

VALIDATION OF MICROBIOLOGICAL METHODS FOR WATER AND ENVIRONMENT: REVISION OF ISO 13843

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The ISO/IEC 17025:2005 standard requires the use of standardized methods which are validated following the development process or the implementation of a proper validation procedure for in-house developed methods [1].

For water and environmental fields, ISO/TR 13843 (2000): *water quality – Guidance on validation of microbiological methods* is a rough guide [2], which describes how to apply the common characteristics of analytical methods performances to the specificity of the microbiological determinations:

- The analyte is a living microorganism which is taxonomically defined, or in some cases defined by a group designation less accurate than taxonomic definitions (i.e.: coliforms)
- The particle nature of microorganisms and their random distribution even in perfectly mixed waters, lead to peculiar statistical considerations and inexorably limit the enumeration precision
- The measurand (Colony-Forming-Unit) is based on the cultivability of the microorganisms which can be in a large panel of different physiological states depending on the matrix (disinfectant stress in chlorine water, nutrient depletion in oligotrophic waters...)

After the publication of ISO 29201:2012, which gave the baselines of the uncertainty of measurement of microbiological enumeration methods [3], and the revision of ISO 17994:2014 for the comparison of the relative recovery of two microbiological methods [4], the challenging revision of ISO/TR 13843 aims to give a more precise determination of the parameters useful for the characterization of microbiological methods in order to achieve an ISO standard status.

In water and environmental microbiology, the characterization is seen as an exploratory process with the aim of establishing the likely set of performance characteristics of a new or a modified method, under a specific set of circumstances. The sensitivity, specificity and selectivity are determined by verifying presumptive counts compared to confirmed counts. The linearity is seen as an aspect of trueness, it is studied with a view of determining the maximum upper limit of counting. At the other extreme of the working range, the definitions of the detection level and the limit of determination need to take into account the random distribution properly, using appropriate statistical models. The recovery is always relative as the true value of the measurand remains unknown. As to precision parameters, repeatability and reproducibility of the methods can be assessed following the ISO 5725-2:1994 guidelines with some adaptations as the basic principles originally applied to continuous data and not to discrete data such as colony counts.

[1] ISO/IEC 17025:2005 - General requirements for the competence of testing and calibration laboratories.

[2] ISO/TR 13843:2000 - Water quality - Guidance on validation of microbiological methods.

[3] ISO 29201:2012 - Water quality - The variability of test results and the uncertainty of measurement of microbiological enumeration methods.

[4] ISO 17994:2014 - Water quality - Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods.