The CDC estimates that annually 70 million illnesses and 5,000 deaths are related to food-borne pathogens. Salmonella is estimated to cause 1.4 million illnesses per year with 33,000 hospitalizations and 230 deaths. The economic impact of Salmonella infections has been put at $4.3 billion on the cost of medical care and premature loss of life. 8 Fatal cases are estimated to cost from $95,000 to nearly $6 million. 9 The need for a rapid, reliable method for identifying Salmonella in food products is paramount to maintain safety in our food supply and rapidly identify sources of contamination. Currently, the identification of Salmonella in food safety testing involves traditional biochemical tests followed by serotyping of the somatic and flagellar antigens. 1 Culture time required for biochemical analysis may be lengthy and anti-sera for complete antigen typing are costly. Repeated sequence-based PCR (rep-PCR), has shown to be a reliable method for the identification of Salmonella. 10 Additionally, rep-PCR is an excellent method for strain differentiation within Salmonella serotypes. The ability to identify Salmonella serotypes and obtain strain analysis in a single test could greatly reduce the time and resources required to identify potential food contaminants and circumvent the spread of these contaminants. 1 We propose a rapid, easy to use alternative to biochemical analysis and antigen testing using rep-PCR in combination with an automated detection method utilizing microfluidics. This study compares the results obtained using the automated DiversiLab System 11 to those by serotyping for the identification of Salmonella.

METHOD

One hundred and ten well-characterized Salmonella isolates representing the most commonly isolated serovars in raw meat and poultry processing plants were analyzed. Figure 1 summarizes the distribution and common sources of the serovars tested. The isolates were cultured on tryptic soy agar containing 5% sheep blood. The cultures were incubated at 37°C for 24 hours. DNA from each culture was extracted using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., CA). The DiversiLab Salmonella Kit was used for rep-PCR amplification of intergenic repetitive elements in the genomic DNA. The amplions were separated using the Caliper® 1000 Analyser (Fig. 2, Caliper Technologies, Inc., CA). The Caliper 1000 utilizes a DNA microfluidics chip for DNA fragment separation and detection. Data archiving and sample analysis were performed using the DiversiLab System (Fig. 2 Bacterial Barcodes, Inc.), that included Pearson’s correlation coefficient for similarity calculation and VUPMA for creating the dendrogram.

RESULTS

The samples tested represent some of the most commonly isolated serotypes contaminating raw meats including turkey, broiler chickens, ground beef and market hogs (Fig. 3). All samples were successfully fingerprinted using the DiversiLab Salmonella Kit. Cluster analysis using the DiversiLab System software identified all serotypes tested by well-defined clusters showing 100% concordance with serotyping. Strain discrimination was also demonstrated within a serotype; S. infantis as seen in Figure 3. Nine S. infantis isolates were separated into two groups. Bands that are common to both groups most likely demonstrate relatedness within the serotype. Distinct band differences denoted on the gel image and the sample overlay graph demonstrate strain discrimination. Figure 4 shows a dendrogram and gel-like images of the consensus fingerprints for each group found among the entire sample set. Serotypes are clearly distinguished, and some serotypes contain multiple strains. The DiversiLab System analysis and report were completed in less than 4 hours (per sample set of 12).

SUMMARY

- Results obtained using the DiversiLab Salmonella Kit were concordant with serotyping.
- The time required to obtain results was greatly reduced (2.5 working days to process all 110 samples) with the automated system when compared to gel-based fingerprinting and traditional serotyping methods.
- Automated fragment analysis and reporting have been shown to be equivalent or superior to gel-based analysis. 12
- Data analysis was automated, requiring limited user intervention, which minimized the risk of technical errors. The DiversiLab System is an accurate, reproducible method for identification of Salmonella serovars.
- For groups of organisms with nomenclature that is unstable, such as Salmonella, the DiversiLab System provides a database platform which may be easily updated with changes in nomenclature.
- As demonstrated in Figure 3 rep-PCR further discriminates potential strains within serovars. These and other data show that rep-PCR is an excellent method for tracking the source of contamination in food-borne outbreaks. 4

REFERENCES