Poster Session 1 – Thursday, 8 October

P1-01 A New "Next Day" Method for Detection of *Listeria monocytogenes* in Food

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Introduction: Detection of *Listeria monocytogenes* in foods with traditional methods is time-consuming, taking up to five days to obtain a negative result.

Rationale and Objectives: the objective of this study was to determine the performance of a new immunoassay method, VIDAS *Listeria monocytogenes* Xpress (LMX), for the next day detection of *Listeria monocytogenes* in food samples.

Methods: The detection method, is associated with a specifically formulated LMX broth containing optimized concentrations of selective agents to inhibit competitive bacteria.. For the food study, samples were culturally enriched for a total of 26 h in LMX broth, before testing in the VIDAS instrument. Positive results were confirmed by streaking enrichment broths onto selective chromogenic agar. The new method was compared to ISO 11290-1 reference method.

Results: the detection limit, established with 50 *L. monocytogenes* strains was found to be between 2.103 and 3.105 CFU/ml in LMX broth. No cross reaction was observed with 30 potentially interfering strains at the growth level reached in a non selective medium.

The food study included 370 food products, 238 meat, 87 dairy and 45 seafood products. 153 samples were confirmed positive by one of the methods, 23 by the immunoassay only, 17 by the cultural method and 113 by both methods.

Sensitivity was respectively 88.9% for the immunoassay and 85.0 % for the reference method. Difference observed between the two methods was not statistically significant. Agreement between the two methods was 89.2%. As all positive results were confirmed after subculture, the test specificity was 100%.

Conclusions: this study demonstrated that the VIDAS LMX method is comparable to the ISO 11290-1 method for the recovery of *Listeria monocytogenes* in meat, dairy and seafood products. It provides a very rapid, sensitive and convenient method allowing a presumptive result within 27 h of sample set up.

P1-02 Evaluation of Two New Alternative Methods for the Detection of *Campylobacter* spp. in Food and Environmental Samples

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Introduction: *Campylobacter* spp. are the most frequent cause of acute intestinal infections in developed countries. Human Campylobacteriosis can be reduced by better control of foodborne contamination.

Rationale and Objectives: This study compares the performance and ease of use of two new alternative methods for detection of Campylobacter spp with the reference method NF EN ISO 10272-1. Forty-five samples (twenty-five food matrices (including twenty-two poultry meats), eight production environment and twelve breeding environment) were analyzed. For these two tested methods a 1/10 dilution of samples is performed in the new ready to use medium: CampyFood Broth (CFB) (bioMérieux). CFB is incubated 44–52 h at 41.5°C under microaerophilic conditions using an innovative Combibag system.

For the simplified conventional detection method, CFB isolation is carried out on the optimized formulation CampyFood Agar (CFA) (bioMérieux). CFA is incubated for 48 h at 41.5°C under microaerophilic conditions. Typical colonies of presumed *Campylobacter* (deep-red (burgundy) to orange-red) are confirmed by respiratory type, oxydase testing, and microscopic morphology.

For the VIDAS CAM protocol, 1–2 ml of the CFB enrichment is heated at 95–100°C for 15 min and 500 µl then transferred to a VIDAS CAM strip for testing. VIDAS CAM positive samples are streaked onto CFA. Typical colonies are confirmed by the same procedure used for the simplified detection method.

Results and Findings:
- Sensitivity: globally, sixteen true positive samples were detected with the reference method NF-EN-ISO 10272-1, twenty-six with the simplified conventional detection method, and twenty-seven with the VIDAS CAM protocol.
- Specificity: no significant statistical difference was shown between the three methods.

Conclusions: These results demonstrate that the two alternative methods have higher recovery for

Campylobacter spp. compared to the NF EN ISO 10272-1 method. The new bioMérieux alternative methods for detection of *Campylobacter* spp. significantly improve ease of use, time to result, and laboratory throughput.

P1-03 Application of an Automated MPN System for Enumeration of Bacterial Counts on Food Contact Surfaces

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Will be presented by Geraldine Ramage

Introduction: The microbiological examination of food-contact surfaces is a recognized tool to assess the efficacy of cleaning and disinfection in food plants. Commonly tested indicators are total viable count (TVC) and enterobacteriaceae. Neutralizing agents can be used to prevent "false-negative" results due to the antibacterial activity of residues.

Rational: The application of an automated MPN system (TEMPO®) for enumeration of bacteria for testing of the cleaning and disinfection efficacy of food contact surfaces was studied. In a first trial, 5 neutralizing agents [Eugon LT100, Difco neutralizing buffer; ISO 18593 buffer; mod. Letheen broth; commercial swab with neutralizer (Quantiswab, Coban)] were tested for possible interferences on the enumeration of selected bacterial strains (*E. coli* ATCC25922; *Lb.plantarum* DSM20174; St. aureus ATCC25923; *C. freundii* NCTC9750; *B. cereus* NCTC7464) by the automated MPN system and cultural ISO or BAM methods. Then, 40 surfaces (stainless steel=22, plastic=15, other=3) in 5 different food premises were tested by the MPN system (TVC, *E. coli*, coliforms, *St. aureus*, Lactic acid bacteria) and cultural reference methods.

Results: As there was no significant difference in the enumation of cultures between the different neutralizing agents, the commercial swab kit (Quantiswab) was chosen for surface sampling. For the 40 surface samples, results by the automated MPN method agreed well with those obtained by cultural methods. For results in the detection range, average differences were 0.06 and 0.12 log for TVC (n=22) and Enterobacteriaceae (n=6), respectively. For coliforms, similar results were obtained. For *E. coli*, 38 samples were below detection range, and differences for the remaining two samples were 0.5 and 0.7 log.

Conclusions: Results obtained for cleaned and disinfected food contact surfaces did not differ significantly from those obtained by reference methods. Depending on the microbiological parameter, results were obtained 24–48 earlier by the MPN system than by reference methods.

P1-04 Use of a Novel Device to Enable Irradiation of Fresh Cantaloupes by Electron Beam Irradiation

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Introduction: Cantaloupes have been associated with numerous *Salmonella* outbreaks. Different studies have addressed the development of methods for cantaloupe disinfection, and their results seem to indicate a limited effectiveness of sanitizing rinses at reducing pathogens. Irradiation may be a solution, although the appropriate treatment conditions are still not established.

Rational: Due to their unidirectionality, electron beams are considered unsuitable for irradiating foods of irregular shapes. However, we have developed the Maxim Chamber, a new application that benefits from the innate scattering behavior of electrons when impacting a solid mass. Inside the chamber, a metallic mesh promotes infinite electron bounces, creating a cloud of electrons around the target object. When using this device, a uniform dose delivery over irregular shape objects such as rabbit carcasses, was achieved. Inoculated *E. coli* O157:H7 was reduced by > 5 log cycles onto rabbit carcasses. The objective of this work was to further investigate the Maxim Chamber capabilities in delivering dose uniformity and penetration using cantaloupes as a model.

Results: Average doses at the cantaloupe surface and at depths of 1 and 2 cm were 3.13 kGy, 2.27 kGy and 1.42 kGy, respectively. A stack of 6 allanine dosimeters immersed into the flesh showed max/min dose ratios from 1.19 to 1.61 kGy. Average log reduction of *Salmonella* Poona on cantaloupe after irradiation in the Maxim Chamber were > 4.7 and > 4.0 when inoculated on the rind and stem scar, respectively, and 4.7 and 3.9 when inoculated in the flesh at 1 and 2 cm under the surface, respectively.

Conclusions: The Maxim Chamber seems to be an effective tool to uniformly deliver e-beam irradiation to products with spherical shapes. Additionally, the advantage of reducing pathogen subcutaneously is highly remarkable due to current food safety concerns.

Acknowledgements: We thank Dr. Mayra Marquez and Mr. Josue Morales for providing technical assistance.

P1-05 Testing for *Salmonella* and *Escherichia coli* O157:H7 from a Single 8-h Enrichment

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will be presented by THOMAS B. MOELLER

Introduction: Introduction: Most *Salmonella* and *E. coli* O157:H7 outbreaks are linked to two food types: fresh produce and beef. Traditional testing protocols call for separate enrichment methods when testing for *Salmonella* versus testing for *E. coli* O157:H7 in these matrices.

Rational: Purpose: The objectives of this study were two-fold; one was to investigate using an established 8-h beef enrichment method with the BAX® system PCR method for detecting *E. coli* O157:H7 in fresh produce, and the other was to evaluate the same enrichment with the same PCR method for detecting *Salmonella* in both beef and produce. Methods: Produce was spiked with *E. coli* O157:H7, and beef and produce were spiked with *Salmonella* at target levels set to yield fractional positive results. Samples were evaluated using the appropriate culture-based reference method and the PCR test kit method following the 8-h enrichment protocol. Twenty spiked and five unspiked samples per food type per method were tested and compared.

Results: Statistical analysis on both *E. coli* O157:H7 and *Salmonella* in all matrices indicated the test method performed as well as or better than the reference method for detecting both *E. coli* O157:H7 and *Salmonella*.

Conclusions: This approach demonstrated that both *Salmonella* and *E. coli* O157:H7 can be detected from the same 8-hour enrichment, which may save food companies cost, time and labor through reductions in sample preparation, media preparation, incubator space and waste streams.

P1-06 Development of a Scorpion™ Probe-based Real-time PCR Assay for Genus *Salmonella*

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will be presented by THOMAS B. MOELLER

Introduction: The use of PCR-based methods for *Salmonella* spp. detection and monitoring have shown tremendous growth in recent years. One commonly used commercial method, the BAX® system, uses end-point PCR based on melting curve analysis. Although this method features excellent performance characteristics for sensitivity and specificity, it can require nearly 3.5 h to complete the cycling and melt curve analysis.

Purpose: The purpose of this study was to evaluate the use of probe-based Scorpion(TM) technology with existing primer sequences to develop a faster real-time PCR assay that would maintain performance identical or superior to the current end-point PCR assay. The use of probe detection allows for much more rapid cycling (< 1 h) and eliminated the need for a melt curve analysis. Methods: Studies comparing the sensitivity and inclusivity of the new real-time assay with the current commercial PCR assay were conducted, using both purified DNA and select *Salmonella* spp.

Results: Results using liquid real-time PCR reagents versus the tableted commercial PCR kit reagents showed equivalent sensitivity using both DNA (5 to 50 fg) and cells (~104 cfu/mL). Inclusivity using a small panel of 48 diverse *Salmonella* spp. was also identical with both assays, showing 100% detection of the strains tested.

Significance: These results demonstrate the feasibility of developing a novel real-time PCR assay for *Salmonella* spp. that allows for cycling and detection in less than one hour, with the same performance characteristics of an existing well-characterized, commercial assay.

P1-07 Validation of a PCR Assay for Screening *Listeria* spp. in Foods and Environmental Sponges

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will be presented by THOMAS B. MOELLER

Introduction: Since other *Listeria* species can out-compete *L. monocytogenes* in culture, potentially leading to false negative results for the pathogenic species, some food producers are testing for *Listeria* spp. instead. Well-validated rapid methods for the detection of *Listeria* species as an indicator of possible product adulteration with *L. monocytogenes* are needed because culture-based take four to seven days to deliver a result.

Purpose: This study evaluated the inclusivity, exclusivity and effectiveness of the PCR-based BAX® system approach to screening of artificially introduced *Listeria* in spinach, processed cheese and frankfurters, and naturally occurring *Listeria* in smoked salmon and drain sponges.

Methods: Inclusivity testing was performed at ~1 log over the claimed product sensitivity of 10^5 CFU/mL, while exclusivity testing was performed at 10^8 CFU/mL. For method effectiveness, foods were spiked with *Listeria* at target levels set to yield fractional positive results and were evaluated using the appropriate USDA, FDA or AOAC culture-based method and the PCR test kit method, with twenty spiked and five unspiked samples per food type per method. Frankfurter testing was repeated at an independent laboratory. One food type, smoked salmon, and one environmental sample type, drain sponges, were testing using naturally occurring *Listeria* with twenty paired replicates.

Results: All 50 *Listeria* in the inclusivity panel were found to be reactive, whole the 30 non-*Listeria* strains were non-reactive using the assay. Comparing effectiveness results for PCR and plating, results for the three inoculated sample types demonstrated Chi-square values of 0.1 to 0.46, indicating no significant difference in method performance. For the naturally occurring *Listeria* contamination of salmon and drain samples, Chi-square comparison of PCR and reference culture methods demonstrated values of 1.26 and 0.10, also indicative of indistinguishable method performance.

Significance: This data indicates that this PCR method for the detection of *Listeria* is as effective as culture-based methods whole providing significant time savings.

P1-08 Exposure Assessment to *Cronobacter sakazakii* in Powder Infant Formula in Ireland

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Introduction: *C. sakazakii* represents a significant risk to the health of neonates. Although, the organism natural habitat is currently unknown, PIF has been identified as a source and vehicle of neonatal infection.

Rationale and Objectives: The objective of this study is to consider some statistical aspects, as the probability of accepting and rejecting a lot, considering two surveys carried out in Ireland to detect the contamination of *C. sakazakii* in PIF. The assumptions and the method used to calculate the probability of accepting or rejecting a lot are the ones adopted by WHO risk assessment model for *Enterobacter sakazakii* in powder infant formula. Calculation of rejection rates requires the mean log concentration of *C. sakazakii* across all lots of PIF (CFU/g), which is estimated from C= l n [1- P > 0] / S where C is the concentration (per gram), P > 0 is the prevalence, and s is the samples size (grams).

Results and Findings: True prevalence is estimated (2.9 and 0.14%) from apparent prevalence using the Bayesian approach based on beta (1, 1) and assuming the microbiological analyses without error, thus considering sensitivity and specificity equal to 1. In this study lots are simulated using the Montecarlo software @Risk and tested against the microbiological criteria established in the EC 2073/2005 (absence in 10 g, 30 samples per unit). The outputs obtained are the probabilities of accepting/rejecting a lot calculated assuming different values for the within and between lot variability. Rejection rates are also presented graphically considering uncertainty distributions around prevalence data.

Conclusions: As Ireland supplies 15% of PIF in the world, monitoring the contamination of the product using an appropriate sampling plan and t he application of microbiological criteria represents an important first step in reducing the risk of contaminating PIF product.

P1-09 Modeling the Concentration of *Salmonella* in Irish Fresh Pork Sausage from Most Probable Number (MPN) Results

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Will be presented by ARIANNA MUSSIDA

Introduction: It has been estimated that 10–30% of food-borne salmonellosis had pork and pork products incriminated as the actual source. In Ireland, a pork product that deserves attention for being a raw comminuted product that is widely consumed is the fresh pork sausage.

Rational: As part of a risk assessment of *Salmonella* in pork sausage, a methodology for modeling the concentration of *Salmonella* (CFU/g) in pork sausage at retail from MPN data was assessed.

Results: From a retail survey study, MPN result triplets were available for each of the sausages (6) from 10 contaminated packs. The conditional probability l(x|?) of observing the tube counts X={xi} given the true *Salmonella* concentration ? was fitted to the MPN triplets for every sausage within a pack. Considering that the sausage production involves meat grinding and thorough mixing with other

ingredients, each of the small portions of sausage mix stuffed into casings (and subsequently packed) can be assumed to be Poisson (?) distributed. Thus, to obtain a better estimate of the uncertainty about the true ? (CFU/g) of each contaminated pack, a posterior distribution f(?|X) was modeled by combining the six l(x|?) functions and a Jeffreys' prior distribution. In order to model the variability of ? in contaminated sausage packs, the uncertainty around the 10 f(?|X) distributions was propagated to a lognormal distribution by calculating its parameters for 10000 Bootstrap samples.

Conclusions: The mean and standard deviation of the lognormal distribution (CFU/g) fitted a normal (4.0389, 0.1039) and a normal (0.6389, 0.0591), respectively, and, in log terms, the initial *Salmonella* concentration of contaminated packs at retail had an expected value of 1.84 log CFU/g with a 95% CI of 1.19–2.30 log CFU/g. This approach provided a more informed second-order distribution of CFU/g than would have been possible by the common practice of fitting directly a distribution to the MPN/g.

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P1-10 Detemination of Microbial Contamination Sources at Sausages Processing Line

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Introduction: Effective HACCP systems must be based on accurate baseline data on the levels of contamination at each stage of production. It is important to provide baseline data on the levels of bacterial contamination of sausages at processing line.

Rationale and objectives: This study has been conducted to determine microbial contamination sources during sausage processing. Minced meat, sausage batter, stuffed sausage, cooked sausage, peeled sausage and pasteurized sausage samples have been examined microbiologically. Moreover, spices and ice water used in production, personnel hands and equipment have been examined.

Results: Counts of total aerobic mesophilic bacteria, *Staphylacoccus aureus, Escherichia coli,* yeasts and molds in minced meat were found to be 7.02, 3.83, 4.42 and 1.62 log CFU/g respectively. *E. coli* and yeast-mold counts in sausage batter reached 3.99 and 1.72 log cfu/g respectively. Heating for cooking was effective in reducing microbial counts. Total plate (3.93 log cfu/g) and *S. aureus* (1.08 log cfu/g) counts in cooked sausages decreased, *E. coli* and yeast-molds were not detected.

Conclusions: According to the results, raw material and spices have been found as primary contamination sources. Personnel hands and equipments have been found as secondary contamination sources. Microbial counts in personnel hands showed significant correlations with the counts of the samples taken from all processing stages. Microorganism counts determined in overall processing were not at harmful levels for human health and microbial load of final product was within critical limits.

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P1-11 Determination of Polycyclic Aromatic Hydrocarbons Profile in Portuguese Traditional Dry Fermented Sausage

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Introduction: Over the past years, growing concerns about polycyclic aromatic hydrocarbons (PAH) carcinogenic activity and their presence in food have been reported. Food industries largely use wood smoke due to its preservative and sensorial properties. Nonetheless PAH contamination may occur in meat products exposed to this practice, especially when smoke generation is not controlled.

Rational: The aim of this work was to study the prevalence of the 16 referred PAH by Environmental Protection Agency (EPA), in 9 samples of a portuguese traditionally dried fermented sausage, in regard to ripening time and also the respective PAH diffusion within the product. The analysis was performed by HPLC-UV/Vis-FLD.

Results: The total PAH content (dry matter basis) in the final product was 626.86μg.kg^{-1} prevailing low molecular weight compounds, namely acenaphthene, fluorene and phenanthrene (53.77, 89.23 e 273.68μg.kg^{-1}, respectively). Naphthalene, acenaphthylene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene were not detected in any sample. In relation to PAH considered as carcinogenics, such as benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene (BaP), the higher content was verified for the benzo[a]anthracene reaching 8.07μg.kg^{-1}. BaP did never exceed 1.60μg.kg^{-1} (fresh weight, FW) which is below the 5.0μg.kg^{-1} (FW) limit established by European Commission for meat and meat products (Regulation (EC) Nº 1881/2006 of 19 December 2006). PAH migration from products surface to internal layers was observed. Total PAH

content reached 3883.89µg.kg^{-1} on the outer layers in comparison to 1092.72µg.kg^{-1} detected in the inner ones.

Conclusions: Our results show that, despite of BaP content being in agreement with the legal limit, all samples revealed PAH contamination that could be minimized by the optimization of the smoking process.

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P1-12 Novel Sample Preparation Solutions for Highly Sensitive and Accurate Detection of Foodborne Pathogens from Complex Food Matrices

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Introduction: Detection of low levels of pathogenic microorganisms in food is often difficult due to the complexity of food matrices. A successful sample prep method should have a simple workflow, give high recovery, and be able to efficiently remove inhibitors that would otherwise interfere with detection.

Rationale and Objectives: We have developed two separate sample preparation procedures that enable sensitive detection of foodborne pathogens using real-time PCR. Each of these novel sample preparation methods allows separate detection of 1 CFU of *Salmonella* spp., *Listeria* spp., or *Escherichia coli* O157:H7 following enrichment in a variety of food matrices.

Results and Findings: Foods from a variety of categories were addressed with the sample preparation procedures including chocolate, shrimp, and chicken wings for *Salmonella* spp detection; ice cream, brie cheese, chocolate and milk for *Listeria* spp. detection; and ground beef for *E. coli* O157:H7 detection. Following enrichment, samples were prepared by either of two methods: (1) total DNA capture method using magnetic beads or (2) clarification spin column. The samples were assayed using specific real-time PCR assays run under fast conditions (<1 hr). An internal positive control was included in the assays to assess inhibition.

Two sample preparation methods were validated for detection of *Salmonella* spp, *Listeria* spp. and *E. coli* O157:H7. Both methods were efficient at detecting 1–3 CFU of each pathogen in all food matrices. The results from the total DNA capture method correlated with results from the column clarification method. The consistent signal from the internal positive control indicated that both methods adequately removed inhibitors.

Conclusions: The novel sample preparation described here are highly efficient when used in conjunction with FAST real-time PCR detection. The methods are robust enough to allow for time-to-result in as short as 8 h, as in the case of *E. coli* O157:H7 detection. The DNA capture method is adaptable to high-throughput automation. The advantage of the column clarification method is a simplified workflow.

P1-13 Validation of a New TaqMan® Real-Time PCR Method for the Detection of *Salmonella enterica* in a Variety of Food Samples Using a Single 18-h Enrichment Protocol

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Will be presented by Olga Petrauskene and Christina Boss

Introduction: *Salmonella* is one of the largest causes of food-related illnesses worldwide and is associated with a wide variety of matrices. The need for the provision of a reliable rapid method which is sensitive, specific and robust has been identified.

Rational: Numerous methods are available for the detection of *Salmonella* in foods, but often take up to four days to confirm a negative sample. As a result, a reliable rapid method which is sensitive, specific and robust is in demand.

Results: The TaqMan® *Salmonella* enterica detection kit from Applied Biosystems is a genetic-based detection kit using TaqMan® chemistry, offering results after just 18 hours enrichment in Buffered Peptone Water (BPW). The method was certified by AFAQ AFNOR validation according to the ISO 16140 standard, analysing naturally and artificially contaminated raw poultry, raw meat, raw fish, milk, cheese, frozen vegetables, raw egg and pet food amongst others comparing TaqMan® Salmonelle enterica detection method with the reference method ISO 6579. Positive results were confirmed by performing the ISO 6579 standard. A total of 333 samples were analyzed, 38.1% of which were

contaminated naturally. Statistical analysis of the data showed that the relative accuracy of the alternative method was 98.5%, the relative specificity 99.4% and the relative sensitivity 97.4%. During the specificity study all 58 *Salmonella* target strains gave positive results, and all 36 non-target strains gave negative results. Non-target strains are commonly found in food and show no cross reactivity with our detection method. Ten laboratories from 7 countries in Europe participated in the inter-laboratory study, giving comparable results which illustrates that the method is reproducible. The practicability of the method was found to be better than the reference method, requiring less than one day of training for technicians with no experience. Negative results were obtained in less time than the reference method and the software gives permanent traceability.

*Conclusion*s: The validation demonstrated that the TaqMan® *Salmonella enterica* detection kit from Applied Biosystems is not only rapid and easy to use, but also selective and specific, offering the food industry high accuracy and sensitivity.

P1-14 Characterization of Surface Properties and Biofilm Formation of Four *Listeria monocytogenes* Strains

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Introduction: *Listeria monocytogenes* is a human pathogen that is implicated in several food-borne disease outbreaks. This bacterium can form biofilm on many food industrial working surfaces. The speed of bacterial attachment depends on the cell surface properties which are af fected by several environmental conditions.

Rationale and Objectives: The aim of this study was to determine the effect of the cultivation conditions of the inoculum (BHI agar slants and BHI broth at 5°C and 37°C) on surface properties of *L. monocytogenes* strains by microbial adhesion to solvents (MATS) and to describe the biofilm formation at 5°C. Stainless steel surfaces in BHI broth and UHT milk media were used for the analysis.

Results and Findings: The cells cultivated on the BHI agar surfaces showed more hydrophilic properties at both temperatures than in the broth cultivated ones. The cells had more pronounced electron donor nature in this case.

The number of attached cells was higher in case of BHI broth inoculated by cell from agar surfaces regardless of the cultivation temperature. The number of attached cells increased by time in all experimental conditions. In case of BHI broth the development of biofilm increased after 48h while in case of UHT milk media the development of biofilm decreased. However the difference between the initial (24 h) and final (168 h) cell number of biofilms was 1.2-1.5 log irrespectively of the past of cells and the media where the biofilm was developed.

Conclusions: The history of bacterial cells have great effect on the properties of cell surface and therefore affects the attachment to stainless steel surfaces. The media where the biofilm was formed affects the speed of biofilm growth.

P1-15 Differential Expression of Genes in *Listeria monocytogenes* under Thermo-tolerance Inducing, Heat Shock and Prolonged Heat Shock Conditions

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Introduction: Recent research has shown that bacterial pathogens can exhibit enhanced survival and virulence especially under sub-lethal heating. Thus, in addition to the issue of pathogen decontamination of foods, the physiological response of foodborne pathogens to stress such as heat stress needs careful examination.

Rational: The underlying hypothesis was that *Listeria* monocytogenes elicits unique transcriptomic responses as part of its overall stress adaptation in response to sub-lethal temperature stress. Microarray analysis was performed to identify the differentially expressed genes during heat stress by comparing the transcriptome of L. monocytogenes ATCC 43256 under varying experimental temperature conditions. The four different experimental conditions namely: (i) 37°C (control), (ii) heat shock at 60°C (for 0 minute), (iii) prolonged heat shock at 60°C for 9 min, and (iv) thermo-tolerance inducing treatment at 48°C for 30 minutes followed by exposure to 60°C for 9 min were performed in a calibrated water-bath. The standard operating protocols of The Institute of Genomic Research (TIGR, USA) were followed with slight modifications for cDNA synthesis, labeling, and hybridization.

Results: The transcriptome has very distinct patterns under the three temperature conditions. When L. monocytogenes was exposed to: (i) 60°C heat shock conditions, 91 out of 6347 genes were differentially

expressed, (ii) 60°C for 9 minutes (prolonged heat shock), 80 out of 6347 genes were differentially expressed, (iii) thermo-tolerance inducing conditions (48°C for 30 minutes prior to 60°C for 9 minutes), 71 out of 6347 genes were differentially expressed.

Conclusions: Overall, the results support the original hypothesis that *Listeria* monocytogenes elicits unique transcriptomic responses as part of its stress adaptive response when exposed to sub-lethal temperature stresses. Since temperature is one of the key stressors that is widely employed in the food industry as a "hurdle" to prevent microbial growth or eliminate microbial populations, these results highlight the critical importance of understanding how L. monocytogenes responds to varying temperature ranges.

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P1-16 Effect of NaCl and pH Stresses in *Listeria monocytogenes*

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Introduction: Number of human outbreaks caused by *Listeria monocytogenes* has increased over the last few years. This bacterial pathogen is capable of growing in a wide range of environmental conditions and capable of surviving a variety of food processing conditions or capable of contaminating processed foods. Understanding the stress adaptive responses of *Listeria monocytogenes* that allow it to withstand environmental stresses (especially pH and NaCl) can help in developing effective food processing techniques to produce safe food.

Rational: In this study, seven *L. monocytogenes* strains of different origins (culture collection, dairy isolates, meat isolates) were examined. The organisms' stress response and adaptation to pH and NaCl stresses were examined in broth media using different combinations of pH and NaCl. The results were evaluated using Response Surface Method (RSM) and analysis of variance.

Results: The results showed that only few of the tested pH and NaCl combinations inhibited the growth of this pathogen at optimal growth temperature conditions. These results point out that the studied *L. monocytogenes* strains have the ability to survive pH and NaCl stresses. There were differences in the resistance even among strains from similar origins. Though there were differences in the inhibitory NaCl concentrations, there was no significant difference in the pH range that was effective at inhibiting the strains. Sodium chloride had a significant effect on growth, pH 4 was effective at inhibiting the growth of the pathogen.

Conclusions: There is a need for a comprehensive study to verify these results, using more strains from different origins (especially with extreme salt and acid tolerant strains) to determine an applicable pH-NaCl combination for the food industry. After an examination in Modell-broth media, there is a need for studies in foods with a possibility of growth of *Listeria* monocytogenes causing a potential risk for human health.

P1-17 ceeramTools Molecular Detection Systems for Foodborne Viruses Detection

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Introduction: Foodborne viral infections are a common source of gastrointestinal disease. The proportion of outbreaks attributed to viral agents, predominantly noroviruses, increased regularly every year.

Rational: To meet the needs for the food industries in term of viral risk control, the commercial solutions, ceeramTools molecular detection systems, were developed. The targeted human enteric viruses were norovirus genogroup I and II, hepatitis A and E, rotavirus, enterovirus, astrovirus, adenovirus 40/41, sapovirus, aïchi virus.

Results: One step real time RT-PCR was developed for each viruses. The sensitivity was tested with other human enteric viruses and potential food pathogens. Amplification conditions and reaction mixes were optimized to obtain a high sensitivity with a very easy protocol. A robustness study was performed for each kit. Internal controls, positive and negative controls were developed for each target. The developed methods were then tested on artificially and naturally contaminated matrices (shellfish, fruits, vegetables). Finally, the different ceeramTools molecular detection systems were evaluated by different reference laboratories. Using the ceeramTools kits, a high specificity was observed for each virus. A sensitivity of 1 to 10 genome copies for each virus was observed at a confidence level superior to 95%. Thanks to internal control, negative and positive controls including in the kits, reliable results are obtained in less than 2 h 30 min. The ceeramTools kits were adaptative to different thermocyclors. Using our concentration and extraction methods, positive results were obtained on artificially

contaminated matrices, even at low contamination level. Results obtained on naturally contaminated shellfish during winter 2008-09 using the ceeramTools molecular detection systems will be presented.

Conclusions: Associated with our extraction protocols, the different ceeramTools molecular detection systems allow a rapid detection, identification of foodborne viruses for different types of food in 24 to 48 h depending of the urgency. This constitutes a real advance in food safety control and public health protection.

P1-18 Flow Cytometry Detection of *Escherichia coli* O157:H7 Stressed by Chemical and Physical Treatments

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Introduction: Food processing and preservation treatments can render injured microorganisms which are not detected by conventional plate count procedures. Flow cytometry combined with bacterial viability stains allow detection and quantification of injured cells.

Rational: The objective of this study was to test flow cytometry and fluorescent dyes SYTO9 and propidium iodide (PI) as a method to identify live, injured and dead *Escherichia coli* O157:H7, in potassium citrate buffer suspension, exposed to chemical (sodium benzoate, vinegar, sodium hypochlorite) and physical (freezing, refrigeration, heat and high isostatic pressure) stresses. The technique was compared to plate counting on nonselective medium: tryptic soy agar with yeast extract (TSAYE); and selective media: TSAYE with sodium chloride (TSAYE+NaCl) and sorbitol MacConkey agar (SMAC).

Results: While *E. coli* O157:H7 counts declined from 7.0 to ca. 6.0 log CFU/ml with sodium benzoate (0.1%, 42 h) and vinegar (to reach pH 4.0, 42 h), the organism was totally inactivated by sodium hypochlorite (150 ppm free chlorine, 30 s before sample processing). Heat (68°C, 15 s) caused a ca. 3.5-CFU/ml reduction, whereas high pressure (400 MPa, 2 min, 20°C) decreased counts by 3.0 log CFU/ml. In general, counts on selective media were similar to those on TSAYE, except for the pressure-treated sample, whose counts on TSAYE+NaCl and SMAC were, respectively, ca. 1.5 and 1 log CFU/ml lower than those on TSAYE, which suggests a large injured cell population that agrees with flow cytometry results. SYTO9-PI staining enabled good separation and identification of live, injured and dead bacteria; and gave counts (determined by means of a calibrated microsphere suspension) higher than those estimated by plate counting.

Conclusions: Flow cytometry can improve the knowledge about physiological state of pathogenic bacteria after food processing and preservation treatments.

P1-19 Evaluation of a Rapid Two-day Isolation Method for *Salmonella* Using Oxoid ONE Broth-*Salmonella* and Brilliance *Salmonella* Agar

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Introduction: *Salmonella* is a Gram-negative, rod-shaped, motile bacterium with a widespread occurrence in animals, especially poultry and swine. Additional sources of this organism include raw meat, raw poultry, and raw seafood, to name only a few. Detection is critical as *Salmonella* is the most frequently reported cause of food borne illness (within the USA, 40,000-50,000 cases are reported annually) and the infectious dose can be as low as 1-10 cfu/g. This study evaluated the Oxoid *Salmonella* rapid culture method which combines Oxoid ONE Broth-*Salmonella* and Brilliance™ *Salmonella* Agar in a simple protocol, for the detection of *Salmonella* in food within 2 days.

Rational: 25g samples of minced beef, minced chicken, lettuce, shrimp and shell eggs were inoculated with *Salmonella* serovars at a level of ~1 CFU/25g. Samples were then enriched in Oxoid ONE Broth-*Salmonella* at 42°C for 16–24 h before plating a 10µl loop full onto Brilliance *Salmonella* Agar and incubating at 37°C for 24–26 h. The Oxoid *Salmonella* rapid culture method was compared to either the USDA or FDA established protocols depending on the food matrix. The specificity of the methods was evaluated using multiple *Salmonella* serovars (n = 100) or closely related bacterial species (n = 30).

Results: When selected foods were inoculated with *Salmonella* serovars at a level of ~1 CFU/25g there was no difference in sensitivity between the Oxoid *Salmonella* rapid culture method and the reference methods. When specificity was evaluated 96/100 *Salmonella* serovars were identified using this method and 29/30 of the non-*Salmonella* showed no growth or atypical growth.

Conclusions: Enriching samples using ONE Broth-*Salmonella* and plating on Brilliance *Salmonella* Agar reduced time to detection to as little as 38 h, compared to the FDA and USDA reference methods which

took 4 days. Identification of presumptive positive colonies on Brilliance *Salmonella* Agar can be conducted rapidly using the Oxoid *Salmonella* Latex kit, to give a confirmed result in 2 days.

P1-20 An Improved Medium for the Enumeration of Coagulase-Positive Staphylococci from Foods: Oxoid Brilliance™ Staph 24 Agar

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Introduction: Contamination of foods both pre- and post-production with coagulase positive staphylococci (CPS) is a potential cause of serious food poisoning. Although Baird-Parker Agar with Egg Yolk and Tellurite (BPA) is traditionally the most commonly used medium for the enumeration of CPS from foodstuffs according to ISO 6888-1, the 48 h incubation period and highly variable colony morphology of typical and atypical isolates are widely seen as disadvantages. Oxoid Brilliance Staph 24 Agar, a new chromogenic medium for the enumeration of CPS within 24 h was evaluated as an alternative to BPA.

Rational: Eighty routine food samples covering cooked vegetables, sweets and chocolate, powdered flavourings, meat sauce and cheeses were analysed. Samples were prepared and decimally diluted according to the laboratories standard method before spreading 0.25 ml of 10-1 dilutions over single plates of Acumedia BPA and Brilliance Staph 24 Agar. Inoculated BPA was incubated at 37±1°C for 48± 2 h. Brilliance Staph 24 Agar was incubated at 37±1°C for 24± 2 h. Typical and atypical colonies on BPA and presumptive positive (blue) colonies on Brilliance Staph 24 Agar were sub-cultured onto Plate Count Agar (37±1°C for 24± 2 h) before confirming CPS by the tube coagulase test.

Results: Seventy-four of the food samples were negative for CPS with BPA. Typical colonies were identified from the remaining six samples. These isolates were shown to be coagulase-negative organisms. A single colony of presumptive growth was recorded from one sample with Brilliance Staph 24 Agar, which was identified as a coagulase-negative organism.

Conclusions: Brilliance Staph 24 Agar proved to be a suitable alternative to BPA for the enumeration of coagulase-positive staphylococci within 24 h. It resulted in significantly fewer presumptive positive isolates compared to BPA, which required confirmation, and accurate results were available within 24 hours.

P1-21 ISO16140 Expert Laboratory Evaluation of a Novel Medium for the Enumeration of Coagulase-positive Staphylococci

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Introduction: Oxoid Brilliance™ Staph 24 Agar is a new chromogenic medium for the enumeration of coagulase-positive staphylococci (CPS) from foods within 24 hours. Traditionally, Baird-Parker Agar supplemented with Egg Yolk and Tellurite (BPA) has been used for enumerating CPS. Isolates on BPA are often difficult to interpret because of the presence of both typical and atypical colonies of staphylococci, which both require confirmation.

Rational: Brilliance Staph 24 Agar was evaluated against BPA for the enumeration of CPS from the five identified food categories detailed in ISO16140:2003. Testing was performed according to the method detailed in ISO6888-1:1999, with the exception of Brilliance Staph 24 Agar which was incubated at 37oC for 24 hr. The Expert laboratory evaluation was conducted in full accordance of the quantitative methods validation section of ISO16140:2003.

Results: Brilliance Staph 24 Agar showed good equivalence to BPA with dairy, meat, sea-food, bakery products and composite/ready to eat food samples. Equivalence of the media was not shown with CPS from sugar snap peas. Results for inclusivity, limit of detection and quantification limit (LOD=2, LOQ=4) were equivalent for the reference and alternative method. Exclusivity testing of Brilliance Staph 24 Agar showed it was more specific than BPA, with no false positive results (0/48) compared to BPA, where 13/48 non-CPS gave typical/atypical colonies. Statistical analysis demonstrated that Brilliance Staph 24 Agar showed excellent linearity and accuracy. Linear regression analysis (GMFR and OLS2) demonstrated that the relative accuracy of the reference and alternative method was equivalent (R=0.999) for all food categories, giving an overall regression of (y=0.9918 + 0.0335).

Conclusions: Brilliance Staph 24 Agar proved to be a suitable alternative to Baird-Parker Agar with the five food groups analysed during the Expert lab phase of the ISO16140 validation. Brilliance Staph 24 Agar showed greater specificity than Baird-Parker Agar and gave results within 24 h.

P1-22 Evaluation of a Novel Medium for the Enumeration of Thermotolerant *Campylobacter* spp.

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Introduction: Traditional media for the enumeration of thermotolerant *Campylobacter* spp. often lack specificity and individual colonies are difficult to count, due to the swarming nature of campylobacters and the presence of blood and charcoal in the media. Oxoid Brilliance™ CampyCount Agar is a novel defined medium for the direct enumeration of *Campylobacter* spp. which was evaluated as the first part of an ISO16140:2003 validation of the medium. Colonies of *Campylobacter* spp. grow as distinct dark red colonies against a clear background.

Rational: Brilliance CampyCount Agar was evaluated against modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) for the enumeration of thermotolerant *Campylobacter* spp. from poultry samples, according to the method detailed in ISO10272-2:2006. Both media were incubated at 41.5°C for 40–48 h in a microaerobic atmosphere. The evaluation was conducted in accordance with the quantitative methods validation section of ISO16140:2003. In addition to the confirmation requirements of ISO10272-2, presumptive colonies on Brilliance CampyCount Agar were confirmed using the Oxoid Dryspot Campylobacter Latex kit and O.B.I.S Campy test.

Results: Brilliance CampyCount Agar was shown to have comparable performance to the reference method (mCCDA) in terms of inclusivity, exclusivity and to limits of detection and quantification (LOD=3.3, So=0.309). Statistical analysis of the linearity, in accordance with ISO16140 showed no statistically significant evidence of lack of fit (P = 0.56). Linear regression analysis (GMFR) showed that the relative accuracy of the reference and alternative methods was equivalent (R=0.82, y=-0.22+1.09).

Conclusions: Brilliance CampyCount Agar was shown to be comparable in performance to mCCDA. Colonies of *Campylobacter* spp. were easier to enumerate as they were distinct dark red colonies on a clear background. The Oxoid Dryspot Campylobacter Latex kit and O.B.I.S Campy tests were found to be accurate methods for the confirmation of presumptive growth on Brilliance CampyCount Agar.

P1-23 Advancing Access to Global Food Safety Research Information: The Research Projects Database

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Introduction: The Food Safety Research Projects Database (http://fsrio.nal.usda.gov/advsearch.php) was created by the Food Safety Research Information Office (FSRIO) at the USDA National Agricultural Library (NAL) (http://www.nal.usda.gov/). The FSRIO program is a collaborative project with the USDA Agricultural Research Service.

Rational: FSRIO's vision is to provide a publicly accessible and searchable database that showcases food safety research projects funded by both United States and International government agencies, as well as educational institutions and other private or non-government organizations. The information provided by this database can assist in the assessment of food safety research trends, identification of research gaps and avoidance of unnecessary duplication, as well as provide a valuable tool to the food safety community and policymakers.

Results: The Food Safety Research Projects Database currently provides access to more than 4,000 food safety research projects, and is the largest searchable collection of research conducted among government agencies. The projects are organized by food safety categories, including risk assessment, on-farm food safety, food defense, and sanitation and pathogen control, which capture the broad concept of the research data and are indexed with key terminology from the NAL Thesaurus (controlled vocabulary) developed by experts. These features result in faster access to the information.

Conclusions: The Research Projects Database uses both cutting edge technology and library resources to leverage access to the breadth of food safety information. Future efforts will focus on expanding collaborations with both International and U.S. agencies, further enhancing the collection and establishing it as the central place to access global food safety research initiatives.

P1-24 WITHDRAWN

P1-25 Evaluation of a New Swab-based Test for *Salmonella* on Food Contact Surfaces

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Introduction: *Salmonella* remains a significant cause of foodborne illness around the world, and food manufacturers must take measures to control its presence of within production areas. Traditional

methods of surveillance require samples being sent for laboratory testing, and can take several days for results to be reported, by which time the food product may have been consumed

Rationale and Objectives: The *Salmonella* Detection Transwab (SDT) is a self-contained swab-based kit for the detection of *Salmonella* on food contact surfaces. The kit consists of a premoistened swab, together with a tube of red *Salmonella* Detection Gel. The swab is used to sample the test surface, then placed into the tube of red gel and incubated at 37°C (a small portable incubator would be suitable). *Salmonella* is confirmed within 24 hours by the development of a black colouring around the swab, eventually spreading through the whole gel. Early development of the black colour is clearly seen at the tip of the conical base of the tube, and can allow detection of *Salmonella* in less than one day.

This study was designed to show the performance of the device, firstly by challenging with known dilutions of *Salmonella* Enteritidis, *Salmonella* Typhimurium, and also *Citrobacter freundii*, an organism which gives false positive reactions in some *Salmonella* media. In addition, known amounts of *Salmonella enteritidis*, *Salmonella* Typhimurium, and also *Citrobacter freundii*, together with various other species of bacteria were spread onto food grade stainless steel plates, before sampling with the swab.

Results and Findings: In both experiments *Salmonella* was clearly detectable by the presence of a black colour in the gel at both 24 and 48 hours, while there was no change with other species, including Citrobacter.

Conclusions: It is concluded that SDT could offer a suitable method for the detection of *Salmonella* in food production facilities.

P1-26 Comparison of a Self-contained *Listeria* Detection Test against USDA and ISO Reference Methods

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Introduction: *Listeria monocytogenes* is a life-threatening foodborne pathogen that causes much illness and many deaths (up to 500 annually in United States). It is important for food manufacturers to have effective control measures in place, including measures for testing food contact surfaces.

Rationale and Objectives: The present study was designed to measure the effectiveness of the *Listeria* Isolation Transwab, a self-contained swab-based test kit that can be used on site, and to compare its performance with two traditional reference methods (USDA and ISO)

The *Listeria* Isolation Transwab (LIT) includes a dry swab, together with a tube of straw coloured gel medium. The swab is rubbed across a test surface, inserted into the gel and incubated at 37°C for 24–48 hours. Blackening of the medium indicates a positive result due to aesculin hydrolysis. Both the reference methods require primary and secondary enrichment stages in the laboratory before a final result is obtained.

For this study, a number of organisms known to hydrolyse aseculin were used. Suspensions of each organisms at different dilutions were used to inoculate either the LIT, swabs which were tested according to the two reference methods.

Results and Findings: LIT showed equivalent sensitivity to the two reference methods for *Listeria monocytogenes*, with a minimum detection level of 15 cfu per swab. Other *Listeria* species were also detectable at low levels. In contrast false reactions were only detectable for very high levels of other aesculin hydrolysers, such as Enterobacter aerogenes (over 43000 cfu).

Conclusions: From the study, LIT appears to be capable of alerting users to the presence of low levels of *Listeria monocytogenes*.

P1-27 Prevalence of *Salmonella* spp. in Porcine and Bovine Raw Meat and By-products in Southern Germany

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Introduction: Salmonellosis is, after Campylobacteriosis the second main cause of bacterial human enteritis in Germany. *Salmonella* spp. is known to colonize the gastrointestinal tract of animals without producing any clinical or pathologic-anatomic signs. Therefore, carcasses of asymptomatic animals can be contaminated with *Salmonella* spp. at the time of slaughter. Contaminated raw or undercooked meat is considered an important factor in transmitting this food-borne pathogen. Thus, this study was undertaken to contribute to the understanding of the actual risk potential of raw meat and by-products originating from pigs and cattle. A further aim was to find out if a seasonality could be observed.

Rational: From March 2008 to January 2009, a total of 4172 beef and pork raw meat samples and by-products were tested qualitatively for *Salmonella* spp. using the VIDAS system. Positive samples were confirmed by isolation of the agent with cultural methods. The samples, composed of 1368 beef and 2804 pork samples, were obtained from seven different slaughterhouses in Southern Germany.

Results: The overall prevalence of *Salmonella* spp. in pork and beef was 1.1% and 0.1%, respectively. The highest contamination rate of porcine samples was found in tongues and livers with 5.0% and 4.5%, respectively. The prevalence of *Salmonella* spp. in pork carcasses amounted to 1.1%, while no *Salmonella* spp. could be found in porcine kidneys and lungs. As for the bovine samples, *Salmonella* spp. were isolated only from tongues at a rate of 2.2%. With the exception of May, June and July, positive porcine samples were detected all over the year, observing a slight seasonal increase in the colder months. From bovine samples Salmonella spp. were isolated only in June.

Conclusions: Although the *Salmonella* spp. prevalence found in this study was relatively low compared to previous surveys, the risk of transmitting this pathogen cannot be neglected in terms of preventing food-borne zoonoses.

P1-28 Comparison Study to Demonstrate the Equivalence of the SimPlate Total *Campylobacter*-CI Method to the Reference Culture Method for the Enumeration of Total *Campylobacter jejuni* and *Campylobacter coli* in Food

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Will be presented by Klaus Lobeck

Introduction: The SimPlate for Campylobacter Color Indicator (C-CI) method allows for the quantitation of total *Campylobacter jejuni* and *Campylobacter coli* in poultry meat and poultry meat rinses after 48 to 52 h of incubation in a microaerophilic environment.

Purpose: A study was undertaken to compare the SimPlate C-CI method to the reference culture method for the quantitation of total *Campylobacter jejuni* and *Campylobacter coli*.

Methods: Target *Campylobacter* and non-target microorganisms were tested for inclusivity and exclusivity by the SimPlate method. 37 strains of *C. jejuni* and *C. coli* were enriched in Bolton broth, diluted and plated onto SimPlate devices and 3 selective agar plates (Abeyta-Hunt-Bark (AHB) agar, Campy CEFEX agar and Line agar). Finally, a field trial comparison of the performance of the SimPlate C-CI method to the Campy CEFEX method was performed. Lab personnel at 3 poultry processing plants analyzed 168 BPW carcass rinse samples with both methods.

Results: There was good correlation for the quantitation of *Campylobacter* from all three plating methods to the SimPlate method; only 2 strains for AHB and 1 strain for Line agar demonstrated greater than 0.5 log difference between both methods. For exclusivity, the C-CI method detected none of the 27 non-target organisms tested. Regression analysis of the results from the field trial comparison showed a correlation of 0.96.

Significance: These results indicate that the SimPlate C-CI method and the reference culture method are comparable for enumeration of *Campylobacter jejuni* and *Campylobacter coli* in poultry meat and poultry meat rinses.

P1-29 Detection of Shiga Toxin-producing *Escherichia coli* (STEC) with the Assurance GDS for STEC assay

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Will be presented by Klaus Lobeck

Introduction: Recent reports of illnesses caused by food-borne non-O157:H7 Shiga Toxin-producing *Escherichia coli* (STEC) have led to increased awareness of their threat to public safety.

Purpose: A rapid screening assay has been developed to detect *E. coli* STEC isolates with the following O-serotypes: O26, O45, O103, O111, O121, O145.

Methods: Immunomagnetic beads are employed to specifically isolate and concentrate bacteria that express these O-antigens during a sample preparation step. DNA from the samples is then amplified and identified using primers and probes directed against conserved, specific, virulence-associated DNA sequence targets in these bacteria.

Results: The assay was able to detect 30/31 *E. coli* strains that expressed one of the O-antigens in question. The one undetected strain did not contain either the stx1 or stx2 gene and is not considered a STEC. An additional 40 bacteria, including 15 *E. coli* strains that express different O-antigens, were not detected.

Significance: The data show that the combination of an immunomagnetic sample preparation step and a specific DNA amplification-detection step yield a screening assay specific and sensitive for the top 6 *E. coli* STEC strains known to cause human disease.

P1-30 Development and Validation of a Real-time PCR Assay for Detection of *Mycobacterium avium* subsp. *paraturberculosis* from a Range of Dairy Matrices

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Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (Map) has concerned the dairy industry by reports of its possible association with Crohn's disease in humans. The 'gold standard' for Map detection remains isolation in culture, however, due to its slow growth rate efforts have been made to use quantitative PCR methods to detect Map in dairy products, particularly milk.

Rational: A key factor in the development of a PCR assay for the detection of Map from dairy matrices is the use of an effective method to recover Map from the matrix and subsequently extract DNA from the cells. The objective of this study was to develop and assess the potential of real time PCR assays coupled with a standardized magnetic bead-based Map DNA extraction method, to quantify Map in a range of dairy products including milk, yogurt and a range of cheeses.

Results: Map DNA extracted from homogenized cheese and milk samples using the Adiapure® kit (Adiagene, France) was subjected to two independent TaqMan®-based real time PCR assays targeting different gene sequences. The IS900 based real time assay was more sensitive (approx. 10-fold) than the assay targeting the F57 sequence, detecting < 4 CFU ml^{-1} in artificially contaminated milk and < 30 CFU g^{-1} in spiked cheese and milk powder samples. In an EU organized dairy products ring trial, involving 12 laboratories, average sensitivities of the prescribed Adiapure/IS900 TaqMan®-based qPCR method across a range of artificially contaminated dairy matrices were 85–100%. Specificities for all matrices were in the range 95–100%.

Conclusions: The real time PCR assay combined with the Adiapure Map-DNA extraction kit represents a reproducible, sensitive and convenient method for detection of Map DNA from a range of raw and pasteurized dairy products. Its robustness has been confirmed through a ring trial within the FP6 EU ParaTBTools project.

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P1-31 A Method for the Laboratory-Scale Manufacture of UK Semi-Hard and Hard Type Cheeses from Milk Contaminated with *Mycobacterium bovis*

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Introduction: Although the UK has established control and monitoring mechanisms for tuberculosis in cattle the prevalence of tuberculosis amongst dairy herds continues to increase. Concomitantly there is a growing market for artisanal cheeses produced from raw milk. The absence of a pasteurisation process removes a major barrier to possible Mycobacterium bovis contamination of raw milk cheeses. A link between M. bovis zoonotic infections and the consumption of raw milk and related products has been established.

Rational: Currently a lack of data exists regarding the survival kinetics of *M. bovis* during the manufacture, ripening and storage of raw milk cheeses. To address this knowledge gap it was essential to devise a protocol for the production of semi-hard (Caerphilly) and hard cheese (Cheddar). The cheesemaking procedure must satisfy stringent health and safety criteria regarding manipulation of Hazard Group 3 organisms and produce on a laboratory-scale cheese comparable with commercial products.

Results: The devised procedure mimics the cutting and stacking of curd, traditionally used to develop the characteristic texture of cheddar cheese. Equipment developed facilitates the application of constant, measurable and reproducible pressure during pressing. All cheesemaking manipulations can be conducted within the confines of a Class I safety cabinet. Containment measures allow for the safe collection of whey and subsequent disposal. Cheddar and Caerphilly cheeses prepared from raw milk artificially contaminated with M. bovis have been produced and *M. bovis* has been enumerated on selective media post manufacture.

Conclusions: This procedure allows the investigation of *M. bovis* survival kinetics in raw milk cheeses. Furthermore the technique could be used with other Hazard Group 2 & 3 pathogens and adaptation is possible for production of alternative cheese types. As a result this protocol is a tool for the production of microbiologically contaminated cheese which can facilitate investigation of food protection relating to cheese.

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P1-32 Qualitative Methods for the Detection of *Listeria monocytogenes* and Other *Listeria* Species – Strategies for Comparison Studies

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Introduction: Current approved methods allow the detection of the food pathogen *L. monocytogenes* from various food matrices. For the detection three general procedures can be described: (1) direct plating on selective media (2) immediate selective enrichment (3) pre-enrichment steps. Of course the choice of the "ideal" method for specific purpose is dependent on different parameters (e.g. number and stage of microorganisms, inhibitory aspects of the media, incubation time and conditions). On the other hand the treatment of samples for efficient detecting of especially low levels and inhomogeneous spreading of microorganisms is also essential.

Rational: The preparation and design for the comparison of qualitative methods based on different methodologies for detection of the emerging food pathogen *Listeria monocytogenes* and other *Listeria* species is described. The matrices were naturally contaminated and sampled in different food factories (seafood, cabbage, ready-to-eat food) and therefore reflect the real situation.

Results: This protocol was successfully applied for comparison studies with VIDAS® LDUO (bioMérieux), IQ-Check® *Listeria* (Biorad), and BAX® *Listeria* PCR (Dupont) run in parallel with the reference method according to ISO 11290:1. It allows the evaluation of these methods by the use of current parameters as specifity, sensitivity, false-positive and false-negative rate for alternative methods.

Conclusions: For estimating the prevalence of *Listeria* spp. and *Listeria monocytogenes* e.g. in neuralgic areas in the food production and in food products it is essential to combine methodologies for an effective detection. The problem of obtaining a representative sample of food or environment is persistent due to uneven spread of contamination. Comparison studies with alternative methods are difficult to perform because they rely on different detection principles (e.g., monoclonal antibodies, virulence genes, enzymes, and ribosome) and the sensitivity is rising with upcoming new methods. Even though the analyzing time and information content about the presence of *Listeria* can be fulfilled with the use of alternative methods.

P1-33 Environmental Monitoring for Noroviruses in UK Food Outlets

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Introduction: Noroviruses are the commonest cause of acute gastroenteritis outbreaks world-wide. Norovirus outbreaks are frequently associated with semi-closed or closed institutions such as hospitals, and homes for the elderly. However, outbreaks have also been associated with eating establishments, cruise ships, a concert hall and schools.

Rational: A proposed route of transmission is cross-contamination from hard surfaces such as fridge door handles to food samples. This study investigated the potential cross-contamination of toilet to food contact surfaces in food businesses. Environmental swabs were taken from to sites within toilet facilities in food outlets (e.g., toilet flush handle, toilet door handle, wash basin taps) and kitchens (e.g,. fridge door handles and other food preparation sites). Nucleic acid was extracted using the guanidinium thiocynate method. Norovirus RNA was identified using real-time PCR, genotyped by PCR and sequencing.

Results: Norovirus was detected in 40/193 (20%) swabs including: 10 refrigerator door handle, 3 food preparation sites, 7 toilet door handles, 12 wash hand basin taps and 8 toilet flush handles. Norovirus was typed as genogroup II (GII) in 39/40 and 1/40 genogroup I (GI). Strains were further differentiated as GII-4 (17.5%), GII-3 (7.5%), GII-UT (Untyped) (72.5%) and GI-4 (2.5%). The seven GII-4 strains were variant typed, with 2/7 v2 strains, 4/7 v6 and 1/7 was vUC (variant unclassified).

Conclusions: The findings indicated that food businesses without a HACCP in place, poor food safety management were more likely to have norovirus present and that environmental swabbing is an effective means of monitoring during outbreak investigations.

Acknowledgements: The authors would like to thank the Environmental Health Services at Local Authorties in London, Hertfordshire and Buckinghamshire for providing the samples.

P1-34 Development of Mathematical Model to Predict the Outgrowth of *Listeria monocytogenes* in Ready-to-Eat Food Products

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Introduction: EU regulations state that the food industry can use predictive models to document that outgrowth of *Listeria monocytogenes* in RTE foods is controlled (EC 2073/2005). The Codex Alimentarius Commission recently agreed on similar criteria. The development of reliable models, based on challenge studies in food products using outbreak strains therefore continues to be important.

Rational: The objective of this study are to: (1) Develop a mathematical model for *Listeria monocytogenes* that predicts its potential for growth in food products as a function of temperature, pH, water activity and the concentration of organic acids and their salts. (2) Demonstrate the efficacy of lactic acid, acetic acid and their salts against *Listeria monocytogenes* and spoilage microorganisms in food products.

Results: The model shows that the addition of lactic acid, acetic acid or their salts to food products significantly retards the outgrowth of *Listeria monocytogenes*. Addition of 0.75 % PURASAL Powder S98 to a Carbonara type of pasta sauce (pH 5.6, moisture 80 %, a_w 0.986) was simulated at 95 % confidence level. At 7°C, for the treated sample, the time for 2 log growth for *Listeria monocytogenes* was increased from 3 days to 8 days compared to that of control. The developed model describes the data very well, including independent challenge studies, and generally gives fail safe predictions.

Conclusions: The developed model gives reliable predictions of the potential for outgrowth of *Listeria monocytogenes* in RTE foods. This model can be used by food processors to evaluate how they can control *Listeria monocytogenes* by addition of lactic acid, acetic acid or their salts to their formulations, and by adequate temperature control.

P1-35 The Inhibitory Effect of the Lactoperoxidase System on the Survival of *Campylobacter jejuni* in Pasteurized Skimmed Milk Incubated Aerobically at 25°C

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Introduction: Campylobacter jejuni and *Campylobacter coli* are the most common bacteria isolated in animals and humans suffering from diarrhea. These organisms can also be found in animals which are clinically healthy. The most common alimentary sources of *Campylobacter* infection include undercooked meat, especially chicken, contaminated water and unpasteurized or raw milk.

Rational: The effects of storage temperature on the survival of *C. jejuni* were examined in pasteurized and UHT skimmed milk. It was found that, *C. jejuni* NCTC 11168 survived for more than 5 days at 4°C in both UHT and pasteurized milk. At 25°C it survived for 3 days in UHT milk but was not detectable after 6 h in pasteurized milk. *C. jejuni* was found to die at the same rate in pasteurized milk that had been filter sterilized to remove the residual micro-flora. This result indicates that the antimicrobial effect was not due to the natural flora but to a natural component in the milk.

Results: The inhibitory action of pasteurized milk was prevented by boiling and by addition of 1 mM of sodium meta-bisulphite (an inhibitor of lactoperoxidase) suggesting that lactoperoxidase in the pasteurized milk was responsible. This was confirmed by restoration of the inhibitory effect in UHT milk by addition of components of the lactoperoxidase system.

Conclusions: A similar effect was not seen with other pathogens such as *Salmonella*, *Listeria monocytogenes* and *E. coli* O157 indicating the unique sensitivity of *Campylobacter* to the lactoperoxidase system.

P1-36 Rapid Isolation and Detection of *Salmonella* serovars from Pre-enriched Pooled Food Samples using an Automated PATHATRIX Re-circulating IMS (RIMS) Coupled to, Real-Time PCR and Selective Agar Plating

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Introduction: Re-circulating Immuno-magnetic Separation (RIMS) is well established as a robust and versatile approach to pathogen isolation from food and environmental samples; combining straightforward analysis of large samples and removal of potential PCR inhibitory compounds with the ability to detect initially low levels of target pathogens.

Methods: A range of food samples including peanut butter, confectionary and cocoa products, fresh produce, tomatoes, almonds and milk powders of various sample sizes (25g–1875g) were inoculated individually at low level, (1–10 CFU/sample), with a range of *Salmonella* serovars. Samples were pre-enriched for 8–18 h as appropriate to sample size and food type. After pre-enrichment, identical duplicate pooled samples were created; consisting of single aliquots from inoculated samples and 4-9 aliquots from uninoculated samples. Pooled samples were processed in parallel using a novel automated RIMS device alongside manual RIMS. Recovered Pathatrix beads were analyzed using real time PCR and selective agar plating as the Salmonella detection methodologies.

Results: The detection of a range of *Salmonella* serovars from wide variety of pooled food samples using Pathatrix RIMS linked to agar plates and /or real time PCR was achieved. There was 100% correlation between the recovery and detection of target organisms by the automated and manual RIMS systems. For peanut butter a detection level of 1–10 CFU in 1875 g for all *Salmonella* serovars tested was achieved in less than 24 h.

Conclusions: The study showed that it is feasible to fully automate the Pathatrix RIMS procedure for use in a wide variety of food types, with no loss of bead recovery or target pathogen capture functionality. This RIMS method provides the capability to quickly identify sources of *Salmonella* contamination in both routine food pathogen surveillance regimes or in outbreak scenarios.

P1-37 Rapid Detection and serotyping of *Salmonella* isolates

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Introduction: Serotyping is a classification system based on differences in structures on the surfaces of bacteria or other disease-causing agents. Serotyping divides Salmonella into more than 2500 different serotypes. Salmonella serotyping is an important tool for classification of strains, epidemiological purposes and the id entification of contamination sources. Successful Salmonella reduction programs within the food industry rely largely on information about the serotypes encountered in the production chain.

Rationale and Objectives: Traditional serotyping, according to the Kauffmann-White scheme (KW), makes use of a range of antisera, directed against the antigens present on the cell surface of the Salmonella. The combination of antigens present determines the serotype. Correct execution of the technique requires a lot of experience and it is a time and resource consuming activity.

Results and Findings: The PremiTest Salmonella is a rapid and robust alternative for traditional serotyping. The technique combines multiplex PCR and subsequent simultaneous detection of the PCR products using a micro array. The Premitest Salmonella uses multiple DNA markers to detect the presence of Salmonella and identify the serotype. Currently, 94 serotypes are being identified by the t e st, based on the genetic pattern generated (genovar). Serotypes not recognized by the test yield other, reproducible genovar scores. These genovar scores may be associated with specific serotypes lateron, but already at this stage allow tracking and tracing of the contamination.

The complete procedure can be executed within 7 hours, enabling a serotype result within one working day after isolation. The test is being used by a range of laboratories, including reference laboratories, and yields reproducible results after a 2-day training.

The test has proven to be independent of the culture media used and is capable of typing rough Salmonella strains (in contrast to KW). The process to obtain external certificates is in progress: The last part for the OIE-validation is completed and the certificate is expected end 2009. The protocol for joint AOAC & MicroVal validation is being finalized.

The Premitest Salmonella has been designed for food processors, as routine use. However, the set-up of the test enables the inclusion of specific DNA markers. In this way, more detailed knowledge on specific serotypes or characteristics (such as antibiotic resistance and the DT104) can be obtained.

Conclusions: The PremiTest Salmonella is a good tool for official labs to get fast and reliable serotyping and the best routine tool for the food industry for serotyping *Salmonella* isolates. It is inevitable in surveillance and outbreak related studies.

Poster Session 2 – Friday, 9 October

P2-01 Stability of Calibration Function (Standards) in Nucleic Acid-based Food-pathogen Detection

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Introduction: Results of real-time PCR depend on a calibration function when using the Ct-method for quantification and the accuracy of this standard is all-important. Long-term storage of standards is saving cost and time, avoiding laborious production on a daily basis in routine application. Nevertheless, aqueous solutions of DNA are prone to degradation during storage.

Rational: The aim of this study was the determination of the causative parameter of DNA-standard degradation and the underlying mechanism impairing the amplification reaction during real-time PCR. Real-time PCR assays targeting the prfA gene of *Listeria* and the fimA gene of *Salmonella* have been used to investigate the influence of long-term storage (>100 d), the GC-value, shear forces, DNA target length, chemical reactions within the storage buffer, glycerolstorage, subsequent thaw and freeze cycles, and the influence of remaining cellular enzymes after isolation, at -20°C, ±0°C, and 4°C. Tests were performed for initial low and high DNA target numbers.

Results: The stability of DNA-standards is influenced by shearing of long DNA fragments (bacterial genomes) if the standard is frozen. Short fragments (~100 bp) are not influenced by shearing during long-term storage or by subsequent thaw and freeze cycles. Depurination of the DNA and following mismatches on the primer attachment sites are biasing real-time PCR results if DNA is stored at 4°C or with glycerol. This effect is increased by primary amines such as Tris or by Mg-ions as included in the PCR buffer. By finally testing the resulting benchmark treatment for DNA-standard storage a deviation of 0.2 Ct-values was obtained by real-time PCR after 100 days storage in H_2O containing 50% glycerol.

Conclusions: Preservation of DNA-standards in 50% glycerol in ddH_2O enables long-termstorage for real-time PCR. Depurination and shearing of the DNA are avoided, thus providing reliable results using the Ct-method for quantification.

P2-02 Application of a Novel Single Bacterial Cell Manipulation Technique

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Introduction: Highly diluted suspensions of bacterial cells are distributed according to Poisson-distribution. Quantitative microbiological methods (Most propable number MPN) are based on this prerequisite. Nevertheless, predictions on the growth performance of single bacterial cells are not possible due to these statistical effects. A new method avoiding these influences was developed which enables the physical manipulation of single bacterial cells. Based on this method the investigation of autonomous growth of low bacterial cell inocula (< 10) was performed.

Rational: The purpose of this study was to evaluate the growth performance of single bacterial cells without the influence of statistics within dilution series. Furthermore the necessity of chemical and physical cell to cell interactions for bacterial growth was investigated. *Listeria monocytogenes* EGDe bacteria from the lag-phase, the mid-exponential phase, and the stationary phase were used to produce single cell inocula with the newly developed single bacterial cell manipulation technique (SBCM). Growth in tryptone soy broth with 0.6% yeast was evaluated after 24 hours at 37°C or 42°C by measurement of optical density and selective- and non selective plating.

Results: For 110 manipulated single cells growth was detectable in 79 samples (71.5%) with a final optical density of 1.21×10^9 CFU/ml (± 9.07%). In 31 samples (28.5%) growth was not detectable. The live/dead ratio of the initial culture was 20.9% (± 20.6 %) as obtained by live/dead staining. These results show a good correlation of live/dead ratios before and after the SBCM indicating the ability of autonomous growth.

Conclusions: These data suggest that the investigated single bacterial cells are able to multiply independently under optimal conditions.

P2-03 *Micrococcus roseus* and *Serratia marcescens* as Coloured Bacterial Indicators: A Simple Strategy during Design and Development of a New Method for Sample Pre-treatment

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Introduction: In the present study, chromogenic (red) bacteria were used to simulate actual target bacteria during setup and optimisation of an isolation process of bacteria, designed for food samples.

Rational: Isolation of bacteria from food in the context of molecular biological detection of food pathogens is a multistep process. Development of such a separation method requires continuous monitoring of the location of the presumable targets in the sample tubes. Therefore, red-coloured pigmented bacteria were used as substitutes for the actual target bacteria, during the establishment of a new sample preparation technique. Visibility of the pigmented bacteria within the complex sample matrices served to allocate bacterial content during the various steps necessary for finalisation of the method protocol. Prior to application, the chromogenic bacteria *Micrococcus roseus* and *Serratia marcescens* were confirmed to withstand the physical (e.g., centrifugal forces) and chemical (e.g. lysis buffer composition) conditions required during establishment of the new technique.

Results: The suitability of these model bacteria to substitute for the actual target pathogens (*Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Listeria monocytogenes*) was assured by testing the physical properties of the model bacteria with respect to the proposed separation methods.

Conclusions: The use of these pigmented bacteria as substitutes for actual colourless target bacteria during design and development of a bacterial isolation method is a simple and inexpensive application. The presumptive bacterial targets can be allocated simply by visualisation of their bright red colour silhouetted against the background sample matrix. Application of coloured bacterial indicators saves a huge amount of time and resources, as the proof of principle of new methods is possible in rapid succession.

P2-04 Statistical Data Analysis of Real-time PCR Results Derived from Single Copy Amplification

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Introduction: The validation of real-time PCR systems and above all the proof of the detection limit of this method is a frequently and intensively discussed topic. We present a statistical method for the accurate determination of DNA amounts < 10 target molecules using real-time PCR. The implication of this method is the possibility of distinct validation of real-time PCR assays and the generation of absolute DNA standards needed for quantification with this enzymatic method in routine diagnostics.

Rational: The purpose of this study was to evaluate a novel validation tool for real-time PCR assays based on the theoretical possibility of the amplification of one single DNA target. The ability to detect such low DNA target concentrations reliable by real-time PCR should be clearly demonstrated. Consequent a validation method based on this pre-requisites should be established which allows the absolute evaluation of real-time PCR assays. Real-time PCR was carried out by targeting a 274 bp fragment of the prfA gene of *L. monocytogenes*. Fit of the empirical data to the theoretical predictions was tested using the Kolomogorow - Smirnov (K-S) test using the SPSS 14.0 statistical software package.

Results: The ability of the prfA real-time PCR assay to detect reliable one target molecule could be clearly demonstrated (pavg.=0.52). The coherence of the results of samples containing < 10 target molecules and samples containing DNA amounts within the range of fluorescent measurement could be clearly demonstrated. The evidence for the accuracy of the newly developed validation-method was shown both statistically and with direct demonstration. The explicit determination of assays with a detection limit of one copy and assays with such a limit of three copies is exemplary demonstrated. We also demonstrate that real-time PCR at best starts from the first cycle with certain efficiency and proceeds with this efficiency until saturation of the reaction.

Conclusions: The results show that an absolute validation of real-time PCR assays is possible. The Ct - values of certain initial target amounts are fixed in dependence of the efficiency of the reaction. An absolute determination of DNA amounts is possible independent of conventional measurement methods. The validation tool also allows on-line monitoring of real-time PCR results in routine diagnosis.

P2-05 The Application of Ionic Liquids for separation and Concentration of Foodborne Pathogens from Food for Subsequent Molecular or Cultural Quantification Methods

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Introduction: Due to the implementation of critical pathogen levels, direct quantification of food-borne pathogens from food is going to become standard in food risk analysis. Until now major challenges for biomolecular detection and quantification (such as real-time PCR) of food-borne pathogens are heterogeneous food matrices and large sample quantities. Therefore a major research topic is the development of sample treatment methods prior to subsequent molecular detection and quantification methods, which allow the separation of the target organisms from the sample matrix. Because of their unique physicochemical properties, ionic liquids offer a promising new approach contrary to classical microbiology.

Rational: The purpose of this study was the development of a new sample treatment method for quantification of food-borne pathogens enabling subsequently both molecular and cultural methods for detection and characterization. Several buffer compositions including ionic liquids were tested for their ability dissolving various edibles, without affecting the bacterial target cells. The toxicity of these buffers towards *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium were investigated. Quantification of both pathogens from artificially contaminated food samples was quadripartite carried out by either real-time PCR targeting the prfA-gene for *L. monocytogenes*, the fimA-gene for *S*. Typhimurium or selective plating methods, respectively.

Results: The application of 1-ethyl-3-methylimidazolium thiocyanate to the lysis buffer system enabled the quantifiable isolation of *S*. Typhimurium and *L. monocytogenes* from different artificial contaminated foodstuffs with decreasing inoculums ranging between 10^5 to 10^2 cells. Recovery for *S*. Typhimurium on selective agar plates varied between 45% (RSD 6%) out of 6.25 g egg and 36% (RSD 19%) out of 6.25g ice-cream. *L. monocytogenes* was recovered with 67% (RSD 26%) from 12.5ml milk and for both pathogens real-time PCR quantification resulted in higher (1.5–2 fold) bacterial equivalent counts in comparison with CFU determination.

Conclusions: Application of ionic liquids permits the separation of food-borne bacterial pathogens from the food of interest for subsequent quantification both with real-time PCR and culture methods. Quantitative results can be obtained within one working day using the new buffer system.

P2-06 Food Safety through a Community of Practice in eXtension

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Introduction: The eXtension Food Safety Community of Practice is unlike any other search engine or information-based website. It's a space where university content providers can gather and produce new educational and information resources on wide-ranging topics. Because it's available to students, researchers, clinicians, professors, as well as the general public, at any time from any Internet connection, eXtension Food Safety Community of Practice helps solve real-life problems in real time. Food Safety is only one of many Communities of Practice that have been established through eXtension.

Rationale: The Food Safety Community of Practice is set up to supply research based Food Safety information to individuals throughout the food chain, from the food grower to the consumer. The Community of Practice is not limited by walls or state and national boundaries but can supply the best information from the top scientist in the field.

Objectives: To bring together food scientists from across the county to deliver research based information to individuals all over the world. The Objective is to bring together food scientists from across the county to deliver research based information to individuals all over the world.

Results and Findings: The Food Safety Community of Practice is made up of Food Scientist from all over the United States. The Food Safety Community of Practice was started by a group of Food Scientist in the Southern Region of the United States. The core leadership is made up of Ph.D. Food Scientist from 5 states. There are at present almost 50 members that participate in answering questions and supplying content to the site. We are actively recruiting more members to participate in the site to supply content. The eXtension website has been recognized by federal granting agencies as a method to bring content to the end user.

Conclusions: Food Safety Community of Practice (CoP) is a newly formed group within eXtension. We plan to bring in new participants and form smaller working groups to work to provide food safety content in their specialty area. The groups that we have established to work within at the present are:

Consumers, Producers, Processors, and Food Service Workers. More groups may be added as interest and expertise is added to the CoP.

Acknowledgements: eXtension

P2-07 Predictive Modeling of Deoxynivalenol Content in Dutch Winter Wheat

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Introduction: Wheat derived feed and food products can be contaminated with mycotoxins produced as a secondary metabolite by a variety of fungi, in particular Fusarium species, causing a potential risk to human and animal health. Deoxynivalenol (DON) is the most implicated mycotoxin associated with Fusarium head blight (FHB) in wheat. Forecasting models for the DON content in wheat at harvest can assist decisions on disease management but can also be useful tools for control authorities and industry to limit potential feed and food safety problems.

Rationale and Objectives: The objective of the current study was to develop a quantitative predictive model for DON content in Dutch winter wheat based on geographic, agronomic and climatic variables.

From 2001 to 2007 winter wheat samples for DON analysis were taken at harvest from in total 264 fields throughout the Netherlands. Geographic, agronomic (resistance, fungicides)and climatic variables (for 48 days period around heading) were recorded for each field. After a univariate pre-selection of variables, multiple regression models were constructed (excluding year) and the model with best set of explanatory set of variables was chosen.

Results and Findings: The best performing predictive model used average 24 days pre- and post-heading climatic variables, heading date, region, variety resistance level, and fungicide use ($P < 0.0001$, R_2 model = 0.59). The predicted DON level increased with higher average temperature, increased precipitation and higher relative humidity, but decreased with increased number of hours with temperature above 25°C. Model evaluation showed little bias and high consistency indicating good statistical performance. In 92.8% of the cases (n = 264) the model predicted correctly whether the concentration of DON was lower or higher than the maximum level of 1250 µg/kg (1.1% false positives, 6.1% false negatives).

Conclusions: We developed a statistically good performing forecasting model for DON in Dutch winter wheat, including agronomic, geographical and climatic variables. We observed a strong regional effect on the levels of DON, which could not be explained by differences in the recorded agronomic and climatic variables. It is suggested that future model improvement might be realized by indentifying and quantifying the mechanism underlying the region effect.

Acknowledgements: This work was financed by the Food and Consumer Product Safety Authority (VWA). The authors thank Don Jansen, Huub Schepers and Harro Spits (Plant Science Group, Wageningen UR) for their contributions.

P2-08 Quantitative Microbial Risk Assessment for *Escherichia coli* O157:H7, *Salmonella* and *L. monocytogenes* in Leafy Green Vegetables Consumed at Salad Bars

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Introduction: Fresh vegetables play an important role in a healthy diet. However, most produce is grown in a natural environment and is therefore vulnerable to contamination with pathogens from multiple sources. In Europe and US both the consumption of fresh vegetables and the number of foodborne disease outbreaks associated with the consumption of fresh produce have recently increased. Several outbreaks of foodborne illness have been associated with consumption from salad bars.

Rationale and Objectives: Temperature is one of the most important environmental parameters from both the food quality and food safety point of view. Respecting the chilled chain is of particular impor tance for fresh produce because of the absence of thermal treatment prior to consumption.

The purpose of this study was to conduct a quantitative microbial risk assessment for *E. coli* O157:H7, *Salmonella* or *L. monocytogenes* infection from consumption of leafy green vegetables based salad from salad bars in the Netherlands. Pathogen growth was modeled in Aladin (Agro Logistics Analysis and Design INstrument), using time-temperature profiles in the chilled supply chain and one particular restaurant with salad-bar. A second-order Monte Carlo risk assessment model was constructed (using @Risk) in order to estimate the public health effects.

Results and Findings: The temperature in the cold-chain was well controlled below 5°C. Growth of *E. coli* O157:H7 and *Salmonella* was minimal (+17% and +15%, resp.). Growth of *L. monocytogenes* was considerably more profound (+194%). Based on first order Monte Carlo simulations, the average number of cases per year in the Netherlands associated the consumption leafy green based salads from salad-bars was 166, 187 and 0.3, for *E. coli* O157:H7, *Salmonella* or *L. monocytogenes*, respectively. The range of average number of annual cases as estimated by second order Monte Carlo simulation (with prevalence and number of visitors as uncertain variables) was 42-551 for *E. coli* O157:H7, 81-281 for *Salmonella* and 0.1–0.9 for *L. monocytogenes*.

Conclusions: This study presented a successful integration of modelling pathogen growth in the supply chain of fresh leafy vegetables destined for restaurant salad bars. We conclude that the temperature in the cold-chain was fairly well controlled and that growth of *E. coli* O157:H7 and *Salmonella* was minimal. Growth of *L. monocytogenes* was considerably more profound but due its low virulence not considered problematic. The estimated number of annual cases were considered reasonable in relation to epidemiological data and in proportion to an earlier risk assessment considering the entire consumption of fresh vegetables.

Acknowledgements: This study was financed by the Dutch Ministry of Agriculture, Nature and Food Quality.

P2-09 Consumer Attitudes and Risk Perceptions Associated with Preparation and Storage of Powdered Formula Milk in the Home: Implications for Microbiological Safety and Education

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Introduction: The risk to infants from powdered infant formula(PIF) milk has received increased attention in recent years due to possible contamination with pathogens such as Enterobacter sakazakii and *Salmonella*. Recommended procedures to safely prepare and use PIF in the home are available to parents; however implementation may be influenced by parental attitudes and risk-related perceptions. For health communication strategies to be effective it is important for them to be relevant. Related psychological constructs need to be identified and addressed.

Rational: This study aimed to determine parents attitudes and perceptions of risk, control and responsibility associated with preparation and storage of PIF in the home. To achieve this, structured face-to-face interviews with 200 parents were undertaken in England and Wales using a Computer-Assisted-Personal-Interviewing technique. Quota controls on age and socioeconomic-grading were applied; the sample was representative of parents who feed their infant(s) with PIF at least once-a-day.

Results: Results indicated attitudes and risk perceptions that may impede implementation of safe preparation and storage behaviours. Sixty-nine percent of parents believed PIF is sterile and the majority were unaware of the association of PIF with *E. sakazakii* and/or *Salmonella* (83%). Ninety-percent of parents believed there was a very-low-risk of infant illness after feeding reconstituted PIF they had prepared; risk of illness was perceived to be greater if feeds were made-up by ‘other parents’, day-nursery staff and hospital staff. The majority (97%) of parents believed they had full-responsibility and full-control of hygiene and safety when preparing PIF for their infant; smaller proportions of parents (44-73%) believed that 'other-parents', day-nursery staff and hospital staff had the same level of responsibility (63-82%) and control (44-73%).

Conclusions: Findings suggest consumer judgements of ‘optimistic-bias’ and the ‘illusion-of-control’ could be a factor in the adoption of appropriate hygiene practices. Data collected will help development of targeted information and messages that address microbial risks of domestic preparation and storage of PIF.

P2-10 Use of Powdered Infant Formula in UK Day Nurseries: Implications for Microbial Safety

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Introduction: Over the past few decades the need for non-maternal childcare has risen as increasing numbers of mothers with infants aged

Rational: This study aimed to understand day nursery nurse (DNN) knowledge, attitudes and behaviours relating to infant feeding with PIF in UK day nurseries. Data from DNN was obtained using focus group discussions across the UK (n = 4) and self-complete postal questionnaires, administered to 10% of UK nurseries with infants aged.

Results: Findings indicated that methods DNN (n = 334) reportedly use to handle, prepare and feed PIF are variable within and between day nurseries. Ninety-five percent of DNN reported feeding PIF according to parent instructions, even if such practices were believed to be inappropriate. Common

practices included (44%) feeding PIF reconstituted by parents and brought to the nursery for use throughout the day (up to 10hours) and (53%) prepared feeds in the nursery using measured PIF, bottle with measured, pre-boiled water provided by the parents. Both practices are contrary to current safety recommendations which indicate it is best to make-up PIF fresh for each feed, using boiled water >70°C. Many DNN believed PIF is a sterile product 'I think it is sterile' and up to 95%DNN lacked of knowledge and awareness of microbiological issues, such as the association between *E. sakazakii*/*Salmonella* and PIF. The majority of DNN reported they had never received training about the microbiological risks associated with PIF.

Conclusions: Findings from this study will help the development of targeted information and national policies that address the microbial risks of preparation and storage of PIF in day nurseries.

P2-11 Applicability of the DHPLC (Denaturing High Performance Liquid Chromatography) Methodology for Fresh Dairy Products Analysis

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Introduction: In the last years, major progress in microbiology analyses has been made thanks to the development of new genomic technologies and especially culture independent detection methods, like DHPLC (Denaturing High Performance Liquid Chromatography). Based on the physical properties of the bacterial DNA sequences, the DHPLC analysis leads to a profile, also named fingerprint, which is a picture of the bacterial community

Rational: The present work describes the DHPLC analysis results of some fresh dairy products: 1 yogurt and 2 probiotics dairy products (still defined as 'yogurt' in some countries). The obtained fingerprints have been compared to those of Streptococcus thermophilus, *Lactobacillus delbruckii* subsp. *Bulgaricus*, *L. casei* and *Bifidobacterium animalis*, isolated from the same products

Results: After sampling, DNA extraction and 16s rRNA gene amplification, amplicons were analyzed with DHPLC. Two different parameter sets were determined: one for the general (and "opened") analysis of the total bacteria population of the products and one for the focusing analysis of the Bifidobacterium phyla. The general analysis gives a fingerprint where appears all the flora as determined with culture. The specific analysis provides a screening method for close *Bifidobacterium* species: *longum, animalis, breve, infantis* and *catenulatum*.

Conclusions: DHPLC analysis provides short turn-around-time reliable results with high-throughput capability for the screening of fresh dairy products. By means of an automated fingerprint fraction collection, DHPLC can be used to describe a microbial population including fraction collection and subsequent sequencing identification of the bacteria, and/or compare its components based on the only comparative analysis of their fingerprints. The latter methodology is applicable for microbial quality product screening using a previously validated gold standard fingerprint from the characteristic native flora of the tested products

Acknowledgements: Thanks to Transgenomic Inc. for technical help on DHPLC analysis

P2-12 Effective Control of Quantitative Food Microbiology Process Variation Using Statistical Process Control Charting (SPCC)

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Introduction: SPCC for quantitative food microbiology is relatively new. Typically, control samples contained high levels of variable target microorganisms from overnight enrichments offering minimal understanding of process variations. Today, SPCC with standardized reference cultures containing stable low levels of microorganisms is used to provide objective evidence of testing process control within the expected variation for a given analysis.

Rational: SPCC data over three years from a US laboratory network were compared and multiple quantitative analyses method variations established. In 2008 the same reference culture material and related SPCC were expanded to the global network in 15 countries. The current work presents expected variation for common quantitative analyses and reviews worldwide network inter-laboratory SPCC data used to drive laboratory process improvement.

Results: Original upper and lower control limits (UCL, LCL) values suggested that some US laboratories had higher variation when compared within the network. Variation was reduced over three years by as much as 30% as demonstrated by the UCL-LCL average differences. Enterobacteriaceae, coliforms and *E. coli* showed significant improvement. A smaller variation decrease was observed in other analyses indicating that the method variation was inherent and not as a result of external sources. UCL-LCL average differences comparison from the global network data showed small variations and equivalent

performances of methods applied, ranging for instance from 0.67+/-0.23 to 0.57+/-013 (APC), from 1.01+/-0.38 to 0.81+/-0.23 (staphylococci), and from 1.13+/-0.47 to 0.87+/-0.19 (coliforms / *E. coli*), in log CFU/g +/-sd, for Europe & Asia-Pacific to North America, respectively.

Conclusions: Standardized control samples SPCC data provide objective evidence of controlled operation within the expected variation for a given analysis. Comparing laboratory network variation on a worldwide basis proved consistency and reliability of process and results. Food microbiology laboratories should demonstrate controlled testing process before releasing results.

P2-13 Effect of Post-incubation Half Fraser Enrichment Broth Refrigeration on *Listeria monocytogenes* Detection

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Introduction: *L.monocytogenes* presence is of major concern in ready-to-eat food products which, due to their nature, require immediate testing upon reception at the laboratory. Food samples arriving on Friday and needing *Listeria* analysis imply continuation of testing operations throughout the weekend which can be sometimes problematic for the laboratory organization. ISO 7218:2007 quotes that "unless otherwise stated, the incubated enrichment broths may only be refrigerated after evaluation of the impact of refrigeration on the results and only if clearly stipulated in the test report". The present study illustrates the preliminary evaluation of weekend post-incubation enrichment broth refrigeration impact on the detection of *Listeria monocytogenes*.

Rational: Results obtained in the Silliker laboratories of France between October 2008 and May 2009 with the AFNOR-validated *Listeria* monocytogenes detection method (BIO 12/14-04/05) using a 24h enrichment in half Fraser broth and isolation on Ottaviani-Agosti agar have been evaluated. A 36 h post-incubation refrigeration was sistematically applied to all half Fraser broths of samples prepared on Fridays. Broths of samples processed on any other week day were not refrigerated after incubation. Rates of confirmed positive samples were then compared between samples with and samples without broth refrigeration.

Results: In the defined period, 2,614 samples were tested in total on Mondays, and 15,641 in total on Thursdays. 12,781 tests were performed in total on Saturdays. Monday confirmed positive samples were 147, Thursday confirmed positive samples were 511, and Saturday confirmed positive samples were 531. Positive rate of confirmed *Listeria* monocytogenes was thus 5.6%, 3.3%, and 4.2 % respectively. The applied statistical model (parametric comparison of 2 proportions) showed a statistical difference (5% risk) between Monday and Thursday samples, but no underestimation due to 36 h refrigeration applied to the half Fraser enrichment broth of the Saturday samples.

Conclusions: The preliminary comparison of a significant number of *L. monocytogenes* tests performed with or without a 36 h post-incubation refrigeration of the half Fraser broth, has shown no negative impact of the refrigeration on the recovery rate and confirmation of positive samples.

P2-14 Evaluation of a New TEMPO Method for Enumerating Yeast and Mold in Food Products compared to ISO 21527 method

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Introduction: Yeast and Mold (YM) enumeration in food is useful in evaluating its quality and the degree of deterioration. It is often an essential component in microbiological quality assurance programs. In this study, we compared the TEMPO automated method for the enumeration of YM to the ISO 21527: Dichloran rose bengal chloramphenicol (DRBC) or Dichloran 18% glycerol agar (DG18) media depending on the food product Aw.

Rational: TEMPO YM enumeration is based on the well known Most Probable Number (MPN) procedure. The method uses a selective dehydrated culture medium and an enumeration card for the automatic determination of the MPN. This method provides a final result in 3 days at 25°C compared to 5 days for the reference methods. For comparative purposes, more than 400 naturally contaminated products were tested. These products represented a wide range of food categories and environmental samples. A combination of regression analyses, difference Log10 distributions and T-tests at the 5% level were used to analyse the data and compare performances. In parallel, the comparison of results before and after confirmation was performed on 30 food products to test the specificity of this media.

Results: This automated method showed similar performances to the ISO method with good agreement on the whole data. Regression analysis and T-test show a slight negative bias due to a better selectivity of TEMPO YM, demonstrated by the complementary tests performed on DRBC.

Conclusions: The results suggest that both tested methods are equivalent for enumerating yeast and mold. The automated method offers food laboratories a rapid alternative for YM enumeration with a time to result of only 3 days compared to 5 days for FDA-BAM.

P2-15 Microbiological Monitoring of Ready-to-Eat food at the Point of Sale

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Introduction: The microbiological quality evaluation is quite important since microbiological hazards continue to be one of the biggest threats to food safety. The interpretation of the results is often a difficult aspect of the food examination process, although using the guidelines suggested by PHLS, UK , this process is manageable and consistent. Checking the compliance with existing regulations is often insufficient and food processors need to verify and validate the efficacy of their food safety systems for that, statistical data of food microbiological analysis could be a useful tool.

Rationale and Objectives: The purpose of this study was to determine the extent to which ready-to-eat meals were contaminated with aerobic bacteria, hygiene indicator bacteria and potential foodborne pathogens at the point of sale.

Results and Findings: To study the microbial flora of the 700 samples of ready-to-eat meals, the following analysis were performed in an accredited laboratory according the NP EN ISO/IEC 17025:2005: Assay Analytical Method
Enumeration of Bacillus cereus ISO 7932:1993
Enumeration of Escherichia coli ISO 16649-2:2001
Enumeration of Microorganisms at 30 ÂºC ISO 4833:2003
Enumeration of Enterobacteriaceae ISO 21528-1:2004
Enumeration of Staphylococci Coagulase positive ISO 6888-1:1999
Detection for *Salmonella* spp ISO 6579:2002
Enumeration of *Listeria* monocytogenes ISO 11290-2:1998

Conclusions: The results obtained were compared with the guidelines for microbiological quality suggested by Public Health Laboratory Services (PHLS) for Ready-to-eat Food (Gilbert et al., 2000). According to these guidelines, the 700 samples were classified in different categories on the basis of their aerobic colony count, according to the type of the food and the processing it was received. Results were statistically analysed in respect to the type of food and usual contamination.

P2-16 Information Technologies to Support Verification in Food Safety Systems

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Introduction: The European Union and other International Organizations have issued several regulations concerning food safety in order to assure food safety of foods and protect the consumer. This has become a fundamental requirement for hotel and catering operators since the approval of the EC Regulation 852/2004.

This regulation lays down general rules for business operators on the hygiene of foodstuffs, through the implementation of procedures based on the HACCP principles.

Studies have shown that implementation of food safety systems has not been very effective, manuals and records are not usually customized, and business operators produce a large volume of paper documents that are difficult to manage and easy to fabricate.

In most cases operators have poor knowledge of procedures and how to control food operations.
As so, we consider has highly important the use of IT technologies to manage and facilitate the control of food safety operations.

Rationale and Objectives: The purpose was to develop an application to automate and manage different tasks related to food safety procedures in hotel and catering facilities.

Results and Findings: The software is composed by different modules that are organized in a relational database, allowing for the validations of CCPs, suppliers control, staff training records and other everyday jobs and controls.

As a database software, it also allows facilitated search of information and building reports. It is also possible to be connected to a network, linked to international and official organizations and therefore send and receive relevant food safety real-time data.

Conclusions: IT simplifies the way procedures and data recording are made. The generated data is

more reliable and it is much harder to alter and fabricate information, and much easier to share and access.

We consider this application to be a valuable asset to all intervenient through the food chain: 1. Producers record easily a more reliable information; 2. Consumers have higher assurance on the safety of foods they eat; 3. Official organizations have real-time information and the possibility to easily send rapids alerts concerning food safety.

P2-17 Traceability in the Mexican Dairy Processing Sector

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Introduction: Traceability has become one increasingly important component of food safety systems within the agri-food system.

Rational: The primary objective of a food traceability system is to generate the ability to identify speedily, and remove from distribution, food which may present a public safety risk. Thus, this study examines traceability in the Mexican dairy processing industry to understand the drivers behind the implementation of product traceability, motivations; challenges faced the industry, and the impacts of traceability system on company performance.

Results: Fieldwork was carried out as a structured questionnaire to the Mexican dairy processing sector. The final survey of 33 processing facilities across Jalisco State in Mexico generated a 69.7% response rate (23 questionnaires). The 85.5% of the firms sold their production within Jalisco State and Mexico City, 14.3% to the rest of the country, and 42.9% exported to the USA, Guatemala and Nicaragua. Around 71.4% of respondents produced only one type of dairy product. The 42.9% of the plants were operating HACCP, 57.0% operated ISO standards, and 42.9% had other food-safety control systems. The 85.7% of the respondents had implemented system of product traceability. Most important motivations for adopting traceability were related with legal responsibility, regulatory requirements, position in current markets and risks/worries of product recalls. Problems were most commonly associated with supplier and customer support and lack of ability to manufacture new products. Impacts reported to product traceability were related to regulatory requirements, enterprises' name perceived by commercial customers, and product recalls/withdrawals.

Conclusions: The study provided the first information in the implementation of product traceability systems in the Mexican dairy processing sector. The results suggest a range of motivating factors. Economic and marketable reasons could be of importance to implement a system of product traceability.

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P2-18 Selection of Strains for Use in Microbiological Challenge Testing to Support Chilled Food Risk Assessments

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Introduction: Sales of chilled foods have been increasing to meet consumer demands for high quality foods that are less heavily processed, contain lower levels of preservatives and require minimal preparation time. Many chilled foods do not have intrinsic properties that will control growth of bacterial pathogens. Their safety is dependant on controlling the initial contamination in raw materials, the possible reduction of pathogens (e.g., by heat treatment) and ensuring that any hazards present are not able to reach levels of concern by the end of the shelf-life at relevant chilled storage temperatures.

Rationale and Objectives: To assess the safety of a product and/or process, microbiological challenge testing is frequently required, often complimentary to the use of predictive models or sometimes to validate/build such models. Challenge tests involve inoculating a product with appropriate pathogens or spoilage microorganisms and assessing their growth, survival or death under conditions that are relevant to the specific product/process. Microorganisms are usually inoculated as “cocktails”, which comprise several strains of the organism mixed in equal numbers to take some account of variability between strains. The objective of the current study was to define safety cocktails of specific pathogens for use in chilled food challenge testing.

Results and Findings: Laboratory studies identified relevant strains that grew under the harshest conditions of low temperature/aw/pH, as well as considering other factors (e.g. heat resistance, growth in the presence of preservatives/modified atmospheres). The Bioscreen microbiological analyser was found to be a useful rapid technique for monitoring growth in aerobic conditions.

Conclusions: Safety cocktails of *Bacillus* spp., *Listeria monocytogenes* and non-proteolytic *Clostridium botulinum* were defined that could be used for challenge testing of chilled foods. An approach was established that could be applied to other food categories.

P2-19 Improved Food Safety Knowledge Obtained by a Simplified Health Information Model

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Introduction: As part of the project CHANCE, taking place in Austria, Germany, Latvia, Romania, Sweden and United Kingdom (Lifelong Learning Programme of European Union 2007-2009) it was shown that the informants (n=202) feel uncertain about food handling. The present intervention was exemplified as a communication tool to improve knowledge and to make social change. In a single meeting, education programs about fruit and vegetable and food safety, respectively, were individually self-administered via computers following discussions in small groups. The outcome was measured through questionnaires, before, immediately after and three weeks after the implementation. The number of participants was 92 (21 to 81+) living or working in the urban area Eriksberg, Uppsala municipality, Sweden. Analysis of data was quantitatively processed using Microsoft Office Excel 2007; McNemans test, SPSS Version 16.0.

Rational: The objective of this intervention was to use a simplified health information model in order to measure improvement of food safety knowledge among consumers and to see if changed behaviour could be reported.

Results: With focus on food safety the result illustrates a statistical significant improvement in knowledge according to the meaning of the expression cross contamination and the recommended storage temperature for smoked salmon and raw minced meat. However, no behavioural change was reported.

Conclusions: This simplified health information program could be a useful tool to improve knowledge about food safety among consumers. For behavioural change the model must be developed. Experiences from this study further illustrates the difficulty to get people interested in participating even though the information is offered in the nearby surrounding.

Acknowledgements: We would like to thank all the consumers participating in the present study.

P2-20 Use of the Qualified Presumption of Safety (QPS) Concept to Prioritise and Harmonise Risk Assessment of Biological Agents within the European Food Safety Authority (EFSA)

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Introduction: EFSA is requested to assess the safety of a broad range of microorganisms in the context of notifications for market authorisation as sources of food and feed additives, enzymes and plant protection products.

Rational and Objectives: The QPS concept was developed by EFSA for its own use to provide a generic safety assessment approach applicable across EFSA's scientific Panels, for all approvals related to the intentional addition or use of microorganisms in the food chain. Unambiguously defined taxonomic groups of biological agents are assessed for potential safety concerns based on a sufficient body of knowledge that covers also the field of application for which an authorisation is sought. Identified safety concerns or gaps in the body of knowledge could be reflected as 'qualifications' of a QPS status as an alternative to an exclusion from it.

Results and Findings: The list of QPS microorganisms is updated annually. The latest revision[3] lists several species from the genera of *Bifidobacterium*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*. *Lactococcus lactis*, *Propionibacterium freudenreichii*, *Streptococcus thermophilus* and *Corynebacterium glutamicum* are included. *C. glutamicum* has as 'qualification' that the QPS status applies only when the species is used for production purposes. The yeast species *Pichia angusta*, *P. anomala*, *P. jardinii*, *P. pastoris* received a similar 'qualification'. *Debaromyces hansenii*, *Hanseniaspora uvarum*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces bayanus*, *S. cerevisiae*, *S. pastorianus*, *Schizosaccharomyces pombe* and *Xanthophyllomyces dendrorhous* have QPS status. Some *Bacillus* species are included with 'qualifications' of absence of food poisoning toxins, surfactant activity and enterotoxic activity.

Conclusions: The QPS approach is currently mainly applied by EFSA's scientific Panel on additives and products of substances used in animal feed (FEEDAP) however it is expected that as a consequence of recent regulatory initiatives the concept will gain increasing importance for EFSA.

Acknowledgements: The QPS working group members and the members of the Panel on biological hazards (BIOHAZ) which are acknowledged in the [3]EFSA 2008 Opinion: The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards (Question number: EFSA-Q-2008-006). The EFSA Journal, 2008, 923, 1 - 48 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902221481.htm

P2-21 Food Surface Decontamination Using Non-thermal Plasma

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Introduction: Special treatments are required to reduce microbial contaminations and to guarantee optimal quality of heat-sensitive products such as fruits and vegetables. Non-thermal plasma is a promising tool for the decontamination of food surfaces due to the various reactive species in the plasma and their associated antimicrobial effects.

Rationale and Objective: The objective of this study was to evaluate the effects of non-thermal plasma on the inactivation of human pathogenic bacteria using flow cytometric techniques.

Results and Findings: E. coli and *L. innocua* on a polysaccharide gel were treated with non-thermal plasma at operating powers of 10 to 40 W which was generated in an rf-driven atmospheric plasma jet. The inactivation of the bacteria were recorded by conventional plate count methods with an detection limit of 10^2 ml^{-1} and by flow cytometry measuring 10,000 cells per sample. Each treatment was performed in triplicate. An energy input of 20 W resulted in a 7 log-cycles reduction (initial count: 10^8 CFU ml^{-1}) after 4 min treatment for both *L. innocua* and *E. coli.* A 10 W plasma treatment led to minor damaging effects (log-cycle reduction < 2) of either tested organisms while a complete inactivation was determined when applying 40 W and 90 s treatment time (*L. innocua*) or 120 s (*E. coli*). Flow cytometric analyses of bacteria cells after plasma treatment at 20 W showed increased membrane permeability with increasing treatment time. The number of slightly permeabilized cells with esterase activity remains almost constant at 15% during the treatment, and the number of cells with intact cell membrane and esterase activity decreased by 70% for both bacteria after 4 min of plasma treatment.

Conclusions: Non-thermal atmospheric pressure plasma inactivates both gram-negative and gram-positive bacteria at temperatures below 40°C. A plasma jet array can be designed enhancing the industrial applicability in food processing.

P2-22 The Efficacy of Mitigation Strategies to Reduce the Norovirus Intake Evaluated by a Risk Assessment Approach

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Will be presented by MIEKE UYTTENDAELE and LIESBETH JACXSENS

Introduction: A risk assessment approach was used to determine the norovirus intake and probability of infection (PI) after the application of decontamination procedures or mild heat treatments on respectively lettuce or raspberries. Therefore, two cases were elaborated to address this issue. The first case represents a worst case scenario as it was assumed that lettuce crops were irrigated with non-disinfected secondary treated effluent. The second case represented a foodhandler introducing viruses onto raspberries during harvesting. The reduction of the viral intake after mildly heating raspberries was assessed (1).

Results and Findings: The application of 200 mg/L NaOCl to treat lettuce, that was irrigated with water containing 2.3 PFU/L (C1), decreased the PI with a factor 7 compared to the washing of lettuce with tap water (case 1). The application of 250 mg/L PAA even reduced the PI to 0%. The use of NaOCl or PAA to treat lettuce, that was exposed to a higher viral load C2 (130 PFU/L), decreased the PI although the PI could not be reduced to 0%.

Accordingly, mild heat treatments (75°C 15 s or 65°C 30 s) in case 2 were more effective in decreasing the PI when raspberries were exposed to a lower viral concentration C1 (2.5×10^4 genomic copies/g) in comparison with the exposure of raspberries to a higher viral load (C2 = 3.0×10^8 genomic copies/g).

Conclusions: Decontamination procedures or mild heat treatments can be useful to lower the viral intake on respectively lettuce or raspberries having an initial low viral contamination level. From the comparison of case 1 and 2, the PI is considerably higher in foodhandler-involved viral contamination of foods compared to irrigation water as a vehicle for viral transmission to foods.
(1) Baert, L. (2009). Molecular detection of, and strategies to reduce Norovirus load or infectivity in foods. Ph D dissertation, Faculty of Bioscience Engineering, Ghent University, Ghent.

P2-23 A Selection Tool for Application of the Most Appropriate Microbial Method of Analysis

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Will be presented by MIEKE UYTTENDAELE and LIESBETH JACXSENS

Introduction: According to the MAS-protocol appropriate methods for sampling and analyses of pathogens and other micro-organisms need to be selected (1). Reference methods for microbial analysis are the internationally agreed ISO standards. However, nowadays numerous and diverse alternative methods for microbiological analysis of foods are currently brought to the market by various suppliers in a variety of formats as a result of recent developments, particularly in the field of biotechnology, microelectronics and related software development, which also can be used. Due to an overload of rapid methods and/or formats on the market, food producers have difficulties in deciding which method is best fit for their purpose in their particular context.

Results and Findings: A decision tree was made that support the selection of the most appropriate method for microbial analysis. The decision tree is based on a techno-managerial point of view and takes into account the context of the analysis, the performance characteristics of the method, the operational requirements etc.

Conclusions: The selection tool helps the end user of the method to obtain a systematic insight into all relevant factors, beyond the inherent performance characteristics of the method, to be taking into account for selection of a method for microbial analysis. In this way this tool helps to select the method which is best fit for purpose for a particular situation.

1. Jacxsens, L., J. Kussaga, P. A. Luning, M. Van der Spiegel, and M. Uyttendaele. 2009. A microbial assessment scheme to support microbial performance measurements of food safety management systems, p. In press. International Journal of Food Microbiology.

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P2-24 Validation of a Norovirus Detection Methodology in Soft Red Fruits

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Introduction: Noroviruses (NoVs) are recognized as one of the most important causes of (foodborne) non-bacteriological gastroenteritis worldwide. Despite these facts, a standardized assay to detect NoV in different food types is still not available.

Rational: In the current study, the robustness of a suggested NoV detection methodology was examined on different soft red fruits.

Results: Ten grams of different food products were inoculated with diluted GI and/or GII NoV stool samples. Virus/RNA extraction was performed as described by Baert et al (2008). A multiplex real-time RT-PCR assay described by Stals et al. (2009) was used for detection of NoV GI, NoV GII and MNV-1 of which the latter served as full process control. MNV-1 ssRNA was added used as reverse transcription control and MNV-1 plasmid DNA was used as real-time PCR internal amplification control. GI NoVs were recovered from deep-frozen raspberry crum samples with efficiencies of 28.11 ± 7.82 % and 20.09 ± 9.40 % (high and low concentrated inoculation). GII inoculations were recovered with efficiencies of 13.82 ± 6.23 % and 7.57 ± 3.79 % (high and low concentrated inoculation).

Conclusions: Results show that the recovery of (genomic material of) GI and GII NoVs from different soft red food products is influenced by the concentration of GI/GII NoVs present on the food sample and the fruit/matrix type. Further validation of the developed method on different food matrices remains necessary, but this assay seems to have perspectives for detection of human GI/GII NoVs in food samples.

P2-25 Simulation Modelling and Risk Assessment as Tools to Identify the Impact of Climate Change on Microbiological Food Safety – the Case Study of Fresh Produce Supply Chain

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Will be presented by MIEKE UYTTENDAELE and LIESBETH JACXSENS

Introduction: The current quality assurance and control tools and methods to prevent and/or to control microbiological risks associated with fresh produce are challenged due to the following pressures upon the food supply chain, i.e. changing consumption patterns, globalization and climate change. It demonstrates the need for scientific research and development of new and/or improved tools, techniques and practices to adapt the current risk management systems.

Rational: A conceptual research approach is presented to analyse the complexity of the climate change and globalization challenge on the fresh produce supply chain taken as a case study. The factors which affect the vulnerability of the fresh produce chain demand a multidisciplinary research approach. The proposed knowledge-based modelling system is believed to be a most appropriate way to identify problems and to offer solutions to monitor and prevent microbiologicalal food safety risks during all phases of food production and supply.

Results: To explore the potential impact of climate change and globalization, baseline information can be obtained by surveillance and performance measurement of implemented food safety management systems. Simulation of climate change scenarios and the logistic chain of fresh produce, along with mathematical models to optimize packaging technology to maintain quality and safety of fresh produce are tools to provide insights in the complex dynamic ecosystem. They are the basis for elaboration of risk assessment studies to scientifically support management options and decisions to new microbiological threats related to globalization and climate change in the fresh produce supply chain.

Conclusions: This research concept as such will contribute to develop strategies in order to guarantee the (microbiological) food safety of fresh produce on the long term.

Acknowledgements: This conceptual research approach will be undertaken in an EU FP7 project Veg-i-Trade coordinated by Mieke Uyttendaele. The Veg-i-Trade project responds to a call by of DG Research of the European Commission and addresses the elaboration of research activities to identify impacts of anticipated climate change and globalisation globalization on food safety, microbiological and chemical hazards, of fresh produce and derived food products. The authors wish to thank all the members of the Veg-i-Trade consortium.

P2-26 Potential Use of Fourier Transform Infrared Spectroscopy (FT-IR) to Assess Pork Spoilage

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Introduction: Quality is a subjective and sometimes elusive term. Freshness of meat muscles is generally considered the most important contributor to quality. It is therefore crucial to have valid methods to monitor freshness and quality. Indeed, methods should be valid for application by industry and consumers in order to obtain reliable information on freshness status when merchandizing and purchasing products. Fourier Transform Infrared (FT-IR) spectroscopy is a rapid, non-destructive analytical technique with considerable potential for application in the food and related industries. FT-IR has been tested for several muscle food analyses and recent studies on meat tissues, stored at ambient temperature, correlate microbial spoilage of meat with biochemical changes within the meat substrate.

Rational: Minced pork meat was stored aerobically at five different temperatures (0, 5, 10, 15 and 20°C) and the microbiological analysis (Total Viable Counts, lactic acid bacteria, pseudomonads, Enterobacteriacaeae) was performed in parallel with FT-IR analysis, pH measurements and sensory analysis. The spectral data collected from FT-IR were subjected to principal component analysis (PCA) to investigate differences between samples and thus reduce the size of the data set. A second PCA with the selected variables (wavenumbers) revealed the principal components (PCs) that significantly contributed to the variance of the data set. These PCs were further subjected to factorial discriminant analysis (FDA) in order to predict the quality of a sample that was pre-characterized as Fresh (F), Semifresh (SF) or Spoiled (S) from the sensory analysis. A corresponding procedure was followed in order to qualitatively predict the storage temperature of a sample.

Results: The FDA exhibited a correct classification of >96% of samples regarding their spoilage status (F, SF, S) and 90% regarding their storage temperature. These data revealed a good correlation between sensory detection of spoilage status and that of chemical metabolites according to storage temperature, as detected from FT-IR. On the other hand, sensory evaluation of spoilage was not always correlated with the same microbial load at the time of the early sensorial detection of spoilage (meat characterized as SF) which was increased with temperature.

Conclusions: Results show that Fourier Transform Infrared (FT-IR) spectroscopy is a rapid, non-destructive analytical technique with considerable potential for application in the food and related industries.

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P2-27 Food Safety and Epidemiological Relevance of Procedures in Cleaning and Disinfection at Household Level

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Introduction: Food borne diseases at household level are considerably frequent, and in many situations associated to cross-contaminations, and deficient hygiene pratices

Rational: This study aimed to undertake a survey to assess the degree of compliance with good hygiene practices at home, and to evaluate the feacal contamination in refrigerators, cleaning sponges and kitchen counters.

Results: Regarding the survey the main evidences are the following: out of the 30 inquires, 62% used a cloth to clean and 49% cleaned only it with detergents; 48% discarded the sponge once a month; 90% washed the dishes with detergents without desinfectant; 63% usually cleaned the refrigerator, 33% desinfected it and finally, 42% did so once a month. Of the 90 analysis carried out in the three refrigeratours zones, *Escherichia coli* was detected in 3%, and always in the meat storage zone. *Enterobacteriaceae* were found in 20% of the samples. Considering the analysis of the 30 kitchen surfaces (100 cm^2) Enterobacteriaceae were detected in 63% of the samples and *Escherichia coli* in 10% of them. Regarding the analysis of the 30 sponges, Enterobacteriaceae were found in 93% of the samples and *Escherichia coli* in 47%.

Conclusions: The sponges are sources of contamination, and a specific factor that can relate the incidence of food borne diseases at home by cross-contamination. The kitchen counters are also sources of contamination, once they contact directly with ready to eat foods. The cleaning procedures of the refrigerators are frequently made without the use of disinfectants, which is a factor that can allow the microbiological survival, and also bio film formation. Considering the analysis of the 30 kitchen surfaces (100 cm^2) Enterobacteriaceae were detected in 63% of the samples and *Escherichia coli* in 10% of them.

P2-28 Development of Artisan Chocolate Confectionery: Microbiological Safety and Chemical and Sensory Stability

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Introduction: Chocolate confectionery is a product consisting of either chocolate mass or a nucleus containing several fillings covered with a chocolate layer. The demand for these products and specially artisan confectionary chocolates is growing in Portugal. Gourmet and chocolate shops are expanding offering new formulas of these products.

Rational: Considering that sometimes chocolate confectionaries are kept for long periods of time, it's essential to assure the microbiological safety of these products and also its stability in terms of chemical and sensorial characteristics, once these are directly related to their acceptance by consumers. The objective of this study was to assess the safety of a new fill dark chocolate confectionary formula, and its chemical and sensorial stability for a four month period, as influenced by the addition of sorbitol to the filling and the storage temperature (18ºC and 25ºC).

Results: Two series of chocolate confectionery samples filled with a vanilla flavoured cream were prepared, one having sorbitol in its formula. After microbiological sampling, both series were divided in two parts and one of each stored at 18ºC and 25ºC. Once a month, for four months, microbiological analyses were performed (total mesophilic counts, *Bacillus cereus*, total coliforms, *Escherichia coli*, *Clostridium*, *Staphylococcus aureus*, *Salmonella* and *Listeria* spp., moulds and yeasts). Also quality parameters of each series kept at both temperatures were assessed - chemical characteristics (pH and a_w) and sensory characteristics (descriptive analysis and triangular test carried out using a panel of 7 to 10 judges half-trained).

Conclusions: Two assays have already been undertaken. By the end of the experiment (September) which started in June 2009, results of the effect of sorbitol and temperature in the developed chocolate confectionary's shelf life are expected and also which product will have a greater potential from a market point of view.

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P2-29 Occurrence and Expression of Toxin Genes in *Clostridium perfringens* Isolates from Healthy Swine

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Introduction: Prevention of food animal diseases is important issue among many elements in animal food production chain. *Clostridium perfringens* is the most important animal pathogen among anaerobic sporulating bacteria responsible for enterotoxemia of many warm-blooded animals. The production of the major toxins is a base for classification into one of the five toxotypes (A - E). Toxin production ability and their level of production decide about pathogenicity these bacteria. Not less important than activity of genes is immune decreasing of animals, which may lead to disease.

Rationale and Objectives: Taking into account that pathogenicity of *C. perfringens* is conditioned by presence and activity of toxin genes the study were undertaken for assessment of toxin genes occurrence and expression of most often detected toxin genes.

There were detected toxin genes of *C. perfringens* isolates from faeces of healthy swine by multiplex polymerase chain reaction. The second step of study was expression checking of alpha and beta2 toxin by reverse transcriptase PCR on the base of mRNA presence.

Results and Findings: Between 354 analyzed isolates suspected of belonging to C. perfringens species, 305 strains were confirmed. Toxin type and its subtype identification revealed that 51.1% of the isolates belong to type A, 48.8 isolates belong to type A subtype beta2. Enterotoxic strains (positive for cpe gene) were detected in 0.6% strains. Additionally, both isolates possessed cpb2 gene. Analysis of isolates (n = 31) for cpa and cpb2 toxin gene expression shows that 71% of isolates expressed both genes and 29% of isolates only cpa gene.

Conclusions: Similar to results of other studies there were noted dominance of isolates type A and among them almost half strains possessed cpb2 gene. Relative low percentage of isolates with enterotoxin gene in our study may be, according to some authors, sufficient reservoir of this gene, taking into account a panmictic nature of *C. perfringens* genus.

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P2-30 Roche Lightcycler TaqMan Methods for Quantification of Genetically Modified Maize and Soybean

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Introduction: Real-Time PCR is the most common technique used for the quantification of genetically modified organisms (GMO) in food and feed analyses.

Rationale and Objectives: The objective was to validate the TaqMan Real-Time PCR procedures for the quantification of genetically modified maize (MON810, Bt11, Bt176 and T2 5) and soybean (GTS 40-3-2) at the capillary Roche LightCycler 2.0. The amplification of reference genes: hmg (MON810, Bt11), zSSIIb (Bt176), adh1 (T25), lectin (soybean) and construct- (Bt176, GTS 40-3-2) or event-specific (MON810, Bt11, T25) GM DNA fragments were done.

Results and Findings: The dynamic range of all methods was satisfactory for GMO analyses but limited by low CRM GMO content.

The trueness of the genes copies quantification was in the range ±25% of true value.
The reaction efficiency reached the lower value 89.6% for Bt11 transgene, and the higher value 99.0% for Bt11 hmg reference gene. For other methods the efficiencies for both reference gene and transgene were very similar. The linearity of reaction was very high, $R_2 > 0.999$.

The limit of detection and limit of quantification was 0.06% for GM maize MON810, Bt11 and Bt176, and 0.05% for T25 event. As regards GM soybean LOD was 0.025%, LOQ was 0.075%. Analyses of CRM maize and soybean samples showed that there is no significant difference between the mean measured value and the certified value. The uncertainty for the quantification of GM maize for all 4 GM event were below or equal ±25%, as regards GM soybean it was ±33% (≤5% GMO content) or ±14% (≥5% GMO content). The last phase of validation proved that robustness of all methods was acceptable.

Conclusions: The results showed that all reactions, optimised for capillary thermocycler, are suitable for the quantification of genetically modified maize and soybean.

P2-31 The Use of Fully Stable Isotope Labeled Mycotoxins as Internal Standards for Mycotoxin Analysis with LC-MS/MS

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Will be presented by GUSTAV KICHLER AND HANNES BINDER

Nowadays, so called LC-MS/MS multitoxin analysis methods allow the simultaneous determination of up to 100 toxins. However, interferences from matrix components can lead to so called matrix effects that cause variations of the ionization efficiency of the analytes in the sample compared to pure standard calibrants, which results in an under- or overestimation of the actual concentration. This is a clear limitation of multi-toxin methods using a mass spectrometer as detector for the determination of mycotoxins.

There are several approaches to overcome these matrix-induced signal suppression or enhancement effects. The application of matrix-matched standards is a common strategy in LC-MS/MS approaches to counteract the adverse effect of co-eluting matrix components on accuracy. However, matrix-matched calibrations are quite laborious and sometimes, depending on the matrix of interest, not applicable due to the lack of a blank sample or large variation between individual samples of the same matrix. An alternative method is the addition of internal standards (IS) to the sample to overcome matrix effects. IS behave similar to the analyte and can therefore correct for recovery losses during the sample preparation process and for ion suppression effects in the MS source. Stable isotope-labeled analogs of natural mycotoxins provide the best IS for these toxins. However, it should be noted that deuterated compounds still run the risk of H/D exchange in protic solvents and retention time shifts relative to the natural toxin. Moreover, partially labeled toxins frequently contain considerable amounts of "lighter" isomers, leading to mass peaks that interfere with natural toxin isotopes. Therefore, fully 13C-substituted compounds can be regarded as the best standard for quantification by LC-MS/MS based methods.

In our work, we demonstrate the use of fully isotope labeled mycotoxin IS to correct for fluctuations that may occur during extraction, clean-up and ionization of the sample in LC-MS/MS methods.

P2-32 Validation Results of New Test Kits for Food Allergens - AgraQuant® Allergen ELISA Test Kits

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Food Allergy, typically an immune system response to a protein present in food that the body mistakenly believes is harmful, represents an important health problem in modern society. Cross-contamination during the production process may occur so that residues of food allergens in different products may be present. Worldwide labeling regulations lead to more accurateness for food manufacturers, although hidden allergens continue to be the largest single cause of global product recalls. With the aim of preventing health hazards by food allergy, Romer Labs® offers AgraQuant® Allergen ELISA Test Kits to sensitively detect food allergens in a wide range of processed foods and raw materials.

The AgraQuant® Allergen Test Kits are sandwich enzyme-linked immunosorbent assays (ELISA). Food allergen proteins, extracted from food products with an extraction buffer, bind to specific polyclonal antibodies pre-coated on the surface of a microwell. After a washing step an enzyme-conjugated antibody binds to captured specific food allergen proteins. The applied enzyme substrate develops a blue color. The reaction is then stopped by adding an acidic stopping solution, turning the color into yellow. Using a microwell reader the color intensity is determined and is directly proportional to the concentration of the food allergen in the sample.

AgraQuant® Peanut and AgraQuant® Hazelnut have quantitation ranges of 1-40 ppm and detection limits of 0.1 ppm peanut and 0.3 ppm hazelnut. The quantitation range of AgraQuant® Gluten is 4–120 ppm gluten and limit of detection was determined to be 0.6 ppm gluten. AgraQuant® Soy has a quantitation range of 40–1000 ppb and a limit of detection of 16 ppb. AgraQuant® Almond and AgraQuant® Egg white have quantitation ranges of 0.4–10 ppm and detection limits of 0.2 and 0.05 ppm. Extensive validation studies indicated low detection limits, good accuracy, precision and recovery of the Test Kits.

P2-33 Multiplex PCR Method for Detection of Pecan and Brazil Nuts Allergens in Food Products

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Introduction: Residues of nut allergens in food products may cause severe allergy in a part of population. European legislation (2003/89/EC) requires labelling of food products with respect to the contents of pecans (*Carya illinoiesis*) and Brazil nuts (*Bertholletia excelsa*).

Rational: The purpose of this study was to develop a multiplex PCR method for simultaneous detection of partial sequence of gene encoding pecan allergen vicilin-like seed storage protein (72 bp) and partial sequence of gene encoding Brazil nut allergen 2S albumin (173 bp) in food matrixes. The specificity of designed primer pairs was tested on a broad range of food ingredients. Food products with various nut declaration and without nut declaration were investigated for the presence of pecan or Brazil nut residues. Universal plant primers were used for the plant matrixes confirmation in food (123 bp).

Results: Twenty eight samples of food products with various nut labelling and eighteen samples without nut labelling were analyzed using developed PCR method. In analyzed samples neither pecans or Brazil nuts were detected. The detection limit of the PCR method was assessed 100 pg/µl.

Conclusions: The presented PCR method is useful for sensitive and specific detection of pecans and Brazil nuts in food products and could therefore prevent the occurence of allergic reaction in context of early nut residues detection.

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P2-34 Compositional Differences in the Lactic Acid Bacteria Flora of Matured Traditional Greek Graviera Cheese as Affected by the Type of Starter Culture added to the Milk Post-thermization

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Introduction: Traditional Graviera cheese is often produced from thermized milk to control undesirable bacterial contaminants. Since thermization also reduces the desirable lactic acid bacteria (LAB) flora of raw milk, natural undefined or commercially defined mixed LAB starters are utilized.

Rational: This study evaluated effects of the type of starter added to thermized (63°C, 30 sec) milk on the numbers and types of LAB dominating in matured Graviera cheese. Eight cheese batches produced either with a natural yogurt-like starter (NS; 4 batches) or with a commercial freeze-dried starter (CS; 4 batches) containing *Streptococcus thermophilus*, *Lactococcus lactis* and leuconostocs were analyzed, and 200 (25/batch) LAB isolates from high dilution agar plates were identified.

Results: Mean populations of total mesophiles (TSAYE; 30°C), mesophilic and thermophilic LAB (MRS; 30 and 45°C), and mesophilic and thermophilic cocci (M-17; 22 and 42oC) in NS-cheeses were 8.7, 8.5, 8.0, 8.1 and 7.8 log CFU/g, respectively, whereas respective populations in CS-cheeses were 8.6, 8.7, 6.6, 8.4 and 8.3 log CFU/g. *Enterococcus faecium* (41%), *E. durans* (35%), *Lactobacillus casei* (9%), *E. faecalis* (8%), *Leuconostoc* (5%) and *Lc. lactis* (2%) were isolated from NS-cheeses. Conversely CS-cheeses contained *L. casei* (46%), *L. plantarum* (17%), *Lc. lactis* (3%) *and S. thermophilus* (3%). *Enterococcus* spp. comprised 31% of LAB isolates from CS-cheeses; 86.2% of them, however, were recovered from MRS plates at 45°C which exclusively contained enterococci at populations ca. 1.5 log lower *(P* < 0.05) than in NS-cheeses.

Conclusions: Replacement of NS with CS suppressed enterococci and favoured mesophilic lactobacilli during maturation. *S. thermophilus* present in both NS and CS, and *Lc. lactis* present in the CS, were overgrown, and *Lactobacillus bulgaricus* isolated from the NS was undetectable, in ripened cheeses. Safety concerns associated with inability of the NS to control *Enterococcus* suggest that concentrated commercial starters should be used in traditional Graviera cheese production.

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P2-35 A New Approach to HACCP for Hospitality: Changing Knowledge, Attitude and Behaviour

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Introduction: The purpose of this paper is to demonstrate an evaluation of the effectiveness of a new method of HACCP for the hospitality industry that was developed, piloted and validated by the UK Food Standards Agency (FSA) and the University of Salford between 2002 and 2006.

Rational: To evaluate the impact of a new approach to HACCP, in-depth case studies using psychological interviews and documentary analysis were carried out in a wide range of hospitality businesses in Greater Manchester. The new approach to HACCP was implemented in these businesses, and the research method was replicated at 6 month and 3 year periods to assess change.

Results: The findings show notable improvements in food safety knowledge, attitude and behaviour, and a reduction or elimination of all previously identified barriers to food safety management, as a result of implementing the new approach to HACCP. They also show how they can be maintained over time with minimum external pressure or involvement.

Conclusions: The results of this study support the FAO/WHO guidance to governments on 'evolving methods' of HACCP for SLDBs, and also provide in-depth psychological and practical insights into how this can be achieved and evaluated.

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P2-36 Could LanguaL be Useful for Food Microbiological Risk Assessment?

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Introduction: Microbiological foodborne diseases are a growing public health problem worldwide. To design public health policies and identify appropriate food safety measures, data on foodborne diseases surveillance and food monitoring systems need to be analysed together. In order to reduce uncertainties in risk assessment, it is very important to improve data quality, particularly regarding food classification and description.

LanguaL (Langua aLimentaria) is a description-classification system that characterizes each food by a set of standard, controlled terms chosen from facets. LanguaL facilitates links to many different food data banks and is currently used in European EuroFIR Composition Databases contributing to coherent data exchange. EFSA's zoonoses reporting system classifies foods according its risk level by hazard.

Rationale and Objectives: To assess LanguaL suitability for food microbiological risk assessment. Foods related to foodborne diseases reported by Portugal in 2008, were classified by LanguaL. EFSA and LanguaL classifications were compared.

Results and Findings: Major contributory factors to microbiological risk like temperature misuse, raw material, are related to Langual facets F. EXTENT OF HEAT TREATMENT, G. COOKING METHOD and J. PRESERVATION METHOD. Also, facets C. PART OF PLANT OR ANIMAL, E. PHYSICAL STATE, SHAPE OR FORM, H. TREATMENT APLLIED, K. PACK ING MEDIUM and M. CONTAINER OR WRAPPING, R. GEOGRAPHIC PLACES AND REGIONS and Z. ADJUNCT CHARACTERISTICS OF FOOD describe food aspects that may be important to risk. The term "canteen" lacks in Preparation establishment descriptor of facet Z.

Conclusions: Results suggest that LanguaL may be adequate for microbiological risk assessment and could facilitate screening emergent problems, because it does not have risk oriented classification limitations. Furthermore, the use of the same Food Description System for composition, consumption and contaminant occurrence databases would allow data combination among networks improving food safety and food security at global level.