Interlaboratory validation of culture-based and flow cytometry methods for quantifying microencapsulated probiotics in complex food matrices

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Objective

To conduct an interlaboratory evaluation and validation of complementary enumeration methods for microencapsulated *Lacticaseibacillus rhamnosus* GG in a yogurt-bite matrix and to assess equivalence between plate count (CFU) and flow cytometry (AFU) approaches for probiotic quantification.

Methods

Both methods met the validation criteria, demonstrating acceptable precision (RSD ≤15%) and no statistically significant differences across operators, days, and experimental conditions. Across all datasets, AFU and CFU values were equivalent within ±0.5 log, supporting analytical comparability. Flow cytometry additionally demonstrated high accuracy (100-104% recovery) and specificity (R2 ≥0.95), while enabling same-day results

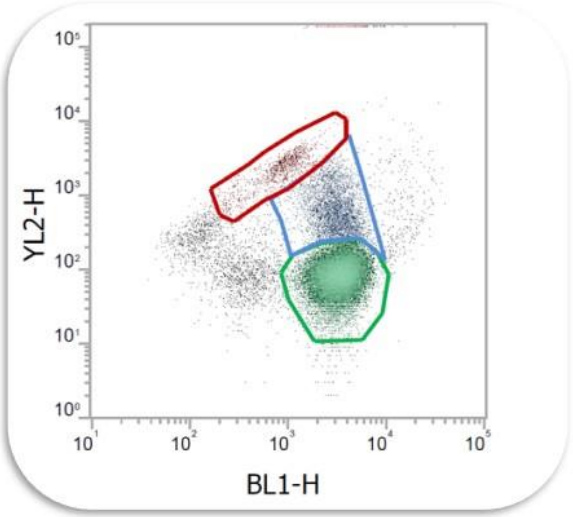

Figure 1. Flow cytometry dot plot example showing Live (green), Injured (blue), and Dead (red) cell populations

Figure 2. The Attune NxT flow cytometer. Copyright 2016, Thermo Fisher Inc. Used with permissions www.thermofisher.com

RESULTS

Both methods met the validation criteria, demonstrating acceptable precision (RSD ≤15%) and no statistically significant differences across operators, days, and experimental conditions. Across all datasets, AFU and CFU values were equivalent within ±0.5 log, supporting analytical comparability. Flow cytometry additionally demonstrated high accuracy (100-104% recovery) and specificity (R2 ≥0.95), while enabling same-day results.

CONCLUSIONS

This interlaboratory study demonstrates that both plate count and flow cytometry methods are robust, reproducible, and demonstrate equivalence for enumeration of microencapsulated probiotics in complex food matrices. The strong agreement across laboratories supports the use of a dual-method approach, where flow cytometry provides rapid, actionable results and plate count serves as the regulatory reference, enhancing confidence in probiotic label claims and quality control.