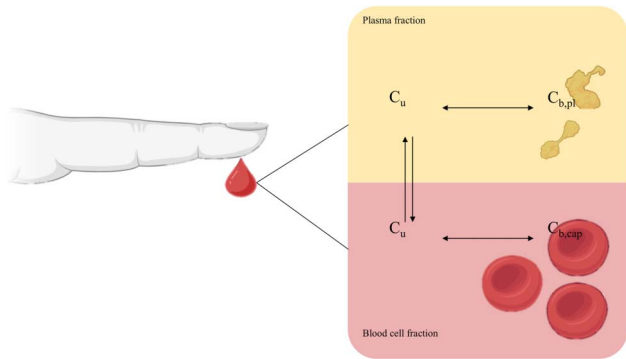


FIGURE 2. Schematic representation of a compound's partitioning in capillary blood at steady-state conditions. $C_{b,cap}$, bound concentration in the capillary blood cell fraction; $C_{b,pl}$, bound concentration in the plasma fraction; C_u , unbound concentration.

$$C_{cap,corrected} = \frac{C_{cap}}{1 - (Hct_{cal} - Hct_{indiv})} \quad (5)$$

where Hct_{cal} and Hct_{indiv} represent the Hct of microsampling-based calibrators and actual microsamples, respectively.^{37,38}

When combining Equation 5 with Equation 4 to correct for the difference in Hct between the calibrators versus real sample and the difference in the matrix, EC_{pl} can be calculated using Equation 6. Details regarding the calculations of the latter approach are provided in Case Example 2.

$$EC_{pl} = \frac{C_{cap,corrected}}{1 - Hct_{indiv}} = \frac{C_{cap}}{1 - (Hct_{cal} - Hct_{indiv})} \times \frac{1}{1 - Hct_{indiv}} \quad (6)$$

Second, for compounds that do partition into the RBCs and/or bind to cellular and plasma proteins, ρ and f_u are also of importance. In this case, the relationship between C_{cap} and C_{pl} can be expressed using Equation 2, and hence, EC_{pl} can be calculated using Equation 3. The application of Equation 3 was reported by Boons et al³⁹ for converting DBS-based nilotinib C_{cap} to EC_{pl} in patients with chronic myeloid leukemia (Case Example 3). Although these authors concluded that the preset analytical acceptance criteria, as proposed by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines, were met,^{40,41} the agreement between EC_{pl} and paired C_{pl} was rather limited, as the results showed a significant bias and a large variability. Because the DBS-based method is intended for the TDM of nilotinib, such bias and variability could potentially influence clinical decision-making. Therefore, beyond meeting the analytical limits, the potential impact on the clinical interpretation should be assessed (section “Evaluation of the performance of the conversion approach”). Interestingly, when using a simplified version of the formula relying solely on Hct (Equation 4), a similar result for EC_{pl} was obtained (Case Example 3). However, this is expected because the value of f_u (0.02) can be considered negligible. In addition, when applying the same approach (based on Hct, ρ , and f_u) to VAMS-based results obtained in a similar

population, Zimmerman et al reported satisfactory results for nilotinib, cabozantinib and dabrafenib, while EC_{pl} and paired C_{pl} were not interchangeable for trametinib and ruxolitinib.²⁰ In contrast to the study by Boons et al, here, ρ was determined using experimental data obtained from an in vitro spiking experiment, which may only partially reflect the in vivo distribution between the RBC and plasma fractions in clinical samples and, consequently, may yield deviating results.^{20,42} Therefore, when aiming to accurately convert C_{cap} based on Equation 3, the values for ρ and f_u should be determined in clinical samples (in vivo versus in vitro established values) and show limited (inter- and intraindividual) variability.¹¹

Alternatively, by replacing ρ and f_u in Equation 3 with the RBC-to-plasma partitioning coefficient ($K_{rbc/pl}$), EC_{pl} can be calculated using the following formula (Equation 7):

$$EC_{pl} = \frac{C_{cap}}{(1 - Hct) + Hct \times K_{rbc/pl}} \quad (7)$$

This approach was applied in the context of TDM for different anticancer drugs (such as vemurafenib, palbociclib, dabrafenib, trametinib, tamoxifen, and endoxifen), where $K_{rbc/pl}$ was either determined based on in vitro data or calculated using data obtained from the literature (Case Example 4).^{43–46}

Finally, compounds that freely enter RBCs and do not bind to cellular or plasma proteins display a C_{bl}/C_{pl} ratio of

TABLE 3. Overview and Classifications of Terms Used in the Equations Outlined in This Guideline

Hct	Low Hct Normal Hct* High Hct	Hct < 0.30 0.35 < Hct < 0.50 Hct > 0.55
f_u	High plasma protein binding Moderate plasma protein binding Low plasma protein binding	$f_u < 0.05$ $0.05 < f_u < 0.2$ $f_u > 0.2$
$K_{rbc/pl}$	Exclusively plasma-distributed Minimally RBC-distributed Equally plasma- and RBC-distributed Moderately RBC-distributed Primarily RBC-distributed	$K_{rbc/pl} \approx 0$ $K_{rbc/pl} < 1$ $K_{rbc/pl} \approx 1$ $1 < K_{rbc/pl} < 1.5$ $K_{rbc/pl} > 1.5$
$\frac{C_{bl}}{C_{pl}}$	Exclusively plasma-distributed Minimally RBC-distributed Equally plasma- and RBC-distributed Moderately RBC-distributed Primarily RBC-distributed	$\frac{C_{bl}}{C_{pl}} \approx 0.55–0.60$ $\frac{C_{bl}}{C_{pl}} < 1$ $\frac{C_{bl}}{C_{pl}} \approx 1$ $1 < \frac{C_{bl}}{C_{pl}} < 2$ $\frac{C_{bl}}{C_{pl}} > 2$

ρ is dependent on f_u and $K_{rbc/pl}$ and can be calculated using the respective values based on.^{9,11,15,153–155}

*Range reflects the reference range for healthy women and men.¹⁵³ The reference ranges can vary slightly among laboratories.

$\frac{C_{bl}}{C_{pl}}$, blood-to-plasma concentration ratio; f_u , unbound analyte concentration; Hct, hematocrit; $K_{rbc/pl}$, red blood cell-to-plasma partitioning coefficient; ρ , red blood cell-to-unbound plasma concentration ratio.

Case Example 1 ³²**1. Given information:**

Analyte: iohexol

 C_{cap} : 100 $\mu\text{g/mL}$ Hct_{mean} : 0.41 L/L $\text{Hct}_{\text{indiv}}$ of three samples: 0.25 L/L ($\text{Hct}_{\text{sample 1}}$) 0.40 L/L ($\text{Hct}_{\text{sample 2}}$) 0.55 L/L ($\text{Hct}_{\text{sample 3}}$)**2. Conversion based on the following formula:**

$$\text{EC}_{\text{pl}} = \frac{C_{\text{cap}}}{1 - \text{Hct}}$$

3. Calculations:

$$\text{Hct}_{\text{mean}} = \frac{100 \mu\text{g/mL}}{1 - 0.41} = 169.5 \mu\text{g/mL}$$

$$\text{Hct}_{\text{sample 1}} = \frac{100 \mu\text{g/mL}}{1 - 0.25} = 133.3 \mu\text{g/mL}$$

$$\text{Hct}_{\text{sample 2}} : \text{EC}_{\text{pl}} = \frac{100 \mu\text{g/mL}}{1 - 0.40} = 166.7 \mu\text{g/mL}$$

$$\text{Hct}_{\text{sample 3}} : \text{EC}_{\text{pl}} = \frac{100 \mu\text{g/mL}}{1 - 0.55} = 222.2 \mu\text{g/mL}$$

Case Example 2 ^{37,38}**1. Given information:**

Analyte: vitamin D

 C_{cap} : 10 ng/mL Hct_{cal} : 0.40 L/L $\text{Hct}_{\text{indiv}}$ of three samples: 0.25 L/L ($\text{Hct}_{\text{sample 1}}$)0.40 L/L ($\text{Hct}_{\text{sample 2}}$)0.55 L/L ($\text{Hct}_{\text{sample 3}}$)**2. Conversion based on the following formula:**

$$\text{EC}_{\text{pl}} = \frac{C_{\text{cap,corrected}}}{1 - \text{Hct}_{\text{indiv}}} = \frac{C_{\text{cap}}}{1 - (\text{Hct}_{\text{cal}} - \text{Hct}_{\text{indiv}})} \times \frac{1}{1 - \text{Hct}_{\text{indiv}}}$$

3. Calculations:

$$\text{Hct}_{\text{sample 1}} : \text{EC}_{\text{pl}} = \frac{10 \text{ ng/mL}}{1 - (0.40 - 0.25)} \times \frac{1}{1 - 0.25} = 15.7 \text{ ng/mL}$$

$$\text{Hct}_{\text{sample 2}} : \text{EC}_{\text{pl}} = \frac{10 \text{ ng/mL}}{1 - (0.40 - 0.40)} \times \frac{1}{1 - 0.40} = 16.7 \text{ ng/mL}$$

$$\text{Hct}_{\text{sample 3}} : \text{EC}_{\text{pl}} = \frac{10 \text{ ng/mL}}{1 - (0.40 - 0.55)} \times \frac{1}{1 - 0.55} = 19.3 \text{ ng/mL}$$

Case Example 3³⁹**1. Given information:**

Analyte: nilotinib

 C_{cap} : 500 $\mu\text{g/L}$ Hct_{mean} : 0.41 L/L ρ : 0.29 f_u : 0.02**2. Conversion based on the following formula:**

$$\text{EC}_{\text{pl}} = \frac{C_{\text{cap}}}{(1 - \text{Hct}) + \text{Hct} \times \rho \times f_u}$$

3. Calculations:

$$\text{EC}_{\text{pl}} = \frac{500 \text{ } \mu\text{g/L}}{(1 - 0.41) + 0.41 \times 0.29 \times 0.02} = 844.1 \text{ } \mu\text{g/L}$$

Calculated solely based on the Hct:

$$\text{EC}_{\text{pl}} = \frac{500 \text{ } \mu\text{g/mL}}{(1 - 0.41)} = 847.5 \text{ } \mu\text{g/L}$$

approximately 1. In such cases, neither the Hct, nor ρ and f_u , are expected to affect the relationship between C_{cap} and C_{pl} . Therefore, C_{cap} and C_{pl} can actually be considered interchangeable ($C_{\text{cap}} \approx C_{\text{pl}}$) without the need for a conversion factor, provided that a proper clinical validation to evaluate the agreement is performed (sections “Clinical validation” and “When is conversion to venous plasma-based results not recommended?”).^{9,15}

Approaches Based on Experimental Data

In addition to the previously discussed theoretical approaches, a conversion factor or formula can be established using experimental data obtained from clinical validation

samples. Specifically, when using empirical data, other clinical variables inherent to the study population are included in the conversion process. However, because these approaches significantly depend on the population characteristics, its performance may be population-dependent and vary in a different or variable population.¹⁵ Furthermore, in contrast to the previously discussed approaches, the performance of an experimentally determined conversion formula or factor should be validated with a second independent data set. Consequently, this experimental approach typically requires more samples, including a set of samples to establish the conversion factor (“test set”) and a validation set, than the approaches discussed in the previous section, which only

Case Example 4⁴³**1. Given information:**

Analyte: tamoxifen

 C_{cap} : 100 ng/mL Hct_{mean} : 0.41 L/L $K_{\text{rbc/pl}}$: 0.461**2. Conversion based on the following formula:**

$$\text{EC}_{\text{pl}} = \frac{C_{\text{cap}}}{(1 - \text{Hct}) + \text{Hct} \times K_{\text{rbc/pl}}}$$

3. Calculations:

$$\text{EC}_{\text{pl}} = \frac{100 \text{ ng/mL}}{(1 - 0.41) + 0.41 \times 0.461} = 128.4 \text{ ng/mL}$$

Case Example 5⁵⁰**1. Given information:**

Analyte: imatinib

 C_{cap} : 700 ng/mL $C_{\text{bl}}/C_{\text{pl}}$: 0.73**2. Conversion based on the following formula:**

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{bl}}}{C_{\text{pl}}}}$$

3. Calculations:

$$EC_{\text{pl}} = 700 \text{ ng/mL} \times \frac{1}{0.73} = 959 \text{ ng/mL}$$

require a test set.¹ Indeed, both sample sets should include minimum 40 participants when a single sample is collected per participant or 25 participants when multiple (2–3) samples are collected per participant. Paired samples for the test and validation sets can be collected from the same participants at different time points to avoid the need for a new cohort of participants. If the number of paired samples in a sample set is too limited for the preferred independent test and validation sets, a statistical resampling method, such as the jackknife approach, may provide a feasible, albeit less precise, alternative that can offer clinically relevant insights.¹ In short, this nonparametric statistical approach uses the original sample set consisting of n samples to generate n distinct “resamples”, each formed by systematically excluding 1 sample ($n - 1$). Subsequently, a conversion formula is derived from each of

the “resamples”, which is then applied to the excluded sample for validation.^{1,47,48}

The first experimental factor that can be applied to convert C_{cap} to EC_{pl} is the in vivo-derived $C_{\text{bl}}/C_{\text{pl}}$ ratio, which is typically determined by the analysis of paired venous liquid blood and plasma samples collected from the target population; the venous plasma sample is obtained after centrifugation of the whole-blood sample. Depending on the data distribution, the mean or median ratio can then be used to easily calculate EC_{pl} based on the following formula (Equation 8):

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{bl}}}{C_{\text{pl}}}} \quad (8)$$

Because this formula involves venous blood instead of capillary blood, this approach should be applied only when

Case Example 6⁵¹**1. Given information:**

Analyte: creatinine

 $C_{\text{cap},t5\text{min}}$: 150 $\mu\text{g/mL}$ $C_{\text{cap},t120\text{min}}$: 50 mcg/mL $C_{\text{bl}}/C_{\text{pl},t5\text{min}}$: 0.79 $C_{\text{bl}}/C_{\text{pl},t120\text{min}}$: 0.95**2. Conversion based on the following formula:**

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{bl}}}{C_{\text{pl}}} (T_x)}$$

3. Calculations:

$$EC_{\text{pl},t5\text{min}} = 150 \text{ } \mu\text{g/mL} \times \frac{1}{0.79} = 189.9 \text{ } \mu\text{g/mL}$$

$$EC_{\text{pl},t120\text{min}} = 50 \text{ } \mu\text{g/mL} \times \frac{1}{0.95} = 52.6 \text{ } \mu\text{g/mL}$$

Case Example 7¹⁸**1. Given information:**

Analyte: lacosamide

 C_{cap} : 10 $\mu\text{g/mL}$ $C_{\text{cap}}/C_{\text{pl}}$: 1.14**2. Conversion based on the following formula:**

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{cap}}}{C_{\text{pl}}}}$$

3. Calculations:

$$EC_{\text{pl}} = 10 \mu\text{g/mL} \times \frac{1}{1.14} = 8.8 \mu\text{g/mL}$$

C_{bl} and C_{cap} can be considered interchangeable based on clinical validation. The performance of the mean $C_{\text{bl}}/C_{\text{pl}}$ ratio as a conversion factor for estimating EC_{pl} from C_{cap} was recently assessed by Verougstraete et al in an independent validation set conducted in the context of TDM of different tyrosine kinase inhibitors using VAMS (Case Example 5).^{49,50} Acceptable results were obtained by comparing the EC_{pl} and paired C_{pl} for imatinib.⁵⁰ Importantly, if the determined ratio exhibits wide variability or large discrepancy compared with the ratio obtained from the validation data set, accurate conversion using a venous $C_{\text{bl}}/C_{\text{pl}}$ ratio may not be possible. This issue became evident when the approach was applied to convert VAMS-based concentrations into EC_{pl} for dasatinib.⁵⁰ Here, the $C_{\text{bl}}/C_{\text{pl}}$ ratio showed a large variability (coefficient of variation, 24.3%) and differed from the $C_{\text{bl}}/C_{\text{pl}}$ ratio derived from the validation data set (1.40 versus 1.50, respectively), which may have impeded accurate conversion using the venous $C_{\text{bl}}/C_{\text{pl}}$ ratio. On the other hand, both the variability in the determined ratio (coefficient of variation, 8.1%) and the difference in ratio obtained from the validation data set (0.73 and 0.72, respectively) remained limited for imatinib, yielding acceptable results.⁵⁰ These examples demonstrate the importance of a consistent $C_{\text{bl}}/C_{\text{pl}}$ ratio, along with the conditions under which it is determined, when aiming for reliable conversion. If this ratio is characterized by a large spread, it is logical that the converted results will also be characterized by a large spread.

Interestingly, instead of applying an overall $C_{\text{bl}}/C_{\text{pl}}$ ratio to the entire data set, the time-dependent $C_{\text{bl}}/C_{\text{pl}}$ ratio may be used as a conversion factor. This is relevant only when multiple samples are collected per participant after administering a compound. Specific recommendations and considerations regarding this topic are provided in section “Collection of multiple samples per individual.” Time-dependent $C_{\text{bl}}/C_{\text{pl}}$ ratio use was recently explored in a formula used to derive creatinine EC_{pl} from VAMS-based C_{cap} , as the endogenous creatinine $C_{\text{bl}}/C_{\text{pl}}$ ratio was affected after intravenous administration of exogenous creatinine. After an initial decrease, the ratio gradually increased to baseline, indicating a time-dependent creatinine distribution in the

RBCs.⁵¹ As deduced from Equation 9, EC_{pl} were calculated based on the mean $C_{\text{bl}}/C_{\text{pl}}$ ratio obtained at different time points during sample collection (T_x ; Case Example 6). An important limitation of this approach is that deviations in the collection time points between paired samples may substantially affect the accuracy of the converted results (EC_{pl}). This also implies that if one aims to adapt the study design or sampling protocol (such as sampling at a different time point), previously determined time-dependent factors cannot be reproduced because the time-dependent $C_{\text{bl}}/C_{\text{pl}}$ ratio cannot be interpolated. This is particularly important when the $C_{\text{bl}}/C_{\text{pl}}$ ratio is not yet stable, which is dependent on the drug dose. Moreover, applying a time-dependent factor can be challenging for remote or home-collected samples, because the crucial importance of accurate timing may be underestimated by patients or health care providers.

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{bl}}}{C_{\text{pl}}} (T_x)} \quad (9)$$

Instead of using the venous ratio, the mean or median $C_{\text{cap}}/C_{\text{pl}}$ ratio can also be used as a conversion factor, with the benefit of including the possible variability in C_{cap} . This ratio is typically derived from paired capillary blood and venous plasma samples, which should ideally be collected nearly simultaneously (section “Clinical validation”).¹ Similar to Equation 8, EC_{pl} can be calculated using the following formula (Equation 10).

$$EC_{\text{pl}} = C_{\text{cap}} \times \frac{1}{\frac{C_{\text{cap}}}{C_{\text{pl}}}} \quad (10)$$

The details of the calculations are provided in Case Example 7. Acceptable results were obtained by Hagemann et al¹⁸ when this approach was applied to convert the VAMS-based C_{cap} of lacosamide and levetiracetam; however, it was inadequate for lamotrigine. In addition, Raymundo et al⁵² observed a time-dependent change in the $C_{\text{cap}}/C_{\text{pl}}$ ratio of docetaxel (similar to the venous ratio after creatinine

TABLE 4. Overview of the Advantages and Limitations of the Regression Models Discussed in Section “Approaches based on experimental data”

Regression Approach	Advantages	Limitations
Standard linear regression	Simple and easy to compute and to interpret Only applicable when the x variable is measured without error (ie, reference value)	Not suitable for method comparison if both x and y variables have inherent measurement variability Sensitive to outliers and heteroscedastic data
(Weighted) Deming regression	Suitable for method comparison if both x and y variable have inherent measurement variability Weighted regression allows modeling of heteroscedastic data	Assumes normally distributed measurement error (such as parametric approach) Estimation of variances required for both methods More specialized statistical software required
Passing–Bablok regression	Suitable for method comparison if both x and y variable have inherent measurement variability Does not assume normally distributed measurement error (ie, nonparametric approach) Robust to outliers	May be less powerful than Deming regression when assumptions regarding error distribution and variance are met More specialized statistical software required
Mixed-effects modeling	Covariates influencing the capillary-to-plasma relationship (‘random effects’) can be evaluated	Establishing regression model and interpreting data is less simple More specialized statistical software required

administration), which complicates the use of a single capillary ratio for accurate conversion. Therefore, two distinct C_{cap}/C_{pl} ratios were used for the samples collected at two different time points. These examples highlight the importance of evaluating the performance of each conversion approach on a case-by-case basis.

One of the common methods to obtain EC_{pl} from C_{cap} is through application of an experimentally derived (linear) regression equation. A benefit of this conversion approach is that the relationship between C_{cap} and C_{pl} covers the entire concentration range of interest. In the first step, C_{cap} is plotted as a function of the paired C_{pl} .¹ Because C_{pl} and C_{cap} , the x and y variables, respectively, have some inherent variability, the use of weighted Deming or Passing–Bablok regression rather than standard linear regression is strongly recommended to establish the conversion formula, as standard linear regression does not consider possible variabilities in the x or y variables.^{1,23,53,54} Other advantages and disadvantages of the regression models mentioned in this guideline are summarized in Table 4.^{23,53–55} In the next step, the slope and intercept obtained from the regression equation are used to calculate EC_{pl} . If a proportional (the 95% confidence interval of the slope does not include 1) but no systematic difference (the 95% confidence interval of the intercept does include 0) between C_{cap} and C_{pl} is observed, the slope should be considered only when calculating EC_{pl} (Equation 11). However, if both proportional and systematic biases are observed, the slope and intercept should be considered when calculating EC_{pl} (Equation 12).

$$EC_{pl} = \frac{C_{cap}}{\text{slope}} \tag{11}$$

$$EC_{pl} = \frac{C_{cap} - \text{intercept}}{\text{slope}} \tag{12}$$

Examples of analytes for which this approach has been applied are summarized in Table 1 and the details of the

calculations are presented in Case Examples 8 and 9 of Equations 11 and 12, respectively. Interestingly, instead of using C_{cap} , Vethe et al²⁶ designed an algorithm for converting creatinine concentrations (for both VAMS and Capitainer DBS samples) based on linear regression between C_{bl} and C_{pl} . However, when applying this formula to obtain EC_{pl} from clinical capillary blood microsamples, suboptimal results were obtained for samples collected using the Capitainer device. To achieve a mean bias of zero, the slope of the applied conversion formula was adjusted based on the data obtained from capillary microsamples, which were then successfully applied to an independent set of capillary samples.

In addition, particular attention should be paid when extrapolating the formula to a different or more extended population, as the experimental data used to derive the conversion formula are dependent on the characteristics of the population.¹⁵ Hence, to increase the applicability of the conversion factor or formula to a diverse population, other variables that can potentially affect the capillary-to-plasma relationship should be considered. Recently, a linear mixed-effects model was explored for converting VAMS-based C_{cap} to EC_{pl} in a PK study of padsevonil.⁵⁶ C_{cap} was considered a fixed independent variable, while other factors such as dosing regimen, age, sex, study population, and sampling time point were statistically evaluated as random covariates. An overview of the factors that could be evaluated as covariates affecting the capillary-to-plasma

TABLE 5. Overview of Covariates to Consider When Evaluating the Capillary-To-Plasma Relationship (Nonlimiting)

Hematocrit
Dosing regimen (eg, dose, time)
Sampling time
Age
Gender
Weight
eGFR

Case Example 8**1. Given information:**

Analyte: creatinine

 C_{cap} : 5 $\mu\text{g/mL}$

Slope (95% CI): 1.87 (1.50–2.20)

Intercept (95% CI): 0.04 (–0.1–0.1)

2. Conversion based on the following formula:

$$EC_{\text{pl}} = \frac{C_{\text{cap}}}{\text{slope}}$$

3. Calculations:

$$EC_{\text{pl}} = \frac{5 \mu\text{g/mL}}{1.87} = 2.7 \mu\text{g/mL}$$

relationship is summarized in Table 5. Interestingly, in the padsevonil study, both dosing regimen and sampling time point were the most significant covariates, which were subsequently included in the model to calculate EC_{pl} .⁵⁶ Although the performance of the conversion might be improved by including other variables, this further complicates the calculation of EC_{pl} than the preceding methods, as statistical software and models are often required. Hence, to determine the most suitable conversion approach for a given microsampling-based method, the potential performance improvements of more complex methods should be weighed against the simplicity of alternative approaches.

Combinatorial Approach

As proposed by Antunes et al for anticancer drugs,⁵⁷ both the Hct and an experimentally determined conversion factor can be used to calculate EC_{pl} (Equation 13):

$$EC_{\text{pl}} = \frac{C_{\text{cap}}}{1 - \text{Hct}} \times \text{conversion factor} \quad (13)$$

Because Equation 13 is partially based on experimental data, both a test set and independent validation set are required to establish and validate the factor.¹ This approach is particularly relevant when the Hct is one of the primary factors influencing the difference between C_{cap} and C_{pl} (section “Approaches based on the Hct and PK parameters”), with an additional factor added for accurate conversion. Different conversion factors have been used. For example, in addition to the Hct, an experimentally determined factor for the conversion of VAMS-based iohexol C_{cap} to EC_{pl} was recently explored by De Baets et al.⁵¹ This factor was introduced to address the slight methodological bias observed when comparing the results obtained from liquid and dried venous blood samples. As previously mentioned, the results obtained from a venous liquid or dried matrix should, in essence, be

Case Example 9**1. Given information:**

Analyte: creatinine

 C_{cap} : 5 $\mu\text{g/mL}$

Slope (95% CI): 1.87 (1.50–2.20)

Intercept (95% CI): 0.34 (0.10–0.55)

2. Conversion based on the following formula:

$$EC_{\text{pl}} = \frac{C_{\text{cap}} - \text{intercept}}{\text{slope}}$$

3. Calculations:

$$EC_{\text{pl}} = \frac{5 \mu\text{g/mL} - 0.34 \mu\text{g/mL}}{1.87} = 2.5 \mu\text{g/mL}$$

Case Example 10⁵¹**1. Given information:**

Analyte: iohexol

 C_{cap} : 100 $\mu\text{g/mL}$ Hct_{mean} : 0.41 L/L

Conversion factor: 1/0.88 (significant slope when comparing results obtained from venous liquid and venous dried blood samples)

2. Conversion based on the following formula:

$$\text{EC}_{\text{pl}} = \frac{C_{\text{cap}}}{1 - \text{Hct}} \times \text{conversion factor}$$

3. Calculations:

$$\text{EC}_{\text{pl}} = \frac{100 \mu\text{g/mL}}{1 - 0.41} \times \frac{1}{0.88} = 192.6 \mu\text{g/mL}$$

similar. However, if they do not match, this indicates an effect of the dried matrix approach in se, which will also be present when analyzing dried capillary samples and, hence, affect the accuracy of the converted results (section “Clinical validation”).¹ When including the significant slope derived from the Passing–Bablok regression analysis when comparing results obtained from liquid and dried venous blood samples into the conversion model, successful agreement between EC_{pl} and paired C_{pl} was achieved (Case Example 10).⁵¹ Alternatively, other conversion factors to account for the remaining bias between C_{cap} or EC_{pl} and C_{pl} have been used.^{45,52,57–59} Importantly, a general prerequisite for a successful conversion is that the spread on the data is not too large; alternatively phrased, whereas a conversion factor can cope with bias, it cannot cope with issues related to (im) precision.

WHEN IS CONVERSION TO VENOUS PLASMA-BASED RESULTS NOT RECOMMENDED?

While accurate conversion of microsampling-based C_{cap} to EC_{pl} is required for most compounds to allow successful interpretation and comparison with results obtained in the reference plasma or serum matrix, there are some exceptions for which translation to EC_{pl} is deemed undesired.

As mentioned in section “Approaches based on the Hct and PK parameters”, under steady-state conditions, C_{cap} and C_{pl} can be considered interchangeable for compounds that freely enter RBCs and do not bind to a relevant extent to cellular or plasma proteins (such as caffeine, methanol, and ethanol).^{9,11,60} However, as most substances tend to interact with blood proteins, compounds showing equal distribution between the RBC and plasma fractions are relatively exceptional.

Furthermore, for compounds that are routinely measured in whole blood as a reference matrix instead of in plasma, the conversion of C_{cap} to EC_{pl} is not clinically relevant. Components for which reference ranges or cutoffs are established in whole-blood and microsampling-based methods have already been

successfully applied include hemoglobin A_{1c}, vitamin B₁, phosphatidylethanol, lead, and immunosuppressants such as tacrolimus, everolimus, sirolimus, and cyclosporin A.^{61–66} Hence, as the results are obtained from a similar matrix (venous blood versus capillary blood), the use of blood-based microsampling approaches is particularly interesting for compounds routinely measured in whole-blood samples. As the capillary blood composition differs from that of venous blood, analyte concentrations may be affected. Therefore, the agreement between the results obtained from capillary blood microsamples and conventional liquid blood samples should be evaluated in clinical validation and a conversion factor or formula should be established, if needed.¹ Recommendations provided in section “Considerations and recommendations to convert capillary blood concentrations to venous plasma concentrations” might in this case be followed. For example, some authors have identified significant and clinically relevant differences in the determination of tacrolimus from dried finger-prick microsamples (DBS and/or VAMS) and liquid venous blood samples.^{67–72} Hence, the mean $C_{\text{cap}}/C_{\text{bl}}$ or the equation obtained from the linear regression analysis was applied as a factor to match the results obtained from the finger-prick samples to those from the venous blood samples.

Instead of collecting dried capillary blood samples, collecting liquid capillary blood samples (for example using Minicollect tubes) has attracted much interest, offering the benefit of directly generating capillary plasma or serum, depending on whether the collection tube contained an anticoagulant (Fig. 1). In addition, the results obtained from paired capillary and venous plasma samples should be compared in a clinical validation study to assess analytical and clinical interchangeability (section “Clinical validation”). This has already been successfully executed by different research groups, allowing accurate quantification of (therapeutic) proteins and biomarkers without the need for conversion.^{73–77}

From an analytical perspective, converting microsampling-based concentrations is not recommended when there is large variability in any factor used in the conversion formula, such as the mean Hct value, mean $C_{\text{bl}}/C_{\text{pl}}$, or

mean $C_{\text{cap}}/C_{\text{pl}}$ ratio, as discussed in the previous section. In these cases, alternative conversion approaches should be considered. If this is not feasible because of the wide variability in one of the conversion factors, conversion is theoretically discouraged, as it will negatively affect the data quality and/or lead to a large uncertainty in the converted data. Indeed, an accurate and precise determination in blood, followed by a conversion accompanied by large variability or uncertainty, may result in poorly converted plasma concentrations. In these cases, interpreting capillary blood concentrations against existing reference ranges in plasma remains challenging, hampering the broader application and implementation of the methodology. In such instances, setting up a reference interval in blood may be required, with associated disadvantages and challenges, as outlined in section “Introduction”. Moreover, capillary microsampling may not be suitable because large intra- and/or interindividual variations in the $C_{\text{cap}}/C_{\text{pl}}$ ratio may intrinsically hamper the interpretability of C_{cap} .⁷⁸ For example, large and inconsistent within-participant variability was recently observed in the determination of uracil in finger-prick DBS, hampering the use of finger-prick samples for this specific purpose.⁷⁹

CONSIDERATIONS AND RECOMMENDATIONS TO CONVERT CAPILLARY BLOOD CONCENTRATIONS TO VENOUS PLASMA CONCENTRATIONS

This section provides step-by-step guidance on how to analytically and clinically evaluate the conversion of C_{cap} to EC_{pl} . As elaborated in detail below, specific recommendations are provided for microsampling-based methods involving the determination of endogenous compounds and biomarkers (section “Endogenous compounds and biomarkers”) or exogenous or administered compounds (section “Exogenous or administered compounds”). These recommendations are primarily based on the IATDMCT guideline for DBS-based method validation,¹ recently published studies comparing paired capillary blood and venous plasma samples (Table 1), and expert opinions of the authors. It is important to mention that as the aim is to consolidate the basic principles of capillary $C_{\text{bl}}/C_{\text{pl}}$ conversion, the following guidance is intended to serve as a basis for (fit-for-purpose) conversion of C_{cap} to EC_{pl} . However, because the performance of the applied conversion approach is contingent on the analyte of interest, its context of use, and the accuracy and precision of the microsampling-based assay (and thus should be evaluated on a case-by-case basis), the recommended approach may not be entirely suitable for certain analytes or methods. Hence, additional conversion factors or alternative approaches should be explored.

For successful conversion, two key prerequisites should be met. First, the analytical method should be successfully validated in accordance with the IATDMCT guideline for developing and validating DBS-based methods, including the assessment of microsampling-specific parameters, such as the Hct effect on recovery and matrix effects.¹ Depending on the analyte of interest and intended use, other (more stringent) limits for analytical validation may be preferred. For instance,

a between-day imprecision of $\leq 10\%$ or even $\leq 6\%$ is recommended when monitoring immunosuppressants,⁸⁰ which is more restrictive than the 15% imprecision threshold suggested in the IATDMCT, EMA, and FDA validation guidelines.^{1,40,41} Alternatively, compound-specific analytical performance specifications and acceptance criteria regarding imprecision and bias may be defined. Different approaches to establish these limitations, such as models based on biological variation, known PK principles, or therapeutic range, have been described for liquid blood- and plasma-based methods.⁸¹ If preferred, these methods could also be extended to establish compound-specific analytical performance criteria for microsampling-based methods. Second, upon the analysis of clinical samples, the quality of the quantitative results should be ensured. If these prerequisites are not met, none of the conversion methods can cope with the issues related to the quality of the analytical method or clinical data.

Endogenous Compounds and Biomarkers

Microsampling-based applications involving the determination of endogenous compounds and biomarkers include diagnostic applications, such as follow-up of creatinine concentrations as an indicator of kidney (dys)function, exposure monitoring, and assessment of nutritional and vitamin status, such as vitamin D level determination.^{26,63,82,83} A schematic overview of the different steps and recommended approaches for converting C_{cap} to EC_{pl} is shown in Figure 3. Compared with the recommendations given in section “Exogenous or administered compounds”, here, conversion can be considered relatively straightforward, as analyte concentrations tend to remain stable or follow predictable patterns, such as circadian rhythms, on sample collection.

As previously mentioned, the agreement between paired capillary blood and venous plasma concentrations, obtained from at least 40 or 25 participants when, respectively, a single sample or multiple samples are collected per participant (“test set”), should first be evaluated using appropriate statistics (**Step 1** in Fig. 3).¹ On one hand, Passing–Bablok regression analysis should be performed to determine proportional or systematic differences between the two matrices (95% confidence interval of the slope or intercept does not include 1 or 0, respectively).⁵⁵ On the other hand, a Bland–Altman comparison of the results should be performed to assess the mean bias, and whether it is significantly different from 0, and the span between the limits of agreement (LoA). The LoA span covers 95% of the results and is thus an indicator for data variability.⁸⁴ Importantly, both the bias and span between the LoA should be checked against the preset analytical acceptance criteria when evaluating whether C_{cap} and C_{pl} yield interchangeable results. For example, although the results obtained from microsampling- and plasma-based methods may have no bias, the span of the LoA may be too wide to be acceptable, implying large variation in the data.¹ Hence, preset analytical criteria defining which bias and corresponding LoA are still considered acceptable are required. In this phase, the clinical acceptance criteria should already be defined to evaluate the impact of C_{cap} (or later EC_{pl}) on the clinical end point(s). These criteria depend on the purpose of the method, together with the clinical setting and the

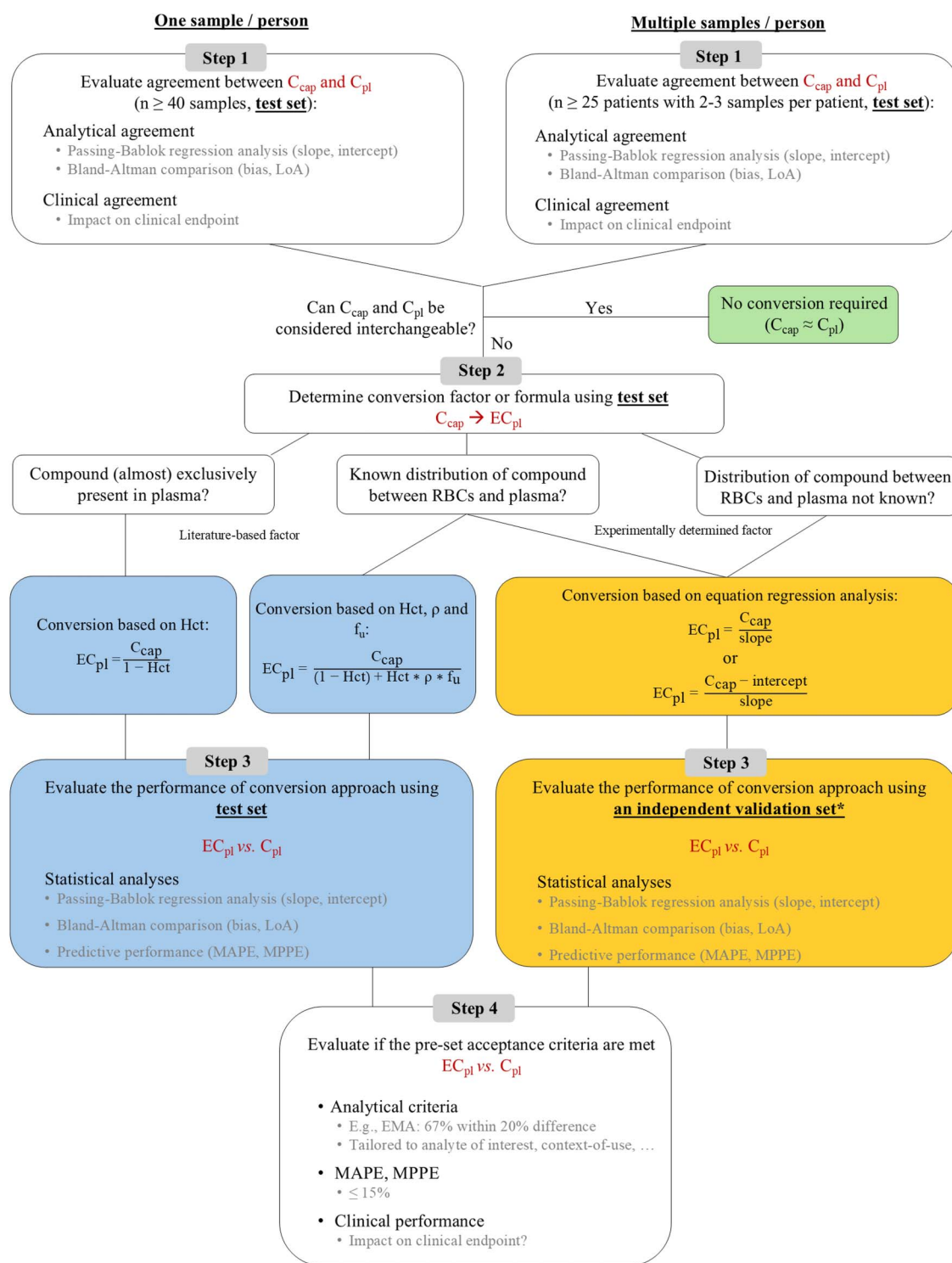


FIGURE 3. Flowchart depicting the different steps and recommended approaches for converting capillary concentrations (C_{cap}) to estimated plasma concentrations (EC_{pl}) for endogenous compounds and biomarkers. The blue and yellow boxes indicate conversion based on a literature-based or experimentally determined factor, respectively. The asterisk indicates that a distinct number of samples is required for the validation data set when a single or multiple samples per person are collected ($n \geq 40$ samples and $n \geq 25$ participants with 2–3 samples per participant). Paired samples collected for the test and validation set can be collected from the same participants at different timings, avoiding the need for a new cohort of participants.

availability of guidelines.¹ Further details on the analytical and clinical acceptance limits are provided in section “Evaluation of the performance of the conversion approach”. If C_{cap} and C_{pl} are considered interchangeable based on the preset acceptance criteria, no conversion to EC_{pl} is required. However, as mentioned in section “When is conversion to venous plasma-based results not recommended?”, this was rather exceptional. Therefore, for most components routinely measured in plasma, a conversion factor or formula to translate C_{cap} to EC_{pl} should be established following the next steps (Fig. 3).

Depending on the knowledge of the distribution of the analyte of interest in the RBC and plasma fractions, a different conversion approach is recommended (**Step 2**).

First, for compounds that are predominantly present in plasma, such as vitamin D and iohexol, EC_{pl} should be calculated solely depending on the Hct (using Equation 4). When a Hct-dependent conversion approach is applied, a fixed population-based Hct or individually measured or predicted Hct value can be used.¹ Although a fixed population-based Hct is beneficial because the individual Hct of a microsample should not be known, it requires a population with a narrow Hct range. However, for populations with more variable Hct values (such as critically ill patients or transplant recipients), the individual venous or capillary Hct should ideally be used when calculating EC_{pl} . The feasibility of using the individually measured or predicted Hct depends on the setting and application of the microsampling-based assay. Although obtaining an individual venous Hct value will not pose issues in a clinical PK trial conducted at a hospital, which typically involves venous blood sample collection at the beginning of the study, obtaining a Hct value in the context of home sampling for patient follow-up is more challenging. Interestingly, different methods exist for deriving a Hct value from dried capillary blood samples (DBS and VAMS samples) instead of relying on venous samples or historical Hct results. These include procedures based on potassium^{85–87} and the hemoglobin content, with the latter being determined through direct measurement,⁸⁵ the use of a sulfolyser reagent,⁸⁸ or spectroscopy.^{89–93} Alternatively, other internal markers (such as the albumin content) have been explored to estimate the serum fraction in dried blood samples to convert C_{cap} to EC_{pl} .⁹⁴ Because these methods require additional manual handling or dedicated instrumentation, their widespread use in routine care is limited. Therefore, if Hct-based conversion in previously published studies was performed using the individual Hct value, the Hct was—with a few recent exceptions^{38,95}—typically obtained from a venously collected blood sample, requiring a venous blood draw to enable calculation of EC_{pl} .^{19,32,96,97}

Second, if the analyte of interest is distributed between the RBC and plasma fractions, the conversion of C_{cap} can be based on the Hct and theoretical PK parameters or experimental data (section “Overview of the different approaches to convert capillary blood concentrations to venous plasma concentrations”; Fig. 3). The choice of the conversion approach will likely depend on the availability of stable PK parameters (namely ρ and f_u) or enough clinical samples. If values for ρ and f_u are known and show a low intra- and

interindividual variability, EC_{pl} can be calculated based on Equation 3. In addition, the application of a fixed or individual Hct value depends on the variability of Hct levels in the intended population. However, if sufficient clinical samples are available to establish a test data set and an independent validation data set, the slope and intercept derived from Passing–Bablok regression analysis can be used to determine EC_{pl} (Equation 11 and 12). Alternatively, the equation derived from weighted Deming regression analysis can be used. If both literature- and experiment-based approaches (Fig. 3) can be applied to the intended microsampling-based assay, the method for which the highest predictive performance is obtained should be considered the best fit for the purpose. For example, in the context of DBS-based determination of tyrosine kinase inhibitors, Zimmerman et al observed a comparable predictive performance regarding bias, variability, and number of samples within the analytical acceptance criteria for conversion based on the equation derived from Passing–Bablok regression analysis than that for conversion based on the Hct, ρ , and f_u .²⁰

Third, if the distribution of the analyte of interest between the RBC and plasma fractions is not specifically known, the conversion should be based on an experimentally derived formula or factor (section “Approaches based on experimental data”). Here, a Passing–Bablok- or weighted Deming-derived conversion factor is preferred over the application of the mean or median $C_{\text{bl}}/C_{\text{pl}}$ or $C_{\text{cap}}/C_{\text{pl}}$, because it may overlook some inherent variabilities.

In the next step (**Step 3** in Fig. 3), the performance of the applied conversion approach should be evaluated using data from the test set when calculating the EC_{pl} based on the Hct and PK parameters or using data from an independent validation set (‘literature-based’ or ‘experimentally derived’ factor or formula, respectively; Fig. 3). In the last step, whether the preset analytical and clinical acceptance criteria have been met must be verified (**Step 4** in Fig. 3). As both **Step 3** and **Step 4** are applicable to microsampling-based assays involving exogenous or administered compounds, these steps are collectively discussed in section “Evaluation of the performance of the conversion approach”.

Exogenous or Administered Compounds

For purposes including administration of compound(s) in general, paired plasma and capillary blood samples for both the test and validation sets must be collected nearly simultaneously to correctly compare the results obtained from both matrices.¹ This is particularly important for compounds with very short half-lives, such as 5-fluorouracil, which has a half-life of approximately 15 minutes,^{78,98} or when samples are collected shortly after dose administration, as drug concentrations change rapidly during the adsorption and distribution phases.^{1,12,28} In the following two sections, recommendations for collecting a single sample per individual (section “Collection of a single sample per individual”) and multiple samples per individual (section “Collection of multiple samples per individual”) within the dosing interval are provided.

Collection of a Single Sample per Individual

One of the best-known microsampling-based applications is TDM.^{3,4,17,67,99–105} Here, samples are preferably

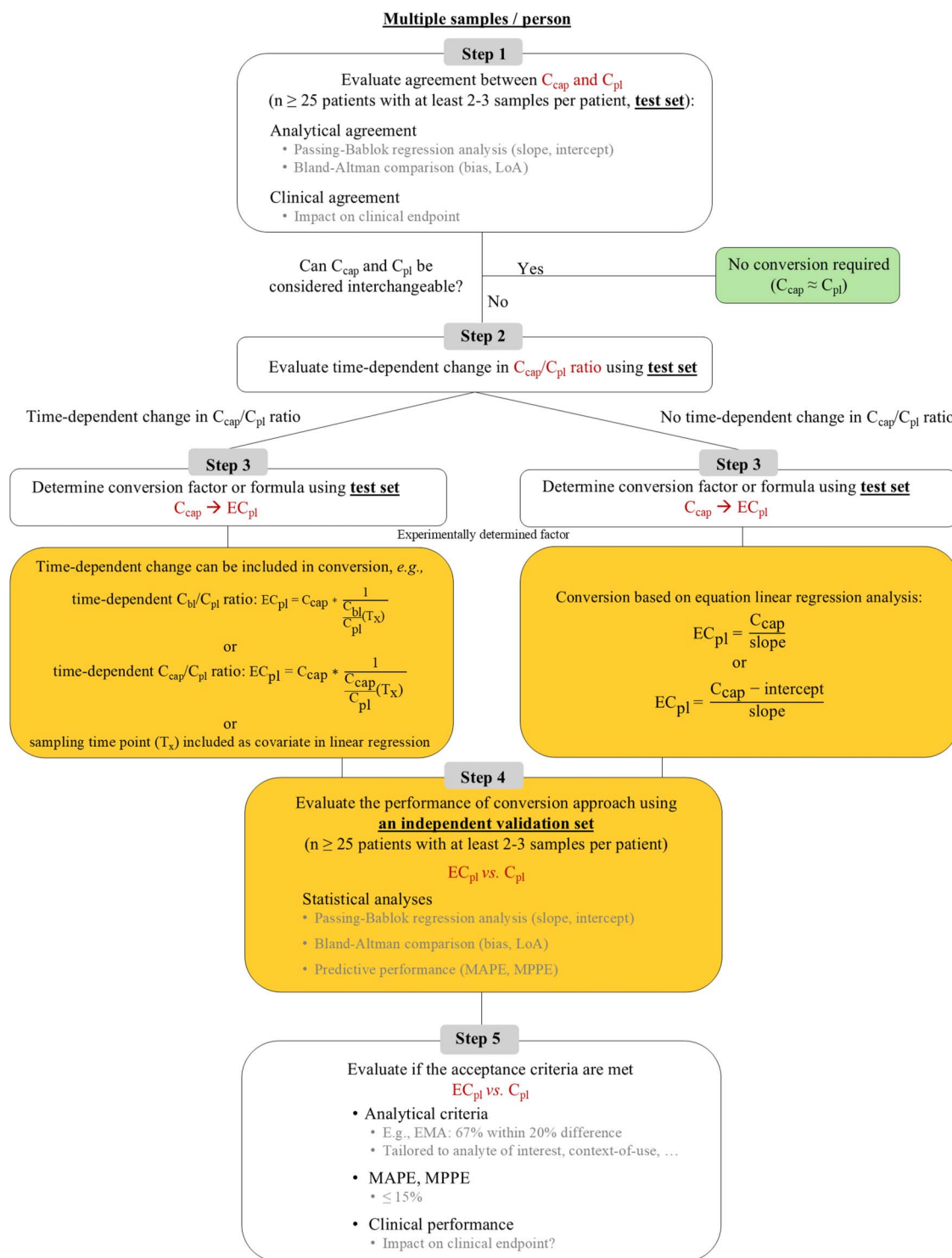


FIGURE 4. Flowchart depicting the different steps and recommended approaches for converting capillary concentrations (C_{cap}) to estimated plasma concentrations (EC_{pl}) for exogenous and/or administered compounds. The yellow boxes indicate that the conversion is based on an experimentally determined factor. Paired samples collected for the test and validation set can be collected from the same participants at different timings, avoiding the need for a new cohort of participants.

collected at a fixed time point within the dosing interval, which is generally at trough concentrations ($C_{\min}$, just before the next dose) or for a limited number of compounds at peak concentrations ($C_{\max}$).⁹⁹ For these and other purposes involving the collection of a single sample within a dosing interval, recommendations for the conversion of C_{cap} to EC_{pl} as shown in Figure 3 should be followed.

Collection of Multiple Samples per Individual

Alternatively, TDM can be based on a compound's area under the curve (AUC), which is recognized as a reliable drug exposure indicator and clinical outcome predictor. This approach generally uses a limited sampling strategy, requiring 3 or less samples per dosing interval.^{8,67,99} Other examples of microsampling-based applications involving the collection of multiple samples per individual after administering a compound include (investigational) drug (PK) trials^{56,97,106} and diagnostic assessments, such as renal function assessment based on the estimation of the glomerular filtration rate (GFR) on administering iothexol.^{30,32,51,56,97,106,107} A schematic overview of the different steps and recommended approaches for converting C_{cap} to EC_{pl} is shown in Figure 4.

First, Passing–Bablok regression analysis and Bland–Altman comparison should be performed to assess the relationship regarding bias and variation between paired C_{pl} and C_{cap} (**Step 1** in Fig. 4). The collection of samples covering the entire concentration–time profile from at least 25 participants is recommended to account for variations in matrix effects.¹

In the next step, whether the $C_{\text{cap}}/C_{\text{pl}}$ ratio differs over time, and thus, is time-dependent, should be evaluated (**Step 2** in Fig. 4). As mentioned previously, capillary and venous blood concentrations may differ, which is particularly important during the distribution phase of a compound. For example, differences in C_{cap} and C_{bl} were noticed for paracetamol in samples collected shortly after intravenous administration, reaching an equilibrium after approximately 1–2 hours.^{28,106,108} In addition, time-dependent capillary-to-venous differences were observed for other analytes after drug administration, including oseltamivir carboxylate, artemisinin, olanzapine, and paliperidone.^{109–111} Obviously, the capillary-to-plasma relationship will also be different when the compound distribution between the capillary and venous blood is not yet stabilized compared with when equilibrium is achieved. Therefore, for microsampling-based assays involving multiple sample collection within a dosing interval, whether the $C_{\text{cap}}/C_{\text{pl}}$ ratio changes time-dependently and at which sampling time point this ratio can be considered stable should be evaluated. This can be assessed by comparing the mean or median $C_{\text{cap}}/C_{\text{pl}}$ ratio, or alternatively, the mean or median $C_{\text{cap}}/C_{\text{bl}}$ ratio, obtained at different sampling time points using appropriate statistical methods such as one-way ANOVA or Kruskal–Wallis as a parametric or nonparametric test, respectively.¹⁰⁶

If a time-dependent change is observed, its impact on EC_{pl} should be evaluated with and without accounting for this effect. If the time-dependency significantly affects the results, it should be incorporated into the conversion method (**Step 3** in Fig. 4) by including the mean or median $C_{\text{cap}}/C_{\text{pl}}$, obtained at different time points during sample collection (T_x). EC_{pl}

can then be calculated using Equation 9. One limitation of this approach is that different ratios, and thus different conversion factors, should be calculated for each sampling time point. However, this can be simplified if the venous or capillary ratio reaches equilibrium. On the other hand, more advanced conversion approaches can be used to cope with time-dependent changes in the $C_{\text{cap}}/C_{\text{pl}}$ ratio, such as the inclusion of the sampling time point as a random covariate in the regression model, such as a linear mixed-effects model.⁵⁶

Alternatively, if no time-dependent change is observed in the $C_{\text{cap}}/C_{\text{pl}}$ ratio, conversion should be performed based on the Passing–Bablok- or weighted Deming-derived slope (and intercept) using the test data set.

As discussed in detail in section “Evaluation of the performance of the conversion approach”, the final steps involve evaluating the performance of the applied conversion approach (**Step 4** in Fig. 4) and comparing it with the preset analytical and clinical acceptance criteria (**Step 5** in Fig. 4).

Evaluation of the Performance of the Conversion Approach

As previously mentioned, it is essential in the first place that the analytical method performance evaluated during method validation meets defined acceptance criteria to ensure accurate and precise microsampling-based results. If this requirement is not fulfilled, the conversion method, which will possibly also introduce its own variability source, will not be able to cope with the issues related to the quality of the analytical method.

After establishing the conversion method outlined in sections “Endogenous compounds and biomarkers” and “Exogenous or administered compounds”, it can be applied, followed by a statistical assessment of the agreement between paired EC_{pl} and C_{pl} . Similar as in **Step 1** (evaluation of the agreement between C_{cap} and C_{pl}), Passing–Bablok regression analysis should be performed to determine any significant proportional and/or systematic differences between EC_{pl} and C_{pl} , on one hand. On the other hand, a Bland–Altman comparison should be performed to assess the overall bias and variability of the data after conversion. To estimate the predictive performance of the conversion approach, the median percentage predictive error (MPPE) and median absolute percentage predictive error (MAPE) can be calculated using Equation 14 and Equation 15, respectively.

$$\text{MPPE (\%)} = \text{median} \frac{EC_{\text{pl}} - C_{\text{pl}}}{C_{\text{pl}}} \times 100\% \quad (14)$$

$$\text{MAPE (\%)} = \text{median} \frac{|EC_{\text{pl}} - C_{\text{pl}}|}{C_{\text{pl}}} \times 100\% \quad (15)$$

MPPE and MAPE are measures of bias and imprecision, respectively.^{1,112,113} Preset acceptance criteria should be used to determine whether the applied conversion approach yields analytically and/or clinically acceptable outcomes, and hence, whether it can be considered fit for the purpose.

The analytical acceptance limits specifying the maximum allowable analytical uncertainty of the converted

microsampling-based results must be defined in advance. As these criteria are highly dependent on the availability of guidelines, internal policy of the laboratory, and purpose of the method, variable acceptance limits have been proposed. For example, because microsampling-specific guidelines for this purpose are lacking, Capiou et al suggested applying the criterion for incurred sample reanalysis proposed by the EMA to evaluate the results from a newly developed microsampling method comparatively with those from a reference method using plasma.^{1,40} This implies that the difference between the two values should be within 20% for more than 67% (2/3) of the paired samples. This acceptance limit has been applied in a multitude of microsampling-based studies to assess the performance of the applied conversion approach.^{18,20,26,45,114–116} The fulfillment of the analytical acceptance criterion based on the difference between EC_{pi} and C_{pi} can be easily assessed using the results obtained using Bland–Altman analysis. As already mentioned, the variation of the results must be assessed and, if possible, compared with preset acceptance criteria based on the span of the LoA obtained using the Bland–Altman comparison. For example, 70% study samples may meet the abovementioned criterion of maximum 20% difference when comparing EC_{pi} with C_{pi} . However, in this scenario, the span covered by the LoA, and hence, data variability, may be too wide to be acceptable.⁴⁰ Unfortunately, a correct and critical interpretation of LoA is often lacking, leading to an overly optimistic estimation of the analytical performance of the applied conversion approach. In addition, the MPPE and MAPE should ideally be below 15% to be analytically acceptable.^{19,20,69,112,115}

The EMA acceptance criterion suggested by Capiou et al¹ may not be appropriate at various instances, particularly when the limits are too broad for clinical decision-making. Hence, stricter acceptance criteria are required for compounds with very narrow therapeutic indices. Alternatively, depending on the laboratory's internal policy, a maximum absolute deviation instead of a relative deviation, or even a combination of both, may be tolerated. For the latter, the maximum absolute differences are often applied at lower concentrations, whereas at higher concentrations, the maximum allowable percentage deviation is used.^{1,62} In the authors' opinion, acceptance criteria (regarding bias and imprecision) should be tailored to the target analyte and intended context of use. For example, when microsampling is used for TDM of compounds with a narrow therapeutic index, such as tyrosine kinase inhibitors, and where clinical decisions are based on single C_{min} or C_{max} measurements, high analytical accuracy (within $\pm 5\%$) and precision ($\leq 10\%$ imprecision, as recommended for immunosuppressants⁸⁰) are essential. By contrast, if TDM is guided by AUC estimation, a somewhat larger variability may be acceptable, as the variability in individual data will be averaged out in model-based approaches. However, an even wider variability can be justified in various scenarios, such as in microsampling-based screening methods used to assess drug adherence, where simply confirming the presence of a drug within a certain concentration range may be sufficient.^{117,118} In addition, slightly lower accuracy and precision may also be justified if the method offers significant advantages from other perspectives, such as a gain in

logistical feasibility in resource-limited settings, where conventional venous sampling may not be possible, and microsampling enables otherwise inaccessible monitoring. In all these instances, whether and to what extent the variation affects (clinical) decision-making should be noted.

In addition to evaluating analytical performance, determining whether the differences between EC_{pi} and C_{pi} are clinically acceptable, and hence, would affect clinical decision-making, is crucial. Therefore, the clinical acceptance criteria should be defined in advance. Moreover, because microsampling-specific guidelines for clinical acceptance criteria are still lacking, limits can be based on guidelines such as from the Royal College of Pathologists of Australasia (RCPA) or the experience of a multidisciplinary team of experts.^{81,119} Importantly, for each measured sample pair, whether the clinical outcome differs when using microsampling-based concentrations (EC_{pi}) versus the concentrations obtained using the reference matrix (C_{pi}) should be investigated. For example, in the context of TDM, possible differences in dosing recommendations should be assessed when relying on EC_{pi} to estimate drug exposure compared with C_{pi} . This was recently performed by Zwart et al¹²⁰ for TDM of mycophenolic acid. Instead of relying only on absolute or relative differences between EC_{pi} and C_{pi} , the agreement was assessed at the AUC level. A clinical acceptance limit of $\pm 20\%$ was applied, as an AUC divergence $>20\%$ results in a dose adjustment recommendation and is therefore considered clinically relevant. Interestingly, although the EMA-based analytical limits were just met, and the MAPE value exceeded 15% when comparing DBS-based EC_{pi} and C_{pi} , no impact in clinical decision-making was observed, as the AUC estimation was similar when using microsampling-based results versus when using those obtained from the reference method. The differences in (clinical) outcomes when using EC_{pi} compared with using C_{pi} have also been assessed for other purposes. For example, the clinical value of a microsampling-based method for monitoring vitamin status may lie in its ability to accurately classify individuals as deficient or sufficient according to clinically relevant thresholds with the aim of supporting supplementation decisions. This was recently assessed by evaluating the agreement between classifications of vitamin D concentrations derived from both converted capillary blood and plasma measurements using the Cohen kappa coefficient. The results demonstrated substantial to almost perfect agreement with those obtained from the reference (plasma-based) method of clinical classification of vitamin D status.³⁸ Other examples include diagnostic applications such as GFR determination, where decision-making is based on estimated (iohexol- or creatinine-based) GFR values, or the determination of compound-specific PK parameters (AUC and estimated clearance).^{19,30,32,56,107} Interestingly, probabilistic models such as Bayesian modeling can be applied to improve the reliability of clinical decision-making. In doing so, the uncertainty of the result is captured in a clinically relevant manner. More specifically, Bayesian modeling integrates prior knowledge, such as population PK data, with data from this current study, such as individual patient data, to generate a full probability distribution of expected outcomes, rather than a single-point estimate. This

method has already been successfully applied in blood microsampling-based AUC and/or PK parameter estimation for clinical decision-making for therapeutics, such as tacrolimus, mycophenolic acid, adalimumab, and tyrosine kinase inhibitors. A limitation is that Bayesian models often require specialized statistical tools and expertise, which may limit their routine clinical use.^{8,46,121–124}

Finally, if the analytical, but most importantly, clinical acceptance limits are not met, the recommended conversion method may not be entirely appropriate for a certain microsampling-based assay. In this case, additional conversion factors or alternative approaches may be required to develop a microsampling-based method that yields results

that are interchangeable with those obtained for the reference matrix and is ready for implementation.

DRIED PLASMA SPOTS TO ELIMINATE THE NEED FOR CONVERSION?

Dried plasma spots (DPS) have been explored as an alternative to dried capillary blood microsampling to overcome the need to calculate EC_{pl} from dried blood concentrations. DPS can be generated by the volumetric application of venous or capillary plasma on filter paper or venous or capillary blood on dedicated devices.

DPS generation after centrifuging venous or capillary blood samples is not feasible for home sampling.^{2,125,126} However, DPS retains the logistical benefits of convenient sample transport and storage inherent to dried samples, which may be particularly important when the samples must be transported to an external laboratory for analysis, such as samples obtained in a remote or resource-limited setting or in multicenter studies.^{125,126}

To eliminate the need for venous blood collection and facilitate home sampling, microsampling devices that collect dried plasma from a nonvolumetrically applied drop of (capillary) blood have recently emerged. Currently, three microsampling devices allowing dried plasma or serum sample generation following blood application are commercially available, including the Telimmune plasma separation card (formerly known as Noviplex), Capitainer SEP10 device (plasma), and the HemaSpot SE device (serum), although other devices, such as the HemaXIS DX device, are under development.^{2,127–129} In such devices, plasma separation is typically based on membrane filtration and capillary forces. At the time of writing, only two published studies have reported using Telimmune cards for DPS collection after application of capillary blood obtained through finger-prick,^{130,131} while other reports have suggested preparing DPS samples by pipetting venous blood onto the device.^{132–139} Whereas these plasma preparation devices offer both logistical benefits and the potential of home sampling, large blood volumes (typically 40–80 μ L) are often required, compromising its applicability in populations where the collection of larger volumes may be challenging, such as in neonates. In addition, the dried plasma sample itself is often relatively small, ranging from 2.5 μ L to 2 times 3.8 μ L spots for the Telimmune card, and 10 μ L for the Capitainer SEP10 device, necessitating sufficiently sensitive instrumentation for analysis.^{2,128,129} Furthermore, the extent to which the ‘dried plasma’ is equivalent to the conventional plasma remains unclear. Differences in analyte recovery from DPS versus liquid plasma samples,¹³⁷ as well as decreased plasma protein recovery after membrane filtration of venous and capillary blood, have been reported.^{140,141} This is particularly inconvenient for identifying compounds in DPS samples that exhibit high plasma protein binding. Hence, a thorough clinical validation study as discussed in section “Clinical validation” is necessary to assess the concordance between analyte concentrations found in liquid venous plasma samples and (capillary) DPS. Mukai et al¹⁴² observed significant systematic errors when comparing venous liquid plasma and DPS to determine tyrosine

TABLE 6. Compiling Table of all Equations Outlined and Discussed in This Guideline

$\frac{C_{bl}}{C_{pl}} = (1 - Hct) + Hct \times \rho \times f_u$	Equation 1
$\frac{C_{cap}}{C_{pl}} = (1 - Hct) + Hct \times \rho \times f_u$	Equation 2
$EC_{pl} = \frac{C_{cap}}{(1 - Hct) + Hct \times \rho \times f_u}$	Equation 3
$EC_{pl} = \frac{C_{cap}}{1 - Hct}$	Equation 4
$C_{cap,corrected} = \frac{C_{cap}}{1 - (Hct_{cal} - Hct_{indiv})}$	Equation 5
$EC_{pl} = \frac{C_{cap,corrected}}{1 - Hct_{indiv}} = \frac{C_{cap}}{1 - (Hct_{cal} - Hct_{indiv})} \times \frac{1}{1 - Hct_{indiv}}$	Equation 6
$EC_{pl} = \frac{C_{cap}}{(1 - Hct) + Hct \times K_{rbc/pl}}$	Equation 7
$EC_{pl} = C_{cap} \times \frac{1}{\frac{C_{bl}}{C_{pl}}}$	Equation 8
$EC_{pl} = C_{cap} \times \frac{1}{\frac{C_{bl}}{C_{pl}} (T_x)}$	Equation 9
$EC_{pl} = C_{cap} \times \frac{1}{\frac{C_{cap}}{C_{pl}}}$	Equation 10
$EC_{pl} = \frac{C_{cap}}{\text{slope}}$	Equation 11
$EC_{pl} = \frac{C_{cap} - \text{intercept}}{\text{slope}}$	Equation 12
$EC_{pl} = \frac{C_{cap}}{1 - Hct} \times \text{conversion factor}$	Equation 13
$MPPE (\%) = \text{median} \frac{EC_{pl} - C_{pl}}{C_{pl}} \times 100\%$	Equation 14
$MAPE (\%) = \text{median} \frac{ EC_{pl} - C_{pl} }{C_{pl}} \times 100\%$	Equation 15

C_{bl} , venous blood concentration; C_{cap} , capillary blood concentration; C_{pl} , venous plasma concentration; EC_{pl} , estimated plasma concentration; f_u , unbound analyte concentration; Hct , hematocrit; Hct_{cal} , hematocrit of calibrators; Hct_{indiv} , individual sample hematocrit; $K_{rbc/pl}$, red blood cell-to-plasma partitioning coefficient; MAPE, median absolute percentage predictive error; MPPE, median percentage predictive error; ρ , red blood cell-to-unbound plasma concentration ratio.

kinase inhibitors, although both matrices were expected to be interchangeable. This suggests methodological issues. Moreover, in another report, conversion of venous DPS concentrations obtained using the Telimmune device through a mathematical formula including the Hct and an additional factor (Equation 13) was needed to achieve results that were interchangeable with those obtained from liquid venous plasma samples.¹³⁷ Owing to the limited data currently available, further investigation is needed to assess the concordance between the analyte concentrations obtained from liquid versus dried plasma samples. Hence, although advocated as an ideal alternative to dried capillary blood microsampling, thereby eliminating the need for conversion of C_{cap} to EC_{pl} , the overall applicability of dried plasma samples requires further evaluation. Indeed, if conversion is applied, one may wonder about the added value over conventional dried blood sampling.

CONCLUSIONS

The past decades have witnessed a steadily growing interest in capillary blood microsampling as a less invasive and more patient-centric sampling strategy with potential applications in many different fields. However, their widespread use and implementation remain rather limited. One hurdle hindering the further implementation of microsampling-based methods is the interpretability of capillary blood concentrations. Because blood-based rather than plasma- or serum-based results are obtained, the conversion of capillary blood to plasma concentrations is often needed to allow comparability with the current reference ranges in plasma or serum.

Although a guideline for developing and validating high-quality microsampling-based methods is already in place, guidance on how to properly perform and evaluate the conversion of C_{cap} to EC_{pl} is still lacking. Here, the aim was to discuss the “what, when, and how” of converting dried C_{cap} to EC_{pl} and provide guidance on the decision and proper evaluation of a fit-for-purpose conversion approach. In addition, guidance on how to assess C_{cap} when the reference matrix is whole blood rather than plasma and how to assess liquid capillary plasma-based results is provided. A compilation of all the equations outlined and discussed in this guideline is summarized in Table 6.

Several take-home messages are provided. First, a comprehensive and well-executed clinical validation study covering all possible comparisons of different matrices is essential to gain complete insights into the results obtained from capillary microsamples. Second, the effectiveness of the applied conversion method should be evaluated on a case-by-case basis. More importantly, when an initial sample set is used to establish a conversion formula, the performance of the conversion should be validated using an independent sample set. Finally, as with any other method to determine biomarkers, microsampling-based outcomes must be fully guaranteed. Therefore, the results obtained must be compared using predefined analytical criteria, and importantly, clinical acceptance criteria. Despite its importance, proper evaluation of the latter is often lacking. Therefore, to further promote the

implementation of microsampling-based methods, one of the key points in future studies is to focus on the clinical impact of (converted) C_{cap} in comparison with decisions based on concentrations obtained from the reference matrix.

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