Measurement uncertainty in microbiological cultivation methods

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Abstract Microbiological analyses are carried out on clinical, food, feed and environmental samples. The aims of the analyses are diagnostic or estimation of the safety or the quality of the sample. Important decisions are made on the basis of microbiological analyses. Little attention, however, is paid to the uncertainty of measurement of microbiological analyses. In microbiological cultivation techniques the result is obtained by counting individual objects. The normally low number of counted objects strongly affects the result of the analysis and its uncertainty. Because of the importance of the particle statistical variation to the uncertainty, the approaches developed for chemical analyses are not directly applicable to microbiology. This paper discusses microbial analyses and describes a novel guidance document for the estimation of measurement uncertainty in culturing methods [1].

Keywords Microbiology · Cultivation methods · Measurement uncertainty

Introduction

It has been asked whether microbiology is more like the art of cooking than science. Cooking, and doing it correctly (e.g. utilising gravimetric, volumetric, temperature- and time-related metrological information) is an integral part of microbiological cultivation methods. Science is the ever present basis for analytical work in microbiology.

The aim in microbiological analyses is usually to detect and enumerate a known species or group of microorganisms in a measured amount of sample. If measurement means counting and identification, quantitative microbiological analyses belong to the sphere of metrology. Traceability to primary measurement standards can hardly be achieved, but the measurement units are the numbers per gravimetric or volumetric units.

Internationally available reference strains and materials, and international performance tests for microbiology are becoming increasingly available to aid traceability and comparability evaluation of analyses carried out in different laboratories.

In microbiological analysis, culture techniques are important because it is often relevant to detect viable microorganisms. Often the target microorganisms in human, animal, food or environmental samples constitute only a minor fraction of the microorganisms present. Different selection principles and indicator systems are applied in order to facilitate the growth of the target microorganism yielding characteristic reactions, while suppressing the growth of other microorganisms. The primary cultivation result is usually not sufficiently reliable, but necessitates the use of further tests to confirm the identity of the target microorganism. In practice, confirmation tests in routine work do not provide taxonomically valid identification, but rely on a limited set of tests confirming the identity of the target with high probability. The valid identification of microorganisms can be regarded as a rather complex measurement and the uncertainties inherent to microbial taxonomy complicate evaluation of the uncertainty involved. In this paper, the uncertainty of taxonomically valid identification is not discussed. In routine work, identification is usually limited to the agreed confirmation tests. Uncertainty of confir-
The particle statistical variation can be estimated by applying the Poisson theory:

\[ RSD_c = u_c = \sqrt{\frac{1}{C}} \]

Because the microbial detectors function optimally at particle numbers between 25 and 100 per test portion, the particle statistical variation often dominates the uncertainty of the measurement. Therefore uncertainty estimates such as reproducibility and repeatability determined in collaborative efforts are not generally applicable in microbiology and it is not worthwhile investing much effort in calculating values for these parameters (see Type A below). Instead, the uncertainty of the method can be expressed as a formula into which observed values of each measurement can be inserted.

Because the Type A uncertainty estimation (based on replicate measurements) is usually not economically feasible in microbiology, the emphasis in the guidance document [1] is on the Type B approach. Type A: The standard uncertainty is calculated from n independent replicate measurements \( x_1, x_2, ..., x_n \) as the experimental standard deviation [2]:

\[ s_x = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x)^2}{n-1}} \]

The number of replicates must be rather high because even 30 replicates from sample sources following normal distribution yield estimates of the standard deviation with only 13% relative uncertainty.

Type B: According to [2], Type B uncertainty is obtained using other approaches than replicate samples. The uncertainty variance \( u^2 \) or the standard uncertainty \( u \) are based on the whole body of scientific information available (with the exception of replicate measurements) on the possible variation of the measurand. Information of statistical theory, earlier measurements, experience or general beliefs on instruments and materials, specifications of the manufacturer, published reference values in calibration and certification reports, and uncertainty estimates in handbooks can be utilised.

The common sources of uncertainty in cultivation methods are sample stability, dilution, counting (including particle statistical variation and personal interpretation of the target), yield on the medium, crowding effect (coincidence error) and uncertainty of confirmation. The combined uncertainty can be calculated as the quadratic sum of different uncertainty components.

Compilation of uncertainty in cultivation methods

In microbiological cultivation methods only four types of detection systems are used: the one-plate instrument, the set of plates instrument, the one-tube detector (Presence/Absence) and the set of tubes instrument (Most Probable Number). All these classes necessitate different formulas for uncertainty estimates. Different media and incubation conditions, and confirmation and identification tests offer the versatility needed for the enumeration of different organisms. However, this versatility is not reflected in the principles of the estimation of uncertainty.

In microbiological cultivation methods the sample is homogenised, after which a measured amount is diluted or concentrated (e.g. by membrane filtration) to the