A Design of Experiments (DoE) approach to accelerate the optimization of viability droplet digital PCR conditions for probiotics enumeration in blends

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Manufacturers combine numerous probiotic species or strains with defined dosage levels as blends for their synergistic health effects. To ensure the intended efficacy, the dosage levels of each species or strain should be precisely measured and monitored for quality control purposes. However, conventional enumeration techniques, such as plate count and flow cytometry, showed limited specificity in species or strain differentiation. Recently, viability droplet digital PCR (v-ddPCR) has been demonstrated as an improved method for simultaneous strain identification and viable cell enumeration. According to a previous study done on single probiotic strains, the viability dye concentration is one of the critical parameters in v-ddPCR. In this study, we show cell density is another critical parameter that affects enumeration results. Therefore, simply transferring the viability dye concentration optimized for specific strain at a predefined cell density to the quantification of a probiotic blend may result in inaccurate enumeration and presents a challenge, due to the impact of cell density of an individual strain. To achieve accurate enumeration result when testing blends, a Design of Experiments (DoE) approach was used to establish a mathematical model between enumeration accuracy and the above critical parameters. In detail, using specific probiotic strains, central composite design (CCD) studies were conducted to accelerate the process of finding the optimal parameters that not only provide accurate enumeration values but also minimize the influence of cell density variation. In the end, the optimal analytical conditions were calculated and validated in reconstituted probiotic blends.

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