Novel Loop Mediated DNA Amplification (LAMP) Based Bioluminescent Assays for the Detection of Salmonella Enteritidis and Salmonella Typhimurium, Including the Monophasic Variants Salmonella enterica I 4,[5],12:i:- & I 4,[5],12:-:1,2

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The traditional phenotypic method to determine *Salmonella* serotype is laborious. Quick and easy screens for serotypes of particular interest are highly desirable for the poultry industry. *S* Enteritidis and *S*. Typhimurium are two of the most common serovars isolated from salmonellosis outbreaks resulting from poultry products consumption. Here we describe novel rapid molecular-based detection assays to specifically identify these serotypes. The objective of this study was to create and demonstrate the performance of two new Molecular Detection System assays (MDA2-SEST) for detection of *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST), including the ST monophasic variants I 4,[5],12:i:- and I 4,[5],12:-:1,2. Assay specificity was evaluated using cultures of 44 (SE) or 59 (ST) inclusive isolates, and 217 (SE) or 202 (ST) exclusive serovars. All isolates were whole genome sequenced to confirm serotype. Extensive in-silico and reagent pellet testing was conducted during LAMP assay design to confirm serotype specificity. The assay parameters were tuned and evaluated by amplifying a dilution series of target DNA at defined concentrations. Assay sensitivity was tested with post enrichment (nBPW chicken carcass rinse and raw ground chicken) spikes at 5 levels. Our results showed that the two new LAMP assays are able to positively detect SE and ST (including I 4,[5],12:i:- and I 4,[5],12:-:1,2). For SE, 44/44 inclusives were positively identified and none of the 217 exclusive cultures were detected. For ST, 59/59 inclusives were positively identified and none of the 202 exclusive cultures were detected. The assay kinetics are comparable to other commercial LAMP-BART assays. From ground chicken and carcass rinse enrichments, the sensitivity of both assays was comparable to the Neogen Molecular Detection Assay 2 - *Salmonella*. The significance of our findings lies in the high specificity, accuracy, and sensitivity of these new assays. They can be reliably used for the rapid screening of SE and ST in selected poultry products.

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