

# AOAC PTM Extension Study to Validate the Thermo Scientific SureCount Multiplex Salmonella PCR Kit method for a Quantitation Principle

Nikki Faulds<sup>1</sup>, Jessica Williams<sup>1</sup>, Daniele Sohier<sup>1</sup>, Annette Hughes<sup>1</sup>, Dean Leak<sup>1</sup>, Rachael Trott<sup>1</sup>, David Crabtree<sup>1</sup>, Nicole Prentice<sup>1</sup>, Wesley Thompson<sup>2</sup>, Andrew Deterding<sup>2</sup>, Benjamin Bastin<sup>2</sup> and M. Joseph Benzinger Jr.<sup>2</sup> (1) Thermo Fisher Scientific, Wade Road, Basingstoke, United Kingdom (2) Q Laboratories, 1930 Radcliff Drive, Ohio, United States

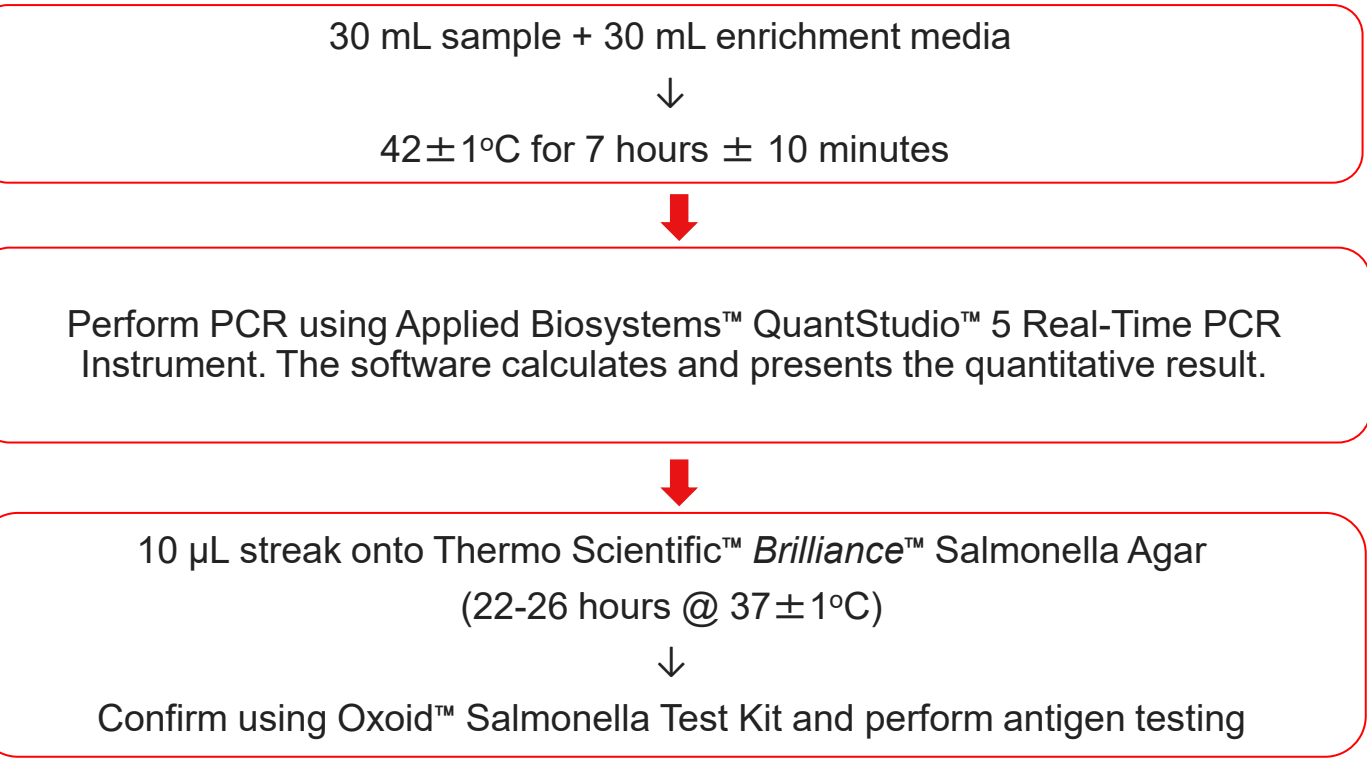
## INTRODUCTION

*Salmonella* species cause an estimated 1.35 million infections annually in the United States according to Centers for Disease Control and Prevention data<sup>1</sup>. Very low levels of *Salmonella* are typically found in food and environmental samples. USDA/FSIS guidelines are continually evolving; cost-effective and easy-to-handle methods capable of enumerating low levels of *Salmonella* are needed to identify critical contamination points together with inactivation process efficiency.<sup>2</sup>

This study evaluated a modification to the detection principle of the Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit for quantitation of *Salmonella* from selected matrices according to the AOAC® Performance Tested Methods<sup>SM</sup> program.

## WORKFLOW

Figure 1: SureCount Salmonella workflow



## METHOD

The validation study was conducted according to AOAC Appendix J<sup>3</sup> requirements.

Matrix Study

- 325 g raw ground meat (turkey, beef, pork) and 30 mL chicken carcass rinse
- Artificial contamination including co-infection strategy of serovars Typhimurium and Enteritidis.
- Ground turkey was also tested by Q Laboratories.
- USDA/FSIS-MLG 4.11<sup>4</sup> reference method.

Inclusivity/Exclusivity

- Total of 200 inclusivity isolates, at least 50 per S. Typhimurium and S. Enteritidis.
- Total of 16 exclusivity isolates
- Tested in both BPW and mTSB

## RESULTS

The matrix study data was analyzed using the Least Cost Formulations AOAC calculator, Grubbs' tests for outliers and linearity plots to assess the relationship of the data using the R<sup>2</sup> value. The difference in means between the candidate and reference method, including 90% confidence intervals, were used to assess the data. For equivalent performance, the confidence intervals must sit between -0.5, 0.5 and the data sets must have a difference in means of less than 0.5.

All matrices demonstrated equivalent or comparable performance (figure 2 and table 1). The candidate method was more consistent method with significantly lower standard deviation compared to the reference method. In addition, all inclusivity and exclusivity isolates were successfully detected and excluded, respectively.

Figure 2: Log<sub>10</sub> mean comparison of candidate method versus reference method. Error bars show standard deviation of each method. Coinfection studies display total S. spp result and as well as specific serovar data.

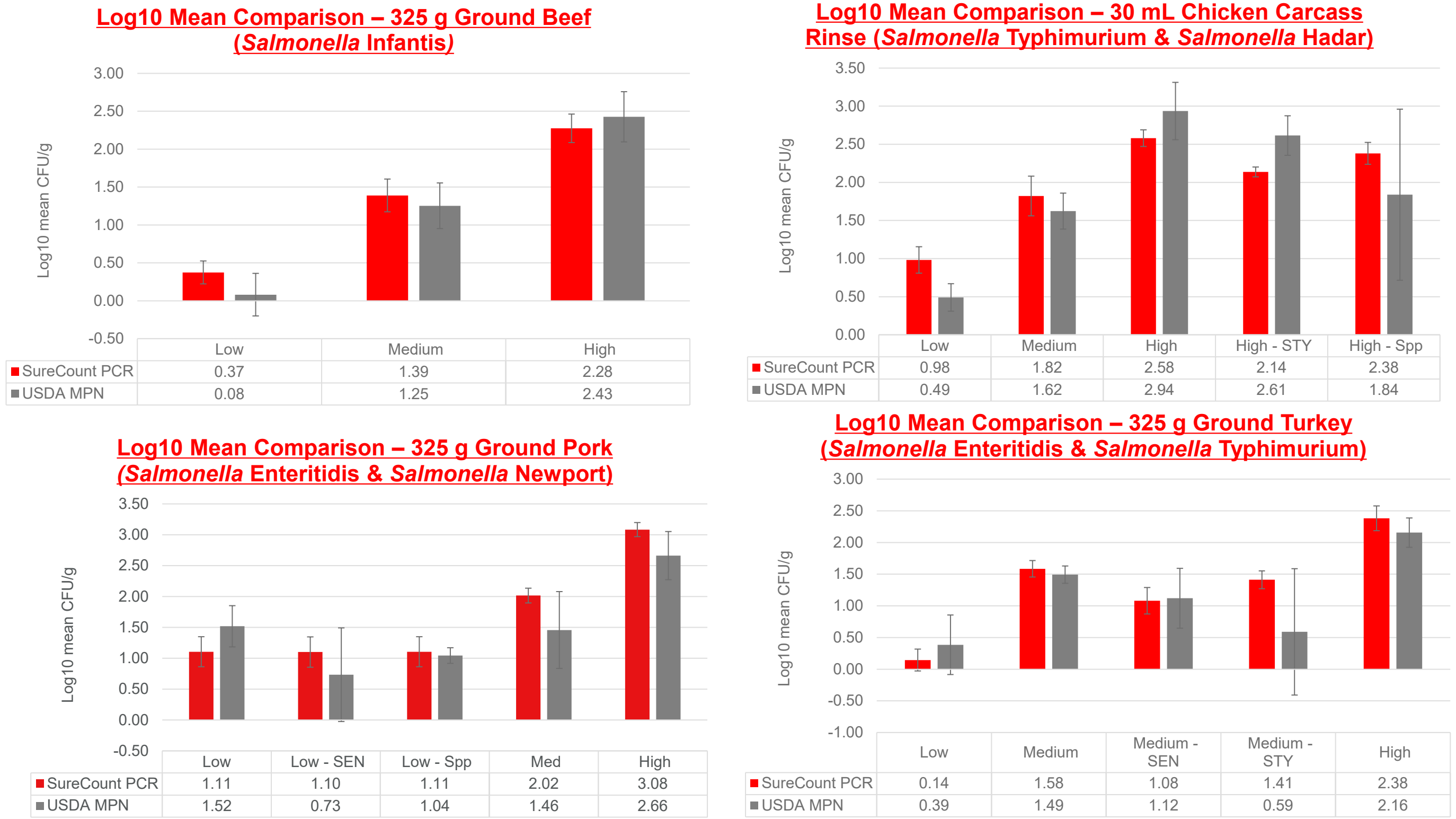


Table 1: Summary of 90% confidence intervals. **Green** = equivalent to reference, **Yellow** = Comparable, **Purple** = Comparable, with candidate method estimating higher and closer to actual spike level

Spike level	Ground Beef	Chicken Carcass Rinse	Ground Pork	Ground Turkey
Low	-0.039, 0.626	0.281, 0.704	-0.763, -0.062	-0.692, 0.210
Medium	-0.177, -0.451	-0.100, 0.494	-0.045, 1.163	-0.067, 0.252
High	-0.438, -0.180	-0.729, 0.017	0.034, 0.809	-0.032, 0.481

## CONCLUSIONS

### ACCURATE AND RELIABLE

- 100% specific and selective
- Equivalent or comparable performance to USDA FSIS-MLG 4.11
- Consistent method with significantly lower standard deviation compared to USDA FSIS-MLG 4.11
- AOAC certification pending

### QUICK AND SIMPLE

- Simple enrichment.
- Results within 8 hours.
- Consistent quantitative result provided for *Salmonella* species, S. Typhimurium and S. Enteritidis
- Simple quantitation workflow for easy implementation for end users.

## REFERENCES

1. Centers for Disease Control and Prevention, (2023) (Accessed June 2023) <https://www.cdc.gov/salmonella/index.html>  
2. Siceloff, A. T., Waltman, D. and Shariat, N. W., (2022) Regional Salmonella Differences in United States Broiler Production from 2016 to 2020 and the Contribution of Multiserovar Populations to Salmonella Surveillance, vol 88(8).  
3. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19<sup>th</sup> Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Rockville, MD, [http://www.eoma.aoac.org/app\\_j.pdf](http://www.eoma.aoac.org/app_j.pdf) (Accessed July 2022)  
4. USDA FSIS MLG 4.11 (2021) *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges* (Accessed June 2023) [https://www.fsis.usda.gov/sites/default/files/media\\_file/2021-08/MLG-4.11.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/2021-08/MLG-4.11.pdf)

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