The Evaluation of Droplet Digital Polymerase Chain Reaction (ddPCR) Methods for The Quantification of Genetically Modified Content in Food

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The labelling of genetically modified (GM) content in food is required for exporting to EU and other countries or is voluntary to provide an informed choice to consumers. The widely used quantitative polymerase chain reaction (qPCR) method has limitations since it relies on standard curves. In the current research, a droplet digital polymerase chain reaction (ddPCR) method is developed for the detection of GM content in food. ddPCR measures absolute copy numbers of deoxyribonucleic acids (DNA) encapsulated in oil-water emulsions.

The goal is to develop and validate a ddPCR methodology to specifically detect genetic markers contained in most widely produced GM crops, including the 35S promoter of the Cauliflower mosaic virus, the Nopaline synthase terminator (TNOS) of *Agrobacterium tumefaciens*, the 34S promoter of the Figwort mosaic virus (FMV), and the Roundup Ready soybean (RRS) gene cassette. The ddPCR assays included an internal control, recombinant *E. coli* which contains an artificial plasmid DNA, to monitor DNA extraction, potential PCR inhibition or amplification failure to improve quantification accuracy and to safeguard against false negative results.

Preliminary results showed that the quantification range of the assays was between 0.1-10% based on certified GM soybean. The lower quantification limit meets the requirement of detection limit below 0.9%, the threshold for mandatory labelling requirement in the EU. The methodology is being validated for specificity, quantification range, matrix effect, repeatability, reproducibility and robustness. The ddPCR method, once fully validated, is expected to offer a rapid and more accurate alternative for GMO testing.