Validation of the Neogen® Molecular Detection Assay 2 – Salmonella Enteritidis/Salmonella Typhimurium Method for Specific Detection of Salmonella enterica ser. Enteritidis and Salmonella enterica ser. Typhimurium in poultry samples.

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The Neogen® Molecular Detection Assay 2 – *Salmonella* Enteritidis/*Salmonella* Typhimurium (MDA2-SEST) method is a rapid, nucleic acid amplification-based test for specific detection of *Salmonella enterica* ser. Enteritidis (SE) and *Salmonella enterica* ser. Typhimurium (ST), including the ST monophasic variant of *Salmonella enterica* I 4,[5],12:i:- in select poultry samples. Results for SE and ST are generated separately. The purpose of this study is to validate the rapid molecular method for detection of SE and ST in chicken carcass rinse (25 g & 325 g), raw ground chicken (325 g), and cooked breaded chicken (25 g) for AOAC *Performance Tested Methods*^{SM} certification. The study consisted of inclusivity/exclusivity testing and independent laboratory testing of chicken carcass rinse, raw ground chicken, and cooked breaded chicken using inoculated matrices. Chicken carcass rinse and fresh ground chicken were compared to the USDA/FSIS MLG 4.14 reference method while cooked breaded chicken and raw ground chicken were compared to the ISO 6579-1:2017 reference method. Our results showed, in inclusivity testing, that all 50 SE strains produced positive results in the SE assay and negative results in the ST assay. Fifty-three ST strains (including the monophasic variant) produced positive results in the ST assay and negative results in the SE assay. Thirty-five exclusivity strains included multiple non-SE group D_1 *Salmonella* serovars, multiple non-ST group B serovars, *Salmonella* spp. from other somatic groups, and other *Enterobacteriaceae* produced negative results in both assays. In matrix testing, results for the candidate and reference methods were in complete agreement for all matrices evaluated. The development of the MDA2-SEST method addresses the increasing interest in specific detection of two *Salmonella* serovars prevalent in poultry environments as well as in human illness. Aditionally, the use of this test reduces the need for laborious conventional serotyping.

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