Instructions

Inlabtec Serial Diluter: Verification by method comparison

Introduction

Alternatives to methods or techniques already used in a laboratory are generally checked by comparison (1). This ensures that the alternative method is used correctly and the results are correct (2).
The introduction of the Serial Diluter into a laboratory is analogous to the introduction of an alternative dilution technique and is therefore comparable to the currently used dilution technique (for example standard test tube technique). This ensures that the Serial Diluter is mastered and the results achieved are correct.
These instructions are a suggestion, how such a comparison can be carried out and evaluated if not already a laboratory internal test and evaluation procedure exists. The protocol and the statistical evaluation of the results are based on the guideline for the validation of microbiological test methods by the Swiss Accreditation Service SAS (1).

Execution of the method comparison

Verification of the serial dilution by plate counting

Based on the results of bacterial counts of food samples (Colony forming units (cfu/g) by the plate count method (pour plate technique, spreading-spatula technique) the equivalence of the Serial Diluter is verified against the dilution method used so far.
Under theoretically ideal conditions without variability and uncertainties, equivalent methods for the same samples would provide the identical bacterial counts and the difference between the two mean values of these methods would be zero. All analytical methods, however, have variability and uncertainty and cause corresponding deviations from the correct mean value.
The difference of the measured mean from the correct mean value is called systematic deviation. When comparing the equivalence of the Serial Diluter to the previously used dilution technique, the systematic deviation of the plate counts in cfu/g should be ≤ 15.4 %. This corresponds to the repeatability of the plate count method of 7.7 % multiplied by the extension factor k = 2 for the 95 % confidence interval (3).
For the verification of measurement methods the sole consideration of the systematic difference is not sufficient. In addition, the random deviation of the values around the mean, the repeatability, must be considered. Based on the experience of interlaboratory tests (proficiency tests), the repeatability of plate counts can be determined and is according to experience ± 0.5 log cfu/g (5, 7).
In addition, if comparing quantitative test methods, two results obtained under repeatability conditions are 95 % likely to differ by less than the repeatability limit $r = 2{,}8 \cdot s_r$ Where s_r = standard deviation of the measurement results obtained under repeatability conditions and factor 2,8 is composed as follows: $2 \cdot \sqrt{2}$ (coverage factor 2 for 95 % confidence interval and $\sqrt{2}$ due to the fact that r refers to the difference between two individual test series) (1). Therefore, for the standard deviation s_r of the measured values, the following condition applies: $2.8 \cdot s_r \leq 0.5$ log cfu/g.
Accordingly, the equivalence of the results after dilutions with the Serial Diluter compared to the dilution technique used until now are confirmed if the difference of the means of the plate counts is ≤ 15.4 % and the repeatability limit r of the measurements is ≤ 0.5 log cfu/g.

Sample type and size

The aim of the verification is to check the equivalence of two mixing techniques. For this purpose a comparison of the dilution techniques with ≥ 5 repetitions of the same 1:10 diluted and homogenized sample should be performed. The sample matrices mainly examined in the laboratory should be used. Normally about 5 different sample matrices are sufficient, since the original sample matrix generally has no influence on the mixing behaviour of 1:10 diluted samples after homogenization.

Evaluation of Measurement Results

The verification is met if for the plate counts obtained the difference of the means of is ≤ 15.4 % and the repeatability limit r of the measured values ≤ 0.5 log cfu/g.

The verification is not met if for the plate counts obtained the difference of the means of is ≥ 15.4% and the repeatability limit r of the measured values ≥ 0.5 log cfu/g.

Template for the evaluation

A suitable verification template (Microsoft Excel) for easy statistical evaluation of the measurement results is available on our website www.inlabtec.com (library). If necessary, adjust the number of samples by deleting and / or inserting rows.

To consider when performing method verification

1. Due to reproducibility (see above) the verification should be carried out by the same person.
2. The correct operation of the serial diluter must be ensured and is described in the operating instructions of the Serial Diluter, Section 9: Verification of dispensed volume.
3. Use the same batch of dilution fluid, culture media and pipettes for both methods.
4. The dilution and plating of the homogenized food sample has to be carried out promptly with both methods (proliferation of microorganisms!) and must be done within 45 minutes after sample homogenization.
5. Based on experience, verification is easily fulfilled if the dilution techniques are mastered. As far as possible, all persons who conduct microbiological analyses and carry out verification should be able to demonstrate their methodological competence (proficiency test and / or in-house comparisons).
6. Sample homogeneity and pipetting accuracy are critical for a successful method comparison.
Investigations showed that 50% to 70% of the total variance in method comparisons is due to the sample inhomogeneity (1). This major influencing factor can be minimized by first transferring the entire sample volume required for the comparison from the initial dilution, for example the stomacher bag into a separate test tube. This minimizes position effects during sampling and secures homogenous samples for an increased the precision of the method comparison.
7. Pipetting accuracy can be checked with an analytical balance. By means of ten 1 ml pipettings, the relative systematic as well as the standard deviation are determined. The maximum permissible error = systematic deviation + 2x standard deviation must be ≤ 2%. If the maximum permissible error is above 2%, the pipette as well as the pipetting technique must be checked.

References

1. Schweizerische Akkreditierungsstelle SAS: Leitfaden zur Validierung mikrobiologischer Prüfverfahren und zur Abschätzung der Messunsicherheit im Bereich Lebensmittel- und Umweltmikrobiologie; SAS Dokument 328.dw, 2013-04, Rev.03

2. ISO/ IEC 17025: 2005 General requirements for the competence of testing and calibration laboratories

3. AOAC INTERNATIONAL: How to meet ISO 17025. Requirements for Method Verification. ALACC Guide, 2007.