

# Validation Study of a Real-Time PCR based Method for the Detection of *Salmonella* in a Broad Range of Food Matrices and Environmental Surfaces.

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## INTRODUCTION

*Salmonella* is one of the most important microbiological criteria inspected routinely by food business operators and national authorities. Since reference methods usually rely on exhaustive and cumbersome procedures, lab operators use alternate methods offering shorter time to results and simpler enrichment and assay protocols. Nonetheless, such alternate analytical methods require official validation studies in order to demonstrate performance equivalency between alternate/candidate and reference methods.

**Purpose:** The objective of this study was to evaluate the performances of the candidate method, Pall GeneDisc® Plate *Salmonella* spp, for 17 food matrices (test portions of 25 g and/or 375 g) and 2 environmental surfaces, in comparison to reference methods (USDA/FSIS MLG 4.05, FDA/BAM Chapter 5, and FDA Guidance Document for Industry for Spent Irrigation Water for Sprouts) following the current AOAC-PTM guidelines.

## METHODS

**Each matrix was inoculated** with a different strain of *Salmonella*. Spiking was done at low level (0.2-2 CFU/test portion, 20 replicates), at high level (10x low, 20 replicates) and 5 replicates for non-inoculated samples. Natural contaminations were used for chicken carcass rinses. Total aerobic count was evaluated for all food matrices.

**Confirmation for the Real-Time Polymerase Chain Reaction (PCR) - based candidate method:** all samples were confirmed for *Salmonella* spp. by direct streaking onto Brilliance *Salmonella* agar (BSA), by direct streaking of the primary enrichment onto the reference method agars (XLT4 and BGSA for the USDA/FSIS-MLG; XLD, HE and BS for the FDA/BAM), and a streak onto those agars after a secondary enrichment in selective enrichment media (TT & RVS).

**AOAC-PTM Statistical Analysis:** For each matrix, the Probability of Detection (POD) with 95% confidence intervals was calculated as the number of positive outcomes divided by the total number of trials for the high, low and the un-inoculated control levels. The POD was calculated for the candidate confirmed results, POD<sub>C</sub>, the reference method, POD<sub>R</sub>, and the difference in the confirmed candidate and reference methods, dPOD. If the confidence interval of a dPOD value did not contain zero, then the difference between the POD values was statistically significant at the 5% level.

## Real-Time PCR-based Candidate Method



## RESULTS & DISCUSSION

For all the food matrices, the low levels of contamination per test portion varied between 0.30 CFU & 0.89 CFU. The high levels varied between 1.32 and 4.95 CFU/test portion.

For all food and surface matrices analyzed in that study, at low level of contamination (Table 1), there was no observed significant statistical difference in the number of positive samples detected by the GeneDisc Plate *Salmonella* spp. with the reference methods.

For the high contamination levels, all food matrices except fresh cut cantaloupe detected 20 out of 20 positive by the candidate PCR screening. Fresh cut cantaloupe showed 19 out of 20 positive, the negative screening result was confirmed negative by culture.

For confirmation of the positive PCR candidate method, the direct streaking on the various agar plates showed some limitations with the following matrices: raw poultry (chicken and turkey), carcass rinses, beansprouts and black pepper. For the poultry & beansprouts, high background flora was observed on the agar plate rendering the identification of *Salmonella* colonies difficult. For black pepper, inhibitory compounds present in pepper might have limited *Salmonella* growth.

For the non confirmed results from direct streaking of the primary enrichment, the subculture in a selective enrichment medium allowed the confirmation of all positive PCR candidate method.

For raw ground beef and beef trim, both enrichment times (10h & 20h) showed equivalent positive PCR candidate results.

## CONCLUSIONS

- ❖ This study shows that the GeneDisc Plate *Salmonella* spp can reliably detect *Salmonella* species in a broad range of food matrices and environmental surfaces, after a single enrichment step.
- ❖ For confirmation of the positive PCR screening results, a subculture of the primary enrichment into a selective medium is recommended for food matrices with high background flora or with inhibitory compounds.

Table 1: POD Summary Results of GeneDisc Plate (GDP) *Salmonella* spp. – Low Level of Contamination

Matrix	Number of Positives GDP <i>Salmonella</i> spp.	POD <sub>C</sub> (95% Confidence Intervals)	Number of Positives Reference	POD <sub>R</sub> (95% Confidence Intervals)	dPOD (95% Confidence Intervals)
Raw Chicken Breast (25 g)	10/20	0.50 (0.30, 0.70)	8/20	0.40 (0.22, 0.61)	0.10 (-0.19, 0.37)
Raw Chicken Breast (375 g)	8/20	0.40 (0.22, 0.61)	8/20	0.40 (0.22, 0.61)	0.00 (-0.28, 0.28)
Raw Ground Turkey (25 g)	12/20	0.60 (0.39, 0.78)	11/20	0.55 (0.34, 0.74)	0.05 (-0.24, 0.33)
Raw Ground Turkey (375 g)	15/20	0.75 (0.53, 0.89)	11/20	0.55 (0.34, 0.74)	0.20 (-0.9, 0.45)
Chicken Carcass Rinsate Lot 1	9/20	0.45 (0.26, 0.66)	9/20	0.45 (0.26, 0.66)	0.00 (-0.28, 0.28)
Chicken Carcass Rinsate Lot 2	3/20	0.15 (0.05, 0.36)	5/20	0.25 (0.11, 0.47)	-0.10 (-0.34, 0.15)
Raw Shrimp (25 g)	7/20	0.35 (0.18, 0.57)	10/20	0.50 (0.30, 0.70)	-0.15 (-0.41, 0.15)
Bagged Mixed Lettuce (25 g)	10/20	0.50 (0.30, 0.70)	10/20	0.50 (0.30, 0.70)	0.00 (-0.28, 0.28)
Fresh Cut Cantaloupe (25 g)	8/20	0.40 (0.22, 0.61)	9/20	0.45 (0.26, 0.66)	-0.05 (-0.33, 0.24)
Whole Cantaloupe	10/20	0.50 (0.30, 0.70)	11/20	0.55 (0.34, 0.74)	-0.05 (-0.33, 0.24)
Non organic peanut butter (25 g)	7/20	0.35 (0.18, 0.57)	8/20	0.40 (0.22, 0.61)	-0.05 (-0.36, 0.21)
Beansprouts (25 g)	8/20	0.40 (0.22, 0.61)	6/20	0.30 (0.15, 0.52)	0.10 (-0.18, 0.36)
Irrigation Sprout Water (375 mL)	8/20	0.40 (0.22, 0.61)	9/20	0.45 (0.26, 0.66)	-0.05 (-0.33, 0.24)
Instant Nonfat Dry Milk (25 g)	10/20	0.50 (0.30, 0.70)	13/20	0.65 (0.43, 0.82)	-0.15 (-0.41, 0.15)
Cheddar Cheese (25 g)	10/20	0.50 (0.30, 0.70)	5/20	0.25 (0.11, 0.47)	0.25 (-0.05, 0.49)
Vanilla Ice Cream (25 g)	8/20	0.40 (0.22, 0.61)	8/20	0.40 (0.22, 0.61)	0.00 (-0.28, 0.28)
Milk Chocolate (25 g)	8/20	0.40 (0.22, 0.61)	6/20	0.30 (0.15, 0.52)	0.10 (-0.18, 0.36)
Dry Dog Food (25 g)	12/20	0.60 (0.39, 0.78)	11/20	0.55 (0.34, 0.74)	0.05 (-0.24, 0.33)
Dry Dog Food (375 g)	8/20	0.40 (0.22, 0.61)	11/20	0.55 (0.34, 0.74)	-0.15 (-0.41, 0.15)
Black Pepper (25 g)	9/20	0.45 (0.26, 0.66)	5/20	0.25 (0.11, 0.47)	0.20 (-0.09, 0.45)
Shell Eggs (25 g)	11/20	0.55 (0.34, 0.74)	9/20	0.45 (0.26, 0.66)	0.10 (-0.19, 0.37)
Raw Ground Beef (375 g) 10 h & 20 h	9/20	0.45 (0.26, 0.66)	11/20	0.55 (0.34, 0.74)	-0.10 (-0.37, 0.19)
Raw Beef Trim (375 g) 10 h & 20 h	9/20	0.45 (0.26, 0.66)	9/20	0.45 (0.26, 0.66)	0.00 (-0.28, 0.28)
Stainless Steel (Sponges)	14/20	0.70 (0.48, 0.85)	10/20	0.50 (0.30, 0.70)	0.20 (-0.10, 0.45)
Rubber (Swabs)	9/20	0.45 (0.26, 0.66)	10/20	0.50 (0.30, 0.70)	-0.05 (-0.33, 0.24)

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