

BACKGROUND

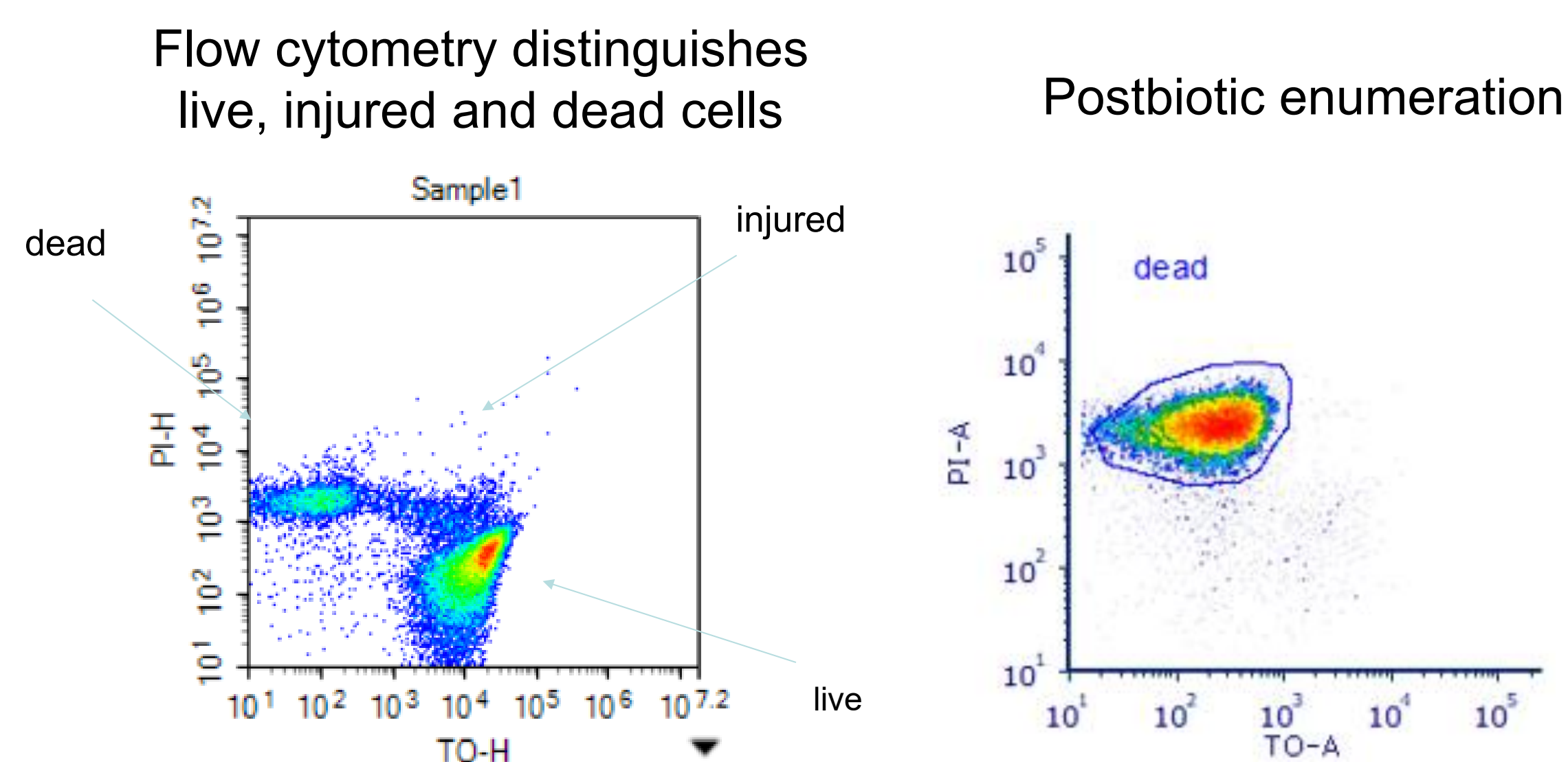
Accurate characterization of probiotics and postbiotics is critical for ensuring product efficacy and consistency. Traditional methods have accuracy and consistency limitations in testing.

Flow cytometry enables rapid, precise viability assessment of probiotics -distinguishing live, injured, and dead cells within 15 minutes of rehydration. This is especially valuable for oxygen-sensitive bacteria in manufacturing workflows. Flow cytometry, as a single-cell analysis tool, can accurately enumerate heat-killed cells in postbiotic formulations by assessing their structural integrity, even when they are non-culturable.

Droplet digital PCR (ddPCR) complements flow cytometry by providing absolute, strain-specific quantification within complex formulations of multiple strains. ddPCR offers actionable insights into overfill requirements and batch consistency.

Together, these platforms deliver a robust analytical framework for optimizing probiotic product development, quality control, and shelf-life assurance.

Flow Cytometry-based Enumeration



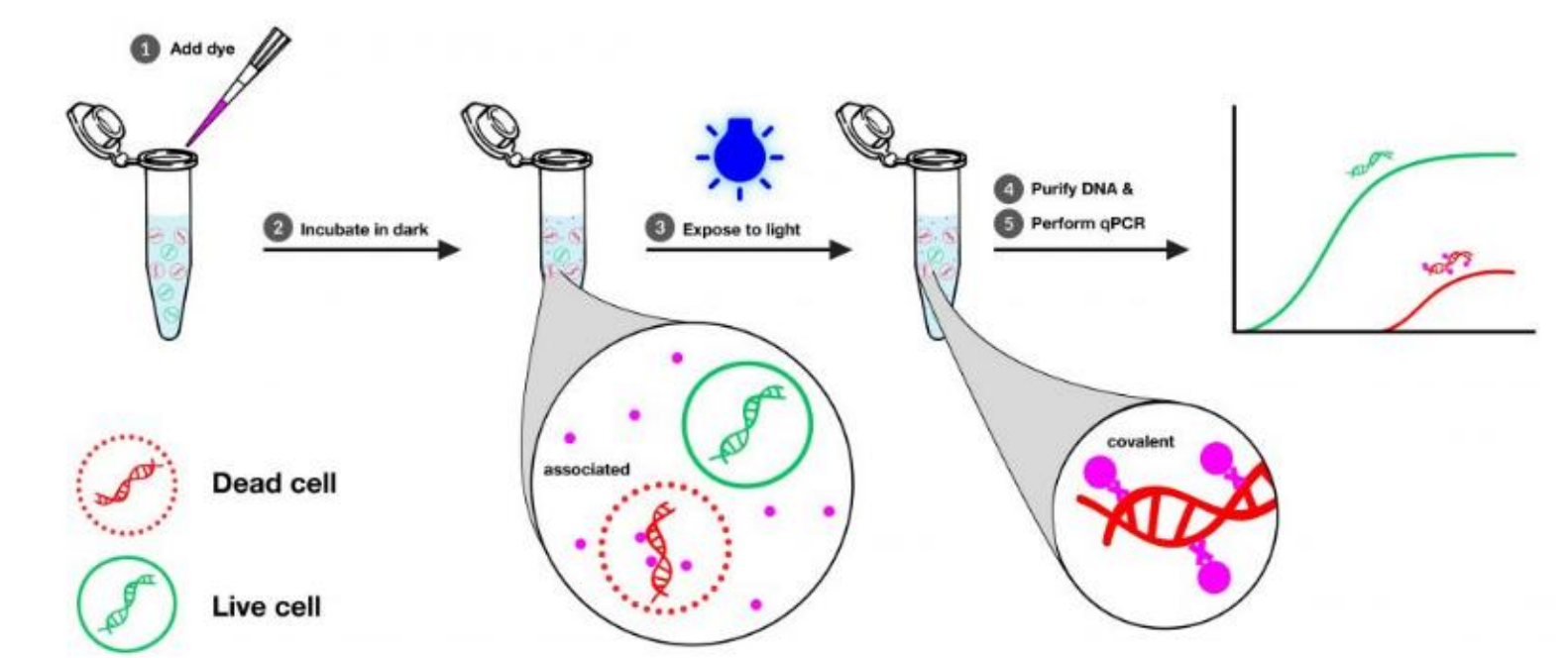
Cell Enumeration:

Flow cytometry enables accurate quantification of live, injured, and dead cells, supporting critical evaluations across raw materials, manufacturing processes, and finished products.

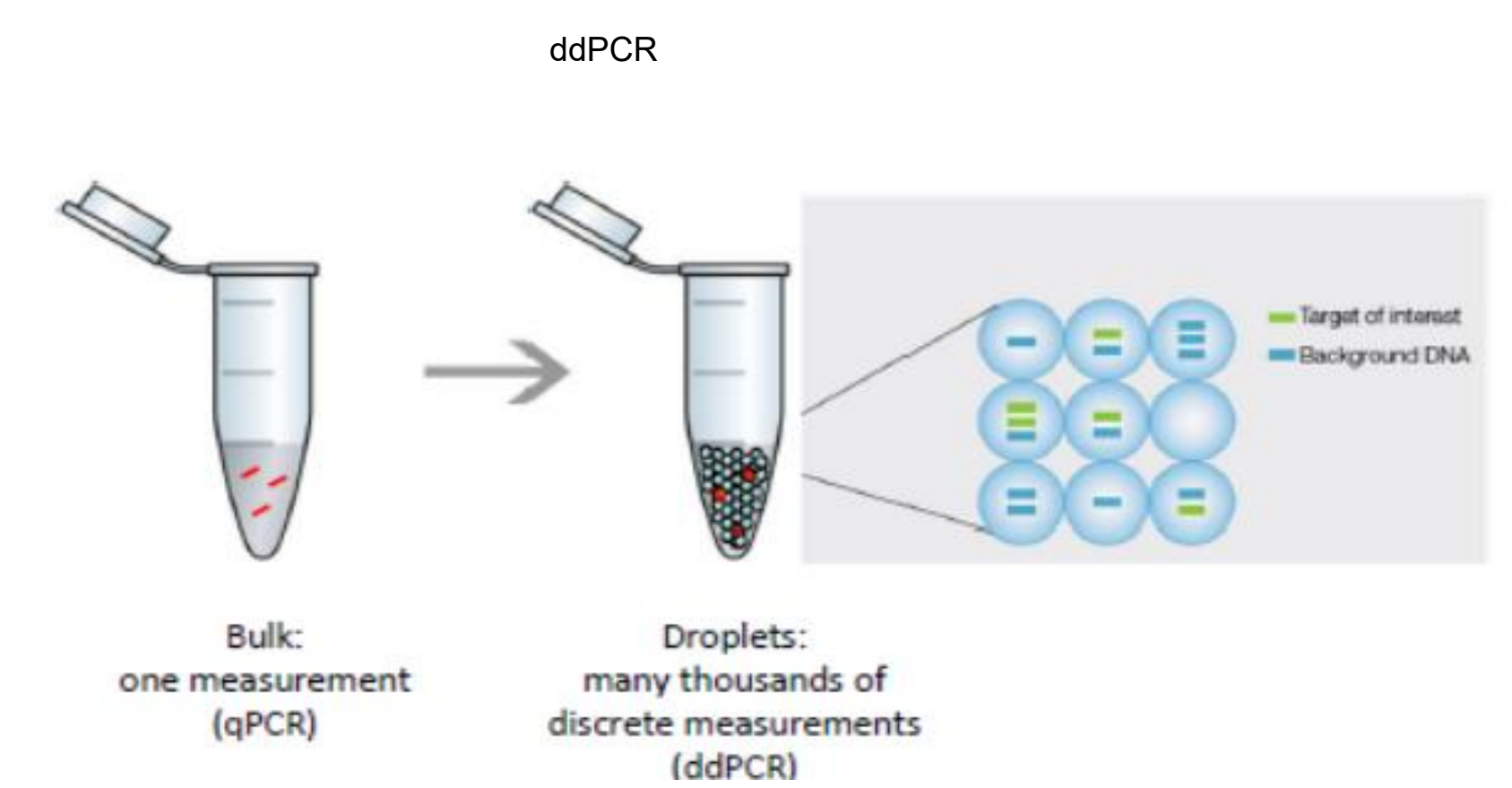
Postbiotic Enumeration Challenge:

Postbiotics, composed of heat-inactivated microbial cells, lack the ability to replicate—making them undetectable by traditional culture-based methods. Flow cytometry overcomes this limitation by assessing structural and functional integrity, allowing precise quantification of non-culturable cells.

Advantages of ddPCR over qPCR



Unlike qPCR, which infers quantity from a standard curve, ddPCR physically partitions the sample into thousands of droplets, enabling absolute and highly accurate counting of strain-specific DNA targets.

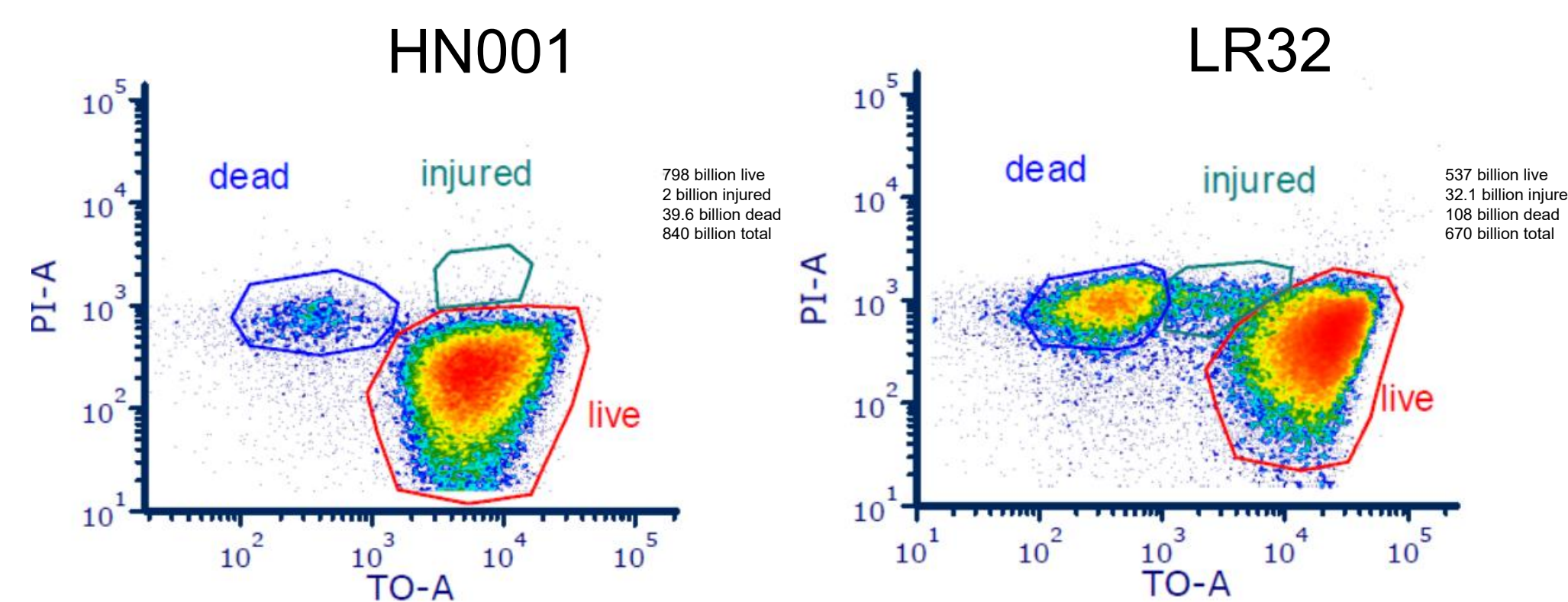


Assay Overview

Flow Cytometry Microbial viability within probiotic products can be assessed using a dual-staining approach with cell-permeant and cell-impermeant DNA-binding dyes. The cell-permeant dye penetrates all cells, while the impermeant dye selectively enters only those with compromised membranes—typically injured or dead cells. Membrane integrity may be affected by various stressors during production, packaging, and storage. This staining strategy enables quantification of multiple microbial subpopulations, providing a detailed snapshot of overall product health.

Droplet Digital PCR (ddPCR) is a highly specific DNA-based molecular technique that can be used to differentiate individual microbial strains. Partitioning microbe DNA into thousands of nanoliter-sized droplets allows for precise separation and absolute quantification of strain-specific DNA. Comparing total and viable cell counts offers valuable insight into the physiological status of each strain within complex probiotic formulations.

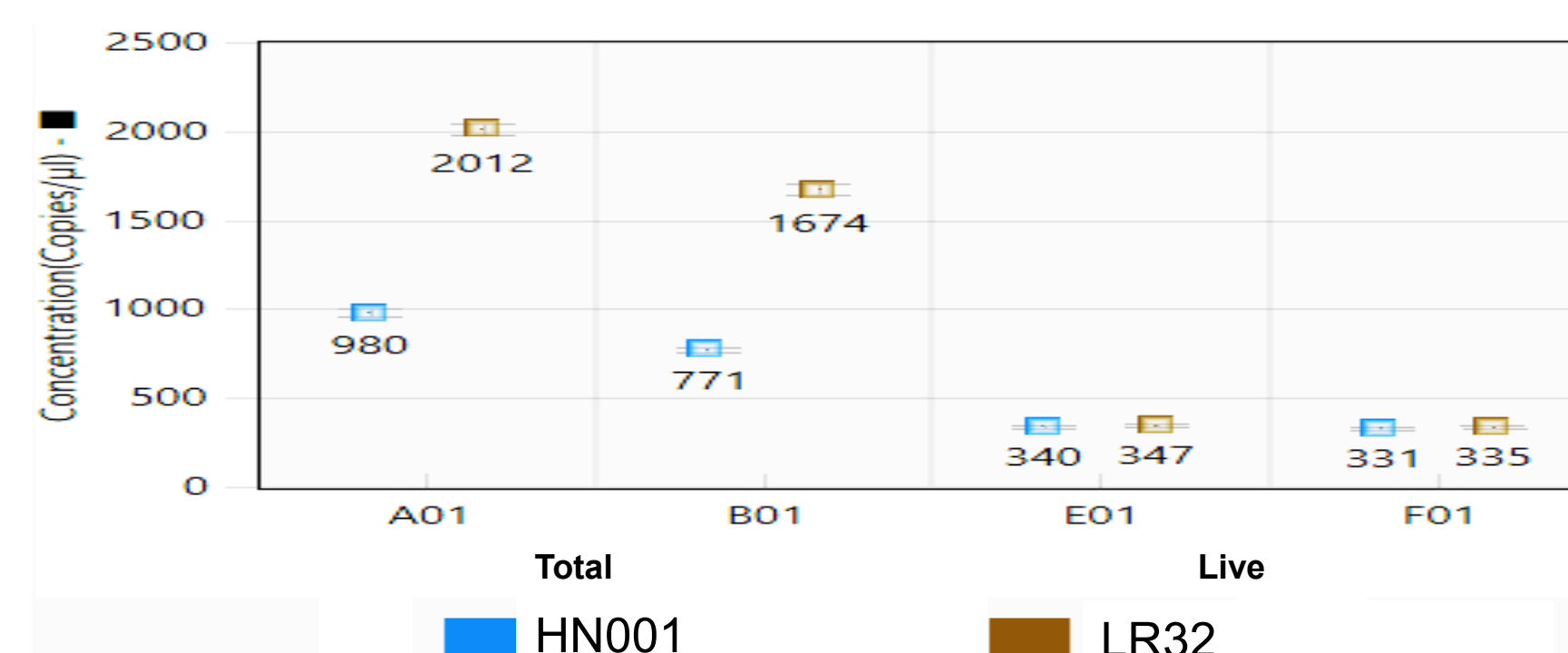
Strain Specific ddPCR-based Enumeration in a Mixed Product



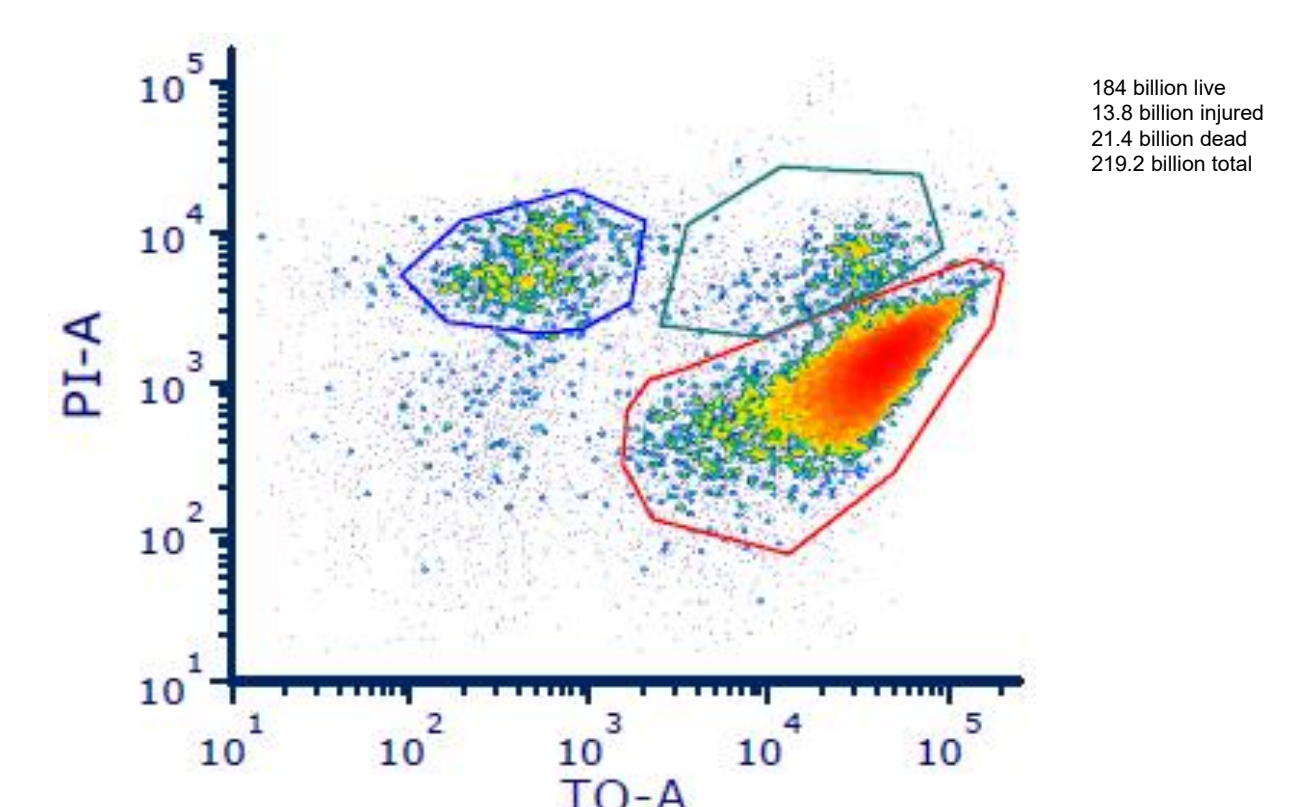
Flow cytometry analysis of two *Lactobacillus rhamnosus* strains, HN001 and LR32, showed distinct proportions of live, injured, and dead cells for each strain.

A 1:1 mixture of live cells from both strains was prepared and quantified using droplet digital PCR (ddPCR). The ddPCR results confirmed equal live counts from HN001 and LR32 in the mixed sample.

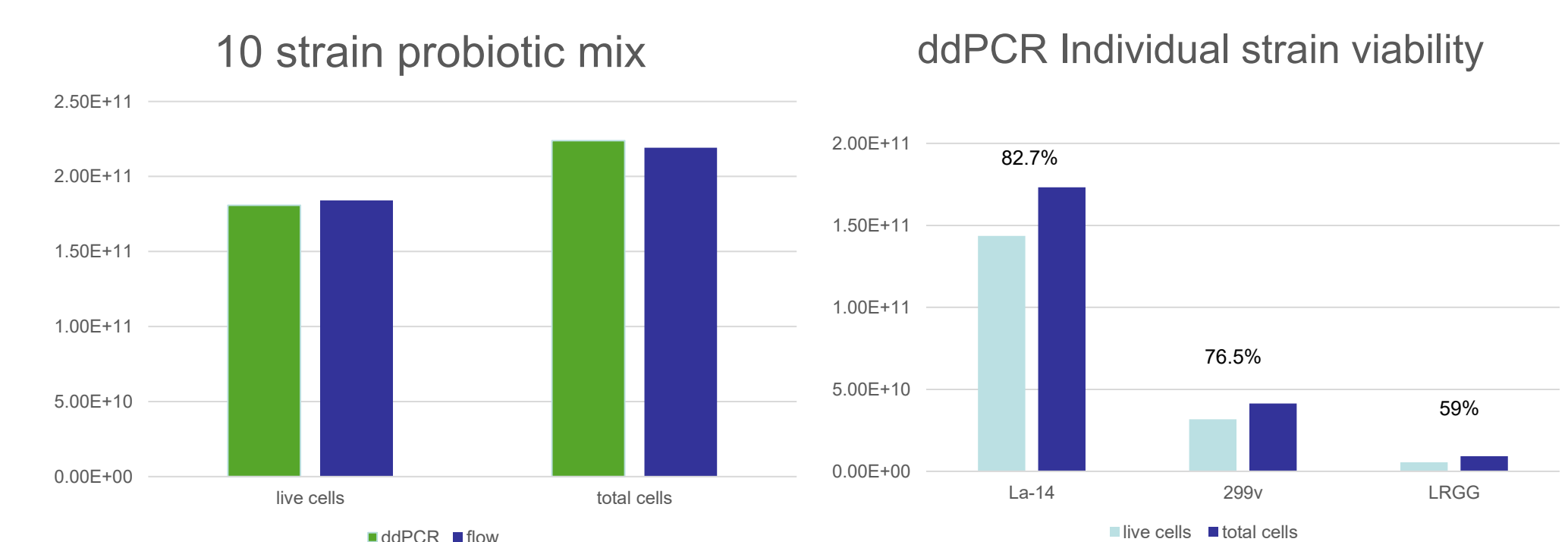
Strain-specific quantification



10 strain probiotic mix



In a 10-strain mixture with a ratio of 10:6:1 of 3 clinical strains, ddPCR quantifies and assesses their viability. Total cell counts obtained via ddPCR align closely with flow cytometry results.



CONCLUSIONS

Using Flow Cytometry and Droplet Digital PCR (ddPCR)

In probiotic manufacturing, precision matters. Traditional culture-based methods fall short when assessing the full spectrum of microbial viability—especially for postbiotics, which are heat-inactivated and non-culturable.

Flow Cytometry offers a powerful solution by directly quantifying live, injured, and dead cells based on their structural and functional integrity. This enables accurate evaluation across raw materials, production stages, and finished goods—even when cells can no longer replicate.

ddPCR complements this by delivering absolute quantification of microbial DNA, without reliance on standard curves. It confirms strain identity, detects low-abundance targets, and monitors genomic stability throughout the manufacturing process. ddPCR proved to be a highly specific and accurate method for analyzing a complex 10-strain mixture. It successfully isolated specific data for 3 clinical targets while matching the overall accuracy of flow cytometry.

Together, these technologies provide a comprehensive and reliable approach to viability testing- ensuring product consistency, regulatory compliance, and scientific rigor.