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# Development of a novel cross-streaking method for isolation, confirmation, and enumeration of Salmonella from irrigation ponds

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## Abstract

The 2013 Produce Safety Rules in Food Safety Modernization Act (FSMA) require regular testing for generic *Escherichia coli* in agricultural water intended for pre-harvest contact with the edible portion of fresh produce. However, the use of fecal contamination indicators frequently does not correctly reflect distribution of foodborne pathogens such as *Salmonella enterica*, and ensuring food safety may require direct detection and enumeration of pathogens in agricultural settings. Herein we report the evaluation of different cost-effective methods for quantification, isolation, and confirmation of *Salmonella* in irrigation pond water and sediment samples. A most probably number (MPN) dual enrichment culture method was used in combination with differential and selective agars, XLT4 and CHROMagar™ *Salmonella* plus (CSP). The necessity for PCR confirmation was evaluated, and methods were compared by cost and performance measures (i.e., sensitivity, specificity, positive predictive value, and negative predictive value). Statistical analyses showed that using XLT4 as the initial selective agar to isolate *Salmonella* colonies improved recovery compared to CSP agar; however, PCR confirmation was required to avoid false positive results on either agar. Therefore, a novel cross-streaking method utilizing CHROMagar™ agar for individual colony confirmation of *Salmonella* presence/absence on XLT4 was developed. This method classifies the colony as positive if typical *Salmonella* appearance is observed on both agars. Statistical analysis showed that this method was as effective as PCR for species confirmation of pure individual strains isolated from enrichment cultures (sensitivity=0.99, specificity=1.00, relative to PCR). This method offers a cost-effective alternative to PCR that would increase the capacity and sensitivity of *Salmonella* evaluation.

**Keywords:** CHROMagar™ *Salmonella* Plus; Cross-streaking; Irrigation ponds; PCR; *Salmonella*; XLT4.

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