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EURL Guidance Document on the Quality control during routine analysis (ongoing method performance verification)

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1. Background and Overview

This guidance is the EURL interpretation of SANTE 11188-2018 which shall support laboratories in the practical implementation of the regulation's requirements. Laboratories operating under SANTE 11188-2018 are not obliged to follow this guidance minutely; different approaches are acceptable, if they offer a comparable level of quality control. This guidance applies to analytical methods designed for confirmatory purposes, and may be used - partly with adaptation - for screening methods[1], as well. However, deviations from the general approach presented in this document may be necessary in order to accommodate for the specific requirements of certain detection modes.

According to ISO 17025:2017[2] chapter 7.7 accredited laboratories shall implement a procedure for monitoring the validity of results. Several possibilities are given as to how this can be accomplished. In order to monitor the method performance continuously with regard to the requirements of SANTE 11188-2018, the recurring analysis of specific control samples is

[1] Additional information specific for screening methods is provided in the guidance "Validation approaches for screening methods"
[2] ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories

recommended. During routine analysis, the analysis of certified reference materials (CRMs) is the preferable option to provide evidence of unchanged method performance. Since CRMs that contain the relevant analytes at the required concentration levels are rarely available, also reference materials provided and characterised by the EURLs, as well as in-house reference materials, which are analysed on a regular basis, may be used as alternatives.

The analysis of quality control (QC) samples and the continued evaluation of calibration curves are also valuable tools for the provision of evidence of method performance in routine analysis. Furthermore, the participation in proficiency tests is required by ISO 17025 as an external means of verification of the suitability of a method.

1.1. Quality control samples

The inclusion of suitable QC samples in each series of analyses is an essential part in ensuring the quality of the test results (internal quality assurance). QC samples are used to detect irregularities during analysis and to prevent erroneous results before they are included in test reports.

A uniform set of QC samples is considered essential for a comprehensive follow-up investigation in case of analytical problems. Hence, within each measurement series ("analyses batch") performed, a set of the following QC samples should be analysed:

1) System suitability control sample / instrument control sample (performance monitoring of representative and critical analytes):
    - serves to check the performance of the instrument, for example using a standard solution. The sample is injected before each analytical series and should be re-injected at the end of the analytical series to demonstrate stable system performance.
    - substance mix of representative and critical analytes, e. g. stable and sensitive analytes, chromatographically challenging analytes (possible characteristics: peak shape, retention time, S/N ratio, peak area, response)

2) Reagent blank:
    - serves to prove that no contaminations have been introduced into the system at any stage of the analytical procedure and can provide helpful information for follow-up investigations in case of anomalies.

- matrix-free sample subjected to the same analytical procedure as the test samples, which therefore contains all the reagents used in the applied method without any matrix interferences

3) Matrix blank (compliant control sample):
   - serves to prove the proper execution of the analytical procedure and the absence of contaminations from matrix or introduced by the analytical procedure. It can provide helpful information for follow-up investigations in case of anomalies.
   - proportion of a sufficiently characterised laboratory sample free of the analytes to be determined
   - sample is ideally the same matrix and species as the unknown samples

4) Matrix blank (compliant control sample, see 3) fortified with internal standard
   - serves to prove the proper execution of the analytical procedure and the absence of contaminations from matrix or introduced by the internal standards or the analytical procedure. It can provide helpful information for follow-up investigations in case of anomalies

5) Fortified matrix blank (recovery control, non-compliant control):
   - serves to prove the ongoing validity of the method performance characteristics and aids in identification of possible interferences
   - matrix blank fortified with single analytes or analyte mixes at relevant concentration levels, e. g. at MRL (confirmation) or STC (screening), preferably using the same or similar matrix as the unknown samples
   - substance mix of all analytes (preferably) or representative and critical analytes of the substance group of interest (stable and sensitive analytes, chromatographically challenging analytes (peak shape, retention time))
   - Ideally, a certified reference standard at several relevant concentration levels is used. If no such material is available, then it is advisable to use independent standard solutions for fortification of the control samples and the calibration solutions.

Even though it is not necessary to provide control charts for all analytes within the scope of the method, the ongoing performance of the method has to be ensured. Respective measures

have to be implemented, e. g. using rolling evaluation programmes for sets of analytes or planned variation of QC samples (see 2).

1.2. Analytical sequence

QC samples should be prepared and processed simultaneously with the unknown samples and measured in a single analytical series using the same instrument. The following order is recommended for every sample sequence evaluated using analytical instruments:

- system suitability control sample (instrument control sample)
- calibration curve (if applicable)[#]
- reagent blank
- compliant control sample (matrix blank)
- unknown samples
- compliant control sample (matrix blank + internal standard)
- fortified matrix blank (recovery control, non-compliant control)
- calibration curve (if applicable)[#]
- system suitability control sample (instrument control sample)
- in-between: injections of solvent/mobile phase (check for carry-over)

[#]) a double injection of calibration samples before and after the injection of unknown sample extracts is mandatory in routine for the calculation of CCα according to ISO 11843

In order to ensure repeatability conditions for all samples, as a rule of thumb an analytical sequence should comprise a maximum of 20-30 evaluations of unknown sample extracts, unless there is experience from preliminary studies or from a ruggedness study guaranteeing system and extract stability and thus justifying the extension of the analytical sequence beyond this number. Injecting QC samples throughout the series of unknown samples is an appropriate way to monitor drift within the analytical sequence.
If samples are analysed using chromatographic systems, it is recommended to analyse multiple solvent/mobile phase runs between samples of a sequence in order to control for potential carry over. The frequency of use of solvent runs should be based on the procedure during the validation and the likelihood for carry-over of analytes.

1.3. Calibration

For quantitative determinations of an analyte, a calibration curve is included in each analytical series. It is preferable to measure the calibration curve in random order so as to detect systematic errors. A calibration curve may be prepared from standards in pure solvent, as a matrix-matched calibration (i. e. fortified in final sample extracts), or as a matrix-fortified calibration (fortified prior to the sample preparation procedure). If matrix effects are expected and no suitable internal standard is available, a calibration curve in pure solvent can generally not be used. In these cases, a standard addition procedure performed on the unknown sample may be an adequate approach.
Calibration curves should consist of at least five levels (including blank level). In the case of semi-quantitative determinations, e. g. for screening purposes, the number of calibration points can be reduced. It is not a requirement that the calibration levels be equidistant. Nevertheless, they should be more or less equally distributed across the entire calibration range.

Calibration standards include the analytes to be determined and, where appropriate, the internal standard used to analyse the samples. Within the curve, single points may only be eliminated if it is scientifically justifiable, i. e. if an error during preparation of the calibration standard or a failure of the measurement instrument is evident.

If a confirmatory analysis is carried out based on an estimated result from a screening analysis, the concentration range covered by the calibration curve should be adapted to the presumed content of the analyte within the limits of the validated concentration range. The presumed analyte concentration should ideally be close to the middle of the selected calibration curve. The distance of the concentration levels of a calibration curve and the calibration range are based on the specifications of the respective analytical purpose (e. g. validation, residue control plan). Matrix-fortified and matrix-matched calibrations may only be used in conjunction with the series of analyses for which they were created, unless a lack of any of these influences is proven during the validation. Otherwise, deviations resulting from different conditions during sample preparation or analyte decomposition in the final extract cannot be excluded. If standard calibrations should be stored and reused, their stability on all relevant concentration levels shall be demonstrated in a stability study[3].

[3] Corresponding data from literature or Reference Laboratories (EURL, NRL) may be used if exactly the same framework conditions apply.

1.4. Evaluation and Documentation

Wherever practicable, recovery on the basis of fortified matrix blanks / non-compliant control samples (or trueness as determined from the analysis of reference materials) of all target analytes shall be evaluated by means of quality control charts in accordance with ISO 17025[1] chapter 7.7. If this requires a disproportionately large number of evaluations, the monitoring by means of control charts may be limited to certain representative analytes (stable and sensitive analytes, chromatographically challenging analytes).
For the QC samples, the criteria for evaluating the results as specified in the method description are to be used. These may include:

- S/N ratio or peak area of the compounds included in the instrument control sample
- slope of the calibration curve
- criteria that characterise the performance of the analytical instrument
- concentration and recovery (fortified matrix blank, recovery control, reference materials)
- differences or deviations, e. g. between two-fold determinations, repeated injections etc.

Templates for selected control charts, e. g. for recovery control, are available from the EURLs.

2. Revalidation

A method revalidation in defined time intervals is not necessary as long as the ongoing performance of the method can be verified by:

- evaluation of control charts of QC samples
- evaluation of control charts of reference materials
- successful participation in proficiency tests

However, it is advisable to plan the analysis of QC samples in such a way that over time a complete set of data for the recalculation of the validation parameters may be obtained. In the case of the alternative validation concept, it would be possible to amend experimental plan data or repeat single runs in selected analytical series with routine control samples.

If methods have not been used in longer periods of time (> 2 years), the method performance needs to be verified prior to the analysis of official control samples, e.g. by the analysis of fortified samples / reference materials, or by participation in a proficiency test.
Should accreditation bodies request different procedures, these need to be considered in the design of all quality management concepts.