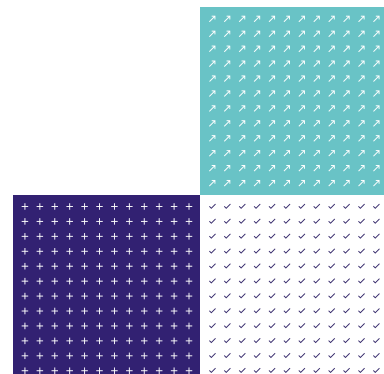# LAB 51

Edition 2 January 2023

UKAS accreditation of laboratories performing analysis of toxicology samples

Contents

Changes since last edition

§1.1 revised to clarify scope of application.
§2.4 revised to provide additional clarity around monitoring for carryover.
§3.5-3.7 revised to clarify calibration requirements.
§6.3 revised to provide more realistic expectation of validation summaries to customers.
§10.9 revised for additional clarity on potential statistical approaches.
§12.14 reference added to ILAC G19.
§13.20 addition of *Limit of identification* into terminology.

1   Introduction

1.1   This publication has been prepared by UKAS and sets out how the requirements of ISO/IEC 17025, ISO 15189, ILAC G19 and UKAS shall be applied for organisations undertaking testing for drugs and drug metabolites in blood, urine, hair, oral fluids and other associated matrices for forensic, workplace, medical legal, or pathology (specialised toxicology) services. Additional requirements to those specified in the standard used for accreditation do *not* apply for laboratories undertaking testing for the purposes of clinical monitoring or medical investigation (which may include post-mortem/cause of death). More information relating to forensic testing is available from the Forensic Science Regulator on the GOV.UK website.

1.2   The publications ISO/IEC 17025 and ISO 15189 (as applicable) remain the authoritative publications and in cases of dispute, UKAS through the appropriate Assessment Manager will adjudicate on unresolved matters.

1.3   Unless specified below, there are no additional requirements to those stated within the authoritative publications ISO/IEC 17025 and ISO 15189 (as applicable).

1.4   This document does not alter or remove the regulatory duties of any company, laboratory, organisation or Regulator nor the right to specify additional requirements under the terms of the agreement between UKAS and the Regulator.

1.5   The definitions of certain terms used in this publication are given in Section 13.

2   Facilities and environmental conditions

2.1   Analysis for **trace drugs** shall be kept physically separate from work involving **bulk drugs**.

2.2   Environmental monitoring **procedures** shall be adopted to determine the presence of any background levels of drugs being tested in the laboratory in which the sample storage, preparation and analysis are undertaken. This should include:

2.2.1   the use of the matrix blank samples

2.2.2   planned environmental swabbing of areas where the potential for contamination is present (e.g. standard preparation areas; storage areas of samples; extraction equipment and standards).

2.3   The detection of a drug in a sample (e.g. Quality Control sample or matrix blank), where that drug should not have been present, will be monitored with a procedure in place that defines the maximum acceptable **concentrations** of each drug for the test to remain valid. The procedure shall also include the actions to be taken when contamination is found at levels above the defined maximum acceptable concentrations for contamination.

2.4   Information relating to the potential for the carry-over of drugs between samples shall be produced as part of the initial validation of the method. This shall then be used to develop a system for the effective monitoring of potential carryover during analytical runs.

3   Equipment

3.1   Instrumentation used for the measurement of drugs shall show fitness for purpose prior to use and on an ongoing basis and shall be capable of achieving the measurement accuracy and uncertainty required to provide a valid response for screening/qualitative, confirmation or quantitative analysis as appropriate.

3.2   Equipment shall be calibrated, ideally with each **batch** of samples, using calibration standards that are traceable to SI units except where this degree of traceability is not possible (see section 4).

3.3   For instrumental analysis methods, calibration solutions may be taken through the entire method or be prepared solely for the determination stage dependent on laboratory procedure. In each case, solutions shall be matched to the sample extract solution to be determined, both in terms of matrix (where possible) and solvent composition. Where the use of the actual matrix is not possible (e.g. for post-mortem samples), the closest available matrix match shall be used. The calibration shall cover the concentration range of interest for the samples being analysed, and ideally should be linear over that range.

3.4 A full calibration for instrumental **presumptive screening** procedures shall be performed at intervals defined on a risk basis to confirm the instrumental suitability at the defined critical and/or cut-off concentration. For instrumental screening procedures, calibrant(s) at the **limit of detection** and/or **critical (decision) limit** or **cut-off concentration** shall be analysed for each individual drug or drug group of interest. This shall be performed at intervals defined on a risk basis but with checks analysed with each sample batch to encompass concentrations either side of the defined critical and/or cut-off concentration.

3.5 Calibration procedures for **confirmatory analyses** for analyses associated with defined cut-off concentrations (e.g. workplace drug analysis) shall include at least 3 calibration points (not including the zero-calibration blank) with each batch analysed. These shall include the defined critical and/or cut-off concentration and concentrations both above and below this (where applicable).

3.6 Calibration procedures for **confirmatory analyses** for analyses where a defined limit of identification (LOI) has been established above which a result of presence/absence is to be reported (e.g. primary confirmation of drugs present prior to a secondary quantitative analysis), shall include at least one calibration point (in addition to the zero-calibration blank) at this LOI with each batch analysed (Note: It is anticipated that linearity of range for such analyses has been established prior to setting of this LOI).

3.7 Calibration procedures for **quantitative analysis** shall include at least 5 calibration points (not including the **zero-calibration blank**) with each batch analysed and shall use a linear fit as the default calibration curve fit (see reference 12.13). The calibration curve shall include and encompass concentrations either side of the defined critical or cut-off concentration. The concentration range shall be appropriate for each analyte and shall encompass the critical level of interest, ideally at approximately 50-75% of the concentration range. A procedure shall be in place to define the acceptance criteria for linearity of the calibration achieved, including policy on the exclusion of calibration points from the curve [Note: if this policy defines that no calibration points shall be removed in **any** circumstances then the linear range may be defined by 4 or more calibration points (not including the zero-calibration blank)]. Any exclusions of calibration points shall be recorded and justified. No more than 20% of calibrators should be removed (not including the zero-calibration blank) although in exceptional circumstances, more than 20% of calibrators may be removed providing that 4 calibration points remain to define the line encompassing the critical level of interest. The justification for these exceptional circumstances shall be documented within the laboratory's non-conforming work process and shall be reviewed (at a minimum of annual review) with other such occurrences to ensure that this has not become a routine practice. Sample results shall fall within the calibration range for a quantitative result to be reported and any samples with concentrations greater than the stated range shall either be quoted as 'greater than the highest concentration calibrator', or where appropriate as indicative concentrations only, or the concentration achieved by re-analysis following appropriate dilution onto the calibration line (see also 6.6.10).

3.8 The response of analytical instruments may change during extensive use due to, for example, contamination of the ion source in a mass spectrometer or deterioration of a detector. This may not be immediately obvious from internal quality control sample results but might coincide with deterioration in both precision and limit of detection of the analytical system. The initial calibration shall, therefore, meet with appropriate predefined system suitability limits (see also 3.11). The **Coefficient of determination [r^2]** shall be greater than 0.990 for linear fit or greater than 0.995 in the exceptional circumstances where a quadratic fit is required for quantitative analysis. In the absence of a **coefficient of determination** the quality of the calibration curve shall be assessed

against defined criteria of maximum error of each calibration point. The signal to noise at the **Lower limit of quantification** [LLoQ] should be at least 10:1.

3.9 Confirmation of the continuing validity of calibration shall be achieved by analysis of **calibration check standards** regularly throughout an analytical batch according to a defined procedure. The frequency shall be on a risk basis dependent on the known stability of the analytical system over time but shall encompass, as a minimum, a check at the end of each batch against defined acceptance criteria. The instrument shall not be recalibrated using the check standard. If a check standard fails to meet appropriate predefined limits, the cause shall be investigated and if necessary, the instrument shall be fully recalibrated and affected samples reanalysed. Where insufficient sample is available for reanalysis then any results reported shall include a deviating sample comment as appropriate.

3.10 Mass spectrometer tuning and mass calibration should be carried out at appropriate intervals using a suitable mass reference standard and the mass calibration fit shall be recorded. The ion source parameters used, including ionisation method [for example electron impact, chemical, atmospheric pressure, electrospray] and the acceptance criteria for the associated parameters, shall be specified within a procedure and the values obtained after tuning and mass calibration also recorded.

3.11 Where high resolution mass spectrometry [double sector, Time of Flight (ToF), Orbitrap or Fourier Transform Ion Cyclotron Resonance (FT-ICR)] is used for confirmation or quantitation, the system suitability checks shall include a measure of the mass resolution [$m/\Delta m$] measured for a given mass at a specified peak height [e.g. at 50% of peak height (full width half maximum), or at 10% of peak height], and the mass measurement accuracy in ppm or other appropriate unit.

3.12 System suitability checks shall be carried out as quality assurance measures to ensure acceptable performance of the analytical system. Where appropriate the results of these checks shall be recorded and monitored. Laboratories shall have fully documented procedures for actions to be taken when system suitability checks fall outside of assigned control limits - such measures may include recalibration of the analytical instrument. Procedures shall be in place to assess trends in system suitability check data and to take action where appropriate. Minimum required system suitability checks include (where applicable):

3.12.1 Requirements for an acceptable 'calibration curve' (see 3.7)

3.12.2 Sensitivity from initial instrument response (peak area or signal to noise ratio) at the critical or cut-off concentration

3.12.3 Matrix method blank for acceptable **determinand** background concentrations

3.12.4 Requirement for relative retention time drift

3.12.5 Chromatographic suitability for acceptable peak shape and peak resolution for closely eluting peaks.

3.12.6 Suitability of **qualifier ion ratios** on a sample-by-sample basis

3.12.7 **Internal standard** recovery on a sample-by-sample basis

3.13 Procedures shall be in place for acceptable chromatographic integration (where applicable). These shall include a policy and/or procedure regarding manual integration of peaks and shall ensure that manual integration of peaks is recorded and justified to safeguard the integrity of the data.

4 Metrological traceability

4.1 Reference standards shall be traceable to the International System of units [SI] through:

4.1.1 Certified values or reference materials provided by a competent producer with stated metrological traceability

4.1.2 Direct rationalisation of the SI units ensured by comparison directly or indirectly with national or international standards.

4.2 When metrological traceability to the SI unit is not possible the laboratory shall demonstrate metrological traceability to an appropriate reference e.g., certified values or **certified reference materials** provided by a competent producer.

4.3 Calibration and Quality Control standards shall be from separate sources wherever possible and traceable to SI units.

4.4 Procedures shall be in place to assess new lots of reference standards prior to introduction into routine use to prevent prolonged step changes in the results obtained.

4.5 Preparations of intermediate standard solutions may include checks of "old" versus "new" to minimise risks of preparation errors and the procedure used shall be documented.

5 Requests, tenders and contracts

5.1 Contracts and associated Service Level Agreements shall clearly and unambiguously define the performance of the analytical procedure(s) on a determinand and matrix basis at the defined critical levels of interest and/or cut-off concentration including:

5.1.1 Measurement uncertainty at these critical levels of interest or cut-off concentration/s

5.1.2 The Limit of Detection and the lower limit of quantification (where applicable)

5.1.3 The calibration range (where applicable)

5.1.4 The **decision rules** regarding statements of conformity for "presence/absence" / "detected/not detected" above a stated cut off value (unless inherent in the relevant regulation)

5.2 Guidance on transport and storage requirements for samples shall be provided to customers by the laboratory through the Service Level Agreement (or equivalent contract).

5.3 Prior to contractual agreement, planning of instrumental capacity and adequate capability of resources shall be recorded and justified.

6 Selection, verification and validation of methods

6.1 The laboratory shall demonstrate and provide justification that suitable methodology (including sample pre-treatment and preparation) has been used in the analysis of a sample matrix and determinand and that it is appropriate with respect to the concentration of the determinand in the sample. This should include a demonstration of the choice of appropriate internal standards, ideally a stable isotope labelled analogue of each determinand for mass spectrometry [MS] based analytical methods.

6.2 For chromatographic confirmatory and quantification analyses, when using multiple-stage MS (e.g. MS/MS), at least two precursor-product ion transitions (i.e. two **SRM** transitions) and their ratio

shall be monitored. (Note: this may not always be possible, for example for low molecular weight compounds, and such cases should be recorded and justified at validation). Where full scan mass spectra are acquired, the acceptance criterion for spectral match against the reference library used shall be defined within the procedure to ensure a consistent approach.

6.3 The laboratory shall demonstrate and provide justification that method validation procedures have been undertaken in such a manner as is appropriate to the sample matrix undergoing analysis. A statement of validation completion shall be made available to the customer if requested.

6.4 Laboratories shall demonstrate that the procedure they wish to employ is adequately validated by reference to published performance data (i.e. acknowledged performance stated within a standard reference method against which verification of performance can be established), or has been validated (see also 6.6) to provide evidence of performance in line with customer requirements.

6.5 Laboratories shall assess the procedure performance under similar conditions to those that will be used when the procedure is in routine use for test samples. Ideally the analytical instrumentation should be dedicated to use for specific matrices and drugs to minimise the potential for compromises between matrix, drug group and sample concentration.

6.6 Prior to validation of the procedure, the validation protocol shall be designed and shall include the required acceptance criteria for the **performance characteristics** of the method (screening, confirmation and quantification), at a matrix and determinand level and shall include, but not be limited to:

6.6.1 Procedural Accuracy [**Bias/Trueness**] and acceptance criteria.

6.6.2 Procedural **Precision** [Coefficient of Variation] (CV) or **Relative Standard Deviation (RSD)**] and acceptance criteria.

6.6.3 Procedural Sensitivity [Limit of Detection] (LoD), Upper and Lower Limits of Quantification (ULoQ and LLoQ)].

6.6.4 Procedural Selectivity (identification of the compound of interest in the presence of other components in the matrix sample and other potential interferences).

6.6.5 Matrix effects, including ion suppression and enhancements (if applicable). This shall include defining the type of ion source used in the case of LC-MS. Matrix effect studies (for example post column infusion/post extraction spike) which quantify the level of matrix effect for all instrumentation techniques where relevant, should be used to estimate the specific level of matrix effect. The laboratory shall quantify the level of matrix effect and the acceptance criteria.

6.6.6 Bias (Recovery) of each drug and acceptance criteria at the cut-off concentration and other critical levels of interest.

6.6.7 Stability of each drug (to ensure that the concentration of the drug of interest is not compromised after sampling). This includes stability during pre-analysis and post-analysis storage (including any freeze-thaw cycles if appropriate) and sample extract stability prior to instrumental analysis. Where studies have already been established within reference documents these may be referred to and the published stability data used, wherever the storage conditions mirrors those used by the laboratory.

6.6.8 Calibration method and acceptance criteria. Where weighted calibration curves [for example $1/x$, $1/x^2$] are used for calibration the laboratory must be able to experimentally justify the weighting used.

6.6.9 Range of application (the validated concentration range of the procedure shall be stated and justified by the validation data).

6.6.10 Dilution – if sample dilutions are undertaken, for example to extend the concentration range of the method, these must be included as part of the validation. These shall include an estimation of the measurement uncertainty where dilution brings the sample into the calibration range for cut-off concentrations (initially outside this range).

6.6.11 Robustness (effect of minor variations of operating procedures, for example variation in analyst, calibration standard or temperature).

6.6.12 Derivatisation Efficiency shall be determined where the analysis is carried out on a derivative of the determinand prepared after extraction from the matrix. Similarly, if the determinand is analysed after hydrolysis [for example glucuronide derivatives in urine samples], the efficiency of the hydrolysis step should be validated.

6.6.13 Procedural carry-over studies.

6.7 These validation experiments shall be carried out by analysing certified reference material (where available), or matrix spiked samples in duplicate in different analytical batches as a minimum at all concentrations reflective of critical levels of interest. For qualitative analyses, the critical level of interest is defined as the limit of detection [LoD] and for confirmation/quantitation this will usually be at cut-off concentration/s. Quantitative analyses shall also require data in support of the entire concentration range and the limit of detection [LoD]. A minimum of 10 degrees of freedom for each validation exercise provides a suitable level of robustness for the data produced and should be obtained using data produced from at least five batches analysed on separate days. It should be noted that where sample results are produced from the mean of duplicate extractions and analyses [for example regulatory S5a drug driving samples] then this shall be replicated within validation experiments.

6.8 Analyses for matrices that cannot be effectively spiked to form homogeneous mixtures (i.e. full interaction of the spike material with the matrix concerned e.g. hair), shall have supplementary validation experiments analysing reference materials (such as **incurred** hair) containing concentrations of the determinands that are ideally at the defined critical levels of interest or cut-off concentrations. The data from these experiments should be incorporated into the final calculation of measurement uncertainty as being representative of precision in the matrix material.

6.9 When a method has been validated, its stated performance [determined from statistical analysis of the validation data] shall reflect the routine capability of the method. That is, when the method is used routinely, its day-to-day performance shall be typical of, and maintained at, the level of the stated validation performance.

6.10 The limit of detection [LoD] of a method used to analyse samples with a complex matrix (such as post-mortem samples) may be higher than the limit of detection of a method used to analyse simpler matrices (such as whole blood). The reported limit of detection shall be fit for the intended purpose and appropriate to the concentration level of interest required of the analysis. The limit of detection shall be calculated by recognised analytical experiments and statistical procedures, with one suggested approach described in Annex A. The limit of detection should never be used in isolation of other method validation data to judge the appropriateness of a method. Note: the maximum value of the limit of detection usually regarded as being fit for purpose is 10 % of the concentration of the critical level of interest or cut-off value and ideally the lower limit of quantification should be at least three times the LoD.

6.11 Assessment of precision and bias/trueness at critical levels of interest and/or cut-off values shall be available for all methods on a matrix and determinand basis and shall provide evidence of fitness for purpose at these concentrations.

7 Handling of test items

7.1 The drugs analysed may be subject to degradation over time both in the sample matrix and after extraction. The laboratory shall use storage methods which minimise such degradation (see also 5.3).

7.2 The laboratory shall demonstrate that the transport and storage procedures being used are appropriate for the stated maximum storage time between sampling and analysis, and where necessary for long-term storage [for example of 'B' samples]. This shall include freeze-thaw experiments if samples are to be retrieved for re-analysis after long-term storage in a freezer. Where these experiments are not possible (e.g. post-mortem samples) an appropriate substitute biological matrix can be used for these studies wherever possible.

7.3 The procedures used for testing the stability of drugs in matrices shall use sufficient replicate analyses, at each analysis time period, to provide a robust indication of the concentrations of drugs present based on the estimated measurement uncertainty of the analytical method used.

7.4 Guidance on transport and storage requirements for samples shall be provided to customers by the laboratory through the Service Level Agreement (or equivalent contract).

8 Technical records and control of data - Information management

8.1 The laboratory shall retain records for a defined period of time which shall take into account the needs of the customer (procurer of the services).

8.2 Laboratory checks completed as part of batch processing of data including, but not limited to, instrumental suitability and acceptable quality control shall be recorded and authorised as having been correctly performed in line with process prior to release of sample results for final reporting.

8.3 Any changes made to the original instrumental data (such as justified manual integration events; calibration point removal) post-acquisition shall be recorded, ideally using the functionality that is available in most MS software packages. This should provide an audit trail that can trace the changes made to the individual analyst responsible for the change. These changes shall be subsequently authorised prior to release of sample data for final reporting.

8.4 Any spreadsheets used during the reporting of results shall be fully validated and controlled for embedded calculations.

9 Measurement uncertainty

9.1 Initial measurement uncertainty shall be calculated at the critical level of interest or cut-off concentration from validation experiments performed for all methodologies (screening, confirmation and quantification) in a manner consistent with accepted guidance such as UKAS, "M3003: The Expression of Uncertainty and Confidence in Measurement" an EURACHEM/CITAC Guide QUAM 2012: Quantifying Uncertainty in Analytical Measurement.

9.2 The expanded measurement uncertainty shall be stated within service levels of agreement (or equivalent contractual documentation) at a minimum of 95% confidence interval (sector dependent).

9.3 Expanded measurement uncertainty shall as a minimum provide a level of confidence at the cut-off concentration commensurate with the market sector. Note: If bias/trueness has not been

accounted for within the expanded measurement uncertainty, then this shall be defined on service level agreements to provide further clarity on the use of the reported result.

9.4 The continued relevance of the expanded measurement uncertainty estimated from validation data shall be determined by an annual statistical review of Quality Control data to provide an on-going estimate of the method precision for each accredited drug/matrix combination. This review should consider results from associated proficiency testing schemes and, where relevant, incorporate these into the UoM estimates.

10 Ensuring the validity of results

10.1 For internal quality control, the performance of each analytical method shall be verified for each batch of samples analysed. Quality Control [QC] samples shall be analysed within the analytical batch with which they have been prepared.

10.2 In each analytical batch, a minimum of 5% of samples shall be laboratory control samples. If the batch size is less than 20, at least one laboratory quality control sample per batch is required.

10.3 Screening techniques shall have quality control samples representative of all drug groups below and above the cut-off concentration (for example +/-25% of the cut-off concentration) to confirm that the determination of presence/absence is being achieved at a level of uncertainty commensurate with that stated within validation data.

10.4 Confirmation and quantitative analyses shall, as a minimum, have quality control samples at the relevant cut-off concentrations and any other critical levels of interest produced using spiked samples. The QC should consist of a sample/s produced by the laboratory containing all determinands of interest and may be produced in a synthetic matrix only if a suitable real matrix material is not available. Note: Standards used for spiking the sample should be from a different source or, if this is not possible, from a different lot number to that used for calibration. Suitable contact times between spiking and extraction should be determined to provide adequate time for interaction between the spiked determinands and the sample matrix, while ensuring that there is no degradation of the determinand.

10.5 Where true matrix-matched QC samples are not available [for example in hair analysis] additional quality control samples prepared from relevant reference materials (e.g. incurred hair) shall be analysed on a regular basis (frequency stipulated based on associated risks) to provide further monitoring of performance within homogeneous matrix material.

10.6 These requirements do not replace other quality assurance and control procedures which shall also be carried out, including system suitability checks (see 3.8).

10.7 To be able to monitor trends in analytical performance, QC data should be plotted on a control chart against statistically derived action and warning limits (see 10.8 below). A minimum of 30 points should be plotted in a 12-month cycle, spread evenly over the period. For analytical procedures that are carried out infrequently, for less frequent analyses, for example those performed once per month, we recommend evaluation after data from 20 control samples have been collected.

10.8 After initial validation experiments have been conducted, laboratories shall have sufficient data available to construct statistically based quality control charts (e.g. Shewhart/Levey-Jennings charts) with 'interim' control limits based on performance seen during validation. Once 60-100 data points have been produced during routine operation of the analytical method, the control limits can be set from statistical analysis of that data. Note: Where customers require that replicate samples

are analysed and a mean result reported, the quality control data shall be produced and monitored on a similar basis.

10.9   A suitably competent member of staff shall review analytical quality control performance regularly, with the timescale dependant on frequency of analysis. All statistically significant changes (which may be identified from relevant F and t-test comparisons or other suitable statistical approaches) shall be investigated. If a statistically significant change has occurred that has been justified through investigation, then the new values are used in the control rules, and new control limits established and drawn on the control chart.

10.10   At a frequency based on the number of data points generated, and at least annually, a comparison of the on-going QC data performance with that of the previous period and that from the initial validation shall be performed on a matrix and determinand level. If no statistically significant changes are detected, then the latest QC data may be incorporated into the calculation of control limits. Any decision made regarding updating of limits shall be justified and recorded. Any significant deterioration of precision and/or trueness affecting overall measurement uncertainty, shall be investigated and where appropriate the customer informed, and related service levels of agreement updated accordingly.

10.11   Laboratories shall have documented procedures that define loss of statistical control and specify actions to be taken (control rules) when control limits are breached. These procedures shall be based on recognised statistical models such as "Westgard Rules'' or those defined within NORDTEST Handbook of Internal Quality Control, NT TR 569 edition 5.1, 2018:09. All rule breaches shall be investigated, and the findings and actions recorded. Samples in an analytical batch where laboratory control samples breach the defined control rules shall be reanalysed, where possible. If this is not possible, then a comment shall be added to the analysis report.

10.12   External proficiency testing [PT] shall be conducted for each determinand/matrix combination wherever a suitable scheme is available. In the absence of an external, inter-laboratory scheme, internal blind testing shall be carried out.

10.13   The laboratory shall have an annual plan providing full details, of the inter-laboratory schemes/supplementary blind spiking exercises including the number of samples, determinands and analyses to be undertaken by the laboratory, on a matrix and determinand basis. The plan shall incorporate information describing how the laboratory rotates these samples through all authorised analysts for each method type to provide supporting evidence of on-going competence within the analytical procedure (Note: The period for this rotation across analysts may be extended over a more relevant time period based on an estimation of the associated risk).

10.14   Proficiency Testing (PT) samples shall be treated, as far as possible, in the same way as test samples. If the laboratory carries out multiple analyses of these samples for training or competency assessment purposes, there should be a defined process for choosing which result/s is reported to the PT provider.

10.15   The laboratory shall have a documented system in operation to review, investigate and address the results submitted to the proficiency testing scheme that are considered, by the scheme organiser, to be unsatisfactory or where they fail to meet regulatory or internal targets for bias/trueness achieved and also to examine trends in performance. If a significant deterioration in method performance is detected and cannot be corrected within a reasonable period of time, then that method shall be re-validated.

10.16   This review procedure should take into consideration the relevance of the matrices and concentrations provided by the scheme, the number of other laboratories participating and whether these laboratories use the same or similar analytical methods.

11   Reporting test results

11.1   The test sample results shall be independently reviewed and authorised prior to release with this review incorporating confirmation that all checks at various stages within the analytical process have been suitably recorded and completed with any deviations from required performance recorded and justified.

11.2   Where results are to be reported from replicate analysis, this shall be the mean of these replicate results with each result being within a range of ±20% of the mean.

11.3   The laboratory shall define within the procedure and related Service Level Agreements the basis for statements of conformity. Confirmation of presence/absence and detected/not detected about a defined cut-off concentration shall incorporate the decision rule employed with this also being recorded on the final report certificate for the associated sample result.

11.4   The decision rule shall consider the level of risk associated with the decision rule employed and apply this to the result (where the decision rule is prescribed by regulation a further consideration of the level of risk is not necessary).

11.5   The nature of the analyses performed defines that where conformity is stated on the final report i.e. presence/absence or detected/not detected; above a cut-off value, a binary decision rule exists with the associated expanded measurement uncertainty at a minimum 95% confidence interval (sector dependent) for the defined cut-off concentration being the appropriate guard band. Simple Acceptance Rules where the guard band width is zero (i.e. measurement uncertainty has not been considered), provides a specific risk of 50% probability of "false accept" and 50% probability of "false reject" when the determinand is equal to the concentration of the cut-off value. Where this rule is applied, the measurement uncertainty measured at the cut-off shall be such that the risks associated with false reporting of presence/absence are minimal.

11.6   Any deviations associated with either the sample integrity or deviations from stipulated method process shall be recorded on the final report.

11.7   LoD and LLoQ shall be adjusted for additional dilutions applied to samples prior to or during the analytical procedure. Quantitative results outside the validated concentration range shall be identified, with these being reported as either greater than values or, where appropriate as indicative concentrations only.

11.8   Quantitative results shall be reported to the number of significant figures commensurate with the measurement uncertainty for the determinand and matrix (or as defined within contractual arrangements where these are stipulated within regulations).

12 References

12.1 ISO 15189 Medical laboratories - Requirements for quality and competence

12.2 ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories

12.3 ISO 17034 General requirements for the competence of reference material producers

12.4 UKAS publication M3003 The Expression of Uncertainty and Confidence in Measurement

12.5 EURACHEM/CITAC Guide QUAM 2012 Quantifying Uncertainty in Analytical Measurement Text

12.6 ILAC G8 Guidelines on Decision Rules and Statements of Conformity

12.7 NORDTEST Handbook of Internal Quality Control, NT TR 569 edition 5.1, 2018:09

12.8 Quality Control Westgard Rules [https://www.westgard.com/mltirule.htm]

12.9 ILAC P10 Policy on Metrological Traceability of Measurement Results

12.10 ISO Guide 80 Guidance for the in-house preparation of quality control materials (QCMs)

12.11 ISO/IEC Guide 30 Reference materials - Selected terms and definitions

12.12 Science and Justice 58 (2018) 335-345 The United Kingdom and Ireland association of forensic toxicologists forensic toxicology laboratory guidelines (2018)

12.13 Statistics and Chemometrics for Analytical Chemistry, J N Miller and J C Miller, 2018. Pearson Higher Education. ISBN-13: 9781292186719.

12.14 ILAC G19 Modules in a Forensic Science Process

13   Terminology

13.1   **Batch** – A number of samples prepared for an analytical run over a time period shown to provide adequate stability of the equipment/assay in use.

13.2   **Bias** – Bias, which may be positive, or negative is the difference (expressed as a percentage) between the mean of a number of determinations obtained under repeatability conditions and the true or accepted concentration.

%Bias = (mean of determinations - true or accepted value) x 100
                    True or accepted value

Bias can be estimated where appropriate certified reference materials are available and a stated (certified) concentration has been quoted. Recovery data can be used to estimate bias via spiking experiments.

13.3   **Calibration Check Standard** – A calibrant analysed at defined intervals to confirm the continuing suitability of the current calibration curve, with a defined maximum allowable error for that calibrant concentration**.**

13.4   **Certified Reference Material (CRM)** – A reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes its traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [ISO/IEC Guide 30].

13.5   **Coefficient of Variance** – See Relative Standard Deviation.

13.6   **Concentration** – Concentration is usually expressed as mass per sample, for example mass per volume (µg/l).

13.7   **Confirmatory Analysis** – The identification a substance with the lowest possible chances of a false positive.

13.8   **Correlation Coefficient [r]** – Is a statistical measure of the strength of the relationship between the relative movements of two variables

13.9   **Coefficient of Determination [r^2]** – The proportion of the variance in the dependent variable that is predictable from the independent variable(s).

13.10   **Critical Level of Interest** or **Cut-Off Concentration** – The concentration value around which a decision is often required. A method is usually deemed acceptable if, when used properly, it is capable of establishing within defined limits of measurement uncertainty, whether a concentration is above or below the critical level of interest. This is generally the Cut-off Concentration specified within Regulations or published Guidance

13.11   **Criterion of Identification** - confirmation methods based on the power of the identification used to identify the compounds present in the sample. The power of identification i.e. the criteria in addition to the retention time used to ensure correct identification shall be documented with this based on at least two identification reference points in addition to retention time.

13.12   **Decision Rule** – Rule that describes how measurement uncertainty is accounted for when stating conformity with a specified requirement

13.13 **Determinand** – Within the sample, this is the measurand, analyte, substance, or group of substances, the concentration of which needs to be determined. It shall be clearly and unambiguously defined.

13.14 **Diagnostic Ion(s)** – Molecular ion or fragment ions whose presence and abundance are characteristic of the analyte and thereby may assist in its identification.

13.15 **Drugs (Trace)** – Includes all main groups such as stimulants, depressants, opium-related painkillers, hallucinogens, psychoactive compounds and all drug metabolites at concentrations anticipated within defined matrices for samples from Forensic, Workplace, Medical Legal, or Pathology sectors

Drugs (Bulk) – Concentrated stock reference materials of drugs of abuse; any materials associated with testing within the Misuse of Drugs Act 1971

13.16 **Incurred Samples** – For hair testing, samples from known drug users may be used for internal Quality Control or Proficiency Testing as these will contain drugs that are incorporated within the hair structure.

13.17 **Internal (Surrogate) Standard** – Relevant standards, usually structural or stable isotope labelled analogues of the determinands, used to account for drug recovery from each sample under test. If the internal standard is added to each sample and calibration and Quality Control standard prior to extraction, clean-up and analysis, it accounts for variations in recovery from samples.

13.18 **Ion Ratio** – Calculated as an intensity (or peak area) ratio of a less intense ion to that of a more intense ion. The reference ion ratio value is calculated as an average of ion ratios of calibration and/or quality control standards in solutions or preferably extracted matrix.

13.19 **Laboratory** – A laboratory, or sub-contracted laboratory, that undertakes the analysis of samples.

13.20 **Limit of detection (LoD)** – Measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence [VIM 2012, 3rd edition International Vocabulary of Metrology - Basic and General Concepts and Associated Terms].

13.21 **Limit of Identification (LOI)** – the minimum concentration of the target compound in the sample at which the criterion of identification (as defined within policy) can be met with a specified level of confidence

13.22 **Lower Limit of Quantification (LLoQ)** – Measured quantity value, obtained by a given measurement procedure, at a concentration ideally at least one fifth the Critical Level of Interest.

13.23 **Performance Characteristics** – Those performance values, such as precision, bias (or trueness, as appropriate), limit of detection and measurement uncertainty that need to be estimated before a method is used routinely.

13.24 **Precision** – The closeness of agreement between independent test results obtained under stipulated conditions. In this document it refers to the distribution of a number of repeated determinations, expressed as the percentage relative standard deviation (RSD).

$$\%RSD = \frac{S \times 100}{M}$$

Where S = total standard deviation, M is the mean of results.

13.25 **Presumptive Screening** – A qualitative analysis that allows identification of the presence of a substance in a sample above a specified LoD or other defined concentration.

13.26   **Procedures** – A series of actions conducted in a certain order or manner.

13:27   **Quantitative Analysis** – measurement of relative peak height/area/abundance from a sample of known concentration across a defined concentration range

13.28   **Relative Abundance** – The abundance of a particular ion relative to the most abundant ion monitored.

13.29   **Relative Standard Deviation** – An estimate of the standard deviation of a population from a (statistical) sample of n results divided by the mean of that sample. Often known as the coefficient of variation (CV).

13.30   **Sample** – That (uniquely identified) specimen submitted to the laboratory for analysis.

13.31   **Selected Reaction Monitoring (SRM)** – A method used in tandem mass spectrometry [MS/MS] in which an ion of a particular mass is selected in the first stage of a tandem mass spectrometer and an ion product of a fragmentation reaction of the precursor ion is selected in the second mass spectrometer stage for detection.

13.32   **Statistical Control** – When the result or results of quality control samples are shown to be within defined limits of recognised acceptability, a method is said to be in statistical control. When these limits are breached, the method is considered out of control.

13.33   **Technical Procedure (Operating Procedure)** – The organisation's detailed written procedures on how to perform a method in line with its quality system.

13.34   **Testing Laboratory** – A laboratory that performs tests.

13.35   **Traceability** – The property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

13.36   **Trueness** – The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value [ISO 3534-1, Clause 3.7]. NOTE The true value of a quantity or quantitative characteristic is a theoretical concept and, in general, cannot be known exactly.

13.37   **Measurement Uncertainty** – A parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand [ISO Guide 98-3 (2008)].

13.38   **Upper Limit of Quantification (ULoQ)** – Measured quantity value, obtained by a given measurement procedure, at a concentration that defines the upper bound of calibration.

13.39   **Zero Calibration Blank** – a measured response from an unspiked matrix (matched to that within the calibration standards) which is used as the zero-calibration point.

Annex A: A suggested method for estimating limit of detection LoD

A.1 Introduction

The definition of limit of detection (LoD) is quite often vague and there is little consistency between standards. In addition, the LoD is widely but inappropriately used as the primary performance measure of an analytical system. It does not indicate whether a method is fit for purpose. For example, a very low LoD value does not mean that the method is suitable for a particular purpose, as precision and bias/trueness could be unacceptable at the critical level of interest.

A.2 Choice of sample and sample pre-treatment

The sample used to estimate LoD shall be a sample containing a small but measurable amount of the determinand of interest. The samples used to estimate the LoD shall consist wherever possible of a matrix as close as possible to those routinely analysed for the specific drug.

Ideally, analysis of the sample, used to estimate the LoD, will produce normally distributed results scattered around zero; both negative and positive results will be generated. It is usually possible for the LoD sample to have a sufficiently small background concentration of the determinand to fulfil this requirement. However, in some analytical systems this may not always be possible because negative or low results cannot be obtained. In these cases, spike the LoD sample with a small amount of the determinand, sufficient to produce a small but significant response from the analytical system, close to the expected LoD.

The sample, used to estimate the LoD, shall wherever possible be put through the entire analytical process. Extraction and measurement based only on reagent blanks is not sufficient for estimating LoDs for satisfying the requirements of this document. The LoD sample shall be processed in the same manner and using the same equipment and reagents as other samples in a batch.

A.3 Calculation

For the purpose of this performance standard, LoD is defined by the equation:

$$\text{LOD} = 2\sqrt{2}.t_{(df,\alpha = 0.05)}.s_w$$

where:

df is the number of degrees of freedom (minimum 10)

t is the one-sided Student's t-test statistic (95% confidence level)

s_w is the within-batch standard deviation of results from samples ideally containing negligible concentration of the determinand of interest obtained from the validation experiments.

Results shall not be rounded before being used for the estimation of LoD.

In the most general case, where m batches of different numbers of replicates n_i give a series of within-batch standard deviations s_i:

The pooled value of s_w is given by:

$$s_w \text{ (pooled)} = \sqrt{\frac{\sum s_i^2.(n_i-1)}{\sum(n_i-1)}}$$

where:

s_i = individual batch standard deviation,

n_i = number of results in the batch.

Where the batches all contain the same number of results, this equation simplifies to:

$$s_w \text{ (pooled)} = \sqrt{\frac{\sum s_i{}^2}{m}}$$ with **m(n-1)** degrees of freedom

for example for 10 batches of 2 blanks:

$$s_w \text{ (pooled)} = \sqrt{\frac{\sum s_i{}^2}{10}}$$ with 10 degrees of freedom

Since $t_{(\alpha = 0.05)}$ for a one sided t-test with 10 degrees of freedom is 1.812

Then   LOD = $2\sqrt{2}.t.s_w = 5.13 s_w$