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Validation of the SIMUL-qPCR *Salmonella* Assay on Cheese/Dairy Products

An unpaired study was conducted to compare the performance of the SIMUL-qPCR *Salmonella* Assay against the FDA BAM Chapter 5 reference method to detect *Salmonella* in cheese. Samples tested in this study were artificially inoculated at two different levels to evaluate the assay's performance on that matrix. The results obtained were analyzed using the probability of detection (POD). The SIMUL-qPCR method demonstrated equivalent performance to the reference method for detecting *Salmonella*

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INTRODUCTION

The purpose of this matrix study was to determine if *Salmonella* could be detected in cheese by the SIMUL-qPCR *Salmonella* Assay without affecting the assay's performance. The assay had previously been validated on other matrices at 16-20 hours of incubation. However, the objective of this study was to see if dairy products, specifically cheese, would have any adverse effects or PCR inhibition. The design of inoculated challenge study are based on FSIS Guidance for Test Kit Manufacturers, Laboratories (10/15/10). The guidelines outline a comparison of the candidate method (in this case the SIMUL-qPCR Assay) to a known reference method (the FDA BAM Chapter 5 method) using a high inoculation set, a low inoculation set, and a set not inoculated. The results should show no statistically significant difference between the candidate and reference method.

The performances (measurement of specificity (false positive rate), inclusivity, exclusivity, repeatability, reproducibility, and ruggedness) of the SIMUL-qPCR Assays have been subjected to evaluation by an independent organization, and by following guidance provided by the AOAC.

Sample Preparation and Enrichment

Salmonella enterica serovar Maregrosso (SGSC 2578) was the strain used for the inoculation of cheese. The strain was heat stressed before being added to the matrix. A bulk sample of cheese was inoculated and a high inoculation level (250 CFU/25g). A second bulk sample of cheese was inoculated at a low inoculation level (25CFU/25g). The level of inoculation was low enough to lead to fractional (5 to 15 positives out of 20 samples) results. Each bulk sample was mixed thoroughly so the inoculum was evenly distributed throughout the sample. After that time, the bulk sample that had the high concentration of inoculum was divided into five 25 gram samples per method. The bulk sample containing the lower (fractional) level of inoculum was divided into twenty 25 gram samples per method. Five 25 gram samples that did not contain any inoculum were also weighed out per method. Once inoculated and portioned out, the samples were held at 2-8°C for 48 hours to allow for stabilization of the microorganisms.

For the candidate (SIMUL-qPCR) method, 225mL of Buffered Peptone Water (BPW) was added to each sample and homogenized by hand, before being incubated at 35°C ± 1°C for 16-20 hours.

For the reference (FDA BAM Chapter 5) method, 225mL of Lactose Broth was poured into each of the 25 gram samples and homogenized. All samples were incubated at 35°C ± 1°C for 22-26 hours.

METHOD

For the SIMUL-qPCR method, after 16 hours of incubation, 5µL of each enriched sample was pipetted into 400µL of lysis buffer in labeled microcentrifuge tubes. The tubes were heated at 95°C ± 3°C for 10 minutes and then cooled at room temperature for 5 minutes. 20µL of the lysate was then transferred to individual PCR tubes, capped, and placed into the PCR machine. The PCR tubes were analyzed using the Pro32 PCR machine using parameters outlined in the "PCR Set Up Guide".

All samples (regardless of the result obtained via the SIMUL-qPCR method) were confirmed using the FDA BAM Chapter 5 method. For the reference method, all samples were run through the enrichment process and then culturally confirmed following the FDA BAM Chapter 10 method.

RESULTS AND DISCUSSION

The probability of detection (POD) and the difference in POD (dPOD) values were calculated with 95% confidence intervals to evaluate the results between the SIMUL-qPCR method and the FDA BAM method. Table 1 shows the presumptive vs. confirmed results of the SIMUL-qPCR method. Table 2 shows the comparison of the confirmed results of candidate vs. reference method.

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Table 1: SIMUL-qPCR Presumptive vs. Confirmed SIMUL-qPCR *Salmonella* Results

				SIMUL-qPCR *Salmonella* presumptive			SIMUL-qPCR *Salmonella* confirmed				
Matrix	Strain	MPN[a]/test portion	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Cheese 16 Hour	*S.* Maregrosso (SGSC 2578)	N/A[h]	5	0	0.00	0.00-0.43	0	0.00	0.00-0.43	0.00	-0.43 – 0.43
		1.27	20	14	0.70	0.48-0.85	14	0.70	0.48-0.85	0.00	-0.27-0.27
		6.45	5	5	1.00	0.57-1.00	5	1.00	0.57-1.00	0.00	-0.43 – 0.43

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Not applicable.

Table 2: SIMUL-qPCR Confirmed vs. FSIS MLG Confirmed *Salmonella* Results

				SIMUL-qPCR *Salmonella* Confirmed			FDA BAM *Salmonella* Confirmed				
Matrix	Strain	MPN[a]/test portion	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Cheese 16 Hour	*S.* Maregrosso (SGSC 2578)	N/A[h]	5	0	0.00	0.00-0.43	0	0.00	0.00-0.43	0.00	-0.43 – 0.43
		1.27	20	14	0.70	0.48-0.85	14	0.70	0.48-0.85	0.00	-0.27-0.27
		6.45	5	5	1.00	0.57-1.00	5	1.00	0.57-1.00	0.00	-0.43 – 0.43

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[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Not applicable.

CONCLUSION

For *Salmonella*, the results of the inoculation challenge show that there was no statistically significant difference between the candidate presumptive and the candidate confirmed results after 16 hours of enrichment. When comparing the candidate to the reference method, the SIMUL-qPCR method performed as well as the FDA BAM Chapter 5 method, with no significant difference in results. The inoculated challenge study demonstrated that *Salmonella* were able to be detected down to fractional levels on cheese after 16 hours of incubation using the SIMUL-qPCR *Salmonella* Assay, and dairy products should not impact the performance of the assay or cause PCR inhibition.