Verification of the Homogenization Performance of the Inlabtec Serial Diluter

1 Introduction

This procedure assesses the homogenization performance of the Serial Diluter. Microbial counts before and after additional manual mixing of the same bag contents are compared. No relevant change after additional mixing indicates sufficient automatic homogenization under the conditions tested.

Equipment that can affect test results must be verified before use in an accredited laboratory. For initial verification, parallel aliquots of the same homogenized sample are tested using both the Serial Diluter and the laboratory's previously established serial dilution method under otherwise consistent conditions.

This internal procedure supports the initial verification and subsequent periodic assessment of homogenization performance in accordance with ISO 7218:2024, Clause 6.4.4.4. The specific procedure and acceptance criterion are internal specifications and are not prescribed by the standard. For periodic assessments, the procedure avoids the need to maintain the laboratory's previously established serial dilution method solely for comparison purposes. Because this method is no longer used routinely, reactivating it only for periodic comparisons could introduce additional variability and increase the risk of error, thereby reducing the reliability of the comparison. The laboratory therefore determines how this internal procedure is applied, including its scope, representative test conditions, acceptance criteria and monitoring intervals, based on the intended use and a risk-based approach.

Mixer speed and mixing time may additionally be checked using a smartphone accelerometer. This provides indirect evidence of correct mechanical mixer function but does not replace the microbiological assessment described here (see "Mechanical Function Check of the Inlabtec Serial Diluter Mixer").

Successful proficiency testing results provide further evidence of the device's suitability within the overall examination procedure and of the laboratory's ongoing control of the method under the conditions assessed.

2 Principle

After completion of the dilution series prepared from a 1:10 initial sample dilution, selected dilution steps plated immediately after automatic homogenization are manually mixed by aspirating virtually the entire remaining bag contents into a sterile 10 mL pipette and dispensing them back at a uniform rate. Flow through the pipette and the resulting liquid jet and recirculation provide additional mixing. The dilution steps are then plated again. If the microbial count does not change relevantly, automatic homogenization is considered sufficient under the conditions tested.

Because the results before and after additional mixing are obtained from the same dilution in the same bag, the procedure provides a simple and targeted assessment of homogenization performance. Other factors affecting microbial count determination are kept largely constant.

3 Additional Materials Required

- Sterile 10 mL pipettes for manual mixing
- Additional culture media

4 Procedure

4.1 Dilution Series and First Plating

- Prepare the sample in accordance with the applicable examination method.
- Prepare the dilution series using the Serial Diluter.
- Plate suitable dilution steps immediately after automatic homogenization.
- Label the plates “before additional mixing”.

4.2 Additional Manual Mixing

- Aspirate the remaining contents of the dilution bags being tested once, as completely as possible, into a sterile 10 mL pipette and dispense them completely back into the same bag at a uniform rate.

Avoid foaming, loss of liquid and contamination during aspiration and dispensing.

If several dilution steps are to be tested, start with the highest dilution, i.e. the lowest microorganism concentration. Then use the same pipette for each successively lower dilution level and repeat the procedure. Before changing to the next dilution level, dispense the contents of the pipette completely.

This sequence minimizes the transfer of higher microorganism concentrations into more highly diluted samples and allows the same pipette to be used efficiently within a dilution series. Use a new sterile pipette for each new sample or independent dilution series.

4.3 Second Plating

- Immediately plate the additionally mixed dilution steps again.
- Use the same plating volume and plating procedure as for the first plating.
- Label the plates “after additional mixing” and incubate all plates under identical conditions.

5 Evaluation

Calculate the microbial counts before (“Before”) and after (“After”) additional mixing in CFU/mL or CFU/g in accordance with the applicable examination method. Only results within the countable colony range specified by the applicable examination method may be used for the evaluation.

The absolute logarithmic difference is calculated as follows:

$\Delta \log_{10} = |\log_{10}(\text{After}) - \log_{10}(\text{Before})|$

Alternatively, the ratio may be calculated:

Factor = higher result ÷ lower result

Calculation Example

Before = 1.2×10^4 CFU/mL
After = 1.8×10^4 CFU/mL

Factor = $1.8 \times 10^4 \div 1.2 \times 10^4 = 1.5$

$\Delta \log_{10} = |\log_{10} (1.8 \times 10^4) - \log_{10} (1.2 \times 10^4)| = 0.176$

The result meets the acceptance criterion.

6 Acceptance Criterion

Homogenization is considered sufficient under the conditions tested if the absolute logarithmic difference does not exceed 0.3 $\log_{10}$:

$\Delta \log_{10} \leq 0.3$

This corresponds approximately to a factor of 2:

higher result ÷ lower result ≤ 2.0

For certain microbiological method comparisons, a difference of up to 0.5 $\log_{10}$ may be used as an assessment criterion. In the present test, both results are obtained from the same dilution steps in the same bags, and additional manual mixing is the only deliberately modified test step. Therefore, the more stringent internal acceptance criterion of 0.3 $\log_{10}$ has been defined.

The relationship between the logarithmic difference and the factor is:

$10^{0.3} = 1.995 \approx 2.0$

ISO 7218:2024 does not specify a numerical acceptance criterion for verifying homogenization performance.

Logarithmic difference	**Factor**	**Assessment**
$\Delta \log_{10} \leq 0.3$	≤ 2.0	Acceptance criterion met
$\Delta \log_{10} > 0.3$	> 2.0	Acceptance criterion not met

For repeated tests, the direction of the difference must also be assessed. A reproducible increase in the microbial count after additional manual mixing indicates that the microorganisms may not have been distributed sufficiently uniformly by automatic homogenization. Random increases and decreases are not considered systematic.

7 Interpretation

If the acceptance criterion is met, the following conclusion may be stated:

The additional manual mixing did not cause a relevant change in the microbial count. Automatic homogenization by the Inlabtec Serial Diluter was sufficient under the conditions tested.

If the criterion is not met, repeat the test under comparable conditions. A confirmed increase of more than 0.3 $\log_{10}$ after manual mixing indicates insufficient automatic homogenization, provided that other causes have been excluded. Check and, if necessary, extend the mixing time and repeat the test.

A reproducible decrease should also be investigated but does not by itself indicate insufficient homogenization. Possible causes include sampling or plating variability, sedimentation, aggregation, time delays or low colony counts.

8 Documentation

- Device identification, date and responsible person
- Sample or test suspension, microorganism and diluent
- Device settings, mixing time and dilution steps tested
- Colony counts, calculated microbial counts, absolute logarithmic differences and assessment
- Deviations, investigations and actions taken

This test demonstrates homogenization performance only under the conditions examined and does not by itself constitute the initial verification of the device for its intended use. Based on the intended applications and a risk assessment, the laboratory shall define the representative samples, microorganisms, acceptance criteria and monitoring intervals.

9 References

ISO 7218:2024. Microbiology of the food chain — General requirements and guidance for microbiological examinations. Clause 6.4.4.4.

ISO 6887-1:2017, including Amendment 1:2024. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1.

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Bergis, H., Bonanno, L., Asséré, A. and Lombard, B. (2021). EURL Lm Technical Guidance Document on Challenge Tests and Durability Studies for Assessing Shelf-life of Ready-to-eat Foods Related to Listeria monocytogenes. Version 4, 1 July 2021.