

INTRODUCTION

Over the past two decades *Salmonella* has been associated with several outbreaks due to the consumption of various low-moisture foods, including cocoa and chocolate products.

The control of this food-borne pathogen in low-moisture foods and the dry food production environments remains a significant challenge, and composite pooling is usually run to improve the sampling plan.

The purpose of this study was to evaluate the Thermo Scientific™ SureTect™ Salmonella Species PCR Assay (candidate method) for detection of *Salmonella* from large test portions of cocoa and chocolate products according to the AOAC® Performance Tested MethodsSM program.



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.

The candidate method was compared to the FDA/BAM Chapter 5 reference method using a 25 g sample size within a paired and an unpaired data study.

Large sample sizes of 375 g were tested for the candidate method, with two enrichment procedures: pre-warmed non-fat dry milk (NFDM) as described in the FDA/BAM Chapter 5 and pre-warmed BPW.

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

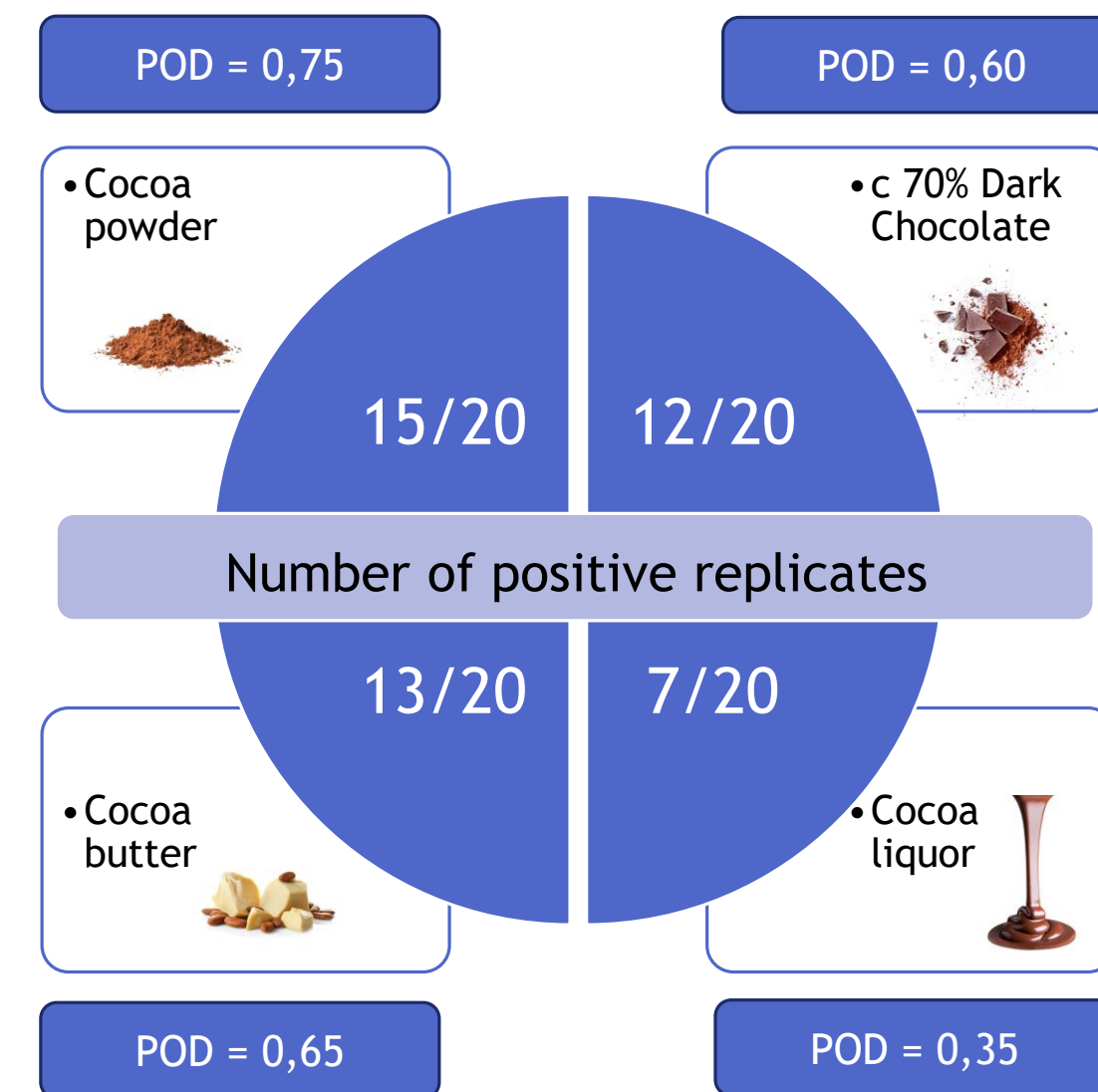


Figure 2: POD results for a 20 hour enrichment in pre-warmed NFDM (low contamination level)

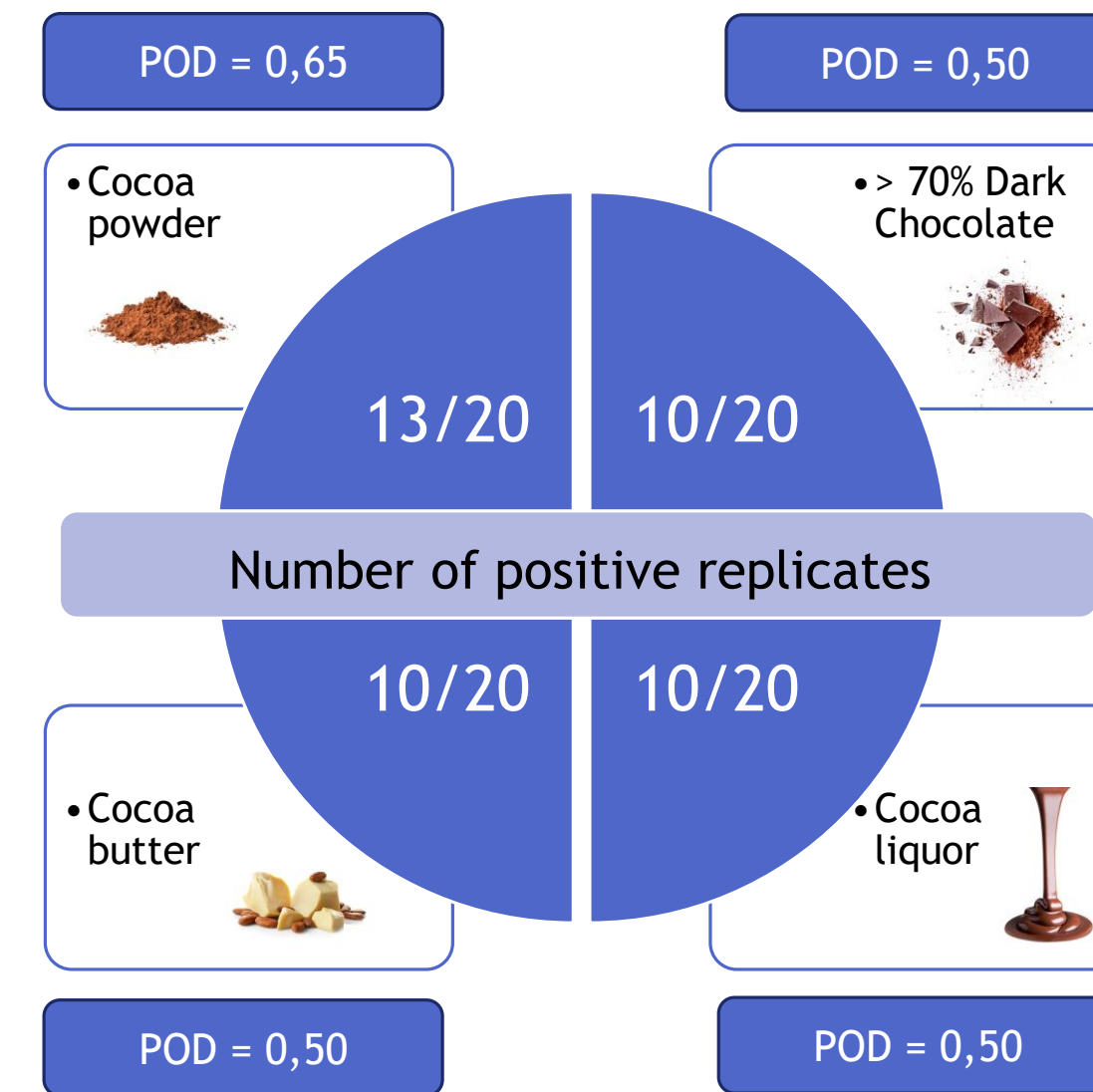
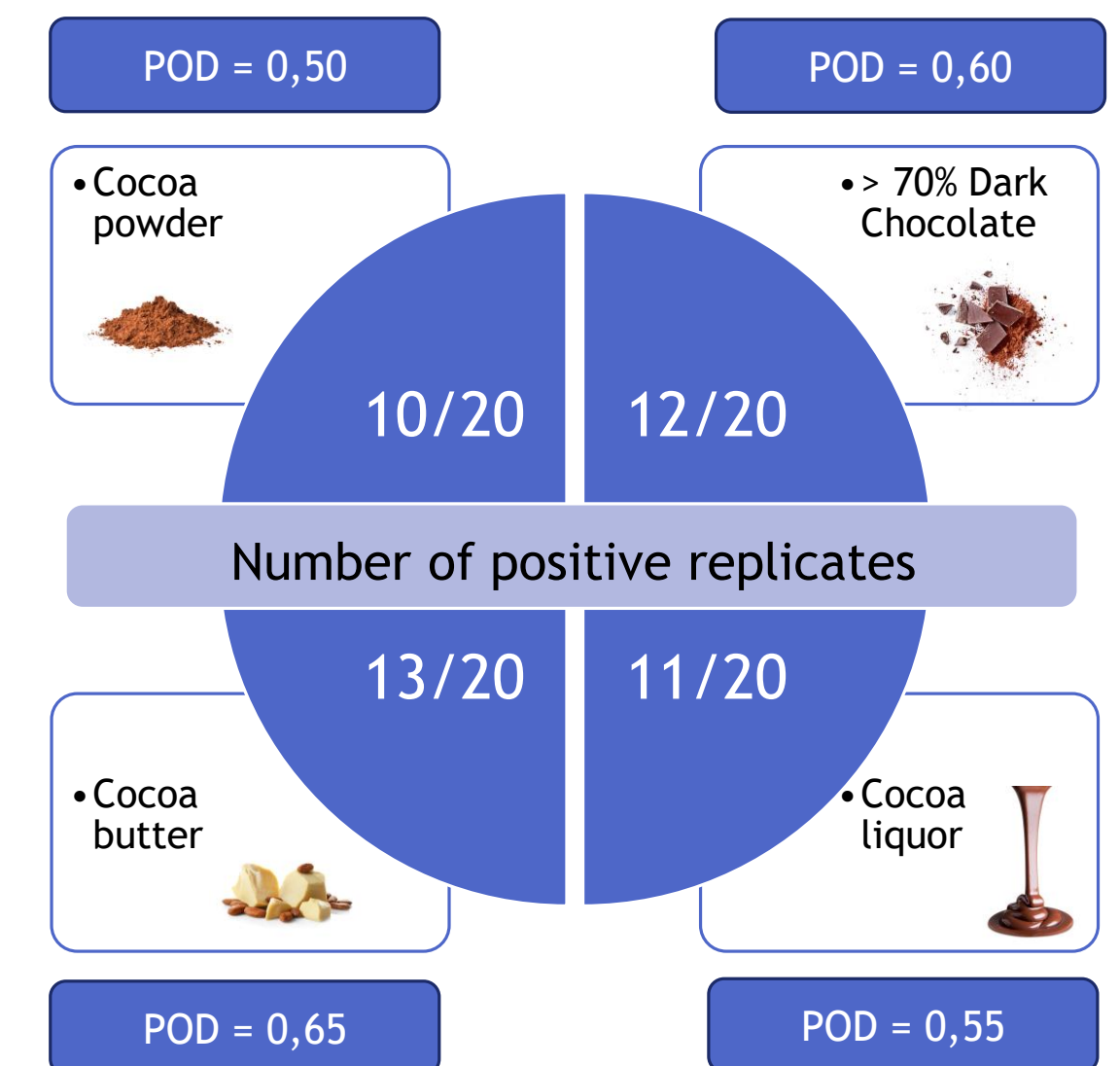


Figure 3: POD results for the FDA/BAM Chapter 5 reference method (low contamination level)



RESULTS

No false positive PCR results and no PCR inhibition was observed with the candidate method. The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. There were no statistically significant differences between the number of positive samples detected by the candidate workflow and the reference methods despite the difference in test portion sizes.



The SureTect Salmonella Species PCR Assay kit contains pre-filled reagent tubes for 96 samples in one box with all components stored at 4-8°C.

The PCR assays were evaluated on the Applied Biosystems™ QuantStudio 5 and Applied Biosystems™ 7500 Fast Food Protection thermal cycle Instruments (Thermo Fisher Scientific).



CONCLUSIONS

Performance: The SureTect Salmonella Species PCR Assay enables sensitive detection of *Salmonella* from 375 g test portions of 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 (Thermo Fisher Scientific).

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

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 375 g test portions performs comparably to the reference method with 25 g test portions.

Simplicity: The workflow is easy to conduct and enables testing samples of 375 g of cocoa and chocolate products.