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### Understanding probiotic cell health using flow cytometry

Probiotics have historically been enumerated using the colony forming unit (CFU) assay which cultures samples on growth media for up to 3 days. This methodology, however, is less accurate when evaluating complex probiotic mixes. Typically, multiple sample dilutions need to be plated on various growth media formulations to facilitate detection of the various strains. Furthermore, evaluating finished goods may present additional challenges as stress of processing, packaging, and environmental conditions affect the ability of probiotics to grow and many cells become viable but not culturable (VBNC) in the plating growth media. Recently, postbiotics have also been utilized in product manufacturing. Postbiotics are heat treated bacteria that maintain their efficacy after death. They can be used as ingredients in many different formulations where a live cell cannot. Enumerating postbiotics presents a new challenge given that they cannot grow in culture. Flow cytometry is a well validated, high throughput, single cell analysis tool that circumvents the issues associated with probiotics, as it does not require culturing to enumerate the postbiotics. This method simply monitors the presence of cells with an intact membrane to enumerate viable cells in a matter of minutes. We previously demonstrated the use of a two DNA-binding dye assay to evaluate the health of probiotics. One dye is cell permeant (gets into all the cells) while the other dye is not cell permeant and only associates with the DNA of injured or dead cells where the membrane is compromised. Using this dye system, the health of the cell can be evaluated with the enumeration of live, injured, and dead cells in a sample. Flow cytometry is a culture independent method that allows enumeration of multiple species and strains of different microbes. This technique is fully validated in an ISO method which can be utilized for all stages of product development through end of shelf life.

#### Biography:

Dana Buckman has over 25 years of experience using flow cytometry for research and industrial applications. For the past 5 years, she developed flow cytometry based assays for the analysis of probiotics as CFU- assays. She founded BioForm Solutions, a San Diego-based contract research organization, to provide such services for the analysis of probiotics in the supplement, food and beverage and agriculture markets. BioForm Solutions is the leader in the flow cytometry-based analysis of probiotics, serving many of the industry's key manufacturers.

dana@bioformsolutions.com

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