Feedback from the European Bioanalysis Forum liquid microsampling consortium: microsampling: assessing accuracy and precision of handheld pipettes and capillaries

Glen Hawthorne¹, Lieve Dillen², Matthew Barfield³, Zoe Cobb⁴, Abdullah Kandira⁵, Katrin Schroeter⁶, Steve White³, Natasha A Karp¹, Iain Love⁷, Valerie Boutet⁵, Morten Rohde⁸, Marion Kranenborgh⁹ & Philip Timmerman^{*,10}

¹AstraZeneca, Cambridge, UK

²Janssen R&D, Beerse, Belgium

³GlaxoSmithKline, Ware, UK

⁴LGC, Newmarket, UK

⁵Sanofi, Paris, France

⁶Sanofi, Frankfurt, Germany

⁷Charles River Laboratories, Edinburgh, UK

⁸Lundbeck, Valby, Denmark

⁹QPS, Groningen, The Netherlands

¹⁰European Bioanalysis Forum, Havenlaan 86c b204, 1000 Brussels, Belgium

*Author for correspondence: chair@e-b-f.eu

Aim: Microsampling in preclinical pharmacokinetics (PK) studies is currently widely adopted across the pharmaceutical industry. **Materials & methods:** The European Bioanalysis Forum liquid microsampling consortium member companies assessed the accuracy and precision of handheld pipettes and microcapillaries at volumes of less than 10 μ l. The following key factors on pipetting performance were also evaluated: Pipette type (positive displacement, air displacement and microcapillary), experience of user and the liquid type. Water was selected as a best-case scenario for accuracy and precision determination and blood plasma as a 'real world' bioanalysis sample type. **Conclusion:** Accuracy and precision on the pipetted volume decreased at lower volumes and experienced laboratory technicians performed better compared with the infrequent users. With respect to the pipetting devices used, microcapillaries showed better or equivalent accuracy and precision compared with handheld pipettes across the volume range 1–8 μ l independent of the matrix used.

First draft submitted: 2 January 2019; Accepted for publication: 22 February 2019; Published online: 11 April 2019

Keywords: accuracy of pipetting • EBF • microsampling

Microsampling of blood or plasma samples was an innovation that enabled serial sampling in small rodents, improved the definition of concentration–time profiles and reduced the number of rodents used [1–8]. The implementation of the methods is challenged by concerns over the technical skills required to maintain adequate accuracy and precision in the analytical assays when handling these small volumes [9,10]. Therefore, the European Bioanalysis Forum established the liquid microsampling (LMS) consortium [11,12]. This consortium has generated two experimental protocols to practically assess various microsampling strategies within member company laboratories. The second protocol assessed analyte homogeneity in plasma generated using capillary microsampling approaches and is the subject of a separate publication to this one [13]. The first protocol, of which this manuscript reports, was built around assessing the precision and accuracy as these are key factors for the successful implementation and acceptance of LMS strategies for bioanalysis. In this protocol, we explore the fundamental issue of the manipulation of small volumes of water and plasma with both handheld pipettes (positive displacement and air) and microcapillaries (1–8 μ l) to give an indication of the limitations of readily available laboratory equipment.

newlands
press

Materials & methods

Protocol: manipulation of small sample volumes

A total of nine independent laboratories engaged with the protocol and contributed data for the evaluation of the manipulation of small sample volumes. Volumes of 4 and 8 μl were selected for evaluation of precision and accuracy as these volumes are often proposed in current microsampling studies. Volumes of 1 and 2 μl were selected to see if the limits of small volume sampling could be stretched further.

Within each institute, two handheld (air or positive displacement) pipettes routinely used in each member company were investigated. Type and brand of pipettes were recorded. The pipettes used were calibrated following manufacturer instructions within the volume range investigated and the acceptance criteria applied by the investigating laboratory to each of the individual pipettes.

Glass capillaries (Vitrex Micropipettes) were supplied from Vitrex (Herlev, Denmark) and distributed to the laboratories after the purchase of one lot to ensure uniformity of the materials. Four versions were studied: 1 μl Plain (165013), 2 μl Plain (165113), 4 μl Plain (165313) and 8 μl Plain (172213). A second source of microcapillaries was also tested (Drummond calibrated micropipettes), and were supplied from Drummond Scientific Company (PA, USA) and distributed to the laboratories after the purchase of one lot. Four versions were studied: 1 μl (2-000-001), 2 μl (2-000-002), 4 μl (2-000-004) and 8 μl (2-000-008).

Within a laboratory, six replicate measurements were executed for each volume and each device. Two matrices were evaluated (water and plasma). At each participating company, the protocol was performed by two operators, a 'trained but infrequent pipette user' (referred to as operator 1) and an 'experienced daily pipette user' (referred to as operator 2).

The gravimetric assessment was conducted using microanalytical balances, (accuracy of 0.01 mg or 0.001 mg), the manufacturer and model were recorded. The balances were calibrated according to internal procedure and preferably on the day(s) of the experiment(s).

The weighing of small volumes is prone to variation due to evaporation of the sample in the weighing cabinet. Therefore, the humidity near the balance was controlled by placing a wet tissue in a beaker within the balance enclosure (or if available an evaporation trap was used). Temperature was recorded at the start and the end of the experiment. The gravimetric assessment of the performance of the pipettes and capillaries with low volumes was initiated when conditions that minimized evaporation were obtained.

A 2-ml vial was used for weighing. All protocol measurements were done at room temperature. Density of water (ultrapure 18.2 M Ω stored in a bottle for 24 h at room temperature) and plasma (anticoagulant, species and source noted) were assessed by weighing 1 ml of each ahead of performing the protocol. For accuracy calculations on the plasma volumes pipetted, the experimentally defined plasma density was used to convert the plasma weight to a volume.

To confirm performance of the weighing equipment and acceptable conduct of the protocol, each operator first demonstrated acceptable performance of a 10 μl volume dispense in replicates of six using pipettes typically used in that laboratory. It was decided between member companies, based on routinely achievable criteria, that the precision and accuracy of the dispense for 10 μl should be <2% to be considered acceptable.

Overview of data obtained

Within a laboratory, two handheld pipettes for each volume being investigated were selected for the study. In some cases where a handheld pipette was not available for the specified volume, no weighings were collected. It was noted that some laboratories used two different handheld pipettes from the same manufacturer, while others switched manufacturer at different volumes such that a mixture of both air displacement and positive displacement pipettes were used. Of the nine laboratories, four used air displacement pipettes only, two used positive displacement only and three used a combination of both positive displacement and air pipettes. In total 3312 weight measurements were made (Table 1).

Two laboratories (A and H) followed an early version of the protocol and collected 7 μl rather than 8 μl for the handheld pipettes. The data from these laboratories for this metric were excluded from the analysis.

The balances used were either Mettler Toledo or Sartorius, in total nine different models were used (i.e., a different balance in every laboratory). Therefore, the effect of balance is confounded with laboratory. Multiple types of handheld pipettes, either air or positive displacement, were used depending on the laboratory meaning that pipette models cannot be separated from the laboratory using them when assessing contribution to overall variability and accuracy.

Table 1. Number of weight measurements recorded per pipette type by each laboratory used in the analysis.

Laboratory	Number of weight measurements		
	Air pipette	Positive displacement pipette	Capillary (two types)
A	84	84	192
B	192		192
C	192		192
D		192	192
E		192	192
F	192		192
G	96	96	192
H	72		192
I	96	48	192
Total	924	624	1728

Data evaluation

Initially, data were visualized using a heat map of mean weights to get an overall assessment of the variability and accuracy of the pipettes and capillaries dispensing. Furthermore, data were assessed statistically to explore the contribution of each pipetting methodology (capillary, positive displacement and air displacement) to the variability of the data using regression analysis (Software package – R version 3.4). Comparison was made between water pipetting performance and plasma.

For each volume dispensed, a linear mixed effect model [14] has been fitted with a model that includes a (pipetting) methodology and operator as fixed effects and laboratory as a random effect (Equation 1). Where methodology had three levels (cap, positive or air), operator had two levels (operator 1 and 2, where operator 2 related to skilled and operator 1 trained but infrequent user) and laboratory had nine levels (Laboratory A to I). Laboratory was treated as a random effect assuming the laboratories are an independent sample from a population with a normal distribution of mean zero. An interaction between operator and methodology was explored and was occasionally statistically significant but the effect was small and therefore the analysis discarded this effect and estimated the methodology effect after accounting for operator and laboratory effects. A covariance structure was selected for each model by assessing whether the variance depended on the methodology and operator group using a likelihood ratio test ($p < 0.05$).

$$y \sim \text{Methodology} + \text{Operator} + (1 | \text{Laboratory}) \quad (\text{Equation 1})$$

This statistical model can be formally represented as in Equation 2

$$y_{ijk} = \mu + \alpha_i + \beta_j + a_k + \epsilon_{ijk} \quad (\text{Equation 2})$$

where $i = 1, 2, 3$ indicates methodology group (microcapillary or positive displacement or air);

where $j = 1, 2$ indicates operator 1 or 2 (1 = skilled, 2 = trained but infrequent user)

where $k = 1, 2, \dots, 9$ indicates the laboratory

In addition, $a_k \sim N(0, \sigma_a^2)$ and $\epsilon_{ijk} \sim N(0, \sigma_e^2)$.

Graphical model diagnostics were used to assess the model fit. A quantile–quantile plot was used to assess the normality of the residuals (difference between predicted from the model and measured) and a plot of residual against the predicted colored by operator/methodology/laboratory asked if there were any systematic patterns in the residuals. An F-test was used to assess the role of methodology and operator within each model.

For plasma, to adjust for differences in matrices, the measured weight was adjusted for density differences by dividing the reading by the relative density measure of the matrix.

To compare estimates across volumes, the inaccuracy (%) was calculated by dividing the estimated effect by the target weight. The exception was for the air methodology, as it was set as the reference in the statistical model, so the intercept value was subtracted from the expected to determine the deviation and then divided by the target weight.

Figure 1. Accuracy and precision obtained for handheld pipettes and capillaries with plasma as matrix. Summary of the accuracy and precision per device (handheld pipettes combined or capillary) and per volume. Data represent the percentage of all the density corrected plasma results with accuracy and precision <5%; between 5 and 10% and >10%. These values were chosen as they reflect commonly applied acceptance criteria present in the standard operating procedures of the individual companies.

Results & discussion

Data were provided by nine independent laboratories using three methodologies of pipetting, positive displacement, air displacement and microcapillaries, on the gravimetric assessment of volume pipetted when sampling either water or plasma. An example of the raw individual weighings for each measure and associated meta data (e.g., model, manufacturer) are reported in Supplementary Table 1 (handheld pipettes) and Supplementary Table 2 (capillaries).

Global assessment of accuracy & precision for plasma pipetting

As an overview, a heat map approach was used to visualize the effect of pipette and volume on accuracy (Figure 1A) and precision (Figure 1B) assessing the occasions a set of data for a pipetted volume performed within defined limits. We observe, for both handheld pipettes and capillaries, the number of individuals generating accurate and precise weighings declines with reduced sample volume. Visual inspection indicates that the capillaries performed better in both accuracy and precision than the handheld pipettes.

Detailed exploration of accuracy & precision as a function of pipette types

A mixed model regression analysis for each matrix and volume was used to assess the bias in volume dispensed as a function of pipette type after accounting for operator skill differences and treating laboratory as a random effect. The percentage inaccuracy for each methodology for each volume was estimated with a 95% CI (Figure 2). For most scenarios, the percentage inaccuracy is within 5%; except for the positive displacement pipettes when dispensing water.

The microcapillaries with water had excellent inaccuracy across the volumes tested with water. When working with plasma, we can see that the capillary methodology tended to over-recover as the volume decreased; however, even at 1 µl it was only 5% above target volume. The positive displacement pipettes, with water, had a systematic pattern in underdispensing which increased as the volume decreased. This trend was not apparent with plasma which is a more viscous matrix. With the air pipettes, no differences in performance could be seen between plasma or water and across the various volumes tested the overall inaccuracy was below <2.5%.

Focusing on precision, the regression analysis found the variability in the volume dispensed depended on the pipetting methodology (Figure 3). When dispensing plasma, the variance of microcapillaries and positive displacement pipettes was similar to air displacement pipettes with the interquartile range spanning the variance of air pipettes. However, when dispensing water, there was a consistent pattern in that the variability was higher for air pipettes.

Figure 2. Inaccuracy of dispensed plasma and water volume as function of pipetting device and volume. A mixed model regression analysis for each matrix and volume, was used to assess the impact of the methodology after accounting for potential operator differences and laboratories differences. The estimated effects were converted to an inaccuracy by subtracting the expected weight from the predicted weight from the model. The error bars indicate the 95% CI in this estimate.

Detailed exploration of operator impact on accuracy & precision

In the experimental setup, within each laboratory two operators were identified to collect data where they differed in pipetting experience. Operator 1 was defined as a trained but infrequent pipette user, while operator 2 was identified as an experienced analyst who regularly performs pipetting activities. A regression analysis, which estimates the effect of operator, after accounting for variation by methodology and laboratory, in general found that for most datasets (5/8) there was no significant effect of operator (Figure 4). For three of the datasets (8 µl plasma, 8 µl water and 1 µl water), at a significance threshold of 0.05, a small statistically significant impact on inaccuracy was detected. The effect size was small but suggested that the experienced operators were tending to pipette a slightly higher volume relative to the infrequent pipette user.

To assess whether operator skill level impacted the precision of dispensing, we explored whether the regression models were a better fit to the data when a heterogeneous variance structure was included. Across all models, allowing the variance to depend on operator was found to be statistically significant. A consistent pattern was seen with a higher variance with the inexperienced operator relative to the experienced operator (Figure 5). This was seen both when working with plasma and water.

Discussion

The manipulations (e.g., pipetting or dispensing) of small sample volumes (<10 µl) with handheld pipettes and capillaries are more challenging compared with traditional volumes (>20 µl). In the bioanalytical community, managing technical innovation can lead to a risk adverse attitude [15] and concerns were expressed which lead to the proposal to gather a dataset to explore the impact in daily practice for a bioanalytical scientist. The European Bioanalysis Forum-LMS investigation aimed to unravel the real and perceived challenges and limitations with respect to accurate and precise pipetting of small volumes as experienced across nine European bioanalytical laboratories (six pharma, three CROs).

Gravimetric evaluation of the 1 and 2 µl volumes certainly has additional challenges with respect to the stability of the balances (humidity, evaporation), this holds for the three pipetting devices used. For the capillaries, the specification of the vendor states an inaccuracy $\pm 0.5\%$; however, information on the methodology applied

Figure 3. Precision differences in dispensed plasma and water volume as function of pipetting device. A mixed model regression analysis for each matrix and volume, was used to assess the impact of the pipetting methodology after accounting for potential operator differences and laboratories differences. The model fitted a heterogeneous variance structure to the model and assessed whether the variance depended on methodology and operator skill level. The plot visualizes the estimated variance obtained in the capillary or positive displacement pipettes relative to the air pipettes. A five-point summary (min, 25th percentile value, median, 75th percentile value, max) box and whisker plot has been used to visualize the data. Values that are three-times the interquartile range away from the median are shown as outliers (dot).

to validate this statement is not included. In our survey across the member companies, it was noticed that the individual laboratories apply different criteria with respect to accuracy, especially in the low volume range (inaccuracy allowed $<2\%$ to $>10\%$ for volumes $<1\ \mu\text{L}$). Other analytical areas (ELISA, PCR) typically also use the low sample volumes as evaluated in this study.

We anticipated that there may be a decrease in accuracy and precision with the more viscous plasma matrix and included water as a best-case scenario. Using the entire dataset to assess the accuracy of each pipetting method shows that, except for positive displacement pipettes with water, the accuracy for every device was estimated to be within 5% of nominal volume across all volumes assessed. Interestingly, capillaries generated the best accuracy with water across the volumes $1\text{--}8\ \mu\text{L}$ with negligible deviation from nominal, but with plasma showed a trend for a positive bias with reduced volume – possibly a consequence of residual plasma on the filling end of the capillary. It should be considered that handheld pipettes involve an aspirate and dispense step whereas the mode of operation with a capillary is to fill (aspirate) and then flush the plasma with a diluent. In the current assessment capillaries were filled only and then weighed.

Precision within devices, across all volumes, were largely independent of device type for plasma but with water as matrix, positive displacement pipettes and capillaries showed better precision compared with air pipettes.

The results presented proved that the majority ($>80\%$) of individuals can pipette plasma volumes of 8 and $4\ \mu\text{L}$ using handheld pipettes or capillaries and achieve, across six replicate weighings, accuracy or precision within a 5% criterion. 8 and $4\ \mu\text{L}$ are the volumes currently typically used within capillary microsample studies [2,4], selecting

Figure 4. Impact of pipettor experience on pipetting accuracy. A mixed model regression analysis for each matrix and volume was fitted to the data and included terms to account for differences in pipetting method and operator skill level differences. The model fitted a heterogeneous variance structure to the model and assessed whether the variance depended on methodology and operator skill level. Operator 1, the less skilled operator was set as the reference and the model evaluated the effect of being skilled on volume dispensed. The estimated effects were converted to an inaccuracy by dividing by the expected weight. The error bars indicate the 95% CI in this estimate.

Figure 5. Impact of pipettor experience on dispensing precision. A mixed model regression analysis for each matrix and volume was fitted to the data and included terms to account for differences in methodology and operator skill level. The model fitted a heterogeneous variance structure to the model and assessed whether the variance depended on methodology and operator skill level. The boxplot explores the relative variance for each dataset that was reported as a function of matrix. A five-point summary (min, 25th percentile value, median, 75th percentile value, max) box and whisker plot has been used to visualize the data. Values that are three-times the interquartile range away from the median are shown as outliers.

volumes less than this show that the chance of accurately and precisely pipetting plasma samples reduces. At the volumes of 1 and 2 μl assessed the chance of pipetting within 5% accuracy or precision declines. The number of individuals pipetting within a wider criterion of 10% is encouraging for capillaries although it must be kept in mind that this would add substantially to the overall variability of the assay and with current bioanalytical acceptance criteria of 15/20% the pipetting step alone can consume a large part of this margin.

The impact of the operator of the pipetting device showed as anticipated that experience matters. For accuracy, there was little statistical effect on the accuracy overall for both water and plasma; however, for precision the experienced individuals outperformed the inexperienced operators by approximately twofold for plasma and 0.5-fold for water. A result which highlights the importance of appropriate verification of equipment. Pipettes are typically calibrated or verified with water, which is an easier matrix to handle; however, acceptable demonstration with water may not reflect the situation with plasma.

All participants in this study were trained bioanalysts, the only difference in the classification of skill level was in the frequency/regularity of routine pipetting. Obviously, new hires cannot be expected to immediately reach the same level of low volume accuracy and precision without substantial training/experience. Familiarization of manipulation of the capillaries is important, it can be tedious at the beginning to use the gripper tool to hold the capillaries and to prevent contamination; however, with time this becomes routine and working practices optimized.

The observation (Figure 1) that more individuals can use capillaries and by far outperform handheld pipettes for low volume pipetting is received as excellent news by the microsampling team. Additionally, in a regulated environment the volume of the microsample is often further increased through a wash-out procedure to enable multiple sampling for re-analysis and/or incurred sample reproducibility. This extra dilution for a microsample assay adds additional pipetting steps (dilution + collecting a subaliquot of the diluted sample) that can add more variability. Consequently, the final sample volumes in the bioanalytical assay are more conventional (usually $>20 \mu\text{l}$) and less challenging for aliquoting in terms of accuracy and precision. However, it is very much dependent on the analytical design for preparation of calibration curves. If calibration curves are prepared without capillaries with handheld pipettes, increased variability can be expected based on the results of this investigation.

Overall conduct of the evaluation

The conduct of an interlaboratory assessment is not without its challenges. For example, each laboratory used a different microanalytical balance; therefore, the differences between laboratory may be influenced by balance. However, to minimize the balance effect each laboratory ensured their balance was calibrated according to local procedures and was verified to be performing within each laboratory's own specification. An optimal balance set up to weigh low volumes of water might explain the observation that manufacturers of low volume pipettes generally specify better performance than that observed here in routine operation. Furthermore, the application of an alternative calibration or verification procedure (e.g., colorimetric or fluorometric analysis) might also improve performance. Whether these alternative verification methods could be applied for capillaries is not clear.

Although some bioanalysts have questioned the achievable accuracy and precision of low volume aliquots ($<10 \mu\text{l}$), the data presented in this study demonstrate that with proper training and experience, laboratory technicians can reliably manipulate these volumes with good accuracy and precision. Although, a volume dependent increase in inaccuracy and imprecision was clearly noticed, the capillaries provide equivalent and, in some instances, better accuracy and precision compared with handheld pipettes. Therefore, microsampling of blood and plasma in *in vivo* studies in small animals can be facilitated using capillaries and comparable accuracy and precision as for regular samples sizes can be assured for the bioanalytical results.

Future perspective

Microsampling continues to be an area of interest beyond and within the bioanalytical community. In particular because of the developments in self-sampling and sampling at home devices for clinical applications such as therapeutic drug monitoring, some of which rely upon the accuracy and precision of capillaries to deliver fixed volumes. One may foresee that to obtain the ultimate accuracy and precision, techniques which remove the requirements for human manipulation, for example, pushing a piston or filling to a certain level, will evolve to enable automated sample collection or preparation.

Refinement and development of microsampling best practices will also continue to advance the 'Replacement, Reduction and Refinement' of use of animals in scientific research (3Rs) [16] by increasing the quality and breadth of information from small volume samples taken from animals.

Continued research in to the application of low volume sampling and the potential advantages that this brings should see an increase in the adoption and the ease at which these technologies can be applied.

Supplementary data

See online at: <https://www.future-science.com/doi/10.4155/bio-2019-0018>

Acknowledgements

The authors would like to thank all European Bioanalysis Forum members for very valuable input, especially the individual laboratory members within companies that performed the analytical work to generate the data – AstraZeneca, Charles River, GlaxoSmithKline, Janssen R&D, LGC, Lundbeck, QPS and Sanofi.

Finally, special thanks to Neil Spooner for his contribution to the development of the protocol while an employee of Glaxo-SmithKline.

Disclaimer

The views and conclusion presented in this paper are those of the European Bioanalysis Forum and do not necessarily reflect the representative affiliation or company's position on the subject.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

1. Barfield M, Spooner N, Lad R *et al.* Application of dried blood spots combined with HPLC–MS/MS for the quantification of acetaminophen in toxicokinetic studies. *J. Chrom. B* 870, 32–37 (2008).
2. Jonsson O, Palma Villar R, Nilsson LB *et al.* Validation of a bioanalytical method using capillary microsampling of 8 µl samples: application to a toxicokinetic study in mice. *Bioanalysis* 5(9), 1131–1135 (2013).
3. Li F, Fast D, Ploch SA. Accurate weighing and dilution-assisted plasma microsampling (AWADA-PM): an easy-to-implement and rugged strategy. *Bioanalysis* 6(6), 805–817 (2014).
4. Verhaeghe T, Dillen L, Stieltjes H. The application of capillary microsampling in GLP toxicology studies. *Bioanalysis* 9(7), 531–540 (2017).
5. Chapman K, Burnett J, Corvaro M *et al.* Reducing pre-clinical blood volumes for toxicokinetics: toxicologists, pathologists and bioanalysts unite. *Bioanalysis* 6(22), 2965–2968 (2014).
6. Bowen C, Licea-Perez H, Karlinsey M *et al.* A novel approach to capillary microsampling for quantitative bioanalysis. *Bioanalysis* 5(9), 1131–1135 (2013).
7. Dillen L, Loomans T, Van de Perre G *et al.* Blood microsampling using capillaries for drug-exposure determination in early preclinical studies: a beneficial strategy to reduce blood sample volumes. *Bioanalysis* 6(3), 293–306 (2014).
8. Jonsson O, Palma Villar R, Nilsson L *et al.* Capillary microsampling of 25 µl blood for the determination of toxicokinetic parameters in regulatory studies in animals. *Bioanalysis* 4(6), 661–674 (2012).
9. Zhang J, Lok D, Gray J, Grossman S, Jones M. Development of a novel noncapillary plasma microsampling device for ultra-low volumes of blood collection. *Bioanalysis* 8(9), 871–880 (2016).
10. Spreadborough M, Day J, Jackson-Addie K, Wilson A. Bioanalytical implementation of plasma capillary microsampling: small hurdles, large gains. *Bioanalysis* 5(12), 1485–1489 (2013).
11. White S, Hawthorne G, Dillen L *et al.* European Bioanalysis Forum – reflection on bioanalytical assay requirements used to support liquid microsampling. *Bioanalysis* 6(19), 2581–2586 (2014).
12. Timmerman P, White S, Cobb Z *et al.* European Bioanalysis Forum – continued plans to support liquid microsampling. *Bioanalysis* 6(14), 1897–1900 (2014).

13. Cobb Z, Rohde M, Love I. Feedback from the EBF liquid microsampling consortium: capillary liquid microsampling and homogeneity of the resultant samples. *Bioanalysis*(2019) (In Print).
14. Jiang J. *Linear and Generalized Linear Mixed Models and Their Applications*. Springer-Verlag, NY, USA (2007)
15. Timmerman P, Henderson N, Smeraglia J *et al.* Managing scientific, technical and regulatory innovation in regulated bioanalysis: a discussion paper from the European Bioanalysis Forum. *Bioanalysis* 5(2), 139–145 (2013).
16. NC3Rs. www.nc3rs.org.uk