Food testing

Evaluation of a PCR workflow for the detection of *Salmonella* from pooled chocolate ingredients

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Introduction

Salmonellosis is a gastrointestinal illness associated with consumption of contaminated foods. In recent years there have been several *Salmonella* outbreaks linked to nuts, dry fruit, and chocolate, which have led to hospitalizations. A reliable detection method is valuable to ensure that chocolate and low water activity chocolate ingredients are safe to eat. Pooling methods offer high throughput testing for matrices with a low incidence of *Salmonella* contamination.

The purpose of the study was to evaluate the Thermo Scientific™ SureTect™ Salmonella species PCR workflow for the detection of *Salmonella* from chocolate ingredients after a post enrichment pooling step.

Materials and methods

A total of 36 samples were seeded and tested, consisting of:


Whole almonds

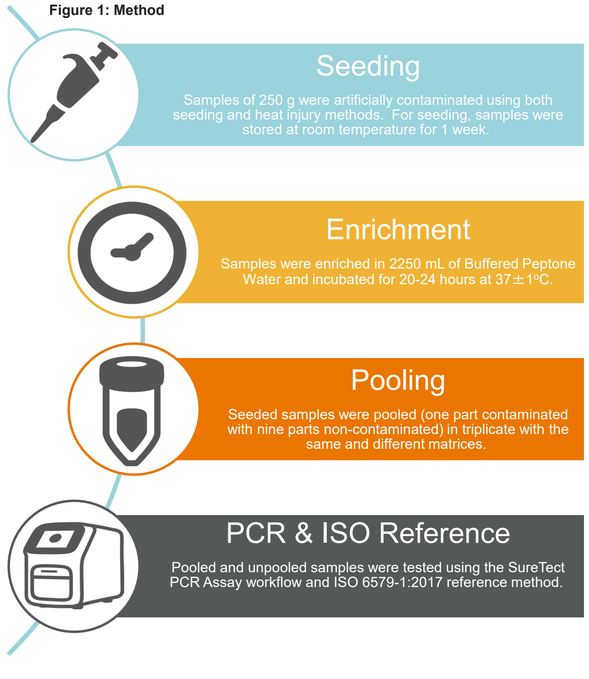

Chopped hazelnuts


Hazelnut paste


Raisins

Samples analyzed as one matrix and combined, pooled and unpooled (Figure 1).

Figure 1: Method



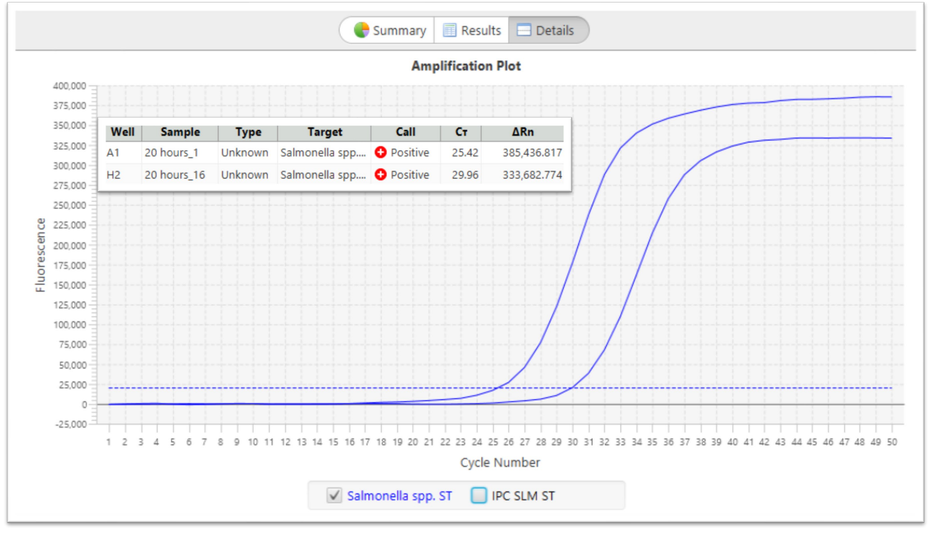
Results

Table 1: SureTect Salmonella species PCR and ISO 6579-1:2017 and culture confirmation results for heat injury and seeding method after 20 hours enrichment

Matrix	Sample	Heat injury method				Seeding method			
		Same matrix pooling		Mixed matrix pooling		Same matrix pooling		Mixed matrix pooling	
		SureTect Salmonella*	ISO 6579	SureTect Salmonella*	ISO 6579	SureTect Salmonella*	ISO 6579	SureTect Salmonella*	ISO 6579
Whole almonds	Unpooled	2/3**	2/3**	3/3	3/3	3/3	3/3	3/3	3/3
	Pooled	6/9**	6/9**	9/9	9/9	9/9	9/9	9/9	9/9
Chopped hazelnuts	Unpooled	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	Pooled	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
Hazelnut paste	Unpooled	3/3	3/3	3/3	3/3	2/3**	2/3**	3/3	3/3
	Pooled	9/9	9/9	9/9	9/9	6/9*	6/9*	9/9	9/9
Raisins	Unpooled	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	Pooled	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9

* SureTect Salmonella PCR results matched the culture confirmation results.
** One seeded hazelnut paste and whole almonds sample was not detected pre-pooling. This was the result of the bag not being inoculated by chance due to the low seeding level.

Figure 2: Amplification plot and Ct values of a unpooled and pooled seeded positive sample



Unpooled Ct value

25.42

Pooled Ct value

29.96

Results Summary

- The results of the study show that the PCR assay was able to successfully detect *Salmonella* from all matrices, following pooling, after 20-hour enrichment (Table 1).
- The assay was able to detect *Salmonella* from the two different seeding methods, when mixed and same matrix pooling was conducted.
- PCR results were in complete agreement with the ISO reference method.
- Ct values, post pooling remained low (Figure 2) and therefore, the risk of missing injured *Salmonella* from chocolate ingredients, using the post pooling method is minimal.

Conclusions

Robust

The data shows that the workflow is a robust method to detect injured *Salmonella* from same and mixed matrix pooling.

Reliable

The data shows that the protocol is a reliable method for detection of *Salmonella* from pooled chocolate ingredients and enables to test 10 test portions of 250 g in one single PCR reaction, improving pathogen screening and risk assessment.

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References

1. ISO 6579-1:2017 Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of Salmonella-Part 1: Detection of *Salmonella* spp.

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