

A control-chart approach for sample homogeneity verification in food and water microbiology proficiency testing schemes

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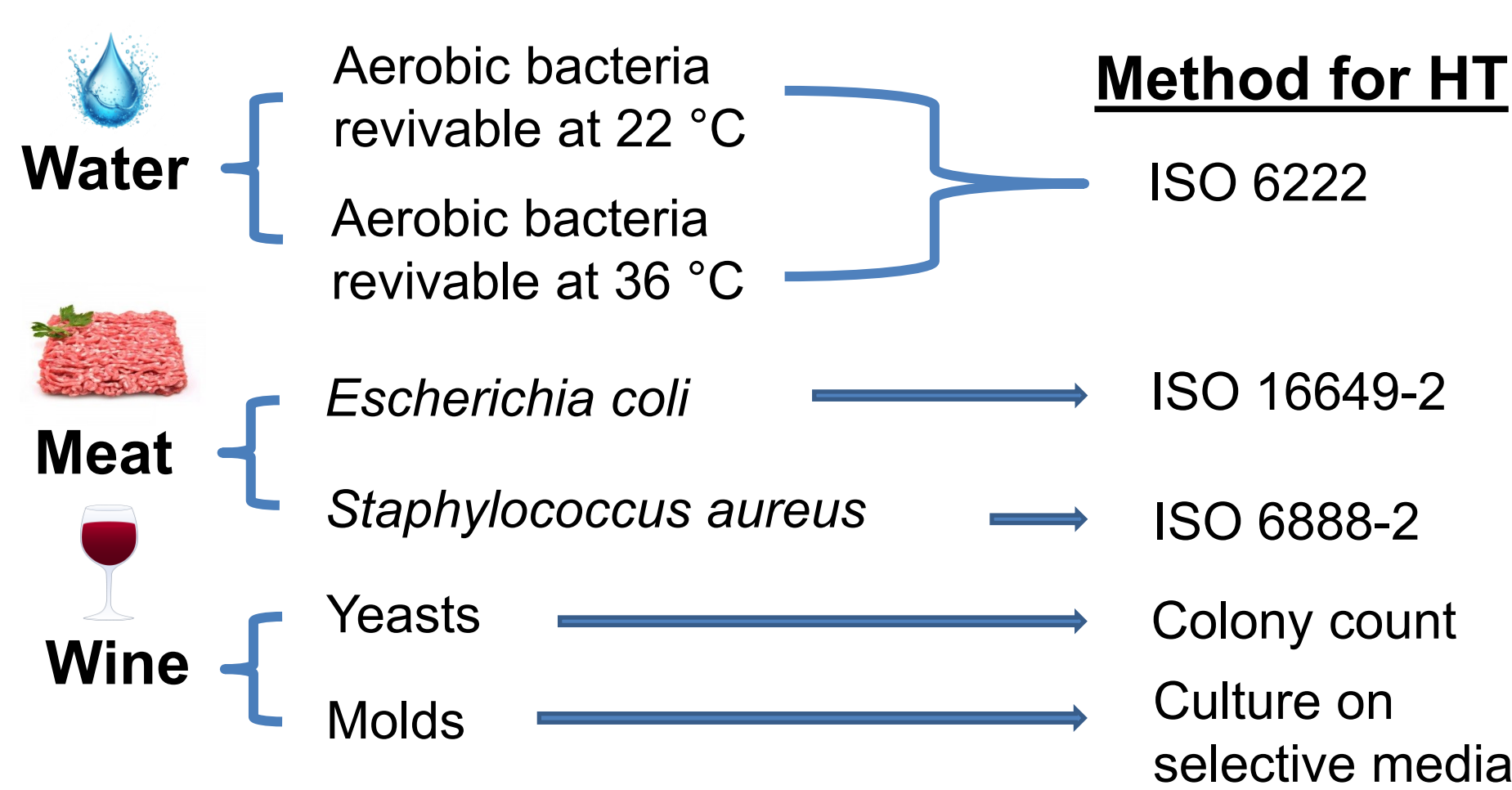
INTRODUCTION

Verifying the homogeneity of the samples used for a proficiency test (PT) is a normative requirement of the ISO 17043 standard to ensure that variation in the results is due to the laboratories' analyses rather than the heterogeneity of the test material [1]. A general method based on the assessment of between-sample and within-sample (analytical) variance components of 10 samples analyzed in duplicate according to the recommendation of the ISO 13528 standard can be strict for microbiological analyses [2]. Microbiological analyses present specific difficulties because microorganisms are living organisms that can behave differently within the same material, for example by dying, reproducing, forming clumps, or experiencing physiological stress. In addition, microorganisms may have an unpredictable and heterogeneous distribution within a material. Consequently, results from quantitative microbiological analyses can be influenced by the analytical method used or the operator performing the analysis. The ISO 13528 standard defines a material as sufficiently homogeneous if the standard deviation between the homogeneity testing (HT) samples (s_g) is less than 30 % of the standard deviation used for proficiency assessment (σ_{pt}). But this system has limitations. Firstly, σ_{pt} is unknown at the time HT is conducted. Additionally, there is a high probability of falsely rejecting homogeneous HT samples when the σ_{pt} estimated from participating laboratories' results is small. Furthermore, in microbiology, replicate analysis not always applicable because of the destructive nature of testing. Finally, HT involves high cost, so the number of samples is usually small. We therefore propose a method for sample homogeneity verification based on **standard deviation (SD) control charts for successive HT and PT rounds**.

METHODOLOGY

Samples and homogeneity control

For this study, results from 3 matrices with 2 parameters each were studied to demonstrate the applicability of the method:



All samples were stored and transported at 5 ± 3 °C, and HT was performed in duplicate on 10 samples of each batch.

Standard deviation control charts

Based on the observed variability of HT and PT results of batches of samples which were concluded to be sufficiently homogeneous, the homogeneity of a new round could be concluded by comparing its standard deviations with those of the previous rounds for a similar level of mean colony counts (for the same microorganism and matrix). All colony counts were transformed to $\log_{10}$ CFU before statistical analysis.

Two charts were used to achieve this objective, by plotting **HT and PT standard deviations** against **mean colony counts** to assess the effect of matrix complexity on the variability of the measurements, and by charting **HT and PT standard deviations** on a **round-by-round basis** to monitor the variability of the colony counts over time. This required calculating:

- Mean colony count of a homogeneity test, $x_{ht,i}$
- Standard deviation of a homogeneity test, $s_{ht,i}$
- Pooled SD of homogeneity tests, $\bar{s}_{ht}$ (the average of all HT SDs over the studied period)
- 95 % and 99 % confidence interval (CI) upper limits for the SD of a HT round
- Advanced Standards Transforming Markets (ASTM)'s upper control limit ($B_4 \bar{s}_{ht}$; $B_4 = 1.490$ for 20 measurements) [3]
- Mean colony count of a proficiency test, $x_{pt,i}$
- Pooled SD of proficiency tests, $\bar{\sigma}_{pt}$
- 95 % and 99 % confidence interval (CI) upper limits for the SD of a PT round (with degrees of freedom corresponding to the minimum and maximum numbers of participants during the studied period)
- Target SD of $0.25 \log_{10}$ CFU proposed in the ISO 22117 [4]

RESULTS and DISCUSSION

Two examples of standard deviation control charts are presented here to show the wide applicability of the method and illustrate key results: aerobic bacteria revivable at 22 °C in water (Fig. 1) and mold in wine (Fig. 2).

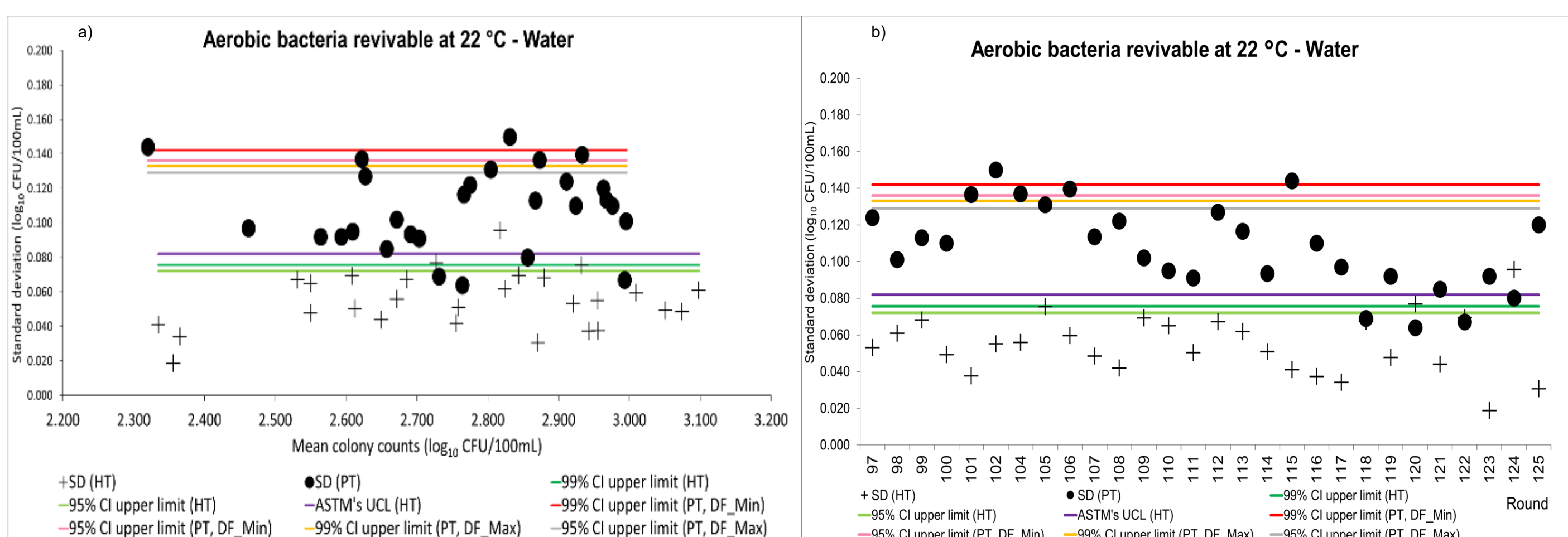


Fig. 1 Standard deviations of 28th successive homogeneity tests and proficiency tests on the enumeration of aerobic bacteria revivable at 22 °C in water from 2017 to 2024 (a) against mean colony counts and (b) for individual rounds. * No HT was scheduled for the round No. 103

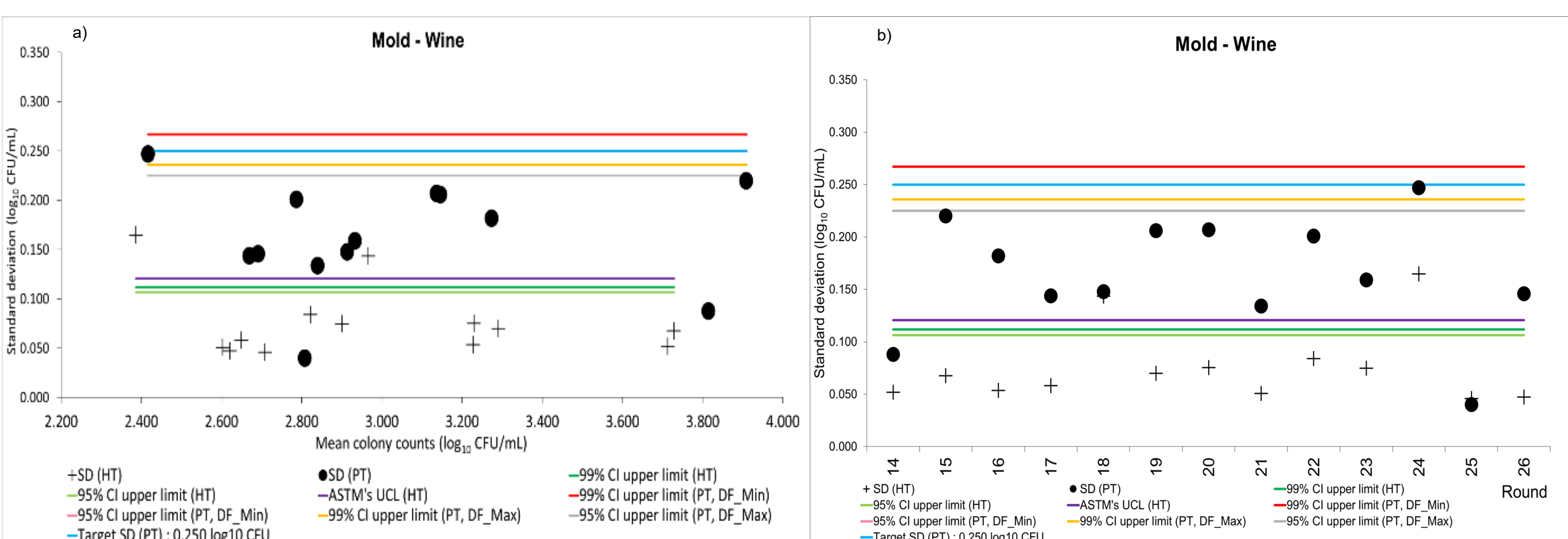


Fig. 2 Standard deviations of 13 successive homogeneity tests and proficiency tests on the enumeration of mold in wine from 2017 to 2024 (a) against mean colony counts and (b) for individual rounds. * The upper limit of the 95 % confidence interval for the minimum number of participants overlaps with the target SD line

Key results:

- Constant standard deviations are observed across different levels of mean colony counts. This homoscedasticity justified the assumption of constant variance underlying the use of SD control charts, and the consistent variation in the laboratories' results over time confirmed the homogeneity of the test materials.
- Outlying data points are easy to detect. For example, in round 24 of mold in wine (Fig. 2B), the HT lay above the 95 % and 99 % CI upper limits and the σ_{pt} was slightly higher than in other rounds. However, this point was still under the target SD, and the observed variability was taken into account in the application of a tolerance value of $2\sigma_{pt}$ from the mean colony count in assessing laboratory performance.
- The target SD, ASTM's UCL, and the 95 % and 99 % CI upper limits can all be useful for homogeneity verification and should be considered. For wine (Fig. 2) the different limits are similar, while for water (Fig. 1), the 95 % and 99 % CI upper limits (0.129 to $0.150 \log_{10}$ CFU/mL) were significantly lower than the target SD for food PT schemes ($0.250 \log_{10}$ CFU/mL), which is unsurprising given the homogeneity of the matrix itself. The 95 % and 99 % CI upper bounds can therefore be used as reference limits in the absence of a normative target SD for proficiency testing.

Additional advantages:

- SD charts can help correct "Type 1" errors (false rejection of homogeneous samples). For round 120 of aerobic bacteria revivable at 22 °C in water (Fig. 1B), the small variance of both HT and PT results did not meet the ISO 13528 criteria for homogeneity testing, but the charts demonstrated that the samples were sufficiently homogeneous.
- SD charts can diminish the requirement for replicate analysis of HT samples. When test protocols are in control over a long time, single analysis of samples is sufficient for homogeneity evaluation, leading to a reduction in HT costs.
- SD charts contribute to characterizing reproducibility in the quantitative analyses of microorganisms in a matrix over a long period of time, allowing a σ_{pt} to be established for each microorganism-matrix pair. σ_{pt} can be lower or higher than the target SDs recommended in ISO 22117, taking into account the inherent heterogeneity in microbiological distribution and matrix composition. This approach also allows minimum and maximum SD limits to be defined to ensure consistent evaluation of laboratory performance in a PT scheme, which is a long-term aim of this study.
- SD control charts can be built for each microorganism-matrix combination to better understand matrix effects and construct characteristic functions for each pair.

CONCLUSION

In conclusion, given the limitations of the normative methods, standard deviation control charts can be effective for verifying sample homogeneity in microbiological proficiency tests when the variance of results is expected to be constant across a range of concentrations. The pooled standard deviations and control limits obtained from many successive rounds of tests give a reliable assessment of the homogeneity of the samples, and any drifts from the chart limits during homogeneity testing can be investigated to ensure that heterogeneous test materials are not used to evaluate laboratory performance. Additionally, the charts can be progressively improved by incorporating the results of new rounds and reassessing control limits annually. These charts are therefore a useful tool and a cost-saving alternative to homogeneity testing in the long run, when costly tests can prevent laboratories from participating regularly in external quality control through proficiency tests.

REFERENCES

- [1] **ISO/IEC 17043:2023** - Conformity assessment — General requirements for the competence of proficiency testing providers
- [2] **ISO 13528:2022** - Statistical methods for use in proficiency testing by interlaboratory comparison
- [3] **ASTM E2587-16:2016** - Standard Practice for Use of Control Charts in Statistical Process Control
- [4] **ISO 22117:2019** - Microbiology of the food chain — Specific requirements and guidance for proficiency testing by interlaboratory comparison