

performed with MC Media Pad at 30°C (validated against ISO) and 37°C (validated against AOAC) in natural contaminated samples showed relevant differences.

**Significance:** Evaluation of challenging food items from a broad range of food categories is crucial to confirm fitness for purpose of alternative and reference methods, allowing a better understanding of method performance and limitations.

## P2-10 Identification of Animal and Plant Species in Food-Based Products Using Next Generation Sequencing: Results from a European Interlaboratory Study

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**Introduction:** The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients for food production. Although there is no reference method for food authenticity analysis, the introduction of Next Generation Sequencing (NGS) in recent years has demonstrated the suitability of this method to verify species composition of food products.

**Purpose:** An interlaboratory study involving 11 European laboratories from eight countries was conducted to support the implementation of NGS for routine food authenticity analysis. In this study the Thermo Scientific™ NGS Food Authenticity Workflow, was used to determine the species composition in a range of different samples

**Methods:** A total of 72 samples were received by each participant. The targets included meat, fish, and plant. All the experiments were carried out in duplicate. Each participant used the Thermo Scientific NGS Food Authenticity Workflow using the Ion Chef and Ion GeneStudio™ S5 instruments and sequence data analysis was done with the SGS® All Species ID software. Then, the performance of each participant was scored, and the robustness and reliability of the workflow was evaluated.

**Results:** The overall scores calculated ranged from 84.4% to 100% for fish samples, 77.8% to 97.8% for meat samples and 80.8% to 98.1% for plant samples. The real food samples produced the most variable results which can be explained by the possible heterogeneity of the samples. Among artificial DNA mixtures, a total of 10 meat species, 15 fish species and 18 plant species were successfully identified. Some of the species were identified at low concentration levels (1%).

**Significance:** This is the largest NGS interlaboratory study performed that included meat, fish and plants. The results obtained demonstrated the high performance and robustness of the Workflow. This study, together with recent developments at ISO and AOAC about the use of NGS for animal and plant species identification, supports the routine implementation of NGS for food authenticity analysis.

## P2-11\* Optimisation of Culture Dependent and Independent Methods to Detect Pathogens in Infant Food Production Chain

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**Introduction:** The continuous effort for the development of optimum detection approaches of pathogens in food responds to public health and regulatory demands. This is particularly true for infant food indus-

tries given the nature and sensitivity of infants. Therefore, finding the way to explain the microbial prevalence with tangible elements that could enhance the decision support system and risk assessment is essential.

**Purpose:** This study aimed to develop a fast and reliable approach to detect the presence of foodborne pathogens that can cause severe illness and death in infants.

**Methods:** *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella enterica*, *Staphylococcus aureus* and *Clostridium perfringens*, were artificially inoculated at the lowest possible cell concentration, in different infant food matrices. Different generic broths were subsequently employed for enrichment purposes. Detection was performed through microbial counts (culture dependent), Real-Time PCR, and metataxonomic analysis (culture-independent methods). Moreover, different DNA extraction approaches were tested in order to select the one with the better purity and quality of nucleic acids.

**Results:** Real-Time PCR detected all pathogens, but only after one day of enrichment. Detection however varied for the different target pathogens and was influenced by the composition of the microbial community. Based on their detection, a common enrichment broth was chosen for all the pathogens, except for the anaerobic *Clostridium perfringens* that were dealt with separately. Differences were observed in the metataxonomic analysis and the resultant microbial composition of the samples based on the DNA extraction kit used. Therefore, the best-performing kit was chosen for further analysis of naturally contaminated samples.

**Significance:** The optimisation of the multiple enrichment methods and the identification of the most suitable DNA extraction approach feasible for both Real-Time PCR and metataxonomic analysis leads the way to a massive survey of pathogens detection in different foods.

## P2-12 ISO 16140-2:2016 and AOAC-OMA Validation of a Real-Time PCR Workflow for Salmonella Detection in Cocoa and Chocolate Products

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**Introduction:** *Salmonella* is major global foodborne pathogen and has been associated with several outbreaks due to consumption of low-moisture food products, including cocoa and chocolate products. Detecting *Salmonella* in such foods is crucial to preventing infection. The Thermo Scientific™ SureTect™ *Salmonella* species PCR Assay (candidate method) is a rapid method for the detection of *Salmonella* in large sample sizes of cocoa and chocolate products.

**Purpose:** To present the accuracy, reliability and reproducibility of the candidate method for the detection of *Salmonella* in cocoa and chocolate products.

**Methods:** Four matrices were evaluated in the validation studies including 375 g of cocoa powder, cocoa butter, cocoa liquor, and dark chocolate (>70% cocoa solids). Method validation studies were conducted according to ISO 16140-2:2016 (AFNOR) requirements and AOAC Appendix J guidelines (PTM and OMA). In the ISO 16140-2:2016 study, the candidate method was compared to the ISO 6579-1:2017 reference method using UHT milk or non-fat dried milk (NFDM) (paired study) and using pre-warmed Buffered Peptone Water (BPW) (unpaired study). For the AOAC studies, the candidate method was compared to the United States Food and Drug Administration/ Bacteriological Analytical Manual (FDA/BAM) Chapter 5 *Salmonella* reference method using NFDM (paired study) and pre-warmed BPW (unpaired study). Cocoa powder (375 g) was also used in the AOAC OMA collaborative study of the candidate method.

**Results:** The candidate method performed statically equivalently or better than the reference method it was compared to for each enrichment in the respective study designs. The candidate method met all requirements outlined in ISO 16140-2:2016 (AFNOR) and AOAC Appendix J.

**Significance:** The candidate method is an accurate, reliable and reproducible method for the detection of *Salmonella* in large sample sizes of cocoa and chocolate products and now holds NF VALIDATION, AOAC PTM certification, and AOAC OMA First Action.