Evaluation of Molecular Serotyping of Salmonella Using the Luminex® Multiplexing Technology
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Introduction
Salmonella infections are among the leading cause of enteric illness in the United States, with an estimated 1.4 million cases of salmonellosis occurring annually. Currently, there are over 2,400 recognized serovars of Salmonella. Rapid identification of Salmonella serotype would provide food producers valuable information that could be used to identify sources of Salmonella contamination.

Purpose
The purpose of this study was to evaluate the accuracy of a molecular serotyping method using known serovars of Salmonella.

Methods
DNA was extracted from 49 Salmonella isolates with known serotypes. Multiplex PCR was performed using Luminex O, H, and AT primer mixes according to the manufacturer’s instructions. The resulting PCR product was then hybridized with the microspheres specific to select gene targets and analyzed on the Luminex® xMAP™ instrument.

Results
Of the 49 isolates tested, the Luminex xMAP Salmonella Serotyping Assay was expected to completely serotype 41 isolates and provide partial serotype information for the remaining 8. The Luminex xMAP Salmonella Serotyping Assay correctly identified 41/49 isolates. Of the remaining 8 isolates, the assay narrowed the possible serotypes significantly. This would potentially reduce the time and expense associated with conventional agglutination serotyping to identify them.

Significance
These data suggest that molecular serotyping of Salmonella using the Luminex® multiplexing technology can provide an accurate and rapid alternative to traditional agglutination serotyping.

Materials and Methods

Isolate Characterization
Salmonella isolates were obtained from clinical and food sources. Additional isolates were completely serotyped and identified by the Wisconsin State Laboratory of Hygiene. Pure cultures were grown on non-selective media in preparation for DNA extraction.

DNA extraction
DNA extraction was performed from a pure Salmonella isolate using the Bio-Rad iScript™ kit (Cat. No. 72-0460). An isolated colony was picked and the cells were suspended in 200 µl of the lysis buffer. This suspension was incubated at 56°C for 10 minutes followed by a 10 minute incubation at 100°C. The tubes were centrifuged at 10,000 rpm for 2 minutes and the supernatant collected. The extracted DNA in the supernatant was quantified using a NanoDrop (Cat. No. ND1000) and diluted to 100 ng/µl in nucleic free water.

PCR
For each sample, 3 separate PCR reactions were performed – O group, H group, and an Additional Targets assay (AT). To create a master mix, 12.5µl of Qagen HotStar™ (Cat. No. 203443), 2.5µl of the O group primer mix and 8µl of molecular grade water were combined. For the H group or AT assay master mix, the O group primer mix was substituted with the H group or AT assay primer mix. 2µl of the diluted DNA (200 ng) was added to the master mix and the reaction was cycled in a thermocycler (ABI Veriti – 9982) with the following conditions –

1) 95°C for 15 minutes
2) 30 cycles of:
   a) 94°C for 30 seconds
   b) 44°C for 90 seconds
   c) 72°C for 90 seconds
3) 72°C for 10 minutes.

Hybridization and Analysis on the Luminex 200
The 3.75X O, H and AT bead mix was diluted to 1X in the hybridization buffer. 45µl of 1X bead mix was added to the appropriate wells of a 96 well PCR plate (BioRad-MIL606) followed by the addition of 5µl of corresponding PCR product. This was then cycled in a thermocycler under the following conditions –

1) 95°C for 5 minutes
2) 52°C for 30 minutes

Finally, the plate was transferred to a Luminex 200 with the brass block preheated at 52°C. 300µl of 6µg/ml streptavidin-R phycoerythrin prepared fresh in the rotator dish was added. The plate incubated for 10 minutes at 52°C in the Luminex analyzer protected from light, and the results analyzed.

Data Interpretation
Luminex raw data, Median Fluorescence Intensity (MFI) was scored for greater than or equal to 1000. Signal for mAb was also calculated. Serotype was determined from the scored data using the Kullman–White Scheme.

Evaluation of Molecular Serotyping of Salmonella Using the Luminex® Multiplexing Technology

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<thead>
<tr>
<th>Serotype</th>
<th>PCR Target</th>
<th>AT Target</th>
<th>O Target</th>
<th>H Target</th>
<th>Control</th>
<th>Signal</th>
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Conclusions

The Luminex® multiplexing molecular serotyping platform is a useful tool for the rapid identification of Salmonella isolates. The method completely identified 41/49 isolates correctly. Two isolates were identified as one of two distinct serotypes. Six isolates could not be completely identified because their specific O or H antigen groups are not targeted by the assay. Even though some isolates could not be completely identified, the system did narrow the possibilities greatly, creating time and cost savings over traditional serotyping.

References