# A Two-Day Training Workshop

# RECENT DEVELOPMENTS IN QUALITY ASSURANCE

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PANCYPRIAN UNION OF CHEMISTS (PUC)

DIVISION OF QUALITY ASSURANCE

FREDERICK UNIVERSITY
School of Health Sciences-Department of Pharmacy

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## Accreditation for Microbiological Laboratories AML GUIDE



FREDERICK UNIVERSITY
School of Health Sciences-Department of Pharmacy

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**Accreditation for Microbiological Laboratories**

Second edition 2013

This document has been produced by Eurachem. It provides microbiological laboratories with appropriate information and guidance on how to be prepared in order to fulfil the requirements of ISO/IEC 17025.

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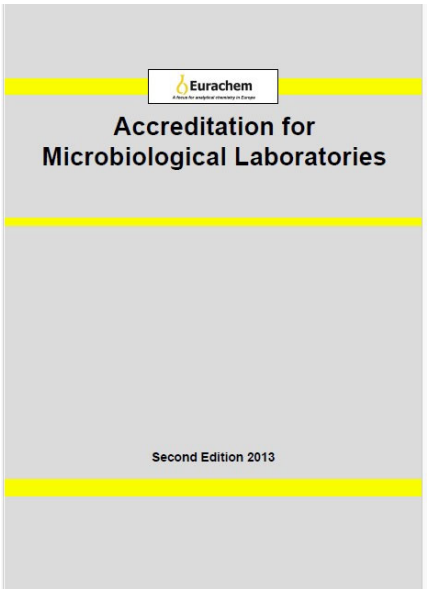




**Accreditation for Microbiological Laboratories**

**Third edition (2023)**

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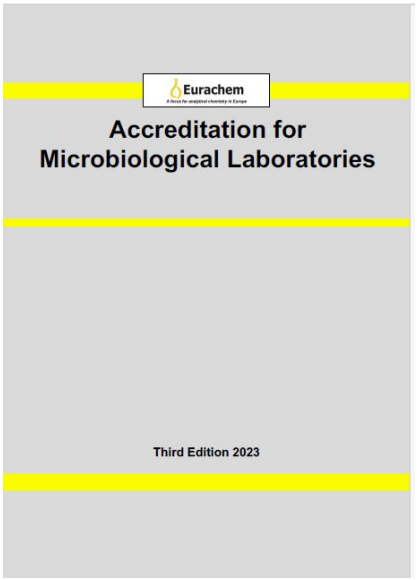

- The Guide focuses on the requirements of ISO/IEC 17025
- The content can be used organizations seeking accreditation or certification against the requirements of standards such as ISO 15189, GLP (Good Laboratory Practice), GMP (Good Manufacturing Practice), and GCP (Good Clinical Practice).
- The Guide provides useful information for laboratories that wish to establish a quality management system but are not seeking formal recognition.
- This revision mainly reflects changes that were introduced with the publication of the 2017 version of ISO/IEC 17025.
- Major changes in the third edition are explained in the following sections

❑ Recent trends in microbiology, e.g. PCR (polymerase chain reaction) techniques for the detection of microorganisms

- 6.2 facilities safety environmental conditions
- 9.7 Metrological traceability
- 10.3 verification of molecular methods
- 12.6 evaluation of measurement uncertainty
- Annex C6: molecular methods MU



## Structure (the order of the sections in adherence with ISO/IEC 17025)

**1 Introduction and Scope**
**2 Standards for accreditation of microbiological laboratories**
**3 Risk-based thinking**
**4 General requirements**
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**12 Evaluation of measurement uncertainty**
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**14 Reporting of results**

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**Annex F – Guidance on equipment validation and verification**
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**List of abb**

| Abbreviations | | Symbols | |
|---|---|---|---|
| ANOVA | ANalysis Of VAriance | $s_{IR}$ | Relative intralaboratory standard deviation |
| AFNOR | Association Francaise de NORmalisation/French Standardization Association | $s_{QC}$ | Relative quality control standard deviation |
| AOAC | International Association of Official Analytical Collaboration | $u_c$ | Combined relative standard uncertainty |
| CITAC | Cooperation on International Traceability in Analytical Chemistry | $u_{conf}$ | Relative uncertainty due to result from confirmation |
| CFU | Colony Forming Unit | $u_d$ | Relative distributional or intrinsic uncertainty due to taking a test portion of a laboratory sample |
| GMO | Genetically Modified Organisms | $u_{matrix}$ | Relative uncertainty from imperfect mixing of the laboratory sample |
| GUM | Guide to the expression of Uncertainty in Measurement | $u_o$ | Relative operational (technical) uncertainty |
| IEC | International Electrotechnical Commission | $\hat{u}_o$ | One-sided upper confidence limit (UCL) for the estimate of the operational uncertainty |
| ILAC | International Laboratory Accreditation Cooperation | $u_{samp}$ | Relative sampling uncertainty |
| ISBN | International Standard Book Number | $U$ | Relative expanded uncertainty |
| MPN | Most Probable Number | $U_{Max}$ | Upper limit of the uncertainty interval |
| PCR | Polymerase Chain Reaction | $U_{Min}$ | Lower limit of the uncertainty interval |
| PT | Proficiency Testing | | |
| VIM | International Vocabulary of Metrology | | |



# Risk-based thinking (addition)

- The main change in the philosophy of the standards used for accreditation
- Provides broader consideration regarding all aspects in the laboratory system and in its everyday operation
- integrated throughout the whole standard (impartiality, statements of conformity, management of non-conforming work and management reviews)
- Consider the probability of a risk and its impact.
- Cases with both high impact and high probability of occurring are given much more emphasis.
- SWOT analysis (strengths, weaknesses, opportunities, and threats) is a useful tool
- Eurolab Cookbook No 18
- The laboratory does not need to have detailed risk management based on relevant standards e.g. ISO 31000 (basic elements)

# 4. General requirements

## 4.1 Impartiality

- Commitment to impartiality
- Should identify risks to its impartiality and demonstrate how it manages in cases when such risks are identified.

## 4.2 Confidentiality

- All information should be kept confidential during the performance of the laboratory activities.
- Special care is required with regard to the release of such information.
- In case this is required by law, relevant provisions shall be followed. In other cases, the customer should be informed in advance.



# 10 Selection, verification, and validation of methods

- Updated sections on method verification and validation to reflect current ISO standards
- Water matrix ISO 13843(Q), ISO/TS 12869(leg) and ISO 29201(ENU)
- Food matrix ISO 7218(GR), ISO 16140(V), ISO 17468, ISO 19036(MU)
- The validation/verification of microbiological test methods should reflect actual test conditions (use naturally contaminated samples or samples spiked with a predetermined level of material (microorganism).
- The spiked samples only mimic the presence of the naturally occurring microorganisms.
- The extent of validation/verification should cover the scope of the method.

# 10 Selection, verification, and validation of methods

- The verification and validation of quantitative microbiological methods include the determination of true and false positives and negatives
- Additionally, the following can be calculated: sensitivity, specificity, false positive and false negative rate, selectivity, and efficiency.



***Example 1***

Verification of a *quantitative method* for a water matrix according to ISO 13843.

Steps followed by the laboratory:

• spike a minimum of five samples and determine the following performance characteristics; sensitivity, specificity, efficiency, selectivity, false positive and false negative rates;

• analyse a minimum of three samples (different sources and levels of target organisms) under repeatability conditions to obtain a set of 10 replicates per sample to determine repeatability;

• perform repeated counting of the same plate or positive tubes for MPN (Most Probable Number), to determine the uncertainty of counting (30 plates or tubes, preferably but not necessary from different samples).

For acceptance criteria for the verification, see the ISO method being used and ISO 13843.

# Annex )



## Annex A – Glossary of terms

Terminology for chemical, biological and clinical measurements is presented in the Eurachem Guide on terminology [61]. Below is presented terminology specific for microbiological measurements.

| | |
|---|---|
| Limit of detection | Applied to qualitative microbiological tests: The lowest number of microorganisms that can be detected, but in numbers that cannot be estimated accurately.<br>See also the definition in ISO 16140-1 [28]. |
| Detection level | Minimum concentration of organisms that produce evidence of growth with a probability of P = 0.95 when inoculated into a specified culture medium and incubated under defined conditions (ISO 16140-1 [28] and ISO 13843 [24]).<br>Note 1: The theoretical level that conforms to this definition is an average of three viable cells in an inoculum volume. |
| Intralaboratory reproducibility (intermediate precision) | Closeness of agreement between test results obtained with the same method on the same or similar test materials in the same laboratory with different operators using different equipment (ISO 8199 [51]). Symbol used is $s_{IR}$. |
| Limit of determination | Lowest analyte concentration per analytical portion where the expected relative standard uncertainty, equals a specified value (ISO 13843 [24]).<br>NOTE: In Eurachem guidance LOQ, limit of quantification, is also used. |
| Negative deviation | Occurs when the alternative method gives a negative result without confirmation when the reference method gives a positive result. This deviation becomes a false negative result when the true result can be proved as being positive. |
| Positive deviation | Occurs when the alternative method gives a positive result without confirmation when the reference method gives a negative result. This deviation becomes a false positive result when the true result can be proved as being negative. |

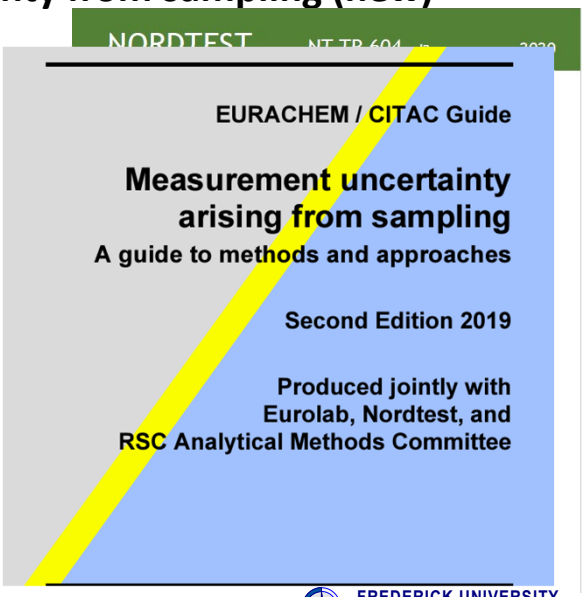


# Annex C on reporting confidence intervals (new)

- Introduce the calculation of asymmetric confidence intervals for microbiological methods.
- The main references are ISO 29201 [MU ENU] and ISO 8199 [guidance] for water matrices, ISO 19036 [MU] for food matrices and G108 for both matrices [internal quality control].
- When the expanded uncertainty is over 30 –40 % it is recommended to state *asymmetric* confidence intervals instead of just giving the expanded uncertainty for the count result in % or log units.
- A confidence interval for the results will be more informative for the client than just giving the result with uncertainty e.g. 50 CFU ± 42 % can also be reported as 50 [33, 76] CFU where 33 – 76 is the asymmetric confidence interval for the result 50 CFU.
- Examples for water and food matrix, CFU and MPN methods

# Annex D on estimation of uncertainty from sampling (new)

- Main scope to briefly introduce the estimation of sampling uncertainty from duplicates following the guidance in the Eurachem Guide *Measurement uncertainty arising from sampling* and Nordtest TR 604.
- Sampling uncertainty due to subsampling (matrix effect) of a laboratory sample is estimated using ANOVA.
- In order to simplify the ANOVA calculations we use an Excel add-in, RANOVA3.






# 14 Use of decision rule

- Quantitative methods (CFU/MPN)
- Annex C: recommends how to report the CL based on calculated MU
- In cases where the specification or the relevant standard does not refer to a decision rule, the laboratory should, when a conformity statement is required, document and apply the decision rule used;
this rule should take into account the level of risk, the acceptance limit and be communicated and agreed with the customer – see ILAC G8 as well as the relevant Eurachem Guide and leaflet.

**Table 1 – Expression of results in CFU/ml or per analytical test portion**

| Counted colonies | Reporting of results | |
|---|---|---|
| | ISO 8199 [51] | ISO 7218 [10] |
| 0 | Not detected or < 1 | < 1 |
| 1-2 | Microorganisms are present | Microorganisms present but < 4 |
| 3 | Report results as an estimate | Microorganisms present but < 4 |
| 4 - 9 | Report results as an estimate | Report results as an estimate |
| ≥ 10 | Report results | Report results |

NOTE 1 Legislation may require different ways of reporting.
NOTE 2 The volume of the inoculum/dish and the eventual dilution must be considered, e.g. 3 CFU obtained in a food sample diluted 10 times (inoculum=1 mg/dish) will be reported as: microorganisms present but < 40 CFU.

https://ilac.org/publications-and-resources/ilac-guidance-series/

https://www.eurachem.org/index.php/publications/guides/uncertcompliance