

# Validation of a PCR Workflow Combining Wet-Pooling and Real-Time PCR for Salmonella Detection in Large Test Portions of Cocoa and Chocolate Products

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## INTRODUCTION

With a water activity below 0.85, low-moisture foods are often considered as low-risk products regarding microbial contamination. However, *Salmonella* is very well-known for being able to survive in dry conditions in cocoa and chocolate products.

Proper monitoring should be ensured while current practices on low-moisture food increases the sample size combined with an appropriate sampling plan and pooling.

The workflow of the Applied Biosystems™ Pathatrix™ 5 Pooling Salmonella spp. Kit (Thermo Fisher Scientific) linked to the Applied Biosystems™ MicroSEQ™ Salmonella spp. Detection Kit (Thermo Fisher Scientific) (candidate workflow) was evaluated through an AOAC-PTM matrix study. The FDA/BAM Chapter 5 was used as the reference method.



The matrix study was run on cocoa powder, cocoa butter, cocoa liquor and dark chocolates (> 70% cocoa)

## METHOD

Four challenging matrices were tested: cocoa powder, cocoa butter, cocoa liquor and > 70% dark chocolate. Two contamination levels were run with bulk inoculation: one low (to achieve fractional recovery) for 20 test portions, and one higher for 5 test positions. Non-inoculated test portions were also included. Large sample sizes of 375 g were tested.

Two enrichment procedures were used for the candidate method: pre-warmed non-fat dried milk and pre-warmed BPW. Post-enrichment pooling was conducted by combining one inoculated test portion from the inoculated bulk with four non-inoculated test portions using the Applied Biosystems™ Pathatrix™ Auto Instrument (Thermo Fisher Scientific) prior to PCR analyses. The FDA/BAM Chapter 5 method was performed on single samples of 25 g.

Figure 1: POD results for a 22 hour enrichment in pre-warmed BPW (low contamination level)

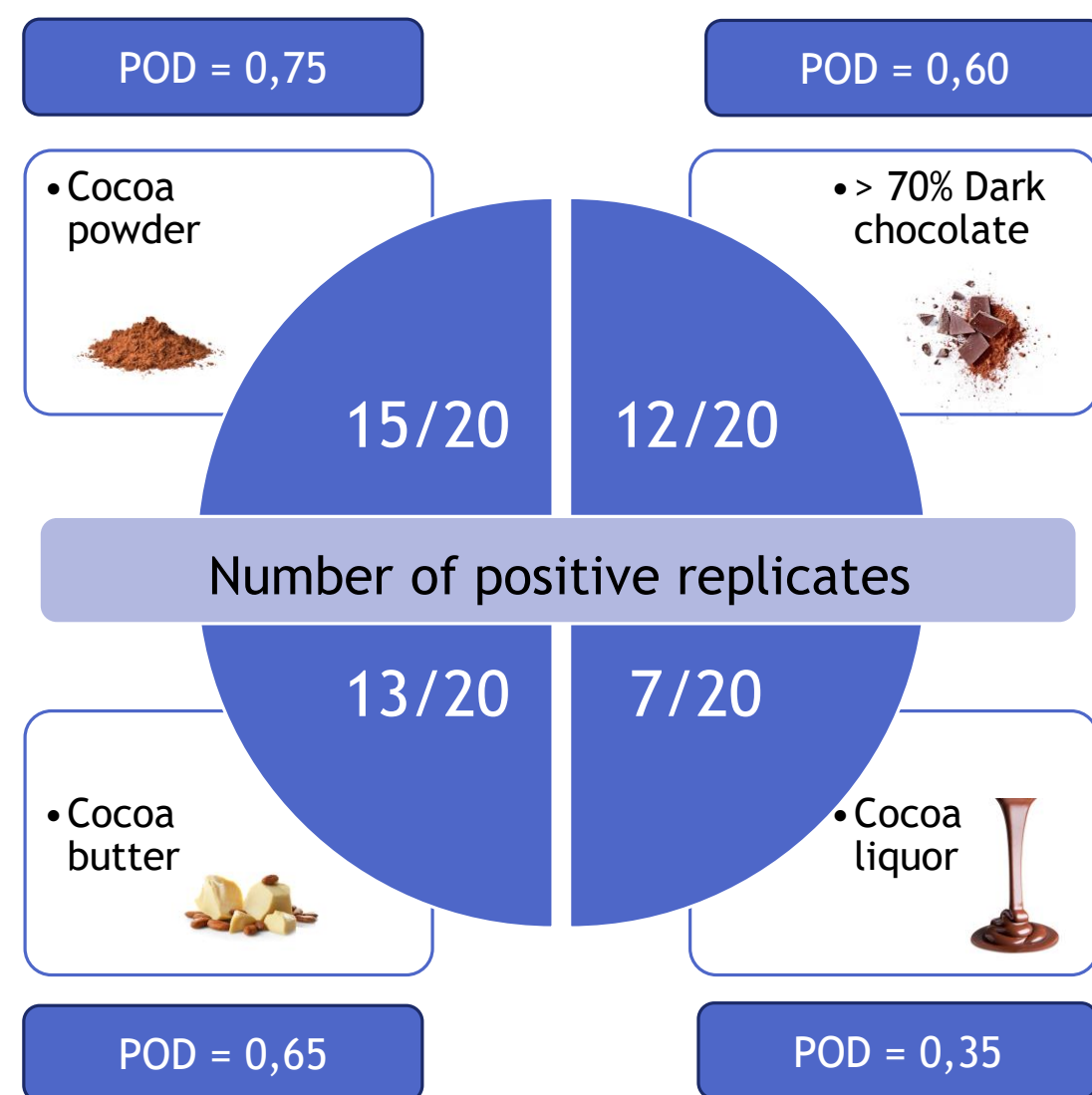


Figure 2: POD results for a 20 hour enrichment in pre-warmed NFDM (low contamination level)

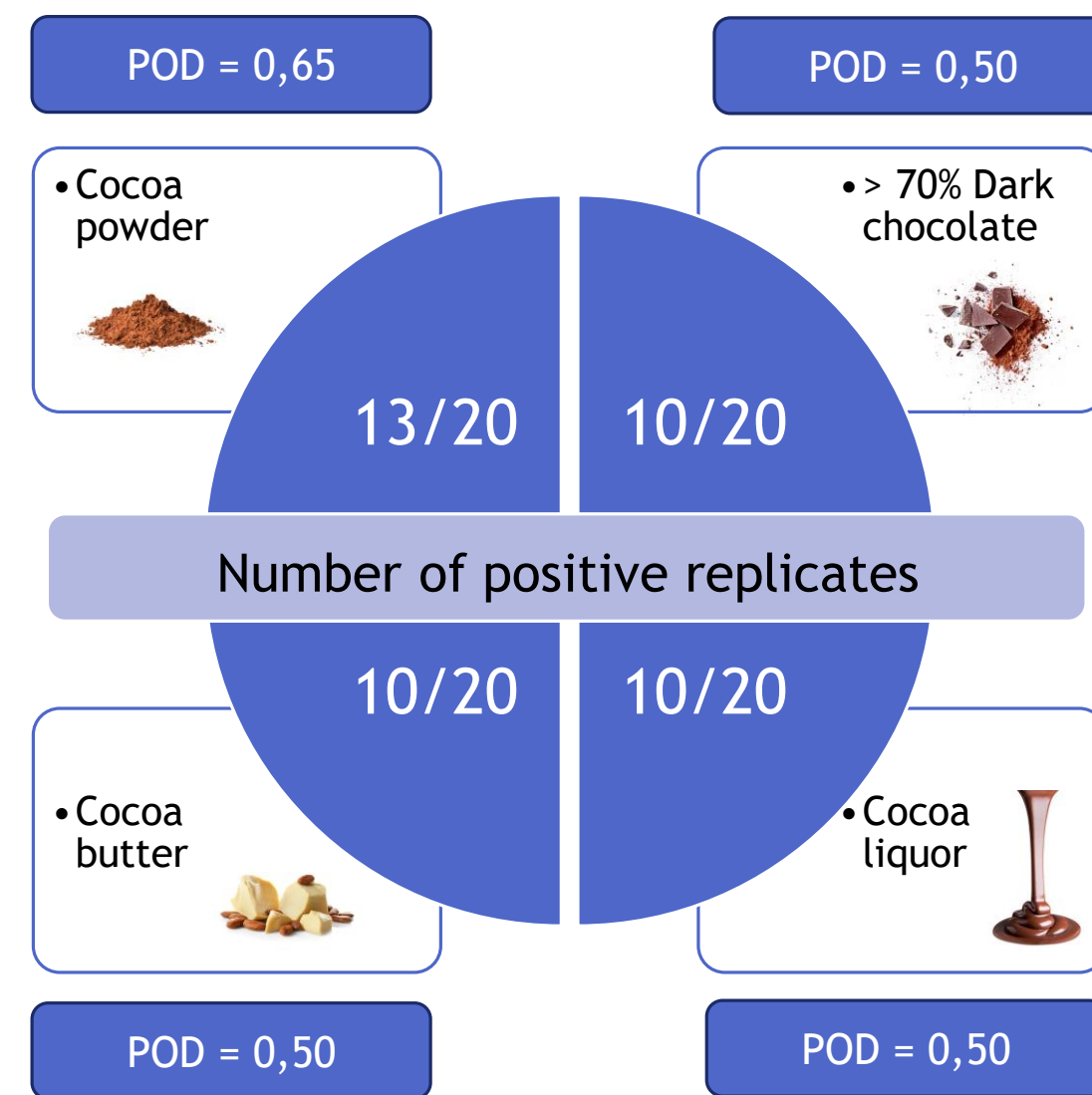
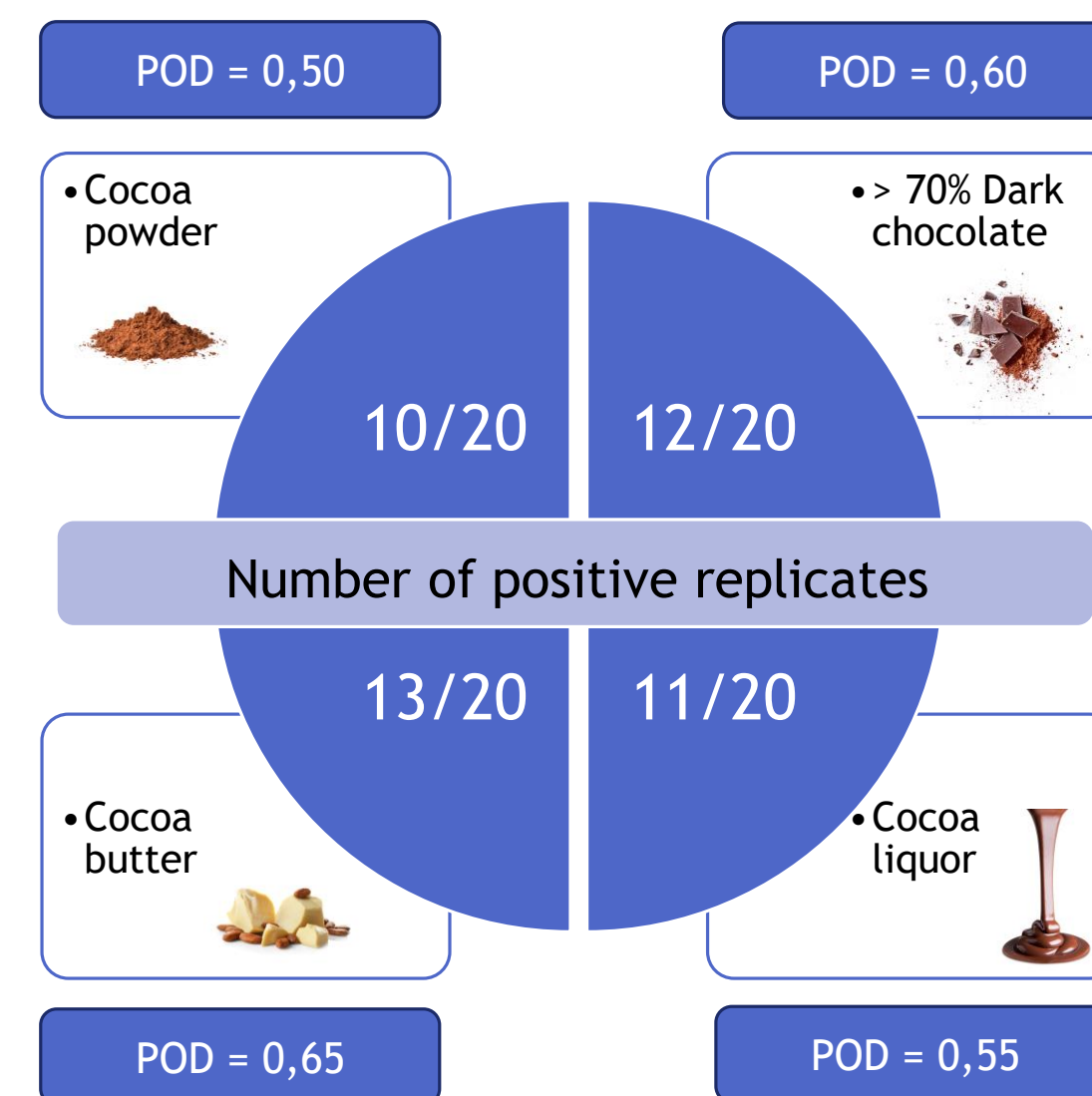


Figure 3: POD results for the FDA/BAM Chapter 5 reference method (low contamination level)



## RESULTS

According to the observed positive replicates out of 20 with the fractional recovery level and the probability of detection (POD) calculations, no statistically significant differences between the number of positive samples detected by the candidate workflow and the reference methods for all four matrices (Figures 1 to 3).



## KEY FEATURES

1. The Pathatrix Auto Instrument utilizes antibody-coated immunomagnetic separation (IMS) beads to clean and concentrate bacteria.
2. This action reduces background flora which can lead to false positives and washes away PCR inhibitors that can lead to indeterminate results in PCR workflows.
3. Additionally, through sample pooling (5x-10x), it is possible to increase throughput and reduce downstream detection costs.

## CONCLUSIONS

**Performance:** the Pathatrix™ 10 Pooling Salmonella spp. Kit linked to MicroSEQ™ Salmonella spp. Detection Kit enables sensitive detection of *Salmonella* with post-enrichment pooling of five test portions of 375 g cocoa and chocolate products within one day.

**Flexibility:** The use of two possible enrichment procedures offers flexibility to end-users.

Positive PCR data can be easily confirmed by directly streaking onto Thermo Scientific™ Brilliance™ Salmonella or XLD agars.

The isolated characteristic colonies can be directly identified using the Oxoid™ Salmonella Latex Test or Oxoid™ Microbact 24E kit.

**Time to Result:** The workflow provides a next-day result on cocoa and chocolate products. Latex tests require only a couple of minutes to confirm isolated characteristic colonies on selective agar plates.

**Large Test Portion:** The candidate method with five test portions of 375 g samples performs comparably to the reference method.

**Simplicity:** The pooling workflow is easy to conduct and enables testing of five pooled samples of 375 g (Σ1875 g), in one PCR test.