Technical Session 1 – Thursday, 19 May, 11.00-12.30

Chair, Lee-Ann Jaykus

T1-01 Preventing Residues from Leaving the Farm

Robert Salter, Charm Sciences, Inc., Massachusetts, USA

Introduction: Animal drug residues and natural toxins are undesirable hazards in food production that can only be prevented through good agricultural practices and early detection and remediation on farm. Simple culture assays for inhibitors have been improved and employed as screening tools but they are still blind to natural toxins, non-antimicrobial animal drugs and are not sensitive to some antimicrobial drugs used in animal treatment. Additional screening methods may complement the gaps in inhibitory method detection.

Purpose: An integrated approach to food residue detection and avoidance, using multiple diagnostic tools, implemented based on farm knowledge will provide greater protection and assurance of food production quality.

Methods: Inhibitory drug detection levels of *Geobacillus stearothermophilus* inhibition assays in milk and tissue are contrasted with MRL levels. Gaps of detection are highlighted and addressed with a series of lateral flow tests.

Results: Farm history with regards to weather/climate, animal health, drugs administered and practices can determine the most likely residues to test for to verify control practices using screening tests. The appropriate tool must be selected based on the residue, its MRL level and the level it could be detected using the screen. Aflatoxin, flunixin, streptomycin, florfenicol and enrofloxacin are potential residues that could escape inhibitor method detection. Results of milk screening tests for mycotoxins are predictive of contamination in feed/grain and screening tests may be employed to eliminate those food sources to the animals and improve their health and milk production. Contents of drug medicine cabinets are indicative of drugs being used and may indicate if additional screening tests should be employed to indicate control of residue on farm. Poor animal drug record keeping may indicate risk that these drugs could be in either milk or tissue and an appropriate screening test selected to mitigate that risk.

Significance: Residues in food create a consumer concern and reflect poorly on the image of the food and the producing corporation. Residue avoidance is most effective and inexpensive early in the food chain. Intelligent application of available screening tools based on audit information can demonstrate that effective controls are in place to minimize residue risks.

T1-02 Dairy Farm Audits Induce a Temporary Improved Bulk Milk Quality

Annet Velthuis, Business Economics Group, Wageningen University, The Netherlands; **Akemi Flores Miyamoto** and **Martine Reij**, Food Microbiology, Wageningen University, The Netherlands

Introduction: Audits aim to ascertain and to improve product quality/safety. But the effect of audits on product quality/safety is hard to demonstrate empirically as data are not easily available. In this study data on dairy farm audits and data about laboratory results of bulk milk deliveries of individual farms are studied to trace a putative relation between audits and product quality.

Purpose: The objective is to study whether the total bacterial counts (TBC) of dairy bulk milk are reduced in the period around a farm audit and/or afterwards.

Methods: The data set contained information about 13,006 audits performed on 12,855 farms as well as TBC counts of bi-monthly tested bulk milk of these farms. A record included: logTBC value, variables indicating the timing of the TBC test relative to the audit date, unique audit number, auditor, type of audit, outcome of the audit, audit checklist items, number of attention points and audit date. A random linear regression model was used to quantify the possible relation between logTBC and the time before, during and after the dairy farm audits and other audit variables.

Results: A significant reduction in logTBC levels were found around the date of the audit and in the period from 1.5 to at least 6 months after the audit. Additionally, logTBC levels were found to depend on: season, total number of attention points given during an audit, audit type (standard, repeated or first audit), audit outcome (approved, rejected or temporary blocked), year of the audit (2006, 2007 or 2008), checklist items related to the maintenance of the milking equipment and/or utility room and tank and some interactions.

Significance: Based on this study we can conclude that there is a temporary decrease in logTBC values due to the audit, whereas this decrease starts 1.5 months after the audit and continues until at least 6 months after the audit.

T1-03 A Novel Technology for Enhancing Sanitization in Food Processing Operations Based on a Renewable Antimicrobial Coating (RAC)

Carl W. Erkenbrecher and **Sherrill Nurnberg**, DuPont Clean & Disinfect, Delaware, USA; **Akintayo Adisa**, DuPont Clean & Disinfect, Suffolk, United Kingdom; **William R. Cahill**, DuPont Clean & Disinfect, Delaware, USA; **Shaun F. Malone**, DuPont Clean & Disinfect, Ontario, Canada

Introduction: A unique quat-based antimicrobial coating (RAC) was developed to meet a growing demand for sanitization of hard to reach, vertical and ceiling surfaces in industrial and commercial operations to provide long-term antimicrobial protection for days to months. The coating is easily applied with a portable sprayer (or brush or roller), can contain a dye for easy visualization and overspray forgiveness, has antimicrobial activity against a variety of bacteria and fungi on hard and porous surfaces, is removable with water while leaving low to no coating residue, and has ultra low VOCs.

Purpose: The purpose of this investigation was to determine RAC's efficacy against key surrogate and food pathogens using a variety of microbiological test methods.

Methods: AOAC, ASTM and EPA regulatory tests (e.g., Non-Food Contact Sanitizer, Food Contact Sanitizer, Residual Sanitizing, and Fungistatic tests for porous and nonporous hard surfaces).

Results: Overall ≥ 3 log reductions were obtained in 5-min exposures for *S. aureus*, *K. pneumoniae*, *E. coli* O157:H7, *S. enterica*, *P. aeruginosa* and *L. monocytogenes*. Antiviral activity and fungistatic activity have also been demonstrated (e.g., bacteriophage and *A. niger*). The effects of organic contamination, relative humidity, temperature, contact time, long-term stability and dye addition on microbial activity were optimized to provide a functionality that makes this coating as versatile and robust as possible.

Significance: RAC is an effective non-food contact sanitizer and fungistat providing long-term efficacy for hard to reach, vertical and ceiling surfaces in industrial and commercial operations. Adjustment of the contact time and/or actives concentration allows for the maintenance of the microbial efficacy of different formulations for a variety of applications. In addition, the basic coating formulation (quat-based) can be modified using a variety of antimicrobial actives (e.g., oxidative chemistries) and could provide a platform for different coating technology applications.

T1-04 Modelling the Use of Different Enforcement Strategies to Improve Food Safety

Esther van Asselt and **Piet Sterrenburg**, NFA, RIKILT - Institute of Food Safety, The Netherlands; **Sjoukje Osinga**, LDI, Wageningen University, The Netherlands

Introduction: According to the General Food Law, food producers are responsible for the production of safe products. Safe in this regard is often interpreted as compliant with EU food safety legislation. The level of compliance between companies differs and can be improved by measures such as education or sanctions. In order to determine the effectiveness of various enforcement strategies on the level of compliance we developed a simulation tool using Agent Based Modelling (ABM) as a method. This ABM tool allows to simulate with actions and reactions between autonomous agents, yielding an emerging overall effect. This emerging effect will give valuable insight in how the overall behaviour of the system and the individual behaviour of agents mutually depend on each other.

Purpose: The aim was to model the effect of different enforcement strategies on compliant behaviour.

Methods: As a case study, we focused on the use of antibiotics within primary pig production. The agents in this case were defined as individual farmers and food safety inspectors. The food safety inspectors could either educate or give sanctions.

Results: The ABM model showed that – given the assumptions – inspection frequency and sanctions had more effect on compliant behaviour than societal control or education. Furthermore, following a risk-based approach in inspections resulted in an overall increase in compliant behaviour.

Significance: The model proved to be a powerful tool in exploring potential effects of different enforcement strategies on compliant behaviour.

T1-05 Prevalence Study of Top Six non-O157 STEC in Raw Beef in the United States

Phil Feldsine, **David E. Kerr**, **Markus T. Jucker**, **Verena Peggion**, **Andrew H. Lienau**, and **Bob Westoff**, BioControl Systems, Inc., Washington, USA

Introduction: As a result of recent incidents of contamination, there is growing interest in the United States in the potential public health risk associated with a broader group of *E. coli* commonly referred to as "the top six non-O157 shiga toxigenic *E. coli*" or "Top STEC." Most surveillance studies of clinical isolates report that the following six STEC O serotypes, in addition to O157, represent the greatest public health risk: O26, O45, O103, O111, O121 and O145.

Purpose: Little data for the prevalence of the Top STEC microorganisms exists in raw beef.

Methods: A surveillance study was undertaken to look at the incidence of Top STEC from raw beef samples. Five beef processors located in various parts of United States evaluated 1800 beef trim and ground beef samples with Assurance GDS® Top STEC and Shiga Toxins for Top STEC assays.

Results: Sixty-three *E. coli* isolates were identified as containing one of the top six serotypes and one of the two targets established by the USDA that define the Top STEC. Among these isolates, 16 contained both targets. At least one Top STEC from each serogroup was isolated.

Significance: This data provides insight into the potential incidence rate of Top STEC in raw beef in the United States.

T1-06 Non-random Distribution of *E. coli* O157 Genotypes among Bovine, Food and Clinical Isolates in The Netherlands

Eelco Franz, Laboratory for Zoonoses and Environmental Microbiology, RIVM – Centre for Infectious Disease Control, Netherlands; **Fimme Jan van der Wal**, Central Veterinary Institute, Wageningen University and Research Centre, The Netherlands; **Angela van Hoek**, Laboratory for Zoonoses and Environmental Microbiology, RIVM – Centre for Infectious Disease Control, The Netherlands; **Annet Heuvelink**, Food and Consumer Product Safety Authority, The Netherlands

Introduction: The relatively high prevalence of STEC O157 in the Dutch bovine reservoir, the high cattle density and the generally accepted high infectivity of the bacterium seems to condradict with the relatively low number of disease cases. This might be explained by the only a small subset of STEC O157 isolates in the bovine reservoir that is transmitted to humans and/or is capable of causing clinical symptoms.

Purpose: The purpose of this study was to evaluate the distribution of various STEC O157 genotypes, as defined by different sets of genetic markers, among bovine, food and clinical isolates in The Netherlands. An additional goal was the comparison with published U.S. data, where the STEC O157 disease incidence is as least four times higher as compared to The Netherlands.

Methods: A set of 75 veterinary (73 cattle, 3 goat, 1 deer), 85 human clinical and 45 food STEC O157 isolates was obtained from Dutch strain databases. Six different PCR-assays designed to detect specific genetic markers were conducted: 1) Lineage specific polymorphism assay (LSPA-6), 2) Shiga toxin-

encoding bacteriophage insertion site assay (SBI), 3) Q-stx2 assay (Q-stx2) 4) Tir polymorphism assay (Tir A/T), 5) stx2/stx2c assay and 6) clade 8 detection.

Results: Dutch bovine isolates were dominated by LSPA-6 lineage 2 (61%) and lineage I/II (38%), while lineage 1 compromised only 1%. Dutch clinical isolates were dominated by lineage I/II (76%), followed by lineage I (15%) lineage II (9%). In contrast, in the U.S., lineage I dominated in among both bovine and clinical isolates (52% and 62%). Dutch bovine and clinical isolates showed a relatively high occurrence of SBI genotype 1, while in the U.S., this SBI genotype 3. Moreover, Dutch clinical isolates showed a greater diversity in SBI genotypes compared to the bovine isolates, while this was the opposite in the U.S. The high toxin producing stx2-Q933 allel was present in only 14% of the Dutch bovine isolates, while 58% of the clinical isolates possessed Q933. In contrast, in the U.S., the stx2-Q933 allel was present in respectively 46% and 91% of the bovine and clinical isolates. The occurrence of the Tir-T allel under bovine and clinical isolates were similar between both geographic regions (approximately 50% with bovine isolates and >90% with clinical isolates). The food isolates showed intermediate frequencier of genotypes. Multinomial logistic regression analysis showed that tirA/tirT and stx2/stx2c were the most distinctive genetic features between human and animal isolates.

Significance: The results support the idea that (potential) clinical STEC O157 isolates form a subgroup of the larger bovine reservoir. The occurrence OF STEC O157 genotypes differed considerably between The Netherlands and the U.S., which might (partly) explain differences in the STEC O157 disease incidence. Current research is focusing on phenotypic differences between the genotypes. The results can be used to build a framework for molecular risk assessment of STEC O157.

Technical Session 2 – Thursday, 19 May, 14.00-15.30

Chair, Stefano Colombo

T2-01 A Bug's Life: Insects as Carriers of *Listeria monocytogene* – Review of the Multinational Listeriosis Outbreak (2009/2010)

Dagmar Schoder and **Martin Wagner**, University of Veterinary Medicine, Austria

Introduction: As a result of an outbreak investigation on a cluster of 34 cases of listeriosis, with 8 fatalities, "Quargel" acid curd cheese was identified as the source of infection (Fretz et al., 2010). The outbreak was dominated by two different *L. monocytogenes* clonal types (type 1 and 2), whereas type 2 was linked to 59% of all clinical cases in Austria, Germany and the Czech Republic and was seen to be more virulent than clone type 1.

Purpose: Immediately after the outbreak became officially known, the Institute of Milk Hygiene, Milk Technology and Food Science was asked to clarify the cause of the outbreak by tracing the contamination routes of *L. monocytogenes* within the cheese production plant and by finding the entry-site and source of contamination.

Methods: The outbreak investigation included (i) the conducting of interviews, (ii) inspection of the cheese production plant, (iii) analysis of the self control data and (iv) sampling of the cheese production chain. Qualitative examination of the cheese samples was performed according to ISO 11290-1. *L. monocytogenes* isolates were genotyped by pulsed field gel electrophoresis (PFGE).

Results: According to the self control data of the company, the cheese production plant was free of *L. monocytogenes* until May 2009. Interviews revealed that crucial parts of the cheese processing plant were under construction in the early spring period of 2009. From May 2009 to March 2010 a total of 1.165 samples were investigated. The contamination route could be traced back to the reconstruction site. Insect traps which were in direct proximity to the reconstruction area, were found to be *L. monocytogenes* positive, showing the same PFGE pattern than outbreak clone type 2.

Significance: This study helps to highlight the role of insects in the transmission of *L. monocytogenes*. Bearing in mind that the environment provides a substantial reservoir of *L. monocytogenes*, the analysis of the whole data set indicates that *L. monocytogenes* could enter the cheese production plant during a reconstruction phase in the early spring period of 2009 using insects as carrier.

T2-02 Modelling the Effect of a_w and Fat Content on the High Pressure Resistance of *Salmonella enterica*

Nicoletta Belletti, **Margarita Garriga**, **Teresa Aymerich** and **Sara Bover-Cid**, Food Safety Programme, IRTA, Monells, Spain

Introduction: High hydrostatic pressure (HHP) is one of the emerging technologies proposed as an alternative to thermal processing for microbial inactivation. The effect of HHP on spoilage and pathogenic microorganisms strongly depends on the magnitude of HHP technological variables, the type of microorganism, and the food composition. Therefore, reliable models quantifying the influence of food characteristics on the pathogen inactivation are needed.

Purpose: The aim of the present work was to model the inactivation of *Salmonella enterica*, on a dry-cured ham system, as a function of a_w and fat content.

Methods: Minced dry-cured ham with adjusted a_w (0.86 – 0.96) and fat content (10% – 50%) was inoculated (at 10^7 CFU/g) with *S. enterica* CTC1003 and submitted to HHP (347-852 MPa, 5 min/15°C), according to a central composite design. Bacterial inactivation was assessed as the difference between *Salmonella* counts after the treatment and the initial inoculum measured on selective media. Multivariate linear regression was used to describe the relationship among studied variables.

Results: According to the best fitting polynomial equation, pressure intensity and a_w exerted a significant influence on the *S. enterica* inactivation, following a linear trend and interacting to each other. Lowering the a_w of the product resulted in a clear baroprotection, which was more evident at increasing pressures. At low a_w (0.88) the level of *S. enterica* inactivation was little affected by increasing pressure (e.g., 2.3 to 3.2 logs at 450 to 750 MPa, respectively), while at the highest a_w the estimated inactivation ranged from 3.3 to 8.9 logs at 450 to 750 MPa, respectively. No effect on *S. enterica* was recorded for the product fat content.

Significance: The relevant influence of intrinsic factors on the pathogen HHP inactivation indicates the need to assess and validate the effectiveness of HHP on specific food products.

T2-03 Strong Inhibition of *Hafnia alvei* on *Escherichia coli* O26:H11 Quorum Sensing mRNA Expression Levels during Growth in Milk in Presence of Model Cheese Microbial Consortium

Florence Postollec and **Nadine Hénaff**, ADRIA Développement, France; **Céline Delbès-Paus**, INRA-UR545, Unité de recherches Fromagères, France; **Françoise Irlinger**, INRA UMR GMPA Agro Paris Tech, France; **Marie-Christine Montel**, INRA-UR545, Unité de recherches Fromagères, France; **Danièle Sohier**, ADRIA Développement, France

Introduction: Quorum Sensing communication in bacteria is mediated through the production, the release and detection of small chemical signal molecules.

Purpose: Within the frame of a collaborative project on the risks and benefits of gram-negative bacteria in a cheese model microbial ecosystem, the aim of this work was to evaluate whether *E. coli* O26:H11 could interact with cheese microflora using QS signals.

Methods: Skimmed milk was inoculated with 10 lactic acid bacterial and technological strains commonly found in the core of raw milk cheese, *E. coli* O26:H11 (10^3 CFU/ml), with or without *H. alvei* (10^6 CFU/ml). Sampling was performed after 4 h at 33°C and 10, 24 and 48 h incubation at 25°C. *E. coli* population was quantified by enumeration on specific agar medium and q PCR. *E. coli* gene expression was performed using RT-qPCR targeting genes involved in QS (luxS, pfs), metabolic activity (16S rRNA, tuf, GAPD) and stress response (uspA, groEL, rpoS). While (RT)-qPCR quantifications of *H. alvei* were performed targeting tuf and luxS genes. All experiments were carried out for 3 independent mixed cultures.

Results: In presence of Gram positive microflora, targeted *E. coli* O26:H11 expression levels mainly correspond to metabolic activities and signals potentially involved in QS activities, with similar expression levels for luxS, pfs and tuf genes. Maximal *E. coli* population reaches 7.83 log and rpoS expression was possible after 4 hours. In presence of H. alvei, *E. coli* growth was strongly inhibited and remained at 5.56 log. Unlike previously observed, *E. coli* luxS and pfs transcripts were largely higher than tuf transcripts. While expression levels recorded for H. alvei clearly showed a predominant metabolic activity.

Significance: This study reports modifications of mRNA expression levels of *E. coli* O26:H11 in cheese model in presence of *H. alvei*, underlining the incidence of signals potentially involved in QS when interacting with complex ecosystems.

T2-04 Effects of Climate Change on the Occurrence of Deoxynivalenol in Wheat

Ine Van der Fels-Klerx, RIKILT, The Netherlands; **Paul Goedhart**, PRI, The Netherlands; **Michel Uitwerwijk**, Alterra, The Netherlands; **Kees Booij**, PRI, The Netherlands

Introduction: Mycotoxins are thermally stable chemicals that pose a serious threat to animal and human health. These toxins are formed by filamentous fungi in plant production under favorable environmental conditions. One of the most important toxins in Europe is deoxynivalenol (DON) produced by *Fusarium* spp. in wheat. Infection of the crops and DON formation is largely affected by the local climatic conditions. Climate change, therefore, is expected to influence the occurrence of DON in wheat.

Purpose: The aim of the current study was to estimate the occurrence of DON in wheat in The

Netherlands, given the projected climatic changes for the future.

Methods: An empirical model was developed for the prediction of DON in wheat at harvest in The Netherlands. The model uses different climatic variables in specific wheat growth stages as input. The model was then used to estimate the occurrence of DON in the future, given the projected climatic changes. To this end, the climatic scenarios of IPCC were used as input to the predictive DON model.

Results: A map was developed with the predicted occurrence of DON – with expected levels on a regional scale in The Netherlands – for the years 2030–2050. This map shows that the levels of DON in wheat are expected to increase in the next 20 to 40 years, but that this increase varies per region.

Significance: The integrated approach of using quantitative models for the prediction of mycotoxins and for climatic conditions is useful to underpin estimations for the future occurrence of this feed and food safety hazard.

T2-05 Modeling Heat Resistance of *Bacillus* Spores as Function of Sporulation Temperature and pH

Eugenie Baril, ADRIA Développement, France; **Louis Coroller**, **Olivier Couvert** and **Ivan Leguerinel** LUBEM, France; **Florence Postollec**, ADRIA Développement, France; **Christophe Boulais**, Danone Research, France; **Frédéric Carlin**, INRA, France; **Pierre Mafart**, LUBEM, France

Introduction: *Bacillus cereus* and *Bacillus licheniformis* spores are able to resist to heat treatments applied in minimal food processing. During the food shelf life, spores germinate and vegetative cells multiplication can be the cause of outbreaks of foodborne poisonings and/or food spoilage. The capacity of spores to resist heat treatments is acquired during the sporulation process during which particular conditions can lead to highly heat resistant spores.

Purpose: The aim of this study was to propose a model describing the heat resistance of spores depending on sporulation temperature and pH.

Methods: The heat resistance of the psychrotrophic *B. weihenstephanensis* KBAB4 and of the mesophilic *B. licheniformis* AD978 were characterized for spores produced at various temperatures ranging from 5°C to 50°C and pHs ranging from 5.2 to 8.5.

Results: The highest spore heat resistance was predicted for both strains when spores were produced at temperature of 24.4°C, pH 8.0 and 49.9°C, pH 8.5 respectively. Above and below those optimal sporulation conditions, the spore heat resistance decreased significantly. Heat resistance data were fitted from a model combining a temperature and a pH cardinal parameter model according to the "gamma-concept". The parameters were defined as the maximum heat resistance, the optimal sporulation temperature and pH, and the minimal and maximal sporulation temperature and pH defined by the equations of the vertical asymptotes of the spore heat resistance. Furthermore, the model was validated by the characterization of spores produced at different temperatures in soil-based medium and in whey. The observed heat resistance values were consistent with those predicted by the model.

Significance: Optimal temperature and pH parameters estimated for the highest heat resistance correspond to values close to those of optimal growth. This suggests that the highest spore heat resistance is acquired when sporulation occurred at optimal growth temperature and pH. This study shows that growth cardinal values could be valuable indications for determining the conditions at which maximal heat resistant spores occurs.

T2-06 Use of ATP as a Field Equipment Cleaning Metric for Leafy Green GAPs

Eric O'Brien and **Robert Salter**, Charm Sciences, Inc., Massachusetts, USA

Introduction: Leafy Green Good Agricultural Practices (GAPs) are a series of U.S. guidelines for fresh produce production implemented to reduce the risk of pathogen contamination. One of these GAPs is cleaning of field-harvest equipment on a daily or semi-day basis and establishing verification/validation metrics for that cleaning.

Purpose: This project evaluated field pre and post cleaning practices with a rapid ATP swab designed

for outdoor applications to establish a cut-off value for acceptably clean harvest equipment.

Methods: Field harvest equipment was evaluated pre-cleaning/sanitization and post-cleaning sanitization using Charm Sciences, Inc. Fieldswab™/FireFly2™ for ATP detection and for APC determination using 3M Petrifilm™. ATP Fieldswabs were tested immediately on-site. Simultaneous lecithin broth swabs were refrigerated and sent to an outside laboratory for APC results. Cleaning was evaluated in the harvest equipment blades and conveyors: header, incline, sorting, crossover, and belt-to-bin.

Results: The mean APC count pre cleaning was 5488 SD 8117 CFU/100 cm^2 and post cleaning was 122 SD 133 CFU/100 cm^2 . The mean ATP RLU pre cleaning was 512591 SD 405920 RLU and post cleaning was 38794 SD 34527 RLU. These metrics represent log reductions of 1.65 for APC and 1.12 for ATP. With additional analysis of post cleaning data a cut-off of 20,000 RLU was implemented as a re-cleaning benchmark and to verify effective cleaning to APC levels typically less than 100 CFU/100 cm^2. Disassembly with scrubbing and soap was more effective than sanitation alone at achieving the cut-off.

Significance: Cleaning metrics are useful in demonstrating log reduction of surface microbial count in contact with produce. Rapid ATP cleaning metrics can improve cleaning practices beyond the practice of visually clean and provide immediate feedback to field operations. This is effective in teaching and improving good harvest practices. It is also an environmentally sustainable practice because effective sSOPS can be verified using environmentally approved chemicals for field cleaning.

Acknowlegement: NewStar Fresh Foods, Salinas, CA for supplying data.

Young Scientists Technical Session – Friday, 20 May, 09.00-10.30

Chair, Christina Harzman

T3-01 Links between Bacterial Tolerance to Biocides, Biocidal Compounds and Clinically Important Antibiotics Explored Using The Proteome

Orla Condell, Centre for Food Safety, UCD, Ireland; **Áine Sheridan**, Food Safety Department, Teagasc-Ashtown Food Research Centre, Ireland; **Shane Cooney** and **Karen Power,** Centre for Food Safety, UCD, Ireland; **Carol Iversen**, Nestlé Research Center, Switzerland; **Ruben Bonilla-Santiago** and **Jarlath E. Nally**, School of Agriculture, Food, Science & Veterinary Medicine, UCD, Ireland; **Seamus Fanning**, Centre for Food Safety, UCD, Ireland

Introduction: Concern has been expressed in regard to the potential overuse of biocides and the imposed selective pressure, leading to reduced tolerance to these agents concomitant with the emergence of cross-resistance to clinically important antibiotics. Our study investigated the tolerance of *Salmonella* to biocides used in the food industry and explored their cross-resistance to a panel of clinically important antibiotics.

Purpose: To determine whether *Salmonella* exposed to sub-lethal concentrations of food industry biocides can develop a tolerance and to explore whether this tolerance is associated with cross-resistance to a panel of clinically important antibiotics. To explore the mechanisms involved using the proteome.

Methods: *Salmonella* mutants tolerant to three biocidal agents common to the food industry, triclosan, chlorhexidine and benzalkonium chloride, were generated. Susceptibility profiles of isogenic tolerant and sensitive strains were determined to a panel of drugs; biocidal agents, industrial biocides and clinically important antibiotics. Mechanisms involved in biocide tolerance were investigated through genetic, proteomic and phenotypic means.

Results: Six *Salmonella* isolates tolerant to three biocidal agents were generated in vitro. Tolerant strains showed no reduction in their susceptibility to industrial biocide formulations nor to the isolated active biocidal agents in these preparations. However changes in antibiotic susceptibilities were recorded for all mutants. Significant alterations in the proteome were observed between isogenic parent and mutant strains, with over 500 proteins showing differential expression and a clear divergence apparent in the response of the proteome following exposure to biocidal active agents.

Significance: This study determined that if bacteria are exposed to sub-lethal concentrations of active biocidal agents common to the food industry tolerant isolates may emerge. Sub-lethal concentrations can induce discernible alterations in the proteome of exposed bacteria. These changes are associated with a reduced susceptibility to clinically important antibiotics.

T3-02 Quality Standards in Quantitative PCR; Specification, Validation, Controls and Standards

Peter Rossmanith and **Martin Wagner**, CD-Laboratory for Molecular Biological Food Analytics, University of Veterinary Medicine Vienna, Austria

Introduction: The implementation of molecular methods such as real-time PCR for food pathogen detection is desired and reasonable. Nevertheless, the obstacles of precise specification and meaningful validation are not yet overcome and therefore, broad range use in food testing is not yet accomplished. Specification is generally based on the determination of the detection limit, the overall efficiency of the reaction and exclusivity and inclusiveness of the assay respectively. These parameters do not provide sufficient information about the real performance of the underlying enzymatic reaction. The validation according to ISO 16140, the validation of alternative methods, has many drawbacks based on its original sense and purpose, the comparison of microbiological methods. Evaluation of real-time PCR is therefore not significant with this process due to the different nature of molecular biological methods.

Purpose: Establishment of a significant specification and validation approach in consideration of the inherent qualities of real-time PCR.

Methods: A validation system including testing algorithms derived from software engineering; per se specification of the enzymatic reaction, controls covering all necessary steps and the investigation of surrounding parameters was designed. The whole approach is based on fundamental principles of systems theory and cybernetics. This alternative strategy includes every necessary detail thus leading to a maximum performance of the assay and most precise specification and validation of the whole analytical chain.

Results: We present the practical application of this new approach by example of an analytical chain for the detection of *L. monocytogenes*, including sample preparation, DNA isolation/purification and real-time PCR detection.

Significance: New approaches for the significant specification and validation of molecular biological methods are necessary to gain confidence in such methods and furthermore support widespread implementation. The whole system approach presented herein is an equivalent attempt, which effectively supports the standard validation method ISO 16140.

T3-03 Is There a Genetic Relationship between Potentially Persistent *L. innocua* and *L. monocytogenes* Strains?

Irene Rückerl, Institut for Milk Hygiene, Veterinary University of Vienna, Austria; **Richard Stabler**, Department of Pathogen Molecular Biology, Faculty of Infectious & Tropical Diseases, London School of Hygiene and Tropical Medicine, United Kingdom; **Sonja Klinger**, **Martin Wagner** and **Beatrix Stessl**, Institut for Milk Hygiene, Veterinary University of Vienna, Austria

Introduction: The genus *Listeria* includes the pathogens *L. monocytogenes* and *L. ivanovii* and non-pathogens *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. marthii* and *L. rocourtiae*. *L. innocua* represents a young species descended from *L. monocytogenes* and comprises four subgroups. *Listeria* spp. are widespread in the natural environment (water, soil) but can be also be problematic contaminants of food processing facilities. Many of the characteristics of *Listeria* spp. such as a tolerance to extreme pH (4.0–9.5), temperature (1°C–50°C), salt conditions (10% sodium chloride) and production of biofilms may have pre-adapted *Listeria* to survive within these facilities. Therefore, the presence of *Listeria* species in food may be an indicator of poor hygiene in food processing facilities.

Purpose: This research was focused on characterization of *L. innocua* strains isolated from food processing facilities by biotyping, serovar-PCR and pulsed-field gel electrophoresis (PFGE).

Methods: Pulsed-field gel electrophoresis (PFGE) was performed according to the CDC protocol. PFGE patterns were compared by means of the Dice coefficient, by using Fingerprinting II software (Bio-Rad Laboratories).

Results: Comparison of the PFGE fingerprints identified short- and long-time persistent *L. innocua* isolates in three dairies. Thirteen potential persisting *L. innocua* and *L. monocytogenes* isolates from the same manufacturers were identified and the gene content surveyed through hybridisation to a pan-species, pan-strain whole genome *Listeria* microarray. Two *L. monocytogenes* (EGD-e, F6854) and one *L. innocua* (CLIP 11262) reference strains were included in the set up.

Significance: Common genetic marker(s) for persistence in *L. monocytogenes* and *L. innocua* will be investigated.

T3-04 The Use of Omic Biomarkers to Track the Survival of *Bacillus weihenstephanensis* KBAB4 Throughout Acid Inactivation

Noémie Desriac and **Florence Postollec**, ADRIA Development, France; **Louis Coroller**, LUBEM, France; **Danièle Sohier**, ADRIA Development, France

Introduction: *Bacillus cereus* is ubiquitously found in soil and may either cause emetic or diarrheal types of food-borne illnesses. To prevent bacterial development, food industries use preservation techniques like low pH and cold temperature storage.

Purpose: in this study, the acid resistance of psychrotrophic *B. weihenstephanensis* KBAB4 strain is investigated using microbiological and transcriptomic methods for various stress adaptations (cold, salt, acid…).

Methods: Standardized protocols were used to determine bacterial inactivation during an acid stress (pH 4.6). Population evolution was followed by CFU enumeration and kinetics were fitted using a mixed Weibull model. In parallel, the expression of fourteen genes, selected as potential biomarkers, was performed using RT-qPCR. Quantification of each gene transcription was related to two housekeeping reporter genes (gyrA and sigA) which showed the most stable expression (geNorm M value of 1.158).

Results: In optimal conditions, exponentially cells submitted to an acid shock could be divided in two populations, i.e., one sensitive (first decimal decrease after 1h00 of inactivation) and one resistant (first decimal decrease after 3h00). In these conditions, gene expression showed up-regulation (9 genes) or down-regulation for 2 genes (narL and napA). Furthermore gene variation profile may vary during inactivation kinetic. For instance, sigB encoding general stress sigma factor, is up-regulated throughout inactivation with a peak after 2h00 (7.4 fold ± 2.3) whereas katB encoding major catalase is up-regulated at the beginning of the kinetic and stays constant from 2h00 to 4h00 of inactivation (around 31 fold).

Significance: Acquisition of gene expressions along inactivation kinetics is in progress for various physiological states. These results will allow to develop a predictive tool that will correlate the genes expression to the bacterial resistance. The prediction of bacterial history-dependent behaviour using gene expression will offer a decision making tool adapted to industrial processes.

T3-05 The Survival of *E. coli* O157 and *Salmonella* in Irrigation Water and the Influence on Their Subsequent Survival on Greenhouse Grown Lettuce

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Introduction: Irrigation water is one of the potential preharvest entry routes of the zoonotic pathogens *E. coli* O157 and *Salmonella* on greenhouse grown lettuce. Until now, the survival and growth of these pathogens on fresh produce has been mainly studied with freshly cultured strains suspended in a standard buffer or distilled water. However, it is known that the pathogens are subjected to physiological changes when introduced in surface water.

Purpose: The present research aims to investigate the impact of the prior history of the inoculum of zoonotic pathogens (overnight lab culture versus survival in irrigation water) on their subsequent survival on plants.

Methods: First, the survival characteristics in irrigation water at 4°C and 20°C of two strains each of *Salmonella* and *E. coli* O157 EPEC were investigated by standard plating techniques. For this study, two types of irrigation water including well water and groundwater samples were collected from four lettuce production sites in Belgium. Second, the growth of the pathogens on butterhead lettuce leaves was investigated. Lettuce leaves were inoculated with a standard laboratory inoculum or with irrigation water in which the pathogen had been present for different time intervals.

Results: In irrigation water, the pathogens survived significantly better at 4°C than at 20°C ($P < 0.01$). When lettuce leaves were inoculated with contaminated irrigation water, the growth capacity of the pathogen on the leaves was negatively influenced by the time that the pathogen had been present in the water. Three days after inoculation of the leaves, differences up to 3 log units were observed between leaves inoculated with pathogens in irrigation water compared to the pathogens' inoculum in phosphate buffered saline.

Significance: Zoonotic pathogens introduced onto lettuce leaves through contaminated irrigation water survive but grow less well than what could be expected from trials performed with standard laboratory inocula. These results also indicate that the risk of pathogen growth on leafy greens and the accompanying food safety risk is influenced by the time spent by the pathogen in irrigation water.

T3-06 Risk Perception towards Emerging Food Safety Risks on Fresh Produce: The Impact of Governmental Trust on Evoked Fear

Melanie De Vocht and **Verolien Cauberghe**, Communication Science, Ghent University, Belgium; **Mieke Uyttendaele** and **Benedikt Sas**, Food Safety and Food Quality, Ghent University, Belgium

Introduction: Fresh produce is an important part of a healthy diet. However, due to recent disease outbreaks and rapid alerts attributed to fresh produce and derived food products, concerns emerged with regard to food safety. The EU Veg-i-Trade project conducts research on emerging food safety risks related to fresh vegetables and fruit due to climate change and global sourcing. Some of these risks cannot be prevented by the consumers (e.g., pesticide residues, mycotoxins,…) which stresses the importance of governmental trust.

Purpose: Risk communication is necessary to prevent people from starting a scare when a foodborne outbreak might occur. The Extended Parallel Processing Model (EPPM) of Witte (1992) is the most appropriate threat/risk related model to explain consumers' reactions to health risk messages. Threat appeals trigger a process by which individuals appraise two perceptions: the perceived threat (susceptibility and severity) and the perceived (self or response) efficacy of the hazard which may result in either message acceptance or avoidance.

Methods: In November 2010 a survey was conducted among 456 participates in Belgium. This survey measured general risk perception, problem awareness, evoked emotions, behavioral intentions and socio-demos.

Results: Besides descriptive results related to socio-demographic differences, a significant interaction effect between susceptibility and governmental trust on the evoked level of fear ($F(1,452) = 5.908$, $P = 0.05$) appeared. Only when the governmental trust is low, susceptibility increases the level of fear. When trust is high, there appeared no effect of susceptibility of the threat.

Significance: This study stresses the importance of governmental trust. In addition, these results show that evoked fear has a significant positive effect on risk perception. This risk perception has subsequently an impact on the intention to rinse fresh produce better ($r = .388$, $P < .001$), but not on the intention to eat it less. Based on these findings further research and risk communication strategies are suggested.

Poster Session 1 – Wednesday, 18 May at 12.00 – Thursday, 19 May at 11.00

Authors will be present at their posters during the conference breaks

P1-01 Farm Food Safety Training Materials for Fresh Produce Farm and Pack-house Operators

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Introduction: Effective food safety training is an important pre-requisite to successful implementation of a food safety management system (Arvanitoyannis and Kassaveti 2009). Training programmes which are more closely associated with the work site are potentially more effective especially if supported by practical reinforcement of the message.

Purpose: To develop effective farm food safety training materials for field and pack-house operators.

Methods: Training needs analysis was conducted with participating fresh produce farms. Only farm workers in direct contact with ready-to-eat (RTE) fresh produce were recruited for the training. Food safety training materials were developed according to Jacob et al. (2010). Training materials were distributed in hand-out formats, video and practical hand hygiene demonstration. Hand hygiene demonstration and practise were carried out to enhance the vivid experience and understanding of cross-contamination.

Results: Training materials consisting of testimonial-based case studies, personal perception of risks via product liability cases and the importance of farm food safety practices were developed. Food safety messages were reinforced through the hand hygiene demonstration using the GloGerm® kit. All farms operators from 6 participating farms agreed that participating in the hands-on hand hygiene demonstration gave them the strongest impact and understanding of cross-contaminations.

Significance: Effective farm food safety training materials are useful to project the importance of food safety practices and hand hygiene to both full-time and seasonal farm workers. Participating in hand hygiene demonstration increases the vivid experience of participants and improves understanding of how cross-contamination occurs.

P1-02 Withdrawn

P1-03 Inhibition of *Alicyclobacillus acidoterrestris* Spore Germination under Most Optimum Growth Conditions Using Lactic Acid, a Natural Antimicrobial

Saurabh Kumar, **Janneke Wijman**, **Renate Zumbrink**, **Diana Visser** and **Edwin Bontenbal**, PURAC Biochem, Netherlands

Introduction: *Alicyclobacillus acidoterrestris* spores have been implicated in the spoilage of commercial pasteurized fruit juices. The growth characteristics of the bacterium like low pH and high temperature tolerance make it a serious spoilage concern during the long shelf life of fruit juices. This study evaluated the antimicrobial efficacy of lactic acid against the bacterial spores under the most abusive storage conditions which can indicate the long term efficacy of the intervention.

Purpose: The objective of this study was to evaluate the antimicrobial efficacy of lactic acid against the spores of *A. acidoterrestris* under optimum growth conditions.

Methods: A three strain *A. acidoterrestris* spore cocktail was inoculated in broth (DSMZ *Alicyclobacillus* growth medium) to a final spore concentration of 2–3 log CFU/ml. The treatments (0, 0.15, 0.30, 0.73 and 0.80% lactic acid concentrations) were prepared in two sets having triplicate tubes for each treatment. Each set of triplicates was incubated at 30 and 50°C, respectively, and growth was measured as absorbance (at 690 nm) using a spectrophotometer.

Results: The data indicate that the *A. acidoterrestris* spores are not able to germinate and grow (OD <

0.01) in the presence of lactic acid, while the control treatments did show outgrowth with normalized optical densities of ca. 0.150 and 0.750 at 30 and 50°C, respectively. The lactic acid treatment consistently inhibited the growth at both incubation temperatures at all concentrations tested.

Significance: This research proves the antimicrobial efficacy of lactic acid in fruit juices under the most challenging conditions. These results provide food industry a natural ingredient to preserve fruit juice compared to the high heat treatment currently used which involves high processing costs and is also detrimental to the juice quality.

P1-04 Renewable Antimicrobial Coatings – A Practical Application of a Novel Technology for Enhancing Sanitation in Food Processing Operations

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Introduction: Cross-contamination and post process re-contamination have been identified as significant contributors to the contamination of food by pathogenic or spoilage organisms. Food contact surfaces are contamination vectors and cleaning and disinfection regimes have been developed to minimize this risk. Despite this, pathogens like *Listeria monocytogenes* and *Escherichia coli* are known to survive in food processing environments as persistent "in-house-microflora", which may re-contaminate food contact surfaces or foodstuffs. These microbial niches may result, from inappropriate cleaning and disinfection or from microbial survival in the wider food processing environment. Temporary, renewable antimicrobial coatings (RAC) are a solution for the reduction of the microbial burden of environmental surfaces. Such coatings are resistant to environmental moisture or condensation, but are removed by water rinses; allowing RAC to be incorporated into routine hygiene programs.

Purpose: The efficacy of an innovative quaternary ammonium based renewable antimicrobial coating (RAC) was tested in two food processing environments on non-food contact surfaces in production and adjacent areas.

Methods: Test sites were cleaned and disinfected before RAC was applied. Adjacent sites were left uncoated after cleaning and disinfection as control. Sanitation level of test surfaces was monitored weekly with contact plates. In total 441 samples were taken from treated and control surfaces over a period of five weeks.

Results: Results show a significant reduction in average colony forming units on treated versus untreated surfaces. Over 80% of control samples were overgrown, whereas about 30% treated area samples showed no microbial growth.

Significance: This study demonstrates the benefits of using RAC, to decrease the microbial burden on surfaces cleaned infrequently. It can help food processors to reduce the risk of indirect microbial food contamination from such sources.

P1-05 Virucidal Efficacy of Vaporized Hydrogen Peroxide Disinfection

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Introduction: Contamination of surfaces by viruses in individual households, food processing facilities, hospitals and health care settings may lead to long-term presence of viruses on such surfaces and is believed to play an important role in their transmission. Chemical disinfection can be an effective means of transmission intervention.

Purpose: To test the virucidal efficacy of vaporized hydrogen peroxide (VHP) disinfection against different human viruses applied to stainless steel, framing panel and gauze.

Methods: VHP disinfection was performed in real-life situation in a room against poliovirus Sabin 1. Additionally, poliovirus Sabin 1, adenovirus type 5, rotavirus SA11, murine norovirus 1(MNV1), influenza A (H1N1) virus and human norovirus (NoV) GII.4 were exposed to VHP in an isolator at room temperature and relative humidity between 65–80%. The viruses were extracted by swabbing.

Results: VHP disinfection at 120 ppm for 1 h at room temperature resulted in complete inactivation of all viruses tested. Complete inactivation was characterized as > 4 $\log_{10}$ viability reduction of poliovirus, rotavirus, adenovirus, and MNV1 on stainless steel and framing panel and >2 $\log_{10}$ viability reduction of all the tested viruses on gauze and influenza A (H1N1) virus on stainless steel and framing panel. Complete inactivation was confirmed under dirty conditions at several locations in a room for poliovirus. Reductions of viral genomes measured in PCR units were minimal on framing panel and gauze carriers, but significant on stainless steel. Comparison of reductions of PCR units on stainless steel shows that human NoV GII.4 RNA was reduced less than the RNA or DNA of the other viruses tested.

Significance: Thus the VHP disinfection at 120 ppm for 1 h is effective against poliovirus Sabin 1, rotavirus SA11, adenovirus type 5 and MNV1 on stainless steel, framing panel and gauze. But effectiveness against human NoV could not be confirmed.

P1-06 Rapid and Simple Pre-treatment Method of Grains and Snacks for Zearalenone Analysis

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Introduction: Due to climate changes and global warming concern, the controls of fungi and mycotoxin were being strengthened. EU regulations concerning mycotoxin dealing with grains and processed grains have been established against mycotoxins and also being tightened its limits in Korea as well. Among the various mycotoxin, zearalenone is a naturally occurring toxin produced by the Fusarium species. Fusarium is so heat-stable that it is hardly removed during processing with heat treatment. The legal limits for zearalenone in snacks (processed grains) were newly established in Korea in 2010.

Purpose: To quantify zearalenone, rapid and accurate analytical method has been required. QuEChERS (quick, easy, cheap, effective, rugged and safe) was introduced by Anastassiades in 2003 as a new approach to extract a wide range of pesticides from different food matrices with high water content. The proposed method was based on liquid partitioning with acetonitrile followed by a dispersive SPE clean-up. In addition, it may have many advantages over traditional techniques such as high simple throughput, the use of smaller amounts of organic solvent without chlorinated solvents. Therefore, a new pretreatment method, QuEChERS method, was applied to detect zearalenone in snacks more efficiently.

Methods: Forty samples of four grains such as wheat, corn, rice and buckwheat and 10 samples of sugar-processed snacks were purchased from retail markets in Seoul. The method uses acetonitrile for extraction of the analytes followed by the addition of high concentrations of MgSO4 and NaCl. The samples were separated by the C18 HPLC Columns, using the mobile phase (Acetonitrile, Methanol, Water(10:55:35,v/v/v)) at a flow rate of 1.0 ml/min for 20 minutes. Detection of zearalenone was carried out at wavelength of 274 nm excitation and 450 nm emission.

Results: The recovery yields of zearalenone from grains and snacks being spiked at levels of 50, 100 µg/L were ranged from 86.0%~99.0% by using QuEChERS pre-treatment method. Likewise, RSD by HPLC- QuEChERS methods could get range of 5%~10% from grain and snacks spiked at the level of 50, and 100 ug/L, respectively. Pre-treatment period for one sample with HPLC- QuEChERS was 0.5 h.

Significance: HPLC-QuEChERS method showed reliable results in recovery yields and

reproducibility. It also needed a small number of samples and much less time to get the result. It was suggested that HPLC-QuEChERS method is an alternative pre-treatment method for zearalenone analysis in grains and snacks. Especially QuEChERS method, because of its advantage of less required time for analysis with less sample quantities, could be applied to industrial fields where a lot of samples have to be analyzed quickly.

P1-07 Chromogenic Indicators for Pathogens Contaminating Food Samples

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Introduction: Commercial indicators commonly seize food spoilage by measuring changes in temperature or pH-value but are not directly dependent on microbial growth. In the present study the applicability of chromogenic bacteria as an `on view` model is shown. Selected chromogenic bacteria placed on filter membranes and exhibiting similar growth characteristics as food pathogens mimic the development of possible pathogen flora. Resulting, a model comprising colored bacteria indicates microbial growth on food stuff.

Purpose: Development of a membrane based approach for a sensitive model to facilitate in situ detection of possible food pathogen growth.

Methods: To proof osmotic nutrient supply for microbial growth but prevent contamination of the food with microbes, semi-permeable filter membranes with pore sizes ranging from 0.1 to 10 µm were used on plate and directly on minced meat or poultry. Controls were plated without filter membranes. As a model organism *Serratia marcescens*, producing the purple-red pigment prodigiosin, was used to test pore size related nutrient flow (colony sizes and numbers), biological integrity (growth on agar without nutrients) and impermeability (growth underneath the filter membrane). Growth was monitored over a period of 48 hours at various temperatures (4°C, 15°C, 25°C, 30°C). To test for minimal sensitivity, total microbial counts were determined for all meat samples (ISO/FDIS 4833).

Results: Similar colony numbers and diameters were detected comparing cell suspensions on filters and controls for pore sizes as far as 0.1µm, indicating a good nutrient diffusion through the membranes. No colony growth was found upon removal of the filter membranes (0.1–0.4µm) and on sole agar plates indicating impermeability and biological integrity. Highly concentrated cell suspensions performed well on minced meat and poultry, developing red cell clusters on the filter membranes within four hours at 30°C. When signal occurrence was compared to total microbial cell counts from meat, a 0.4 log scale change of total microorganisms was detected.

Significance: The study presents a model for a food pathogen growth indicator, which can be adapted to various pathogenic organisms and food stuffs.

P1-08 Ionic liquids for DNA Quantification out of Gram Negative and Gram Positive Bacteria

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Introduction: In molecular biological food pathogen detection an easy and quick method for bacterial cell lysis and DNA purification is of great interest. Time saving is a major reason for the application of molecular methods. In analytical chains, including sample preparation, the DNA isolation procedure is the most promising step for significant reduction of expenditure of time. Gram positive bacteria especially need extensive protocols, including overnight enzymatic digestion to obtain DNA that can be used in sensitive analysis methods like qPCR.

Purpose: Establishing a fast and easy method for bacterial cell lysis and DNA isolation by using a two phase system using a bmpyrr based ionic liquid (IL) and ddH2O. Screening of ILs for DNA isolation out of Gram negative (*S.* Typhimurium, *E. coli*) and Gram positive (*L. monocytogenes*) bacteria. Inhibition of the following qPCR should be excluded.

Methods: As reference method for quantitative DNA isolation the NucleoSpin® tissue kit was used. The quantification of bacterial cell equivalents was carried out by qPCR with the respective protocols

for *S.* Typhimurium, *E. coli* and *L. monocytogenes*.

Results: A ten minute protocol for cell lysis and DNA isolation out of various bacterial species was developed. DNA of *Salmonella* and *Escherichia* was found in high amounts. *Listeria* was isolated with an average yield of only 10% in comparison to the NucleoSpin® tissue kit. A pre-incubation step by using a DMAE based IL was introduced for *Listeria* and resulted in a yield of about 100%. QPCR quantification demonstrated inhibition free amplification after the new DNA isolation protocol.

Significance: The two phase IL cell lysis protocol is able to lyse Gram negative cells within a few minutes and the DNA can be used directly afterwards for qPCR quantification. For Gram positive bacteria a pre-incubation step of ten minutes is necessary to obtain DNA recovery comparable to Gram negative bacteria.

P1-09 Discrimination and Classification of Acetic Acid Bacteria and *Saccharomyces cerevisiae* Strains by Attenuated Total Reflectance Microspectroscopy

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Introduction: In wine processing, there are different microorganisms that are known to have an effect on wine quality (color, taste and chemical composition)and participate during the fermentation process (alcoholic or malolactic). Yeast (*Saccharomyces cerevisiae*, lactic acid bacteria and acetic acid bacteria are the common ones. There is a strong need to rapidly identify bacteria and yeast populations during wine fermentation. The microorganisms involved in a wine fermentation have been studied by conventional culture techniques but are time consuming and may underestimate the number of viable bacteria. Attenuated total reflectance infrared microspectroscopy (ATR-IRMS) provides bands from all the cellular components of microorganism (e.g., cell membrane and wall components, proteins and nucleic acid), giving spectral signatures or "fingerprints" that permit the classification of microorganisms at the strain and serovar level.

Purpose: Attenuated total reflectance infrared microspectroscopy (ATR-IRMS) coupled with multivariate analysis technique was used to discriminate between acetic acid bacteria, (*Gluconobacter oxydans*, CECT 315 and *Gluconacetobacter xylinus*, CECT 473) and yeast (*Saccharomyces cerevisiae* CECT 1327 and commercial).

Methods: Strains were grown in red grape must for 48 h, centrifuged at 6000 rpm for 5 min, washed with saline solution and pellets (10^8 CFU/mL) were deposited onto the grids of hydrophobic membrane filters and dried to produce a uniform and thin film. Spectra were collected in the attenuated total reflectance (ATR) mode in the mid-infrared region (4000–700 cm^{-1}) and were analyzed by a multivariate analysis technique, soft independent modeling of class analogy (SIMCA). *Saccharomyces cerevisiae* and acetic acid bacteria strains were discriminated mainly due to the difference in their cell wall composition.

Results: Our classification models, soft independent modeling of class analogy (SIMCA) obtained from derivatized infrared spectra (1600–900 cm^{-1}), showed that *Saccharomyces cerevisiae* and acetic acid bacteria strains were discriminated mainly due to the difference in their cell wall composition. Discriminating power of infrared spectra analysis of the bacteria and yeast strains showed three strong spectral bands at 1130, 1026 and 1666 cm^{-1} located in the bacteria fingerprint region. These IR peaks were related to $>PO^2-$ stretching of nucleic acids, O-H group of cellulose present in the bacterial cell, and amide I bands linked to the presence of mannoprotiens in yeast or peptidoglycan in bacterial cell walls, respectively.

Significance: This technology would allow identification of spoilage bacteria in wine fermentation with a reduced detection time.

P1-10 The Impact of Four Buffer Systems Derived from a Matrix Solubilisation-based Sample Preparation Protocol for Bacteria on the Integrity of Virus Particles

Susanne Fister, **Sabine Fuchs**, **Patrick Mester**, **Martin Wagner** and **Peter Rossmanith**, CD-Laboratory for Molecular Biological Food Analytics, University of Veterinary Medicine Vienna, Austria

Introduction: Major causes of foodborne illnesses in humans are viruses. Accurate detection of foodborne viruses is limited on molecular diagnostics and existing methods are laborious, time consuming and not standardized. One important demand is the separation of the target particles from the food matrix as obtained by sample preparation protocols. Nevertheless few investigations and protocols covering this topic are published so far. On the other hand there are several newly developed and well established protocols for molecular biological quantification for food-borne bacteria available. Aim of this study was the investigation of the applicability of a recently developed sample preparation method that involves the solubilization of the food matrix (Matrix Lysis) for the separation of viruses from food samples

Purpose: Testing of the impact of several food solubilization buffers as used for Matrix Lysis on the integrity of calicivirus and MS2 bacteriophage particles.

Methods: The influence of the four existing Matrix Lysis buffers on feline caliciviruses and MS2 phages was tested using reverse transcription RT-PCR for quantification. Feline caliciviruses and MS2 phages were used as model for norovirus. The particle count before inoculation was compared with the RT-PCR data after incubation in four buffer systems (A: 8M Urea and 1% SDS; B: 8M Urea and 1% Lutensol; C: 1.5 M MgCl2; D: 7.5% Ionic Liquid: [emim]SCN).

Results: The recovery as obtained for the MS2 and calicivirus particles are in good agreement with the respective values as obtained for bacterial cells. Recovery rates are ranging from 31 to 70% in buffers A and B and from 63 to 86% in buffers C and D.

Significance: The Matrix Lysis system is a promising tool to support molecular biological detection of foodborne viruses. Future work will focus on concentration of viruses subsequent to Matrix Lysis and before RT-PCR detection.

P1-11 Phage Ligand-based Selective Enrichment (AMS) for the Detection of *Escherichia coli* O157 According to ISO-Norm 16654 and with Real-time PCR

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Introduction: In previous studies, highly specific bacteriophage proteins binding to *Escherichia coli* O157 have been shown to be suitable for selective enrichment and detection of foodborne pathogens, allowing reduction in pre-enrichment time as well as lowering background noise in the detection [Kretzer et al. 2008, Rozand et al. 2009].

Purpose: The purpose of this study was to evaluate a commercially available phage ligand-based Affinity Magnetic Separation (AMS) method for the selective enrichment of *E. coli* O157 combined with detection according to ISO-norm 16654 as well as with Real-Time PCR (foodproof® *E. coli* O157 Detection Kit, Biotecon Diagnostics, Potsdam, Germany). Currently, antibody-based Immunomagnetic Separation (IMS) is recommended for the selective enrichment of *E. coli* O157 in ISO-norm 16654, which was applied as reference method in this study.

Methods: The evaluation was conducted using minced meat and fresh mixed salad samples which were artificially contaminated at low and medium inoculation levels (0.9–6.0 CFU/25 g) in order to obtain fractional positive results. 20 meat and 10 salad samples of each 25 g were pre-enriched according to ISO-norm 16654.

Results: The detection according to ISO 16654 combined with AMS gave analogue results to the reference method using IMS in all 30 food samples. The AMS method showed a highly reduced background flora on the selective media plates which simplified the visual identification of suspected *E. coli* O157 colonies. The detection with Real-Time PCR combined with AMS identified the same

samples as contaminated/not-contaminated as the reference method ISO 16654.

Significance: Our results show that AMS is a promising alternative to IMS in the ISO-norm 16654. Additionally, the time for detection using AMS combined with Real-Time PCR can be significantly reduced.

Acknowledgments: We would like to thank Hanna Dietz, Meike Gross and Sybille Holzmann (LMU). The study was supported by the federal state of Bavaria with its project executing organization Bayern Innovativ GmbH.

P1-12 Comparison of SimPlate® *Campylobacter* – Color Indicator and *Campy* CEFEX Agar Methods for the Enumeration of *Campylobacter jejuni* and *Campylobacter coli* from Poultry Rinse Samples

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Introduction: The SimPlate® for *Campylobacter* Color Indicator (C-CI)method allows for the enumeration of *Campylobacter jejuni* and *Campylobacter coli* in poultry carcass rinses in 48 hours.

Purpose: To compare the specificity of SimPlate® *Campylobacter* Color Indicator (C-CI), *Campy* CEFEX Agar, Abeyta-Hunt-Bark(AHB)agar and Line Agar. To compare the recovery and enumeration of *Campylobacter* from poultry carcass rinses using SimPlate C-CI and Campy CEFEX Agar.

Methods: The specificity of SimPlate C-CI was compared to 3 *Campylobacter* selective agar formulations, Abeyta-Hunt-Bark (AHB) agar, *Campy* CEFEX agar and Line agar. A field trial comparison of the performance of the SimPlate C-CI method and the *Campy* CEFEX method was performed at 3 poultry processing plants. A total of 168 carcass rinse samples were analyzed with both methods.

Results: SimPlate C-CI and the 3 selective agar media detected all isolates of *Campylobacter jejuni* and *Campylobacter coli* tested. SimPlate C-CI demonstrated good correlation for the enumeration of *Campylobacter* with all three plating methods. A total of 168 samples were analyzed by both SimPlate C-CI and *Campy* CEFEX methods. The data were plotted on a simple regression graph and the resulting slope and correlation coefficient were 0.91 and 0.96, respectively.

Significance: These data indicate that SimPlate C-CI is comparable to the *Campy* CEFEX culture method for the detection and enumeration of *Campylobacter* from poultry rinse samples.

P1-13 Adjusting Sensitivity of ROSA Beta-lactam Tests to More Closely Detect Cefalonium at MRL

David Douglas, **Say-Jong Law**, **Robert Salter** and **Robert Markovsky**, Charm Sciences, Inc., Massachusetts, USA

Introduction: Receptor-based beta-lactam detection in rapid milk screening assays uses broad-spectrum screening of chemical functional-groups. The sensitivity of any one chemical structure is related to 3-D conformational fit into the ligand binding sites. The Charm ROSA (Rapid One Step Assay) formatted test uses a patented down-regulation with competing ligand-binding agents to adjust hyper-sensitivity to more closely match drug tolerance levels in milk, e.g., maximum residue limits (MRL) or Safe Level (SL). Sensitivity adjustment to cephapirin and ceftiofur has helped farms abide with prescribed antibiotic treatment and withhold times without loss of milk from oversensitive screening tests. Cefalonium with MRL of 20 ppb is a beta-lactam that is hyper-sensitive at 3–6 ppb in receptor binding assays; and it is reported to be a cause of unnecessary milk rejection in countries such as Ireland and New Zealand where the drug is a popular dry-cow treatment.

Purpose: Adjust the sensitivity of the Charm SL Beta-lactam Test to more closely detect cefalonium at the MRL of 20 ppb.

Methods: Cefalonium ligand-binders were produced with beta-lactam-protein conjugate injected into animals. The Charm Kiwi Strip was produced for screening evaluation by applying affinity purified ligand-binders to Charm SL beta-lactam strip. The method was evaluated internally, and in NZ-SAITL

and trialed on dairy trucks to qualify method sensitivity.

Results: The modified Kiwi Test had 90% sensitivity with 95% confidence at 15 ppb compared to the original Charm SL Test 6 ppb sensitivity. Concentration response range of the Kiwi test was 10–14 ppb cefalonium and the original Charm SL test was 3–6 ppb. Selectivity of negatives and sensitivity of the other detected beta-lactam drugs penicillin G, ampicillin, amoxicillin, cephapirin and cloxacillin was not shifted.

Significance: It is possible to adjust sensitivity of broad antibiotic screening tests to be more compliant to specific drug tolerances.

Ackowledgments: Peter Bailey, Food Tech Solutions, Auckland NZ and Paul Jamieson, SAITL, Hamilton NZ

P1-14 Evaluation of the New IQ-Check™ *Cronobacter* spp. Kit for the Detection of *Cronobacter* spp. in Infant Formula and Environmental Samples

Sophie Pierre, **Florence Préaudat** and **Jean-Philippe Tourniaire**, FSD, Bio-Rad, France

Introduction: *Cronobacter*, formerly named *Enterobacter sakasakii*, is an ubiquitous pathogen that has been isolated from foods, environment and clinical sources. It is sadly associated with rare but fatal (case-fatality rate may reach 80%) infant infections linked to the consumption of reconstituted Powdered Infant Formulas.

Purpose: The iQ-Check™ *Cronobacter* spp. method is based on the RTi-PCR technology and consists in a single enrichment step in Buffered Peptone Water followed by an easy DNA extraction and the RTi-PCR detection step using the Double Strand fluorescent Probes.

Methods: The performances of the kit were evaluated.

Results: The kit offers a high level of specificity (50 *Enterobacteriaceae* strains were tested without cross-reaction), a good sensitivity closed to 100 CFU/mL in pure culture and was tested on Powdered Infant Formula and Environmental Samples in the context of an AFNOR validation.

Significance: This method provides faster results than the microbiological reference method which is time-consuming.

P1-15 Preliminary Selection of Yeasts to Reduce the Growth of *Salmonella enterica* serovar Typhimurium in Vitro and on Fresh Cut Lettuce

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Introduction: *Salmonella* is a genus of rod-shaped bacteria, gram-negative, belonging to the *Enterobacteriaceae*. In recent years some outbreaks have been also associated to various plant products. *Salmonella* spp. may contaminate fresh products during the pre- and postharvest phases. The capacity of this pathogen to survive and grow on vegetable products is affected by its ability to adapt to the new ecological environments outside its hosts. The fresh-cut products represent a promising and innovative sector, responding to the customer needs and adapting to his lifestyle. Therefore, it is necessary to ensure the microbiological safety of fresh-cut products, because they go through different steps of processing and manipulation (cutting, washing and packing), and for this reason they are more prone to contamination than whole products. *Salmonella enterica* is the most frequently isolated species in cases of foodborne infections. The complete removal or killing of this pathogen on fresh or fresh-cut products is currently a difficult task.

Purpose: The aim of the present study was to evaluate and select some potential antagonist yeasts against *Salmonella enterica* serovar Typhimurium.

Methods: The antagonistic yeasts tested were *Pichia guilliermondii* strain M8, *Metschnikowia pulcherrima* strains MACH1, BIO126, GS9, 3008 and 3345, *Hanseniaspora uvarum* strain SAL3,

Rhodotorula glutinis strain PW8, *Rhodotorula mucilaginosa* strain PW34 and *Debaryomyces hansenii* strain AR37. In vitro and in vivo experiments were performed.

Results: Among the antagonists tested, the strain M8 showed a potential biocontrol potential against *S.* Typhimurium at room and low temperature and after 17 or 24 hours of incubation.

Significance: Yeast biocontrol agents could be applied as a suspension on fresh cut lettuce during processing before packaging, helping to reduce human pathogen growth on plant products.

P1-16 Survey Conducted by a Consumer Organization for the Presence of Bacterial and Viral Pathogens on High Risk Fresh Produce from the Belgian Market

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Introduction: Although fresh produce is an important part of a healthy diet, vegetables and fruits are increasingly recognised as an important vehicle of foodborne outbreaks and have been associated with alerts. As a consequence, also the consumer starts to concern about the safety of these products.

Purpose: A survey of leafy greens, herbs and soft red fruits commissioned by the Belgian consumer organisation was undertaken in Belgium in collaboration with Ghent University in the framework of the EU FP7 Veg-i-Trade project.

Methods: Sampling took place across the country in different markets in the period around the Christmas holidays of 2010 as in this period of the year many high-risk fresh produce products are globally sourced and imported in Belgium from a variety of countries and companies, each with their own production practices and food safety management system.

The bacterial pathogens *Salmonella*, *Campylobacter*, *Escherichia coli* O157 and hygiene indicator *E. coli* were analysed. Reversed transcriptase (RT) real-time PCR was performed to analyse viral pathogens such as noroviruses (NoV) including murine norovirus as process control. FRNA bacteriophages were examined as a potential viral indicator.

Results: In Belgium a total of 36 fruit or vegetable samples were analysed. None tested positive for any of the bacterial pathogens analyzed, and 11.4% (4/35) tested positive for NoV. Despite the absence of FRNA bacteriophages, one sample was found positive for NoV genogroup I (rocket salad) and three for NoV genogroup II (parsley, basil and rocket salad). According to the hygiene indicator *E. coli*, all samples were of good microbial quality.

Significance: Despite the good bacteriological quality, NoV were detected by RT-PCR. As the relation between the detection of genomic copies and the presence of infectious virus particles is not yet established the risk to human health is still questioned. Further steps will be undertaken for confirmation and sequencing of the NoV positive samples.

P1-17 Prediction of *Listeria monocytogenes* Growth in Italian Crostini Liver Pâté

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Introduction: *Listeria monocytogenes* presence is of major concern in ready-to-eat food products which, due to their nature, might allow growth. EU Regulatory n° 2073/2005 requests the evaluation of the potential growth of *Listeria monocytogenes* in the product in order to determine the adequate criterion applying to the RTE. The use of challenge studies or growth prediction models can be used but prediction is sometimes not corresponding to the reality of the challenge studies.

Purpose: The aim of this study is to compare the results of 6 challenge studies (3 repetitions on 2 batches) performed on Italian crostini liver pâté (traditional recipe with no preservatives) with the prediction made by Combase on these products.

Methods: Challenge studies were performed according to EU Guidance document (AFSSA November 2008) using 16 strains of *Listeria monocytogenes* (isolated in majority from meat) cultivated in post exponential phase. Challenge studies were conducted with an inoculation level between 10 and 100 CFU/g and incubation at 8°C for 10 days. Every day, *Listeria monocytogenes* was enumerated according to ISO 11290-02 (with increased sensitivity of 1 CFU/g), Total plate count was enumerated according to ISO 4833, Lactic bacteria's were enumerated according to NF ISO 15214:1998. Growth rate and lag phase were obtained by ComBase – fitting, physiological state was calculated. For the period when pH is stable, *Listeria monocytogenes* development was predicted through ComBase Predictor with: physiological state as calculated, average pH and w of this time. For the period during which the pH starts to decrease the prediction was based on the following data's pH and a_w averages of 2 adjacent days and with physiological state of 1. Prediction and experimental data have been compared.

Results: The results of the challenge study demonstrate a growth of *Listeria monocytogenes* ranging from 2.28 log to 3.89 log reached within 8 to 10 days. Lag phase ranges from 0.75 to 1.40 days, growth rate ranging from 0.46 to 0.60 log/day. The maximum level *Listeria monocytogenes* obtained in the challenge studies ranges from 3.6 to 5.04 log/g when the prediction model integrating pH reaches 5.95 to 6.71 log/g. Prediction systematically overestimated final level of *Listeria monocytogenes* by 1.05 to 2.4 logs. The correlation of prediction and challenge study is excellent until level of lactic bacterias reaches 8 log/g (stationary phase) corresponding to a pH drop from 6.1 to 5.6. At this stage, *Listeria monocytogenes* stops its growth in all cases.

Significance: These results indicate that prediction with Combase Predictor is confirmed by challenge studies demonstrating traditional Italian Crostini liver pâté is favourable to *Listeria monocytogenes* growth when stored at 8°C. Prediction is overestimating the level of *Listeria monocytogenes* when lactic bacteria reaches the stationary phase (about 8 log/g). The limiting factor is not pH and the influence of lactic flora should be introduced in the model.

P1-18 Preliminary Results on the Spontaneous Loss of *Vtx* Genes after the First Subculturing Step

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Introduction: *Verocytotoxigenic Escherichia coli* (VTEC) is an emerging group of foodborne pathogens, causing a variety of clinical outcomes such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The major virulence factors are the verocytotoxins, which are phage-born. Different studies reported the potential loss of *vtx* genes during multiple subcultivation and storage of isolates.

Purpose: The aim of this study was to determine the frequency of the spontaneous loss of *vtx* genes after the first subculturing step among O157 and non-O157 VTEC strains after isolation from cattle feces.

Methods: For non-O157, the isolation method described by Possé (2008) was used. Isolation of VTEC O157 was performed by an enrichment during 6 hours, followed by IMS prior plating. One O157 and non-O157 of the suspected colonies were subcultured. Consequently, 10 colonies from each subculture were tested by a virulence multiplex PCR to detect the presence of *vtx*.

Results: A loss of *vtx* genes was observed in 3 out of 10 O157 and in 7 out of 15 non-O157 VTEC strains. The frequency of this loss within the 3 O157 strains was rather rare (on average 1 out of 10), while for the 7 non-O157 strains the rate of loss was higher (on average 5 out of 10 isolates). The loss was not related to the *vtx* gene type, namely *vt1* or *vt2*.

Significance: Our results prove that a loss of *vtx* genes in VTEC isolates can already occur during the first subculturing of VTEC isolated from naturally contaminated samples. Consequently this may lead to an underestimation of VTEC in animals, food and humans. Therefore we advice to test different colonies from the subculture for the presence of *vtx* genes in order to avoid false negative results.

P1-19 Improved Rapid Detection of Human Norovirus from Tomatoes by Next (Second) Generation SYBR GreenER Based Real-time RT-PCR

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Introduction: High risk foods that include fresh produce have been frequently implicated in norovirus related human gastroenteritis outbreaks. Therefore, rapid detection of human noroviruses from fresh produce is important to curb outbreaks, but remains challenging due to their low virus load in vegetables, that can still cause infection.

Purpose: Our objectives were to determine and compare detection sensitivity of SYBR Green I and SYBR GreenER fluorescent dye based real-time RT-PCR kits for improved norovirus GI and GII detection from cherry tomatoes, used as a model representative produce item.

Methods: Washed tomatoes (25 g) were spiked with serial dilutions of norovirus GI and GII stool samples in a blind random manner. RNA was extracted using the TRIzol™ method, and samples of undiluted and 10-; 100-; 1.000-; 10.000-fold dilutions of extracted RNA were analyzed by SYBR Green I and SYBR GreenER real-time RT-PCR kits and previously published highly specific degenerate primers for GI (COG1F-COG1R) and GII (COG2F-COG2R). In order to eliminate false negative results, previously designed RNA internal amplification controls (IACs) were included in the assay. External controls included RNA extracts from stool samples used for spiking tomatoes.

Results: End-point detection limits for both norovirus GI and GII with SYBR Green I and SYBR GreenER were 10 RT-PCR units/25 g and 1 RT-PCR unit/25 g, respectively. Higher detection sensitivity of ~1 to 2 log RT-PCR units of all extracted viral RNA dilutions using SYBR GreenER RT-PCR kit was recorded in comparison to SYBR Green I RT-PCR kit. These results were confirmed by *Tm* analysis (product *Tms* for GI, GII and IAC were 81.5°C, 84°C and 83°C, respectively) as well as by agarose gel electrophoresis (product size of GI, GII and IAC were 85 bp, 98 bp and ~155 bp, respectively).

Significance: This improved rapid and sensitive detection assay for human norovirus from tomatoes shows promise for application in routine analysis of outbreak-associated produce.

P1-20 ISO 16140/MicroVal Evaluation of a Defined Medium for Enumeration of Thermotolerant *Campylobacter* spp.

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Introduction: Oxoid Brilliance™ CampyCount Agar is a novel defined medium for the direct enumeration of thermotolerant *Campylobacter* spp. colonies grow as distinct dark red colonies against a clear background, facilitating enumeration.

Purpose: Brilliance CampyCount Agar (alternative medium) was evaluated in an independent MicroVal validation study according to ISO 16140:2003.

Methods: The alternative method was evaluated against modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) for the enumeration of thermotolerant *Campylobacter* spp. from poultry samples, according to ISO 10272-2:2006. 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 ISO 16140:2003. In addition to the confirmation requirements of ISO 10272–2, presumptive colonies on Brilliance CampyCount Agar were confirmed using the Oxoid Dryspot *Campylobacter* latex kit and O.B.I.S Campy test.

Results: In the method comparison study Brilliance CampyCount Agar was shown to have comparable performance to the reference method (mCCDA) in terms of inclusivity, exclusivity and limits of detection and quantification. Statistical analysis of the linearity showed no statistically significant

evidence of lack of fit ($P = 0.25$). Linear regression analysis (GMFR) demonstrated the relative accuracy of the reference and alternative methods to be equivalent (r = 0.99, y = 1.05 × -0.14) for all confirmation methods. The interlaboratory study was conducted with 17 laboratories in 8 countries. Samples of minced chicken meat were artificially contaminated to Low (log_{10} 3.4 CFU/g), Medium (log_{10} 4.7 CFU/g) and High (log_{10} 6.0 CFU/g) levels of contamination. Results showed no significant bias between both methods at those levels (D = 0.08, 0.14, and 0.19, respectively).

Significance: Brilliance CampyCount Agar was shown to be comparable in performance to mCCDA for the enumeration of thermotolerant *Campylobacter* spp. in poultry products. Campylobacters 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 confirming presumptive growth on Brilliance CampyCount Agar. The certificate of compliance (2008-LR12) can be found on www.microval.org.

P1-21 ISO 16140 Preliminary Study of a New Chromogenic Agar Plate Method for Detection of *Listeria monocytogenes*

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Introduction: ChromID™ Lmono (LMO) agar is a chromogenic medium for the selective detection of *L. monocytogenes* species in food and agri-food production environmental specimens. Based on their specific enzyme activity, *Listeria monocytogenes* strains appear as blue colonies on the agar when other bacteria are inhibited or appear as white colonies.

Purpose: This new method was independently validated in Pasteur Institute, for the detection of *L. monocytogenes* in food products and environmental samples, as part of the ISO 16140 validation process.

Methods: Food samples, 1/10 diluted in half-Fraser broth, were enriched for 22–26 hours at 30°C. Then, 100 µl of enrichment broth were streaked onto the chromogenic agar and the plates were incubated for 24–26 h at 37° C. Presumptive *L. monocytogenes* blue colonies were confirmed by the Rapidec™ Lmono assay, The VIDAS® LMO2 assay or by traditional assays.

Results: A comparative study between the new method and the ISO 11290-1 reference method was performed on 426 products distributed over the 5 categories meat, dairy, vegetable, seafood products and environmental samples. One hundred and sixty two products were found positive of which 67% were naturally contaminated. Similar results were obtained by both methods showing a sensitivity of 97% for the chromogenic method and of 98% for the reference method. The 50% detection limit, determined on 6 food products/strains tested at 4 contamination levels, was found to be between 0.2 and 1.9 CFU/25 g for the two methods. Good results were obtained for the inclusivity and exclusivity studies with the analysis of 50 *L. monocytogenes* and 30 non-*L. monocytogenes* strains.

Significance: This new method is a reliable alternative to the traditional method for detection of *L. monocytogenes* in food and environmental samples with positive and negative results obtained after 2 days compared to 5 to 11 days for the reference method.

P1-22 Raw Milk Cheeses Offical Controls during 2008–2010 in Piedmont

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Introduction: Recently the attention on raw milk cheese has increased, as raw milk could be a potential vehicle for transmission of pathogens to the consumer. In Italy, especially in Piedmont, there is a growing market for locally produced raw milk cheese. As a consequence, consumption of raw milk cheese has increased. To verify safety of these products, a monitoring plan against *L. monocytogenes*, *Salmonella* spp. and staphylococcal enterotoxin was conducted in Piedmont.

Purpose: The aim of this work is to report results of monitoring plan in raw milk cheese in Piedmont.

From 2008 to 2010 a total of 663 samples of raw milk cheese were collected according to official sampling plans.

Methods: The cheeses selected were produced by bovine, goat and/or sheep milk. 626 of raw milk cheese samples were analyzed for *L. monocytogenes*, 567 for *Salmonella* spp. and 663 for staphylococcal enterotoxin using official normated laboratory methods

Results: Of the cheese tested 98.3% (CI95%: 97.1%–99.2%) were free of *L. monocytogenes*. No *Salmonella* spp. was found in the selected samples; while staphylococcal enterotoxin was detected in 1.3%. (CI95%: 0.61%–2.53%).

Significance: Results indicate that Piedmont raw milk cheeses are of high microbiological quality; as level of *L. monocytogenes* and staphylococcal enterotoxin in raw milk are low. However, raw milk cheeses safety can be enhanced if producers are aware of the food-safety hazards associated with their industry and therefore implemented hygienic conditions for housing and milking practices.

P1-23 Evaluation of VIDAS® UP *Salmonella* (SPT) Assay for the "Next Day" Detection of *Salmonella* in Foods and Environmental Samples

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Introduction: Because of market demands, *Salmonella* detection analyses are becoming more frequent and being performed in growing number of samples. As a consequence, food industry requires rapid and simple method to increase the sensitivity of the diagnostic process and to reduce the time spent to obtain a result.

Purpose: The objective of this study was to evaluate the performances of a new automated next day method for the detection of *Salmonella* in food, animal feed and environmental samples.

Methods: Samples are enriched in Buffered Peptone Water with the addition of a selective supplement for 18 hours at 41.5 ± 1°C. The enriched samples are heated 5 minutes at 95–100°C, prior to the detection on the VIDAS instrument. The enzyme immunoassay enables the specific detection of somatic (O) and flagellar (H) *Salmonella* antigens by associating monoclonal antibodies with an innovative technology involving recombinant phage proteins. Both motile and non-motile strains are detected. Positive results were confirmed by streaking the enrichment broth onto XLD and/or ChromID *Salmonella* agar. This study compared the new method to the ISO 6579 reference method, according to the ISO 16140 standard requirements.

Results: The comparative study included 478 products distributed as follows: 73 meat, 76 dairy, 62 seafood and vegetables, 68 miscellaneous products, 60 raw milk cheeses, 60 feed samples and 79 environmental samples. Forty-six percent of positive samples were naturally contaminated and 54% were artificially inoculated with stressed *Salmonella* at low level. Two hundred twenty-one samples were confirmed positive by one of the methods, 1 by the immunoassay only, 1 by the cultural method only and 255 by both methods. Mac Nemar's analysis at the 5% level showed no significant difference between the alternative method and the reference method.

Significance: The VIDAS® UP *Salmonella* provided comparable results to the ISO 6579 reference method for the detection of *Salmonella* in food, animal feed and environmental samples. It provides a rapid, sensitive and convenient method allowing the release of a negative sample in less than 20 hours after a simple enrichment procedure.

P1-24 An Irish Foodborne Outbreak — Sequence Assembly and Characterisation of *Salmonella* Agona 0800

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Introduction: Since being cited a "new hazard" in 1972, *Salmonella* Agona, a major foodborne pathogen, has caused global outbreaks. In humans, symptoms of *Salmonella* Agona infection include diarrhea, fever and abdominal cramps, which last between 4–7 days. In 2008, *Salmonella* Agona 0800 and an Irish food producer were responsible for causing a major outbreak. This producer was a major exporter of pre-cooked meats used as sandwich fillers and pizzas toppings (y800 tonnes/week) that were distributed across Europe. Some 163 cases in seven European countries were laboratory-confirmed with cases ranging in age from 3 months to 87 years. Two elderly infected patients died.

Purpose: Due to the significance of the outbreak, the purpose of this study was to sequence, characterise and compare this persistent foodborne pathogen with other *Salmonella* spp.

Methods: As part of the Centre for Food-borne Zoonomics (CFZ) project, *Salmonella* Agona 0800 and R0102, another strain found to contaminate and persist in the factory providing the food, but does not cause infection, were sequenced. Optical maps of the 2 genomes alongside other *Salmonella* species were generated. In combination with assembled sequencing reads, these maps were used to generate a comparative analysis with the only published strain of the same species SL483.

Results: Strains 0800 and R0102 displayed very similar optical map profiles with ~0.5% difference. In order to interrogate these differences further, a bacterial annotation pipeline was then used to predict protein-coding genes to cross-compare results on a gene level. A ~137kb region of interest was detected as significantly different between all three strains. Phage genes contained within this region were found to be a major cause of difference between the 0800 and R0102 in comparison with the SL483 genome and reduced the optical map distance between them from 2.5 to 0.7%.

Significance: The differences observed environmentally between 0800 and R0102 are interesting, however, more sequencing is required to determine other potential genetic differences such as single nucleotide polymorphisms (SNPs) that may contribute to their characteristics. These genomic results might help to explain why this particular serotype is involved in food outbreaks.

P1-25 Survival and Growth of *L. monocytogenes* in Quargel (Acid Curd Cheese) Lots Recalled during the Multinational Listeriosis Outbreak 2009/2010

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Introduction: Contaminated "Quargel", acid-cured cheese was the cause of the most recent multinational outbreak of listeriosis that accounted for 34 clinical cases and 8 deaths. The cheese, which was distributed in Austria, Germany, the Czech Republic, Poland and Slovakia, was recalled January 23, 2010.

Purpose: All recalled lots could be seized and shipped to the Institute of Milk Hygiene, Milk Technology and Food Science. The aim of the study was (i) to detect the contamination rate of all recalled lots and (ii) to conduct challenge tests at 5, 15 and 22°C in order to simulate proper and improper storage conditions.

Methods: Depending on the production date of the lots, cheese samples were analyzed at four different time points: (i) beginning, (ii) mid and (iii) end of shelf life and (iv) ≤ 46 days after exceeding the expiry date. Qualitative and quantitative examination of the cheese samples was performed according to ISO 11290-1 & 2 and a combined enrichment/real time PCR method (Rossmanith et al. 2006).

Results: 260 acid curd cheese samples were investigated immediately after call-back from the retail market. Examination of the samples, according to ISO 11290:1 & 2 resulted in 16 *Listeria monocytogenes* positive and 2 negative lots. These results were confirmed by a combined enrichment/real-time PCR method. The values of contamination obtained by quantitative ISO 11290-2 varied from ≤ 100 CFU/g to 1.2×10^8 CFU/g. Three out of sixteen *L. monocytogenes* positive lots showed a contamination rate of ≤ 100 CFU/g at the beginning of the shelf life when stored at 5°C. Nevertheless by increasing the storage life and/or the storage temperature (15, 22°C) the contamination rate could be raised to 10^5 up to 10^6 CFU/g.

Significance: Our data indicate that 72% (13/18) of the recalled Quargel cheese lots were highly contaminated with *L. monocytogenes*. Obviously Quargel cheese can easily support growth of *L. monocytogenes*.

P1-26 Building a Portuguese Food Microbiological Information Network

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Introduction: The integration of food data from research, microbiological monitoring, epidemiological investigation and disease surveillance is crucial to manage foodborne risk. Consequently, INSA launched the Portuguese Food Information Resource Programme (PortFIR) in a partnership with GS1 Portugal to create national food chain expert networks and sustainable databases on food composition, consumption and chemical and microbiological contamination. Presently, the Food Microbiological Information Network (RPIMA) is being built.

Purpose: The purpose is to build RPIMA including users and stakeholders, food microbiological data producers and regional authorities on agriculture and health to maximize resources (data, knowledge, financial, human, equipment…), spread knowledge and amplify the national capability.

Methods: Potential users, stakeholders and data producers were identified and invited to participate in RPIMA through an e-mail questionnaire. A network meeting was organized to present, discuss and approve RPIMA's goals. Structured brainstorming with experts of all food chain steps was used to define thematic working groups. Terms of Reference of PortFIR transversal working groups (WGs), "Users", "Organization and Transfer of Information" and "Support to Standardization Work" were adapted to microbiology specificities through consensus within the WGs.

Results: Currently the network has 82 members covering activity areas like food production and trade, risk assessment, research and education. RPIMA's goals approved at its 1st annual meeting in October 2010 were a) to collect food microbiological information produced in different contexts, b) to standardize and c) analyse it and d) to make it available to national and international users and stakeholders namely risk assessors and risk managers. The brainstorming meeting took place on January 2011 and lead to the creation of 2 WGs: "Food Chain Microbiological Occurrence" and "Foodborne Outbreaks" whose reference terms are being defined. Presently, the Terms of Reference of the transversal WGs are under discussion.

Significance: The results obtained so far regarding involvement and willingness to share data and knowledge indicate that RPIMA will allow to 1) monitor food microbiological occurrence, 2) biotrace foodborne outbreaks, 3) detect antibiotic resistance emergence, 4) provide scientific evidence for risk management, good hygiene practices and optimization of HACCP systems, 5) quantify the impact of risk management decisions, 6) trace climate change impact and 7) identify information gaps to be researched in order to improve food safety.

P1-27 Evaluating Food Safety Management Performance in a Food Service Establishment According a Microbiological Assessment Scheme

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Introduction: Poor performing food safety management systems (FSMS) and an attendant increase of food safety risks in professional kitchens may impact on a significant number of (sensitive) consumers.

Purpose: The microbiological performance of a FSMS in an institutional catering setting (ICS) was measured, using a Microbiological Assessment Scheme (MAS) vertical through the production process from raw materials to final product.

Methods: MAS supports in deciding on where and how to take a sample, at what frequency, how to conduct microbial analyses, how to interpret results and judge the outcome in perspective of the FSMS. Three different production processes were evaluated: a high risk sandwich (containing raw meat preparation); a medium risk hot meal (starting from undercooked raw materials) and a low risk hot meal (in pack regeneration).

Results: Total aerobic count exceeded guidelines on gloves of foodhandlers and on food contact surfaces. Spoilage indicators, hygiene indicators and relevant pathogens (like *L. monocytogenes* and *Salmonella* spp.), were in accordance with criteria and/ or guidelines.

Significance: Based on the obtained microbial safety level profiles over the three visits during one year, it can be concluded that the FSMS in the ICS is functioning well. The current FSMS can only be improved on the control activities cleaning and disinfection of food contacts materials and personal hygiene to reduce the contamination of total viable count. The MAS allows to get insight in the microbial contamination and the variability of a production process and is enabling to identify weaker points in a FSMS.

P1-28 Withdrawn

P1-29 Withdrawn

P1-30 Withdrawn

P1-31 Determination of Growth/No Growth Boundaries for 5 Species of Molds Commonly Found in Bakery Products

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Introduction: Bakery products are important staple foods in most countries and cultures. However, mold growth is a major problem that limit shelf life even for low-moisture constant products.

Purpose: The aim of this work was to develop a predictive software to determine growth/no growth boundaries of 5 strains of molds and further simulate growth on intermediate moisture content bakery products.

Methods: A total of 5 strains, i.e., *Aspergillus flavus*, *Cladosporium cladosporoïdes*, *Eurotium herbariorum*, *Penicillium chrysogenum*, and *Wallemia sebi* were studied. Growth was recorded for a range of temperature (5–40°C) on Potato Dextrose Agar ($0.8 < a_w < 0.99$) and on real bakery products for lower a_w.($0.7 < a_w < 0.84$) Fungal growth rate was expressed as the increase of colony diameter per day while lag time was reported as mold apparition time. Rosso model (1993) allowed the evaluation of cardinal values for water activity ($a_w$min, $a_w$opt and $a_w$max) and temperature (Tmin, Topt and Tmax).

Results: Simulation showed that growth of *Aspergillus flavus* and *Eurotium herbariorum* was possible for a_w lower than 0.7 while $a_w$min of *Cladosporium cladosporoides* was 0.73. *Wallemia* and *Eurotium* tested strains showed lower awmin value than already published data. Temperature cardinal values determined for the 5 studied genera showed great correlation with values from the litterature. Because the infuence of pH was not significant for the range of studied pastry products, growth boundaries were defined considering only a_w and temperature parameters. Developed models were validated comparing simulated optimal growth rates and observed growth rate on various bakery products during storage at 25°C. Growth simulations were in agreement whatever the product formulation tested.

Significance: This study shows that developped predictive models enable to simulate the influence of temperature and a_w on fungal growth rate on bakery products, for studied conditions. Developped decision making tool provide growth/no growth boundaries as well as fungal growth simulation for bakery products.

P1-32 A Microbial Assessment Scheme for the Cooked Chilled Foods Production Process

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Introduction: Cooked chilled foods, or REPFEDs (refrigerated and processed foods of extended durability), are a heterogeneous group of food products. Depending on the applied heat treatment during the production process, different pathogens pose a risk. The pathogens of concern are *L.*

monocytogenes, *B. cereus* and *C. botulinum* since these can survive the different types of pasteurisation treatments.

Purpose: This research assessed the microbiological safety and quality of REPFEDs to determine the potential for decreasing the pasteurisation-time/temperature without compromising food safety. A decrease in heat treatment can improve sensorial product quality and reduce production costs.

Methods: A Microbial Assessment Scheme (MAS) was performed in 5 Belgian REPFED-producing companies. Each MAS consisted of 18 sampling locations throughout the processing line, which were sampled 1 to 3 times per sampling day. Sampling locations consisted of both high-risk raw materials, half-fabricates, end products and contact materials during processing and shelf life. A total of 696 samples were analysed for 5 microbial parameters (*B. cereus*, *L. monocytogenes*, sulphite reducing *Clostridia*, total aerobic psychrotrophic count and aerobic spore count).

Results: Despite the high contamination (6–7 log CFU/25 cm^2) of contact materials, the microbial safety and quality of the final products is good to excellent. The majority of these products showed absence of *L. monocytogenes*, *B. cereus* and sulphite reducing *Clostridia*. If present, the counts were low and within acceptable limits. The microbial safety of raw materials and half fabricates was more variable, but this situation was corrected by the heat treatment applied in the production process.

Significance: Results indicate that the use of safe harbours delivers safe products and that companies rely to some extent on the heat treatment in the production process to guarantee the food safety of REPFEDs. Yet a reduction in pasteurisation process is possible if the recontamination of the products from contact materials is controlled properly.

P1-33 Microbial Risk Profiling of Cooked Chilled Foods

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Introduction: Cooked chilled foods, more aptly known as REPFED (refrigerated and processed foods of extended durability), are a very heterogeneous group of food products. To assure their microbial safety, the food industry relies on a combination of mild heat treatment (i.e., pasteurisation), refrigerated storage and consumer instructions for handling and preparation.

Purpose: This research assessed the potential risk to the consumer for different types of REPFEDs, based on the production process, labelling and data collection on prevalence and survival of pathogens.

Methods: The production-processes of 5 Belgian REPFED-producing companies were analysed according to their microbial risk profile. Both historic (n = 1533) and new (n = 90) analysis results for 3 pathogens (*B. cereus*, *L. monocytogenes* and sulphite reducing *Clostridia*) were collected for final products on the day of production and for final products at the end of shelf life. The pasteurisation value (*P*-value) for heat treatment at consumer phase was determined for 50 products by simulating the proposed heat treatment as recommended on the label. Finally, one high-risk product (paella) was challenge-tested (in 12-fold) for survival of *L. monocytogenes* during heat treatment at the consumer phase.

Results: Three types of cooked chilled foods could be distinguished based on the heat treatment applied during the production process (P90 = 10, P0 = 2 or no safe harbour), while 5 types of REPFEDs could be distinguished based on the heat treatment applied at consumer phase (Ready-to-eat (s.l./s.s.), ready-to-reheat, ready-to-heat and ready-to-cook). The combination of the production and consumer heat treatment ultimately determines the risk to the consumer. None of the analysed products (n = 1533) carried unacceptable numbers for any of the three pathogens. Only 10 out of 50 products that were reheated obtained a *P*-value sufficiently high to eliminate *L. monocytogenes* (P0 = 2). During the challenge tests of *L. monocytogenes* in paella, the pathogen was able to grow in all 12 replications and remained present in 7 out of 12 replications after reheating at consumer level.

Significance: Results indicate that the current microbial safety of REPFEDs is good, but that a thorough validation of both production-process and final product is necessary to guarantee the food

safety. If reheating by the consumer is necessary for food safety, then this process should also be validated.

P1-34 Risk Profiling of *Campylobacter* Contamination in Broiler Carcasses and Correlation with Slaughterhouses Hygiene and Infrastructure Inspection

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Introduction: *Campylobacter* contamination is a major challenge to the microbiological safety of poultry meat.

Purpose: In addition to the insights gained from microbiological baseline surveys, results from official food inspection could provide additional information about the hygiene related factors that contribute to *Campylobacter* contamination in poultry slaughterlines.

Methods: This study investigates risk-profiles and factors associated with *Campylobacter* contamination in broiler carcasses, using survey data collected from nine Belgian slaughterhouses in 2008 in accordance with a European Union baseline study. We also investigated the results of hygiene and infrastructure inspections routinely performed by the Belgian Federal Agency for the Safety of the Food Chain in correlation with *Campylobacter* contamination status for eight out of the nine slaughterhouses.

Results: *Campylobacter* were detected in 51.29% (202/389) of broiler carcasses. *Campylobacter* count was < 10 CFU/g in 49.6% of carcasses, while 20.6% were contaminated with ≥ 1000 CFU/g. The average *Campylobacter* contamination, as modelled by maximum likelihood estimation for censored data, was 1.78 $\log_{10}$ CFU/g, with a standard deviation of 1.89 $\log_{10}$ CFU/g. There was statistically significant variation between slaughterhouses in incidence and counts of C*ampylobacter* in their sampled carcasses. *Campylobacter* incidence and counts were positively correlated with increase in broilers age and with sampling during the months of June and September. Interestingly, ranking of slaughterhouses based on their hygiene inspection score was strongly correlated with their ranking based on prevalence of *Campylobacter*.

Significance: In the present study we demonstrate that the outcome of a baseline survey could be used to inform national control programs on *Campylobacter* risk-profiles posed by different slaughterhouses. Indicators from this work call for subsequent in-depth research on technical and hygiene management factors that could impact *Campylobacter* contamination levels across broiler slaughterhouses.

P1-35 Optimizing the Use of Peracetic Acid for Sporicidal Activity

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Introduction: Peracetic acid present a bactericidal and sporicidal activity at room temperature and it is used as sanitizer for surfaces as well as disinfectant for fruits and vegetables in the food industry.

Purpose: The aim of this study was to quantify the impact of peracetic acid on spores of *Bacillus*

cereus as a function of environmental conditions.

Methods: The spores of a psychrotolerant *Bacillus cereus* specie were inactivated by a peracetic acid solution. The treatment was done at four peracetic acid concentrations and four temperatures; from 0.25 g/L to 1.05 g/L and from 5 to 20°C, respectively. The surviving spores were enumerated using spiral plate count on Nutrient Agar. The survival curves were fitted by the Weibull model.

Results: The bacterial resistance decreases with increasing peracetic acid concentration and storage temperature. A model was proposed to quantify the treatment efficiency at a given peracetic acid concentration and temperature of use. Even though the synergy between the temperature and the concentration on the bacterial resistance is neglected, this model has a good quality of fit (RMSE = 0.04). But its main advantage remains simplicity and practical interpretation of parameters. A 17.1°C increase reduces ten fold the bacterial resistance like a 1.48 g/L peracetic acid addition.

Significance: The model allows the estimation of bacterial population decrease for given concentration of peracetic acid, time and temperature of treatment. And vice-versa, it might be used also to optimize the process parameters (time, temperature and acid concentration) knowing a targeted value of bacterial decrease. For example, if the objective is a 3 log reduction of the spore population, 1h15 treatment is required at reference conditions (20°C and 1.25 g/L). If the sanitation process is made at 4°C, we can choose to increase either the treatment time up to 10 h 30 or the concentration of peracetic acid up to 2.64 g/L.

P1-36 Measurement of the Virolysis of Human GII.4 Norovirus in Response to Disinfectants and Sanitisers

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Introduction: Noroviruses (NoVs) are recognised as the single most common cause of acute non-bacterial gastroenteritis in the industrialised world. The evaluation of suitable control measures for NoVs is considered limited by the inability to culture human NoVs making assessment of control measures difficult.

Purpose: The aim of this study was to apply a molecular method which measures NoV capsid destruction or 'virolysis' to investigate the stability of the human NoV capsid after exposure to disinfectants and sanitisers. Virolysis can be considered an indirect measure of virus infectivity.

Methods: Human NoVs GII.4 present in dilute faecal samples were exposed to a range of potential virucides based upon a standard method for measuring virucidal activity in plaque assays (EU standard EN14476.2005). The method incorporates a separation or microfiltration step in order to remove the virucide following virucide treatment. This step avoids cytoxicity problems that can prevent the determination of virus survival by plaque assay. Capsid destruction or "virolysis" was measured using the reverse transcribed quantitative PCR (RT-QPCR) reaction in conjunction with RNase I treatment in order to destroy any exposed RNA resulting from capsid lysis. Control spiking experiments were performed in order to determine RNA recovery following microfiltration and test for RNase and RT-PCR inhibition.

Results: Two commercially available alcohol-based handwashes, alcohols (75% ethanol, 75% isopropanol), quaternary ammonium compounds (0.14% BAC, 0.07% DIDAC) and chlorine dioxide (200 ppm) were all ineffective; there was no evidence that these compounds caused virolysis of human norovirus under the conditions tested. In contrast exposure to 0.1M NaOH at 50°C caused capsid destruction. Results showed that recovery of RNA following virucide treatment and microfiltration was frequently inhibited.

Significance: These data show that commonly used virucides do not cause virolysis of NoVs present in dilute stool samples and are consequently likely to be ineffective at controlling NoVs. Since virucide

treatment can inhibit RNA recovery using microfiltration then conclusions regarding the virucidal activity of complex sanitisers obtained by conventional plaque assays using EU standard EN14476.2005 may not be justified.

P1-37 Investigation of Antibacterial and Antioxidant Activity of Shallot Extract (*Allium ascalonicum*) on Rainbow Trout (*Oncorhynchus mykiss*) during Chilled Storage (4 ± 1°C)

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Introduction: The increasing demand for high quality fresh and safe fish has resulted use of new methods and technologies for better storage of fresh fish. Plant extracts such as shallot (*Allium ascalonicum*) are source of natural antioxidant and antibacterial and as natural sources are suitable for shelf-life extension beside the usual methods such as cold chain and packaging.

Purpose: The purpose of this study was to determine the effect of shallot extract application on the shelf life extension of completely gutted rainbow trout (*Oncorhynchus mykiss*) during vacuum packaged storage at 4 ± 1°C by evaluating certain microbiological, chemical and sensory parameters.

Methods: Fresh rainbow trout were purchased from a public market alive and were brought to the laboratory in cold condition, gutted and washed. Fish samples were divided into two lots (control samples and treatment samples in shallot extract solution (1.5% v/v))and then vacuum packed and stored at 4 ± 1°C. Trend of changes in chemical indicators (TVN, TBA, PV), microbial factors (total viable count, psycrotrophic count), pH and sensory evaluation were followed during storage period.

Results: Shallot extract significantly ($P < 0.05$) delayed chemical spoilage reactions such as lipid oxidation and protein degradation in treated samples. Also, spoilage bacteria activity and population significantly ($P < 0.05$) decreased in shallot treated samples in comparison to the control samples. Sensory properties of treated samples were acceptable for consumption during storage period.

Significance: Antioxidant and antibacterial effect of shallot extract during storage period caused extension of Rainbow trout shelf life.

P1-38 Novel Solutions for Molecular Food Safety Testing

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Introduction: Concerns about food safety have steadily increased in recent years and sensitive and reliable pathogen detection constitutes a major challenge for food authorities. Most food testing laboratories still rely on time-consuming traditional methods of bacterial culture, which results in long product approval processes, increasing storage and production costs.

Purpose: To demonstrate that real-time PCR is a powerful tool for the specific detection of foodborne pathogens.

Methods: The efficiency and accuracy of two molecular pathogen assays for the detection of bacterial contamination of food samples were determined. Serial dilution experiments were performed to assess the reproducibility and sensitivity down to low copy numbers. A panel of food-relevant PCR inhibitors was applied to assess the tolerance of the assay to inhibition. DNA was extracted from samples of bacterial enrichment cultures using manual preparation protocols with either thermal or mechanical lysis, This was followed by a test for the presence of *Salmonella* or *Listeria* using two pathogen-specific assay kits (mericon *Salmonella* spp. and *Listeria* spp. Kits, QIAGEN) on a thermal cycler (Rotor-Gene Q, QIAGEN). The DNA was then amplified using a standardized PCR cycling protocol, which is identical for the whole assay portfolio, and the results were analyzed for the two pathogens. Additionally an automated solution (QIASymphony RGQ, Qiagen) was used to isolate highly purified

DNA and reliably detect *Salmonella* from enrichment cultures.

Results: Both systems proved equally efficient at the extraction and detection of *Salmonella* and *Listeria* DNA. Representative data is shown for the extractions and subsequent detections. The assay results were reproducible and sensitive down to low copy numbers, and the assay was tolerant to the presence of food-derived PCR inhibitors. Furthermore, the internal amplification control proved to be an accurate indicator of PCR inhibition. By using the described automated solution, highly pure DNA can be extracted and detected.

Significance: Real-time PCR and the automation of sample preparation can yield significant time and cost savings compared to traditional culture methods while giving sensitive, robust, and reproducible results.

Poster Session 2 – Thursday, 19 May at 12.00 – Friday, 20 May at 11.00

Authors will be present at their posters during the conference breaks

P2-01 Potential Genetic Relationship between *L. monocytogenes* Isolates from Soil, Food Chain, Human and Veterinary Sources

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Introduction: The gram-positive bacterium *Listeria monocytogenes* is a well described facultative intracellular pathogen that causes the severe foodborne disease listeriosis. The latter is able to survive in demanding habitats as soil, water and food-processing plants. The ubiquity of this microorganism makes especially necessary the use of typing methods for the study of its epidemiology.

Purpose: The purpose of this research was to study the possible genetic relationship of *L. monocytogenes* isolated from human, food and environmental origin.

Methods: A total of 79 *L. monocytogenes* strains from contaminated food and food associated samples, soil and sporadic or epidemic outbreaks was selected for phylogenetic and genotypical typing methods. Characterization was performed by serotyping of somatic (O) and flaggelar (F) antigens, pulsed-field gel electrophoresis (PFGE) according to the CDC protocol and multilocus sequence typing (MLST) based on the seven housekeeping loci abcZ, bglA, cat, dapE, dat, ldh and lhkA. PFGE patterns were compared by means of the Dice coefficient, by using Fingerprinting II software (Bio-Rad Laboratories). The sequence types (ST) were determined using the Institute Pasteur Database (www.pasteur.fr/mlst). Furthermore, the phylogenetic trees were created using the maximum likelihood method implemented in PhyML 3.0 (Montpellier bioinformatics platform).

Results: Preliminary results showed that distinct sequence types correlated with distinct serotypes. Furthermore, environmental samples grouped together with outbreak isolates and food plant isolates.

Significance: The outcome of the MLST cluster analysis showed a trend to the classical phylogenetic relatedness of strains of serotypes 1/2b and 4b, which belong to lineage 1, whereas strains of serotype 1/ 2a belong to lineage 2. The results of MLST typing correlated with the PFGE analysis.

P2-02 Separation and Identification of Imidazole-based Antifungal Residues in Pastorized Milk of Iran

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Introduction: Antifungal drugs have an important role for treatment of fungal skin diseases of livestock and prophylaxis. These compounds belong to the different chemical groups but imadazole group is one of the most important and usual.

Purpose: In this study; separation and identification of antifungal compounds consist of Clotrimazole and Ketoconazole were set up and validated and 36 milk samples were analyzed by liquid-liquid microextraction and high performance liquid chromatography. The efficiency of solid phase extraction and high performance chromatography-UV spectroscopy was evaluated in the extraction and determination of Ketoconazole and Clotrimazole analytes in milk.

Methods: In order to obtain the optimal conditions for extraction, the effect of different parameters such as eluent solvent type, composition of solvents, buffer volume, ionic strength and sample loading time on the extraction efficiency were studied. Using optimal conditions, enrichment factor, was 5–250 times and detection limit was 0.01–1 μg.mL-1.

The calibration curve for Ketoconazole and Clotrimazole as analytes in the range of 0.01-1 μg.mL-1 was linear with a recovery for both drugs between 95.9–101.8%. Relative standard deviation (RSD), as

an indicative characteristic of the proper reproducibility of this technique was 1.2–6.8% for Ketoconazole and 0.3–5.3 for Clotrimazole. The optimal chromatographic conditions were investigated by varying different parameters such as detector wavelength, mobile phase composition; pH and flow rate. Sample retention time and system suitability was optimized. Using optimal values for chromatographic procedure, the developed method was fully validated.

Results: In this research, detection and quantitation limits were 0.01 µg.mL-1 and 0.1 µg.mL-1 for both analytes. The linear range of calibration was relatively wide i.e., 0.01-100 µg.mL-1. The relative standard deviation was 0.1–7.5% for Ketoconazole and 0.1–8.5% for Clotrimazole which confirms an acceptable reproducibility for the developed method.

Analysis of 36 pasteurized milk in different months for Clotrimazole and Ketoconazole residue with 0.01 ppb detection limit, demonstrated not detectable amounts of these antifungal residue in pasteurized milk.

Significance: Key words: Pasteurized milk, Antifungal residue, validation, Identification, Separation, Ketoconazole, Clotrimazole

P2-03 RIDA®COUNT Yeast & Mold Rapid – Modern Technology of Cultivation and Detection for Rapid and Easy Enumeration of Yeasts and Molds in Food

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Introduction: In food microbiology it is obvious that fungi need a longer time period to grow as bacteria. On common nutrient media, mainly molds, need 3 to 5 days to grow until they are detectable. Substantial progress was made with the development of the RIDA®COUNT Yeast & Mold Rapid test cards, which can be used to detect yeasts and molds in approximately 48 h. This test medium contains chromogenic substances in order to allow chromogenic detection of target organisms.

In the first product version a chlorimetric system has been used that could lead to heavy background reactions with enzymes containing food matrices. With the development of a new generation of RIDA®COUNT Yeast & Mold Rapid this problem was largely solved. The improved chromogenic detection system uses metabolic enzymes of fungi which will not occur in high amounts in the food matrices. Therefore matrix effects are widely reduced.

Purpose: The purpose of this study was to show the improved performance and applicability of this modern culture based detection method.

Methods: 18 different raw food matrices have been analyzed on their contents of yeasts and molds. The two different product versions of RIDA®COUNT Yeast & Mold Rapid were compared against common YGC agar plates (Heipha, Germany). 10 g sample were diluted with 90 ml of 0.9% NaCl solution were added. The sample dilutions were homogenized and further diluted to 1:100 and 1:1000 using the NaCl solution. The dilution steps were applied to the different ditection media. Media were incubated at 25°C. Evaluation time was set for RIDA®COUNT "old": after 2 and 6 days, for RIDA®COUNT "new": after 2, 3 and 6 days and for YGC-plates: after 3 and 6 days.

Results: For YGC as well as for the new RIDA®COUNT Yeast & Mold Rapid product version, nearly all plates/cards were evaluable. From the previous RIDA®COUNT version only 42% showed heavy colored backgrounds and could not be evaluated. 47 RIDA®COUNT results from different incubation times were compared with 32 YGC results from corresponding incubation times. 31 compared results differed less than one rank of common logarithm (66%). 7 compared results (15%) differed more than one rank and 9 (19%) more than two ranks of common logarithm. The best correlation was between 6d RIDA®COUNT and 6 d YGC, followed by 3 d RIDA®COUNT and 3 d YGC. The correlation between 2d RIDA®COUNT and 3 d YGC differed only marginally and contained most of the differences in more than one rank of common logarithm.

Significance: The data presented in this study prove that the new RIDA®COUNT Yeast & Mold Rapid

good suited for rapid detection of yeasts and moulds in raw food stuffs. It is comparable to common yeast and mold detection.

P2-04 Enhanced Detection of *Listeria* spp. from Environmental Swab and Food Samples within 24 Hours Using PATHATRIX Pooling and Real-time PCR

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Introduction: Listeriosis is an important public health problem that produces high mortality rates. Serious infections occur mainly in pregnant women, neonates, immunocompromised and elderly individuals and result primarily from eating food contaminated with the bacterium *Listeria monocytogenes*. *L. monocytogenes* has a ubiquitous distribution and possesses properties that increases the risk of its persistence and dissemination in food processing facilities. Like other members of the genus, *L. monocytogenes* is psychrophilic and can grow at refrigeration temperatures of 1°C. The potential implications of *L. monocytogenes* being present in ready to eat foods which support growth has led to a zero tolerance approach from both the FDA and USDA FSIS. RTE Food considered to be high risk for *L. monocytogenes* include those with pH > 4.4 and a_w > 0.92.

Purpose: Given the potentially serious implications of *Listeria monocytogenes* being present in RTE foods that support growth or in the food processing environment, the aim of this study was to assess the feasibility of using an enhanced enrichment protocol, recirculating IMS and real time PCR to develop a method which is capable of detecting the presence of *Listeria* contamination, including *L. monocytogenes*, at low level in pooled food and environmental swab samples within 24 hours. It is apparent that a *Listeria* spp. screening and surveillance method such as that described can offer profound benefits to food producers in their hygiene monitoring and the targeting of sanitization regimens.

Methods: A range of *Listeria* species were individually inoculated at low level (1–10 CFU per 25 g food sample) or 1–10 CFU per 10 × 10 cm swabbing area. Food samples and swabs were enriched prior to aliquots being pooled and analysed as single 10–50 ml composite samples. Target capture in a recirculating IMS system employed PATHATRIX IMS particles with proven inclusivity for all *Listeria* species. Aliquots of the recovered IMS particles were taken into a mechanical lysis procedure prior to real time PCR. An unlysed fraction of the beads was streak plated onto selective agar plates (LOx, PALCAM and ALOA) to confirm the real-time PCR results.

Results: The range of *Listeria* spp. were successfully isolated from pooled food samples down to 0.004 CFU/g and environmental contact swabs (1–10 CFU in 100 cm^2) using recirculating IMS. Detection was achieved using real time PCR and this was confirmed by isolation of target *Listeria* on selective agar plating.

Significance: The Pathatrix *Listeria* pooling method described allows food production facilities to increase sample throughput during routine *Listeria* monitoring of both food and environmental contact swabs. The method has the potential to enhance HACCP and pathogen testing regimes and can be employed to validate hygiene practices and sanitizing procedures aimed at reducing the incidence and spread of listeriae in the food processing environment.

P2-05 A Novel Screening Assay for *E. coli* Pathotypes O26, O111 and O157 Based on PATHATRIX Recirculating IMS

John Murray, **Nicole Prentice**, **Katarzyna Brzegowa**, **Paul M.C. Benton**, **Michael F. Scott**, **Ian Sheldrake**, and **Adrian Parton**, MATRIX MicroScience Ltd., United Kingdom

Introduction: Detecting the presence of *E. coli* O157 and other emergent STEC pathotypes in raw beef presents significant challenges. A significant number of *E. coli* O157 outbreaks and food safety recalls have been linked to raw ground beef. Raw beef patties contaminated with *E. coli* O26 recently resulted in two food safety recalls in France and a cluster of foodborne illnesses in the USA were previously linked to *E. coli* O26 adulterated ground beef. Testing for these pathogens contributes significantly to reducing the risk of foodborne illness.

Purpose: This collaborative study examines the feasibility of developing a PATHATRIX screening assay for multiple STEC pathotypes including *E. coli* O157, O26 and O111. The assay is based on specific antibody recognition and capture of the target STEC serotypes during re-circulating IMS.

Methods: PATHATRIX immunobeads were generated using antibodies with proven inclusivity and specificity for *E. coli* O26 and O111 serotypes. The antibodies characterised by the USDA-ARS were linked to paramagnetic beads and their capture efficiency and specificity for each target serotype was assessed in a recirculating IMS assay linked to selective agar plating. A 'gold standard' *E. coli* O157 PATHATRIX assay using O157 specific immunobeads was used to assess how well the O26 and O111 capture reagents performed in the new assay. A number of strains of the target serotypes (food and clinical isolates) were tested and target recovery with the capture reagents was assessed using PATHATRIX linked to selective agar plating.

Results: The capture reagents generated with USDA-ARS anti-O26 and anti-O111 antibodies gave reliable and specific capture of several isolates for each target pathotype. Recovery was comparable to the *E. coli* O157 control assay. It was possible to blend the capture reagents and isolate multiple serotypes simultaneously. Recovery of *E. coli* O157:H7, O26 and O111 colonies on selective agar plates confirmed the screening assay result in all cases. Real time PCR for *E. coli* O157 also demonstrated that molecular detection could be used in conjunction with the PATHATRIX capture assay.

Significance: Immunomagnetic separation (IMS) methods using para-magnetic beads pre-coated with specific antibodies are able to capture and concentrate target pathogenic bacteria. Scientists at the USDA ARS previously demonstrated the superiority of recirculating IMS over other methods for isolating *E. coli* O157 shed naturally in bovine fecal samples. This study provides proof of principle that a screening assay for multiple pathogenic *E. coli* serotypes based on PATHATRIX is feasible. The captured pathotype can be detected by real-time PCR and isolated with standard cultural methods.

P2-06 Evaluation of Three Commercially Available Real-time PCR-based Systems for Detection of *Cronobacter* Species

Nicole Cernela, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Switzerland; **Claudia Fricker-Feer**, QA and Food Safety Department, Hochdorf Nutritec AG, Switzerland; **Sophia Johler**, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Switzerland; **Angelika Lehner** and **Roger Stephan**, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Switzerland

Introduction: *Cronobacter* spp. are Gram-negative opportunistic foodborne pathogens causing rare but live-threatening neonatal infections, which can lead to severe disease manifestations such as brain abscesses, meningitis, necrotizing enterocolitis and sepsis. The current ISO/TS 22964:2006 technical standard method for detection of *Cronobacter* spp. includes a pre-enrichment step in buffered peptone water (BPW), enrichment in modified lauryl sulphate tryptose (mLST) broth, plating on ESIA agar and picking of presumptive positive colonies onto tryptone soy agar (TSA) plates, which are incubated at 25°C for 48–72 h. Yellow-pigmented colonies on the TSA plates are confirmed as *Cronobacter* spp. using biochemical identification kits. This procedure requires up to six days to confirm a positive result. In the last few years, various PCR based methods have been developed that enable fast detection of *Cronobacter* spp. to the genus and species level. Moreover, several real-time PCR-based systems for detection of *Cronobacter* spp. are available.

Purpose: The current study aims to comparatively evaluate three commercial diagnostic systems for the rapid identification of *Cronobacter* spp.

Methods: BAX® System PCR Assay *Enterobacter sakazakii* (DuPont, Qualicon, Wilmington, USA), the Assurance GDSTM *Enterobacter sakazakii* (BioControl, Bellvue, USA) and the foodproof® *Enterobacter sakazakii* Detection Kit (Biotecon Diagnostics, Potsdam, Germany) were compared. Twenty-one target and non-target strains were included in the study and results were compared for specificity and convenience in performance.

Results: A specificity of 100% was observed for two of the three real time PCR systems tested, namely the Assurance GDSTM *Enterobacter sakazakii* and the foodproof® *Enterobacter sakazakii* Detection

Kit for pure cultures as well as artificially contaminated powdered infant formula (PIF) samples.

Significance: This study is the first comparative evaluation of real time PCR based commercial diagnostic systems for *Cronobacter* spp., thus presenting an assessment of potential future alternatives to the ISO/TS 22964:2006 method.

P2-07 Characterization of *Staphylococcus aureus* Obtained from Rabbit Meat by DNA Microarray and Spa Typing

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Introduction: *Staphylococcus aureus* produces enterotoxins causing Staphylococcal Food Poisoning (SFP), one of the most prevalent causes of foodborne intoxication worldwide. Rabbit meat is considered one of the potential sources of SFP, as staphylococcal colonization and infection of rabbit skin is common. However, to date, conclusive data is limited.

Purpose: Therefore, the objective of this study was to compare *S. aureus* isolates obtained from rabbit carcasses at slaughter to isolates associated with cases of SFP in humans.

Methods: Spa typing and DNA microarray analysis were performed with 40 *S. aureus* isolates, comprising 20 isolates collected from rabbit carcasses at slaughter and 20 isolates associated with Staphylococcal Food Poisoning.

Results: Our study showed that *S. aureus* isolates obtained from rabbit meat possessed a multitude of enterotoxin genes, among them the major enterotoxin genes sea, seb, sec, and sed. Microarray profiling revealed clusters of isolates with similar virulence gene profiles, which tended to comprise exclusively rabbit or SFP isolates, respectively. However, five rabbit isolates were assigned to mixed clusters. In the spa typing analysis, rabbit isolates primarily exhibited spa types t179, t741, and an unknown spa type, with the repeat pattern 26-23-16-34-17-20-17-17-16. One rabbit isolate each was assigned to t085, t091, t681, t745, t4770. None of these spa types was found among the investigated SFP isolates (t008, t015, t018, t024, t056, t084, t279, t377, t383, t648, t733, t912, t1239, t1270, t4802, and t6969).

Significance: In our study, several *S. aureus* obtained from rabbit meat possessed genes encoding enterotoxins and exhibited virulence gene profiles similar to SFP isolates. However, as there was no overlap in spa types among rabbit and SFP strains, there is no evidence that rabbit meat represents a common cause of SFP.

P2-08 Same Day – TVC Results in Raw Meat and Fish Including Preparation and Incubation Time

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Introduction: A simple, 'mix and measure', automated assay for the determination of total aerobic viable counts in raw meat and fish samples is presented. The method uses a water-soluble oxygen-sensitive phosphorescent probe (GreenLight™) to determine the microbial load in a sample by monitoring microbial oxygen consumption.

Purpose: The objective was to determine the assays' suitability to assess microbial contamination in foods and to validate the method against the industry standard.

Methods: The samples produce characteristic profiles, with a sharp increase in signal above a baseline level at a specific threshold time, which is correlated with initial microbial load. The higher the initial microbial load of the sample, the earlier this threshold level is reached; expressed as a characteristic onset time (t0).

Results: A strong correlation was observed ($r^2 > 0.90$) when validated against the standard 'aerobic plate count' method (ISO 4833:2003). Contamination levels as low as 1×10^3 CFU/g can be measured

within 12 hours instead of the 72 hours required by the conventional method, while samples at ~1×10^8 CFU/g are identified within an hour. Speed to a CFU/g result is therefore vastly improved. Five meat types and five fish species were analyzed: beef, lamb, pork, chicken, turkey, salmon, cod, plaice, mackerel and whiting. A calibration curve was generated using the conventional agar plate method which established the relationship between threshold time and total aerobic viable counts load (CFU/g). The test was validated with 169 unknown fish samples. The assay is also less labour, materials and energy intensive, with a single 96-well micro-titre plate being the equivalent to 1000 agar plates. Assay ruggedness was also assessed and shown to be excellent.

Significance: The presented rapid, user-friendly TVC test provides a simple, fast, convenient and high throughput alternative to conventional TVC testing.

P2-09 Antibiotic Screening Test Quality Control Testing Compatible with Community Reference Laboratory Validation Guidelines

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Introduction: Community Reference Laboratory Guidelines for Validation of Screening Methods for Residues of Veterinary Medicines (CRL 20/1/2010) detail minimum requirements for demonstrating appropriateness of a method for matrix testing. These guidelines are compatible with previous existing screening test evaluation criteria developed by FDA Center for Veterinary Medicine and IDF Guidelines for standardization of preliminary confirmation test. Data that meet one set of specifications may be analyzed and presented in the CRL Guideline format.

Purpose: Convert quality control data, modeled after FDA-CVM and IDF screening test criteria, into a CRL Guideline compatible report.

Methods: Using qualitative broad screening methods for antibiotics in raw milk, Charm (ROSA) Sulfa Drug Test, Charm Beta-lactam and Tetracycline Test, and Charm II Macrolide Test, concentration-response experiments with n = 60 replicates at Negative Control (blank) and n = 30 at various percentages including 100% of Screening Target Concentration and MRL for each claimed drug are analyzed. An additional n = 20–50 different Negative Controls (random negative producer milk samples) are evaluated to determine drug detection capability relative to the cut-off level. Ruggedness challenges and on-going production tests are compared with a representative drug relative to the MRL.

Results: Drugs 90% positive with 95% confidence detection levels less than or equal to MRL also have an CCβ less than or equal to 5.0%. Negative Controls do not overlap cut-off, meet 90% selectivity with 95% confidence (2 or less positive of 60 negative samples), and have CCα of negatives with no overlap of CCβ of positives. The 90% sensitivity and the 90% selectivity with 95% confidence levels meet the CCβ and CCα level criteria for the CRL Guideline. Some drugs are detected more sensitive than 1/4 MRL in order to detect the difficult to detect drug in the family at or below the MRL.

Significance: Data collected following one set of drug screening test criteria may be analyzed according to CRL guidelines and provide equivalent detection and selectivity results.

P2-10 Farm Inhibition Assay for Antibiotics in Cows Milk with Improved MRL Detection

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Introduction: The Charm Cowside II Test is an improved Geobacillus stearothermophilus calidolactis inhibition assay based on US Patent 7,897,365 that claims class tetracycline detection and ceftiofur metabolite sensitivity without β-lactam hypersensitivity. The assay is designed for on farm use and in milk control stations.

Purpose: To evaluate the Cowside II Test according to the Community Reference Laboratory Guidelines for Validation of Screening Methods for Residues of Veterinary Medicines CRL 20/1/2010.

Methods: The Charm Cowside II Test (Lawrence, MA, USA) has a one step 100 µl milk addition and

is dry-well-incubated at 64 ± 1°C for approximately 3 hours. The test turns yellow if free of antibiotics and remains blue if it has detected antibiotics from different classes of antibiotics covering β-lactams, sulfa drugs, macrolides, tetracyclines and aminoglycosides. A variety of farm milk tanks of various bacterial and somatic cell quality were evaluated and spiked with USP reference antibiotics at MRL to achieve a population of at least 60 negative samples and 30 spiked replicates of penicillin G 3 ppb, sulfamethazine 125 ppb, oxytetracycline 100 ppb, gentamicin 150 ppb, and erythromycin 100 ppb. These drugs represent the difficult to detect antibiotic concentration of each drug family.

Results: The method detected antibiotics at or below MRL as follows: oxytetracycline 100 ppb, chlortetracycline 100 ppb, sulfadiazine 60 ppb, sulfadimethoxine 50 ppb, tylosin 30 ppb, pirlimycin 50 ppb, gentamicin 150 ppb, pen G 3.0 ppb, amoxicillin 4 ppb, ampicillin 4 ppb, cefalexin 100 ppb, cefalonium 20 ppb, cephapirin 10 ppb, cloxacillin 25 ppb and ceftiofur total metabolite 100 ppb. Detected within 2x-MRL were sulfamethazine 125 ppb, erythromycin 100 ppb.

Significance: Broader screening and detection of antibiotics used on farm gives greater assurance that residue control programs are working.

P2-11 The Use of Targeted mRNA Expression as Omic Biomarker to Track Growth of *Lactobacillus helveticus* in Broth and throughout Swiss-type Cheese Ripening

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Introduction: Biomarker is defined as a biological characteristic that is objectively measured and evaluated as an indicator of biological processes.

Purpose: The aim of this work was to monitor *Lactobacillus helveticus* mRNA expression levels associated to metabolic activity and stress response during growth. Biomarker transcriptomic levels were compared in pure culture and during Emmental cheese manufacture and ripening.

Methods: Three semi industrial pilot scale Emmental cheese productions were made from raw microfiltered milk inoculated with *Lactobacillus helveticus* ITG LH56, *Streptococcus thermophilus*, *Propionibacterium freudenreichii* and *Lactobacillus paracasei*. In parallel, three independent cultures of *L. helveticus* ITG LH56 were performed in MRS at 37°C. Population was quantified by numeration on specific agar medium and quantitative PCR while tuf and groL mRNAs were quantified by RT-qPCR. Expression levels were indicated as percentage of targeted mRNAs associated to either metabolic activity or stress response. All experiments were carried out in triplicate.

Results: During pure culture in broth, *L. helveticus* growth was correlated with a high percentage of mRNA expression levels associated to tuf gene, while mid-exponential and stationary growth phase was associated to an increasing expression of groL which reached a maximum of 30% at the beginning of stationary phase. During cheese manufacture, *L. helveticus* reached similar maximal population during the acidification step with groL mRNA expression levels higher than 40% showing a peak at the end of cold room storage. During cheese ripening, culturable population was 1 log lower than Q-PCR quantifications. Entering cold and warm storage were associated with tuf expression of 50% even though bacterial counts were decreasing suggesting possible aroma synthesis.

Significance: This study reports how recording of targeted mRNA expression levels along cheese manufacture and ripening process could enable correlations between bacterial physiology or metabolic activity of interest. The use of biomarkers enables accurate quantifications to better understand and improve online industrial fermentation processes.

P2-12 Group, Trace and Track *Bacillus cereus* Group Foodborne Isolates

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Introduction: *Bacillus cereus* group, including *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis* and *B. cereus* (sensu stricto), gathers closely related Gram positive bacteria exhibiting highly divergent ecological and pathogenic properties. While *B. cereus* food poisoning outbreaks are mostly due to temperature abuse, the presence and survival of spores to heat processes and cleaning procedures yield to their persistence in the industrial environment.

Purpose: The aim of this study was to develop a set of rapid tests to be performed on *B. cereus* isolates showing characteristic colonies on Mossel agar, in order to classify and trace isolates along production chain.

Methods: A collection of 100 *B. cereus* strains from clinical origin (8%) as well as isolated from environment samples (6%), soil (5%), ingredients (8%), vegetables (12%), egg-based-products (17%), dairy products (20%) and RTE (24%) were analyzed. Specific genetic biomarkers (cspA, cspF, panC) were targeted by PCR for classification within the seven phylogenetic groups defined by Guinebretière et al. and bioinformatic comparison of pulsed field gel electrophoresis fingerprints were performed to define clusters. Moreover, adhesion to surfaces was tested for 15 strains using crystal violet assays.

Results: Among the 100 strains studied, most isolates belong to groups VI (27%), IV (27%) and III (25%) with minor contribution to groups II (17%) and V (4%). Psychrotrophic growth was reported for both group II and VI which were composed of strains mainly isolated from vegetables, egg-based product and RTE. Based on 15 strains, isolates from groups II and IV seemed to exhibit stronger adhesion properties. PFGE fingerprints clusters were obtained, even for isolates with high nuclease activity, which enable to trace contaminations.

Significance: Due to heterogeneous properties of *B. cereus* isolates, rapid and accurate clustering is of prime importance to study, track and trace contamination along production lines in food industries.

P2-13 PCR Subtyping of *Listeria monocytogenes* Isolates Retrieved along the Gorgonzola PDO Production Chain

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Introduction: *Listeria monocytogenes* represents a concern in the production of Gorgonzola PDO cheese. Subtyping of isolates retrieved from production environments and raw materials may lead to a better understanding of the reservoirs and contamination pathways.

Purpose: The aim of this study was to investigate the subtypes of *L. monocytogenes* in the Gorgonzola production chain to identify possible reservoirs and dissemination routes

Methods: One producer and its conferring farms (N = 20) were selected and *L. monocytogenes* was isolated from 12% of the collected samples (N = 200) – data presented at IAFP symposium, 2010 P1-10. All *Listeria* strains isolated were typed through ERIC and REP PCRs. For each samples, if possible 5 colonies were selected for typing, for a total of 81 isolates. PCR profiles (combining the Two PCRs) were analysed using Dice coefficient and UPGMA algorithm in Bionumerics software.

Results: The analyses allowed the differentiation of *L. monocytogenes* and *L. innocua* in 9 and 7 PCR profiles (P), respectively. The former showed an overall similarity value of 75.4% and the latter of 81.2%. P1 (N = 3) of *L. monocytogenes* was present in multiple sources (ripening surfaces and salting equipment) and P2 (N = 6) in different sampling rounds (January and June 2009). P1 (N = 25) and P2 (N = 15) of *L. innocua* were found in multiple sources (performing equipment, ripening surfaces, salting equipment, moving carts) and in multiple sample rounds (P1: May 2008, January and June 2009; P2: June and October 2009).

Significance: The presence of a few highly similar strains of *L. monocytogenes* in the production chain, as suggested by the profiles retrieved from different sources/sampling rounds, indicates the presence of persistent and niche-adapted strains. However, no profile was shared between raw milk and

environmental samples, suggesting that the plant environment, not the incoming raw milk, is the source of strains contaminating Gorganzola cheese.

Acknowledgments: Founds granted by Regione Piemonte – Direzione Sviluppo Agricoltura – 2007.

P2-14 Occurrence of Pathogenic and Spoilage Bacteria in Hungarian Raw Bovine Milks: A Comprehensive Survey (2004–2010)

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Introduction: Raw milk of high microbiological quality is an essential prerequisite for production of safe dairy foods.

Purpose: The objectives of this research were to (1) evaluate the efficacy of classical protocols and an automated immunoassay system for detection of foodborne pathogens commonly found in raw milk and (2) determine the occurrence of a wide range of pathogenic and spoilage bacteria in Hungarian ex-farm raw bovine milks.

Methods: The sensitivity, specificity and accuracy of conventional plating techniques and those of the Mini-VIDAS automated enzyme-linked fluorescent immunoassay were compared for detection of *Salmonella* spp., *Campylobacter jejuni* and *Escherichia coli* O157:H7. Then, from 2004 through 2010, over 3,000 raw cow's bulk-tank milk samples were collected from dairy farms located in various parts of the country, and were tested for presence of *Listeria monocytogenes*, *Salmonella* spp., *C. jejuni* and *E. coli* O157:H7. Viable counts of *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis* and mesophilic sulfite-reducing clostridia were also enumerated in the same samples.

Results: The results showed that the Mini-VIDAS system was superior to conventional plating methods in terms of sensitivity, specificity and accuracy. This was especially true for the detection of *C. jejuni* and *E. coli* O157:H7. As to the bacteriological quality of Hungarian raw bulk milks, the overall percentages of samples failing to comply with regulatory standards for *Salmonella* spp., *L. monocytogenes*, *S. aureus*, *E. coli* and *E. faecalis* were 0.15, 0.75, 2.07, 14.48 and 63.06, respectively. By contrast, none of the samples tested contained detectable levels of *C. jejuni*, *E. coli* O157:H7 or mesophilic sulfite-reducing clostridia.

Significance: Viable counts of spoilage bacteria *E. faecalis* reached or exceeded the level of 100 CFU/ml in nearly two-thirds of the 2,951 samples tested between 2004 and 2010, whereas the presence of pathogens was detected in less than 1% of raw milks over the same 7-year period. In conclusion, it is suggested that raw bovine milk, which makes up more than 99% of total raw milk production in Hungary, be tested on a regular basis for a variety of pathogenic and spoilage organisms so that microbiological safety of commercial dairy foods be ensured.

Acknowledgment: Author L. Varga gratefully acknowledges research funding support from the National Development Agency of Hungary (Project No.: TÁMOP-4.2.1.B-09/1/KONV-2010-0006).

P2-15 Tribos Transformation — A Novel Method for Bacterial Transformation

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Introduction: During the past decade Yoshida and co-workers have developed a novel tranformation method to introduce foreign DNA into bacteria using chrysotile asbestos fibers (Yoshida, Ikeda et al. 2001). A colloidal solution containing mineral nanofiber and bacterial cells are mixed together with plasmid DNA and plated on a selective agar. A sliding friction arises between the agar surface and the applied solution when plating with a stir stick. This result in a penetration of the bacterial cells by a phenomenon called the Yosida effect (Yoshida 2007) and the foreign genetic material can enter the cell. Wilharm and co-workers (2010) has tested and adjusted the method. Due to the obvious health risk associated with asbestos fibers, they showed that utilizing the less harmful sepiolite fibers gave similar

results as the Yoshida experiments.

This is a simple, cheap and effective alternative to e.g., electroporation. Why has it not been accepted by the scientific community? Is this method maybe to simple to be true?

Purpose: The purpose of this study was to investigate the possibility to use the tribos transformation in our laboratory. The protocol from Wilharm and co-workers (2010) was implemented.

Methods: To perform a tribos transformation a 0.01% sepolite suspension was prepared in a buffer of 5 mM HEPES and 200 mM KCl (pH 7.4). An overnight culture of *E. coli* DH5α was diluted in LB and grown to an OD600 of 0.5. Cells were pelleted and resuspended in the sepiolite suspension. Aliquots of 100 µl of the solution were mixed with plasmid DNA. Cells were then spread with a sterile glass stir stick on agar plates containing selective antibiotics. Cells were grown at 37°C in 1–2 days.

Results: *E. coli* DH5α as well as commercially electrocompetent *E. coli* (Top10, Invitrogen) were successfully transformed with pUC18. The transformation efficiency was slightly lower than earlier reported. However, the method will be further investigated to achieve better transformation efficiency and also possibly used for bacterial transformation of other bacterial species.

Significance: A novel and promising transformation method, tribos transformation, was successfully tested and resulted in nanopiercing of *E. coli* cells with insertion of pUC18. This method is easy and cheap and will thus enable smaller labs to work with molecular biology. Our intention is to use the method in our work on "tagging" food pathogens as Listeria moncytogenes with e.g., green fluorescent protein for further studies in different food related matrixes.

P2-16 Towards a Unique Data Model for Chemical and Microbiological Food Information

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Introduction: The integration of food data from research, monitoring, control, epidemiology and other sources is crucial to improve food safety and public health. Consequently, INSA launched the Portuguese Food Information Resource Programme (PortFIR) in a partnership with GS1 Portugal CODIPOR to create national food expert networks and sustainable databases on food composition, consumption and chemical and microbiological contamination. Presently, the PortFIR data model is being developed. Existing data models on food information usually refer to either chemical substances or microorganisms. However, for food safety, particularly for risk-benefit evaluation, a unique data model to compile Chemical and Microbiological Food Information (CMFI) would be a huge step forward, regarding data standardization and optimization of resources.

Purpose: The aim of this work is to explore the possibility of creating a unique data model for the compilation, management and use of CMFI compatible with EFSA's chemical and microbiological calls for data and for nutrition applications, namely EuroFIR European Food Composition Data Bank.

Methods: The work was developed in two steps: 1) identification of existing relevant Data Models; and 2) comparison and listing of all required attributes.

Results: The data models identified as relevant were EFSA's Standard Sample Description for Food and Feed, for chemical contaminants, Zoonoses Data Collection for microbiological contaminants and foodborne outbreaks and the CEN/TC 387 prEN_16104 Food Data – Data structure. The reasons to choose these references were the need to report data to EFSA and to update national data in the EuroFIR European Food Composition Data bank whose structure formed the basis of CEN/TC 387 work. All attributes in each model were listed and correspondence among models was cross-referenced.

Significance: This work was the first stage in the development of a unique data model for CMFI. The biggest advantage of such a data model is the ability to store all the information needed in a single database whose compatibility allows to exchange and to share information with national and international partners, contributing to improve food safety at a global level.

P2-17 Modelling Sporulation Rate and Yield of *Bacillus weihenstephanensis* and *Bacillus licheniformis* as a Function of Sporulation Temperature and pH

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Introduction: The physical and chemical environment of the sporulation of *Bacillus* sp. has a major influence on their resistance properties. Environments for sporulation are present at many steps in the food chain, as nutrients are available, as incubation time is sufficient for the completion of the sporulation process and as environmental factors are favourable.

Purpose: Determining the sporulation limits and kinetics contribute to the identification of sporulation niches and may suggest possible points in the food chain where to focus control measures.

Methods: The sporulation of the psychrotrophic *B. weihenstephanensis* KBAB4 and of the mesophilic *B. licheniformis* AD978 was studied from 5°C to 50°C (each 5°C) and from pH 5.2 to 8.5 (each 0.3 pH unit). The sporulation kinetics (changes in concentrations of cells surviving to a 70°C -5 min heat treatment) and sporulation yields (final concentration of spores) were achieved in a sporulation mineral buffer.

Results: *B. weihenstephanensis* was able to form spores from 5°C to 35°C and from pH 5.2 to 8.5. Shorter "time to first spore formation" (8.1 h), higher sporulation rate and final spore concentration (0.58 h -1 and 7.3 log CFU/ml) were observed for a sporulation at 30°C than for lower or higher sporulation temperatures. At 10°C for instance the "time to first spore formation" was respectively 158.5 h, the sporulation rate was 0.07 h -1 and sporulation yield was 6.3 log CFU/ml. Kinetic parameters of sporulation were also affected by incubation in media at pH lower than 7.0 – 8.5 and showed a slower sporulation process and a lower yield. Similar results were observed for *B. licheniformis*. *B. licheniformis* sporulated from 20°C to 50°C and from pH 6.0 to 8.5.

Significance: Interestingly, the range of temperature and pH allowing the spore formation were similar to that allowing growth. Moreover, for both strains, the changes in sporulation rate as function of the temperature or pH was close to the changes in the growth rate of the bacterial population. The temperature and pH appear to affect the sporulation kinetics in the same way as the vegetative cells kinetics.

P2-18 Parameters (Storage Temperature, pH and Preservative Content) Affecting the Shelf Life of Liquid Whole Egg

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Introduction: The shelf life of liquid eggs is relatively short since the proteins responsible for microbial resistance of shell egg is denatured during pasteurization, and in case of the mixture of white and yoke of the egg, provides a medium of excellent composition for microbial growth. Therefore, liquid egg production plants use various preservatives to increase the shelf life of their products.

Purpose: Our purpose with this work was to determine how the total live germ count changes in liquid whole eggs during storage in refrigerator depending on the storage temperature, pH value of samples and their preservative content.

Methods: During our measurements we tested the change in viable cell count in liquid whole eggs. In planning our experiments, central complex rotation design was used and response surface method (RSM) was applied to analyze the effect of each parameter (pH, storage temperature, storage time and preservative content) on the viable cell count.

Results: Based on our measurements beyond the storage time, pH value and storage temperature of liquid egg samples ($P < 0.01$) affect live germ count but inhibitory effect of preservative (Na benzoate,

K sorbate mixture) on microbial growth could not be clearly detected.

Significance: Our results could be described with a secondary polynomial model fit onto our measured results thereby our results hopefully help with the estimation of microbiological status of liquid whole egg products preserved by various methods.

P2-19 Destruction of *Salmonella* in the Function of Treatment Temperature and Heating Rate

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Introduction: Some microbes such as *Salmonella* show an increasing resistance after heat shock. Such a heat shock could be the heating of refrigerated liquid eggs to the temperature of long time (6 – 24 h) heat treatment (50 – 60°C).

Purpose: The aim of our work was to investigate the extent of heat resistance changes of *Salmonella* Enteritidis in liquid egg products during the heating from 4°C to 50–55°C in 5–60 minutes.

Methods: In our study we investigated the effect of heat treatment temperature and heating rate on heat resistance of *Salmonella* Enteritidis. The samples were heated from 4°C to 48, 96–56, 04°C by a heating rate 0.76–9.24°C/min and the changes of the colony counts were determined at given time by plating to XLD agar with overlay. We used Central Composite Rotatable Design (CCRD) in our experiment and Response Surface Method (RSM) was used to evaluate the data.

Results: Our results pointed out that besides the temperature of heat treatment the heating rate have also an effect on the heat destruction of *Salmonella* Enteritidis. In case of heating rate 9.24°C/min the D52.5-value was 2.32 min, however at heating rate 0.76°C/min the D52.5-value was 19.23 min. In our measurements the samples were heated with linear heating rate in laboratory scale, so further studies are necessary to describe the heat resistance changes of *Salmonella* Enteritidis under parameters which model the industrial heating circumstances.

Significance: We can conclude that the heating rate and the holding temperature have an effect on the heat resistence of *Salmonella* Enteritidis in liquid egg white. This should be considered particularly in case of technologies where the refrigerated liquid egg white is heated to heat treatment temperature for a relatively long time.

P2-20 Establishing Process Hygiene Microbiological Criteria for Wild Venison Carcasses in an Irish Game Processing Unit

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Introduction: Foodstuffs should not contain micro-organisms at levels that present an unacceptable risk to human health. Food safety is ensured by a preventative approach such as HACCP. Microbiological criteria can be used to verify HACCP and other hygiene control measures. They also provide guidance on the acceptability of foodstuffs and their manufacturing and handling processes. It is thus appropriate to set criteria defining process acceptability and also food safety criteria setting a limit above which a foodstuff should be considered unacceptably contaminated.

Purpose: Within the European Union, Regulation 2073/2005 establishes the process hygiene criteria (PHC) for carcasses derived from domesticated species. No such criteria exist for wild venison. Notwithstanding this, wild venison must be processed using HACCP principles. Consequently, PHC have a role in its verification. This study proposes to establish PHC for wild venison in an Irish establishment.

Methods: A wild venison processor was selected for the study. On each of 16 processing days 5 carcasses were selected. Each carcass was swabbed in 4 locations (haunch, saddle, neck and shoulder) immediately after dressing and prior to chilling using an abrasive polyurethane swab. The area

swabbed was 100cm^2 delineated using a sterile paper template. Samples were transported at 4^{o}C and were analysed within 4 hours. Each carcase's samples were pooled prior to analysis for Aerobic Colony Count (ACC) and Total *Enterobacteriaceae* Count (TEC) using ISO method 4833:2003 and 21528/2:2004 respectively. Test results for both criteria were reported as CFU/cm^2. The mean log value for each day is calculated by taking the log of each individual result and calculating their arithmetical mean.

Results: The mean log ACC per sampling day ranged from 0.12 – 3.42 $\log_{10}$ CFU/cm^2 with a mean (χ) of 1.76 $\log_{10}$ CFU/cm^2 and a standard deviation (s) of 1.22 $\log_{10}$ CFU/cm^2. Mean log TEC counts per day ranged from 0.00 – 0.86 $\log_{10}$ CFU/cm^2 with χ = 0.28 $\log_{10}$ CFU/cm^2 and s = 0.37 $\log_{10}$ CFU/cm^2. The value for the lower control limit (m) was determined as $m = \chi + ½s$ while the upper control limit (M) was calculated as $M = \chi + s$. The process hygiene criteria for wild venison in the current plant are therefore m = 2.37$\log_{10}$CFU/cm^2 and M = 2.98 $\log_{10}$ CFU/cm^2 for ACC and m = 0.47 $\log_{10}$ CFU/cm^2 and M = 0.65$\log_{10}$ CFU/cm^2 for TEC.

Significance: Comparing ongoing performance to these PHC allows the establishment to implement timely corrective action to its dressing procedures. In the absence of legally mandated PHC for wild venison it is recommended that each operator follows the protocols outlined in this study to establish its own PHC.

P2-21 *Listeria monocytogenes* Contamination from Meat Brining Equipment

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Introduction: During recent years major meat-related listeriosis outbreaks have been traced to the consumption of various processed pork products. *L. monocytogenes* can be continuously recovered from the different meat-processing environments and equipment used for cutting, slicing or dicing and can be transferred among plants by persistently contaminated equipment. In the present study, we analyzed the *L. monocytogenes* contamination in a cold-smoked pork-processing plant associated with brining equipment.

Purpose: Our aim was to analyze *L. monocytogenes* contamination in cold-smoked meat products associated with brining procedures.

Methods: Environmental sampling combined with pulsed-field gel electrophoresis (PFGE) subtyping and serotyping were applied to investigate the genetic diversity of *L. monocytogenes* in the meat-processing plant environment and the RTE cold-smoked pork products.

Results: Of the environmental samples, most contaminated sites were associated with the brining machine. Three different contamination sites were found. The feeding teeth of the brining machine already harbored *L. monocytogenes* before production, whereas an additional two *L. monocytogenes* contamination sites were found on the plastic curtain, smooth surfaces and spaces of the brining machine during production. The number of *L. monocytogenes*- positive pork samples clearly increased after the brining injections or after contact with the brining machine. In all, 60% of the pork samples were positive for *L. monocytogenes* after the brining injections as compared to 18% of the raw pork samples that were positive before brining.

A total of 17 different PFGE types were detected from 66 *L. monocytogenes* isolates originating from the environmental samples and product at different stages of production. In all, four different PFGE types belonging to serotypes 1/2a and 1/2c were found in finished RTE cold-smoked pork products produced. Of these, two PFGE types were also found in the brining machine and on the aprons of personnel working in the brining area. A wide variety of *L. monocytogenes* PFGE types belonging to serotypes 1/2a and 4b was isolated from incoming raw pork. One PFGE type belonging to serotype 4b was found in all processing stages from raw, defrosted pork to finished RTE cold-smoked pork, whereas three PFGE types belonging to serotype 1/2a were found only in raw, defrosted pork.

Our findings revealed that genetically diverse populations of *L. monocytogenes* entered the meat-processing plant with the raw material, where only some of the strains colonized and possibly

established persistent microbial communities.

The feeding teeth of the brining machine harboured three different PFGE types belonging to serotypes 1/2c, 1/2a, and 4b, and were found on the feeding teeth of the brining machine and in finished RTE cold-smoked pork products. Moreover, PFGE type 8 belonging to serotype 1/2c was also found in the finished cold-smoked pork products 5 years earlier, and may indicate the presence of persistent *L. monocytogenes* contamination in the brining machine.

Significance: The brining area and the brining machine caused persistent contamination with *L. monocytogenes* over 5-years. Due to poor hygiene design, removal of the brining machine from the production of cold-smoked meat products should be considered to significantly reduce *L. monocytogenes* contamination in the finished products.

P2-22 A Novel Multiplex PCR Assay for Simultaneous Detection and Identification of *Sarcocystis* spp. in Cattle

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Introduction: The genus *Sarcocystis* consists of cyst-forming coccidian with an obligatory two-host life cycle. Three species of *Sarcocystis* have been described in cattle: *S. cruzi* (bovicanis), *S. hirsuta* (bovifelis), *S. hominis* (bovihominis). *S. hominis* has been implicated as a human pathogen in case of raw or undercooked beef consumption and associated with gastrointestinal symptoms. Besides many authors recognize a role for this protozoan genus in the pathogenesis of eosinophilic myositis in cattle.

Purpose: At present, definitive identification of *Sarcocystis* species requires electron microscopy or DNA sequencing. The usage of both methods is limited by cost and technical considerations. The purpose of this study was to develop a rapid and simple method for the simultaneous detection and identification of Sarcocystis spp. in cattle.

Methods: Based on sequences deposited in Genbank, three forward primers and a common reverse primer were designed in the 18s rRNA coding region. 20 samples of bovine muscle found positive for *Sarcocystis* spp at histological examination were submitted to electron microscopy analysis and DNA sequencing for species identification. Those samples were used as positive controls. Species-specificity of primer pairs was tested and the multiplex assay was subsequently optimized. The assay was then tested for its applicability on 20 unknown samples.

Results: Amplification of simplex primer pairs resulted in a band of 108bp, 182bp and 284bp respectively for *S. hirsuta*, *S. hominis* and *S. cruzi* without cross reactivity for all the positive samples. Multiplex PCR gave the expected results for the positive controls and detected several coinfections in the unknown samples.

Significance: This novel assay offers a rapid, simple, and feasible method for the detection and identification of *Sarcocystis* spp. in cattle. It may serve as a useful tool to determine the prevalence of the zoonotic species as well as to investigate the role of this protozoan genus in the pathogenesis of eosinophilic myositis in cattle.

P2-23 Litter Treatment Reduces Pre-harvest Intestinal *Campylobacter* in Poultry

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Introduction: Campylobacteriosis is a significant human health problem worldwide and poultry products are a significant source of transmission. Treatment of poultry litter with Aluminum Sulfate (Alum) has been reported to possibly reduce enteric *Campylobacter* concentrations in broilers. Little is known about how Alum may reduce enteric *Campylobacter* concentrations.

Purpose: The purpose of this was to determine whether Alum reduces *Campylobacter* colonization in the ceca of broilers by reducing horizontal transmission between birds or by reducing *Campylobacter* concentrations in birds already colonized (therapeutic efficacy).

Methods: Newly hatched broilers were reared in no (controls), or top dressed with low (2.4 kg) or high (4.8 kg) amounts of Alum for each 3 sq m/pen. For the horizontal transmission group, *Campylobacter* negative birds were reared with *Campylobacter* positive birds that served as carriers. The carrier birds were fitted with leg bands to distinguish them from the rest of the birds in the pen. For the therapeutic efficacy group, all birds were inoculated with *Campylobacter* prior to placement in pens. During weeks 1, 2, 4 and 6, cecal *Campylobacter* concentrations were determined in 10 birds from each treatment group. Furthermore, 20 g of litter were also collected weekly from each pen to monitor litter pH.

Results: For both the horizontal and vertical treatment groups, birds reared on high or low Alum also had significantly lower cecal *Campylobacter* concentrations (approximately 2 logs) when compared with positive controls in pens not treated with Alum. The decline was similar for both groups. These changes were associated with a weekly reduction in litter pH in Alum treated pens when compared with controls ($P \leq 0.05$). It is possible that the acidic environment of the litter reduces environmental *Campylobacter* levels and enteric recolonization via litter consumption (pecking). This may be the mechanism by which Alum reduces *Campylobacter* concentrations in poultry.

Significance: Alum can be used as part of a multifaceted approach to reduce *Campylobacter* in poultry and subsequent human foodborne illnesses. The use of Alum caused a two-log reduction in enteric *Campylobacter* which has been estimated to produce up to a 30-fold reduction in human campylobacteriosis cases.

P2-24 Monitoring of Bacterial Growth by Electro-Optical and Flow Cytometric Techniques

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Introduction: The efficacy of inactivation processes is essential in food microbiology to define critical process parameters. The comparison of inactivation treatments and validation of critical parameters require bacteria in a consistent and reproducible physiological state. Electro-optical techniques enable the continuous monitoring of physiological properties of single bacteria cells.

Purpose: The objective of this study was to monitor bacterial growth by electro-optical and flow cytometric techniques to obtain detailed knowledge about bacterial physiological properties at different growth stages. This knowledge should allow the selection of bacteria with defined and consistent physiological properties for examination and comparison of inactivation processes.

Methods: The growth of *L. innocua* in ST-I nutrient broth at 37°C was monitored with the EloTrace® 3.0 (EloSystems GbR, Germany) measuring the cell density, cell size, and the anisotropy of polarisability (AP). Simultaneously, cell density was measured by spectroscopy, cell size and total count were determined by Multisizer™ 3 Coulter Counter® (Beckman Coulter, Germany) and membrane potential (MP) was measured in the Cytomics FC500 (Beckman Coulter, Krefeld, Germany) using the fluorescent dye DiOC2(3). Additionally, the culturability was determined using traditional microbiological culture methods.

Results: *L. innocua* reached the stationary growth phase after 12 h at 37°C. The total count and viable count are comparable with the bacteria count obtained by the electro-optical method. The AP of *L. innocua* decreased during the exponential phase and remained almost at a constant level during stationary growth. The membrane potential of bacteria was low when the AP level was high and increased with decreasing AP level. Similar to the AP level, the MP of *L. innocua* remained almost constant during the stationary growth phase.

Significance: Growth curves of bacteria can be monitored by electro-optical methods to define the optimal and consistent harvest point for evaluation of inactivation efficacy

P2-25 Detection of *Cronobacter* spp.: a Feasibility Study Using the Vidas Platform

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Introduction: *Cronobacter* spp. (formerly known as *Enterobacter sakazakii*) is an opportunistic pathogen that causes meningitis, necrotizing enterocolitis, bacteremia and sepsis, predominantly in infants less than one month of age. Infection has been linked to the consumption of contaminated powdered infant formula (PIF). Culture-based methods currently in use (and which include pre-enrichment, enrichment and selection) require up to 7 days to provide a confirmation. Therefore, a simplified detection platform for *Cronobacter* capable of providing a reliable result within a shorted time frame would be desirable.

Purpose: Develope a simplified reliable detection method for *Cronobacter* spp. in powdered infant formula.

Methods: Using a recently developed detection protocol, based on Vidas technology, using an enzyme-linked immunoassay, we tested its ability to detect a large collection of *Cronobacter* and non-*Cronobacter* isolates. All strains which tested negative were confirmed by real time polymerase chain reaction (RT-PCR).

Results: A total of 180 isolates, including 160 *Cronobacter* spp. cultured from various origins including food and 20 non-*Cronobacter* isolates, were assayed on the Vidas platform. The positive detection rate for *Cronobacter* spp. was 98.75% (158/160) and the negative isolation rate for non-*Cronobacter* strains was 85% (17/20).

Significance: The Vidas platform, which requires a shorter time-duration (within 48 h) for positive confirmation is an effective method for the reliable detection of *Cronobacter* spp. This approach may provide a useful basis upon which to improve positive release protocols for powdered infant formula and other foods.

P2-26 Microbiological Monitoring of Ready-to-eat Food at the Point of Sale

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Introduction: In food microbiology, the interpretation of the results is often a difficult aspect of the food examination process. Checking the compliance with existing regulations and guidelines may be insufficient for food processors verify and validate the efficacy of their food safety systems.

Purpose: 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.

Methods: In an accredited laboratory according to the NP EN ISO/IEC 17025:2005, 227 samples of ready-to-eat meals from restaurants and school canteens were analyzed for Microorganisms at 30 C; *Enterobacteriaceae*; *Escherichia coli*; *Staphylococci coagulase* positive; *Salmonella* spp and *Bacillus cereus*.

Results: According to international guidelines and considering the potential pathogenic micro-organisms in ready-to-eat food, regardless of the number of bacteria present, no *Salmonella* spp. could be found in any of the samples. For other pathogenic microorganisms such as *Bacillus cereus*, less than 1% of the samples were considered unsatisfactory although all the samples analyzed had rice as an ingredient.

Counts for coagulase-positive staphylococci were satisfactory for 81% of the samples and none of the sample had unsatisfactory values for this microorganism. The presence of hygiene indicator bacteria in ready-to-eat food, although not inherently a hazard, can indicate poor practice. *Enterobacteriaceae* and *Escherichia coli* counts did not reach unsatisfactory values in any of the samples.

Another study was conducted in order to find a possible correlation between the increase of contamination and the presence of raw vegetables since 55% of the samples as these ingredients.

Statistical analysis was used to evaluate the significance of the presence of raw vegetables in a ready to eat meal for the total microbial load.

Significance: All the samples were acceptable, considering the parameters analyzed, according to the Regulation 2073/2005 altered by Regulation 1441/2007 however statistical analyses of food microbiological data is a useful tool for assist in the data interpretation.

P2-27 Survival of Moulds in Bottled Waters

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Introduction: Generally, it is thought that moulds do not grow into visible bodies in bottled mineral water products which contain very low amounts of nutrients (organic matters) for the moulds. After all visible growing of moulds in bottled water caused customer complains in several cases.

Purpose: The aim of our study was to verify the microbial stability of 12 types bottled waters with different mineralization and CO_2 level against 4 different molds *Fusarium* sp.; *Cladosporium* sp.; *Penicillium chrysogenum* and *Aspergillus fumigatus*, isolated earlier from different commercial bottled waters.

Methods: Inoculation of water samples was performed from the same suspension at the same time for all samples. The water samples were inoculated with the conidial suspensions to obtain approximately 10, ~10–50 and ~100-500 conidia per 100 ml water sample. The surviving colony forming unit (CFU) numbers and visual growth were monitored during the investigation period (26 weeks). To determine the CFU number membrane filtrations or when expected CFU number could be expected to be higher than 102/10 ml, spread plate method was used on a weekly basis up to week four. Later on it was continued bi-weekly until visible growth was recognized.

Results: Results of membrane filtration and spread plating showed decreased cell numbers in case of carbonated and mid-carbonated waters, while slow decrease, stagnation or even some growth were detected in still waters. Visual monitoring showed significant differences between carbonated, mid-carbonated, and still waters. In carbonated and mid-carbonated water samples visual growth was not detected during 26 weeks, while visual growth was detected in all of the non carbonated samples after 12–26 weeks even if the initial conidial number of mould was rather low (~10 CFU/100 ml). A. fumigatus has been found the most resistant test species. Even after 26 weeks there were surviving A. fumigatus conidia in carbonated and mid-carbonated water samples also. None of the other tested mould strains survived the first 12 week test period in carbonated samples.

Significance: Based on the results the microbiological growth was mostly determined by the carbonation level and the type of the mould strain. Neither the inoculation level nor the mineral content had any significant effect on the survival of the different moulds.

P2-28 Heat Resistance of *Geobacillus pallidus*, a Thermophilic Spore-forming Spoilage Bacterium

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Introduction: Endospores of bacteria are able to survive extreme environmental conditions and highly resist to preservation techniques e.g. to heat treatment, drying, irradiation, pressure treatment, chemicals. Recently several bacteria belonging to the *Bacillus* genus has been reclassified, such as *Alicyclobacillus*, *Paenibacillus*, *Brevibacillus*, *Aneuribacillus*, *Virgibacillus*, *Salibacillus*, *Gracilibacillus*, *Ureibacillus* and finally *Geobacillus*.
Thermophilic bacteria have an optimal growth temperature range of 45–70 °C, therefore they are isolated from hot environments such as hot springs, hot water pipelines, heat exchangers or waste treatment plants. Some of these spore forming bacteria are considered as novel food spoilage organisms.

Purpose: The aim of our studies was to determine the heat resistance parameters of novel thermophylic bacterium, *Geobacillus pallidus* spores.

Methods: Heat resistance of *Geobacillus pallidus* spores isolated from spoiled canned sweet corn was determined in sweet corn brine. Heat treatments were carried out in glass ampoules at 95, 102, 104, 110, 117 and 130°C in glycerol bath. Surviving counts were determined on CASO agar (MERCK) and plates were incubated at 55°C for 48 hours. D10-values and z value were calculated.

Results: Using temperature below 100°C did not cause considerable decrease in the number of surviving cells. At 117°C and 130°C the suspension reached the required value during 10 minutes and there were no survival cells after the first sampling. At 102, 104 and 110°C the following D10-values were obtained: 12.2 min; 7.1 min and 1.0 min, respectively. Z-value of *Geobacillus pallidus* spores was calculated as 7.3°C.

Significance: Compared with literature data the examined strain of *G. pallidus* did not proved to be extremely heat resistant in sweet corn brine and the reason of spoilage was a result of probable post-contamination of the product.

Acknowledgment: The support of the TÁMOP-4.2.1/B-09/1/KMR-2010-0005 project of the National Development Agency of Hungary is highly appreciated.

P2-29 Predictive Model for Mycotoxins in Maize

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Introduction: Climate change is expected to influence mycotoxin levels in maize as temperature increase and changes in rainfall patterns have an impact on the presence and persistence of fungi. Predictive models are, therefore, needed that are capable of predicting mycotoxin levels related to weather parameters. In The Netherlands, Fusarium graminearum is the predominant species producing deoxynivalenol (DON) and zearalenone (ZEA).

Purpose: Currently, there are very few models that predict mycotoxin levels in maize. Therefore, the aim of the current study was to derive a mechanistic model that can predict DON and ZEA levels in maize related to weather conditions in the Netherlands.

Methods: The model included parameters for fungal infection during silking and subsequent fungal growth and toxin formation. Mycotoxin data from Dutch maize samples were used to calibrate the model. As input parameters, weather data (temperature, rainfall, relative humidity and wind speed) from a central location in the Netherlands were used as well as average dates for sowing, emergence, flowering and harvest of maize.

Results: Due to the assumptions made, precise model predictions of DON and ZEA were not possible. However, the results do show that the model is capable of describing the observed trend in mycotoxin levels for 2002 to 2007.

Significance: The model can be used to evaluate the effect of changing weather on mycotoxin levels in maize in the Netherlands on a trend basis. Once the model is calibrated to local conditions, it can also be used for other temperate areas with a predominance of F. graminearum (such as NW Europe),

P2-30 Modelling Contaminant Transfer through the Food Chain — A Case Study on Perfluorooctane Sulphonate (PFOS)

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Introduction: Soil pollution has an impact on the quality of various food products as a result of transfer processes along the soil-crop-animal-food chain. The extent to which a pollutant is transferred from

soil to consumer can be assessed using a chain model based on the farm-to-fork approach. Such an approach links the occurrence of substances in the soil with uptake by crops, animals and ultimately transfer to food products. As a case study, we applied the approach on Perfluorooctane Sulphonate (PFOS).

Purpose: The aim was to quantify transfer of PFOS throughout he food chain.

Methods: A literature search was performed to determine the availability of data needed to develop a food chain model. The model approach developed in this study starts with the transfer from soil to arable food and fodder crops and surface water. Subsequently, animal intake is quantified using animal feed consumption data and intake of soil and drinking water. Finally, an animal carry-over model is used for the transfer of PFOS to kidney, liver and meat. Each model compartment is validated with measurements from a local contamination in North Rhine-Westphalia in Germany.

Results: The literature review showed that, in general, data are available for PFOS levels in surface water and fish, whereas data on levels of PFOS in the terrestrial food chain are lacking. Field data confirmed that model predictions for crops and surface water were within the range of measured concentrations.

Significance: The modeling approach followed enables the user to determine the effect of local pollution events on consumer products. Based on the model, the impact of intervention measures can be evaluated. Furthermore, various food groups can be compared in their contribution to PFOS exposure, which can result in strategies to eliminate the high risk products from the menu.

P2-31 Food Safety in Schools Supported by the National School Food Program in Brazil: What is Revealed by Scientific Literature Published between 1990 and 2009?

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Introduction: National School Food Program (PNAE) is a major public policy of Food Security in Brazil. Existing for over half of a century, it aims to provide children and young people with a perspective of right of a healthy, adequate and safe food, respecting feeding and culture diversities. Despite increased regulation over the last decade food safety remains a concern, as there is no efficacious mechanism of both control and assessment. Previous researches have indicated high rates of inadequacy of food safety.

Purpose: This study aimed to characterize the food safety in the context of the Program, based on scientific literature published between 1990 and 2009.

Methods: Fifty-three papers were reviewed. These included reports from different parts of the country over the last nineteen year. Four dimensions were considered: general conditions of the production unities, water, foods and foodhandlers.

Results: Overall, it was noticed the use of different investigation procedures, what impaired a comparative analysis between the studies' results. Regarding production unities general conditions, problems related to infrastructure, facilities, equipments, utensils, hygiene and documentation were found. As to the water, the situation revealed itself worrisome, as the researches registered non-adequacies related to potability and stocking conditions and to the insufficiency of registrations. For foods, nonconforming products were found, as for the presence of dirtiness and indicator and/or pathogenic microorganisms, as for irregularities in operational procedures throughout the whole production chain. In relation to food handlers, the studies pointed out the compromise of the quality of the food served, considering different aspects – health conditions, knowledge acquisition and hygiene practices, emphasizing the relevance of training programs to improve the scenery of the canteens.

Significance: This study is indicative that the perspective of food safety by PNAE remains limited. We provided suggestions on regards the management of food safety by the Program. The method used provided systematic information on food safety in schools supported by PNAE and allowed both

characterization an identification of gaps and strengths, important to support the development of science and the Program management.

P2-32 Street Food in Salvador's Carnival, Bahia, Brazil: Study in Social and Health Perspective

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Introduction: The Carnival of Salvador, at the same time that reunites more than one million of people in search of pleasure and entertainment, attracts thousands of street food vendors that occupy the streets in anticipation of financial gains. In parallel, there is a growing concern about the safety of food, since the increase in demand for these products is accompanied by the sprawl of trade, expanding health risks.

Purpose: This work aimed to characterize the activity of street food on Carnival circuit of Campo Grande, Salvador-Bahia, Brazil, in social and health perspectives.

Methods: This cross-sectional study, exploratory, descriptive, quantitative in nature, carried out with participation of 264 sellers and application of semi-structured forms filled out through interview and observation on site.

Results: Trade street food was characterized by the predominance of females (69.7%), at economically active age, with elementary schooling (64.8%) and with family income less than 3 minimum wages (97.3%). The main reason cited for insertion in the activity was the income supplementation (73%), with estimated average fundraising with work during the period of approximately two minimum wages. Most individuals (57.2%) used fixed sales structure with predominance of coolers (67%). As to the origin of foods and beverages marketed, 90.5% were industrialized. Among the drinks predominated beer, soda and mineral water and as to foods, hotdogs, barbecued meat and meals. In points of sale, food and beverages in contact with the ground were observed (30.1%), concurrent manipulation of food and money (34.3%), in addition to the finalization of products (17.4%). Sellers reported washing their hands more than five times a day (54.9%) and the utensils (26.1%), point of sale, equipment, and/or environment (42.8%), once a day. The water used came mainly from residences (24.8%) and shops (24.4%) and was stored in plastic bottles or jars (44.2%). Sanitary-hygienic conditions were classified as bad or very bad for (36.9%), packaging utensils (26.1%), stands (17.5%) and sellers (50%).

Significance: The results indicate the need for municipal planning of Carnival, with views to the development of intervention programs, contemplating the provision of adequate infrastructure and promoting health education for vendors, to enable higher levels of food safety.

P2-33 Assessment of Parents' Powdered Infant Formula Preparation and Storage Behaviours Using Observation: Implications for Microbial Safety

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Introduction: Powdered infant formula (PIF) is a non-sterile product that has received attention in recent years due to possible contamination with *Cronobacter* spp. and *Salmonella*. It is the parents' responsibility to minimise microbial risks and ensure preparation of safe PIF feeds. However, cognitive data detailing parents' knowledge, attitudes and self-reported-practices related to PIF suggests recommended PIF safety practices are not always implemented.

Purpose: The purpose of this study was to assess parents' risk-related PIF practices and hygiene behaviours and link with microbiological risks.

Methods: Parents (feeding infants aged < 12 months with PIF) prepared PIF feeds alongside a chicken salad in a model domestic kitchen. Risk-related PIF and hygiene behaviours were recorded using an observational checklist.

Results: Cumulatively, hygiene and preparation practices of 296 'used' infant feeding bottles (including 118 reconstituted feeds) were observed. Although all parents (n = 50) used fresh, hot water

with detergent for cleaning 'used' feeding bottles, many (42%) failed to scrub key bottle locations which are known harbour food residues and micro-organisms if inadequately cleaned e.g. inner screwcap/outer-rim thread. Failure to clean and rinse feeding bottles/components may result in inadequate disinfection. 34% of parents failed to rinse all bottles/components before disinfection. More than 46% of parents demonstrated practices illustrating that PIF feeds are not prepared with boiled water > 70^{o}C; 40% of parents demonstrated reconstituting feeds for feeding > 2 hours after preparation. Although 96% implemented adequate handwashing/drying before starting food and PIF preparation, 32–40% washed/dried hands thoroughly immediately before removing items from disinfection and before PIF reconstitution. Indirect cross-contamination behaviours between raw chicken and PIF feeds/feeding bottles were observed during 46% of preparation sessions.

Significance: Cumulatively, data indicates that parents implement variable PIF preparation and storage practices; many are contrary to recommended guidelines and increase the potential risk for microbial survival and growth in reconstituted feeds. This data is part of an ongoing risk assessment which will be used to inform the development of risk communication strategies to increase implementation of risk-reducing behaviours during PIF preparation and handling.

P2-34 Monitoring Allergens in Foodstuffs: Results from Piedmont Region (2007–2010)

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Introduction: Food allergies are a major health issue as each year the number of people suffering from allergic reactions to food is estimated to be about one million. Undeclared food allergens in the label represent a risk for consumers, as there is no cure for food allergies. According to Directive 2003/89/EC declaration of all ingredients and derived substances in the label is mandatory.

Purpose: To protect food-allergic consumers, a monitoring plan on allergenic ingredients in foodstuffs was settled in the Piedmont (Northwestern Italy) in 2007–2010. The aim of this work is to analyze 4 years data in order to assess the presence of undeclared allergenic substances on label in food placed on the market and check the conformity of labelling of food allergens.

Methods: In Piedmont from 2007 to 2010 a total of 1,749 products were collected and tested by ELISA according to standard criteria. To test association between variables as labelling conformity, eggs and milk proteins and allergenic food a chi square test was performed.

Results: A total of 66 samples out of 1,749 showed the presence of milk and egg proteins. Milk proteins were found on 34 out of 899 samples (3.8%; CI95%: 2.5%–5.03%) and eggs proteins were found on 32 out of 850 (3.8%; CI95%: 2.5%–5.04%). Milk proteins and eggs proteins were found on meat preparations (45 samples out of 904), on meat products (17 out of 734) and others products, especially minced meat and wafer (4 samples out of 60). Chi squared test showed no association between the presence of proteins and type of food product; while the presence of eggs proteins was associated with meat (OR=4.9%; CI95%: 1.9%–12.9%).

Significance: Results confirmed the importance of monitoring allergenic food and labelling. Therefore more attention should be put on food labelling especially for meat products.

P2-35 FP7 EU Veg-i-Trade: Assessment of the Global Food Market of Fresh Produce: Production and Trade in Representative EU and EU Trading Partners

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Introduction: Global sourcing and climate change influence trade flows in the fresh produce food market. Veg-i-Trade is an EU FP 7 collaborative research consortium of 23 worldwide partners assessing the production and trade of fresh produce in representative EU and EU trading partners to evaluate the status of the fresh produce supply chain of different countries subjected to different climate conditions.

Purpose: To determine the functioning of the fresh produce supply chain, the first objective of the Veg-i-Trade project is to map the economic structure and organization of this supply chain at both European and global levels.

Methods: A systematic, comprehensive compilation and analysis of available economic data from various institutional databases was done. Three economical mapping conceptual frameworks were used: Conceptual framework 1, for geographical settings (for the world, regional, EU-27 and third countries); Conceptual framework 2, for production components (production volume and production area); and Conceptual framework 3, for trade components (export volume, export value, import volume and import value). The different frameworks enable us to identify the most important production and trade flows of the top 10 fresh fruits and vegetables. In mapping the top 10 products in production and trade against different region/countries four colours were specifically used; Green: top 3, Orange: 4th-6th, Red: 7th-10th, and Grey: not in top 10.

Results: The top 10 fresh fruits and vegetables in production and trade for the world, regions (Africa, Americas, Asia, Europe and Oceania), EU-27, and selected countries (including the nine Veg-i-Trade country partners and selected third countries) were identified for the most recent available year in the FAOSTAT database (2007). Precisely, apples and grapes (fruits) and tomatoes and cabbages (vegetables) are the most important in production volume around the world, EU-27, and at selected country level. Apples and tomatoes were the fruit and vegetable that had the highest frequency of the Top 3 green colour respectively and in many region/countries they also appeared in the top 3 rank for trade. From the comparison of production and trade data of different countries it has become clear that many regions may have agreeable climatic conditions and production area available but only those countries that have the necessary infrastructure, human capacity and policy support in place to implement the various technical and managerial requirements for exploiting trade opportunities emerge as the key players.

Significance: The mapping of the economic structure of the fresh produce supply chain contributes to the understanding of the complexity of the global food market and supports the selection of case studies and logistic chains in Veg-i-Trade for further research activities in assessing vulnerability of fresh produce to bacterial contamination, mycotoxin production and pesticide residues

P2-36 FP7 EU Veg-i-Trade: Assessment of the Organization Structure of the Global Fresh Produce Food Supply Chain at the EU and Global Level

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Introduction: The growing importance of healthy food in the daily diet at both European and global levels has increased demand for fresh fruits and vegetables. This trend has not only resulted in increased importance of global sourcing for these products but also spurred the emergence of particular dynamic and complex supply chains that are able to continuously match supply capabilities to the changing demand requirements.

Purpose: EU FP 7 Veg-i-Trade aims, among the other goals, to provide a review of and gain insight in the generic organization structure of the highly globalised food market of the fresh produce supply chain.

Methods: This study is a desk-based research in mapping various layers of chain organization. Based on the farm to fork concept, food supply chain components at the micro, meso and macro levels are

identified. Schematic flow charts of various activities and stakeholders involved in the organizational structure and relevant legislations, quality standards (Non Tariff barriers) for fresh produce at the EU and international levels were mapped in a theoretical framework.

Results: Specific information relevant to the fresh produce supply chain (from inputs, production, processing, wholesale and retail trade) was assembled in schematic diagrams. In particular these are; a conceptual map of an integrated fresh produce supply chain at the country, EU and global level, key handling activities, chain operators, chain supporters, chain enablers along the fresh produce supply chain, international hygiene requirements, international food safety legislations, EU hygiene legislations, EU food safety legislations, EU phytosanitary legislations, EU trade norms legislations, Quality assurance standards, and finally private & brand labels along the fresh produce supply chain. Analysis of the organization of supply chains of product case studies from Uganda for passion fruit and Serbia for berries are performed as an example of the different levels which need to be elaborated before a non EU country can export to EU.

Significance: The complexity of the supply chain shows the need to identify and distinguish each actor's task and responsibility in guaranteeing food safety in the globalized food supply. This study also underlines the need and importance of strengthened international collaboration and mutual communication on standards and demands as currently applied in global food trade.

P2-37 FP7 EU Veg-i-Trade: Compliance to Food Safety Standards in the Fresh Produce Chain: Increasing Pressures and Challenges for Third Party Countries Supplying the European and U.S. Markets

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Introduction: Global trade in fresh and processed fruits and vegetables, has increased by 30 percent since 1990. In both Europe and the United States, one of the fast-growing product segments is semi-prepared and packed fresh produce including preassembled salads, vegetable dips and sliced or mixed fruit salads. Factors such as consumer pressure, protection of brand image, global sourcing of raw materials, stricter food regulation and outbreaks pointing out the vulnerability of the fresh produce chain has culminated in the introduction of strict food safety standards which challenge third party countries and may affect their food sovereignty.

Purpose: EU FP 7 Veg-i-Trade work is addressing the concept of food sovereignty in the context of a highly globalised fresh produce supply chain.

Methods: This study is a desk-based research on the degree and manner in which global trade flows in the fresh fruit and vegetable logistic chain has been influenced by these food safety standards e.g., changing priorities of key stakeholders, transformation of the outputs of the chain, increasing importance of traceability, food safety requirements etc. Overall criticism of the typically weaker compliance capacity of third party countries is expressed among other issues.

Results: In many respects, this study analyzes the level of variability in the quality and safety of fresh produce in the logistic chain and its determinants in primary production. These include agricultural practices, harvesting and/or on-farm post-harvest handling in addition to the organization of logistic facilities and chain governance. It lists up the challenges these third party countries face with regard to vulnerability to food safety issues (with special focus on viral and microbial pathogens), vulnerability to climate change, difficulties associated with existing and emerging supply chain characteristics.

Significance: Veg-i-Trade aims to provide a platform for international collaboration which may turn the demands of food safety standards into an opportunity for enhancing dialogue with all trade partners to guarantee food sovereignty of producer countries for fresh produce.

P2-38 Foodborne Pathogen Detection and Identification Using Verefoodborne™ Chip Technology

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Introduction: Because of rapid changes in our lifestyle, current and future outbreaks require new technologies to provide fast and specific methods for the detection of dangerous pathogenic bacteria in food. An integrated PCR-DNA microarray method is a good example for identifying the presence of several virulence genes for many bacterial strains simultaneously.

Purpose: The purpose of this study was to compare and contrast the currently accepted standard of the USFDA's Bacteriological Analytical Manual (BAM) for the detection of *Salmonella*.

Methods: Veredus Laboratories has developed a novel Lab-on-Chip, VereFoodborneTM for this purpose. This fast, user-friendly and low cost device combines an ultra-fast miniaturised PCR for amplification of nucleic acids and a high quality microarray for detection in a single chip. Customised spotting on the chip means that the chip can be easily reconfigured to expand its range of detection to detect other pathogenic targets of interest. This device allows users to apply the full benefits of DNA testing in real-world conditions, at a fraction of the time, cost and complexity. The bio-chip is able to rapidly detect, differentiate and identify more than 9 bacteria and 2 viruses including *Salmonella*, *Vibrio*, *Staphylococcus*, *E. coli*, *Shigella* and Noroviruses in one test. The bio-chip was tested recently to identify *Salmonella* in lettuce and tomatoes. The chip was able to reveal promising results when it was compared to the Bacteriological Analytical Manual (BAM) methods.

Results: Results show that the bio-chip has similar sensitivity with the BAM method for the detection of *Salmonella* in lettuce, and higher sensitivity in tomato. For the BAM method with lettuce samples the results were 100% (22/22) where the bio-chip detection results were 95.5% (21/22). For the BAM method with tomato samples the results were 16.7% (4/24) where the bio-chip detection results were 43.5% (10/23). A highlighted difference between the two methods was sample-to-answer time. The bio-chip sample-to-answer time was 2–3 hours after enrichment compared to 72 hours after enrichment using the BAM method. Furthermore, other bacteria, including *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium perfringens* were detected simultaneously on the bio-chip.

Significance: By providing answers to foodborne pathogen risks in hours rather than days, this technology will significantly decrease the reaction time needed to address a potentially deadly threat.

We would like to thank the Singapore National Environment Agency and the Singapore Agri-Food and Veterinary Authority for participating in this study.

P2-39 Antibacterial Effects of Two Iranian Plant Extracts and Their Synergistic Effect on the Growth of *Enterococcus faecalis* in Laboratory Medium

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Introduction: The food industry has tended to reduce the use of chemical preservatives in their products due to increasing pressure from consumers or legal authorities. Natural extracts from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens including *Enterococcus faecalis*.

Purpose: The aim of this work was to evaluate the antimicrobial effects of two Iranian plant extracts, garlic (*Allium sativum*) and Mint (*Menth spicata*) and their combination on *Enterococcus faecalis*.

Methods: In this work the antimicrobial effects of garlic (*Allium sativum*), Mint (*Menth spicata*) and their combination on *Enterococcus faecalis* were evaluated using disk diffusion method as a preliminary step and microdilution method. The extracts of garlic and Mint were introduced into TSB Broth at eight concentrations from 50% (v/v) to 0. 39% (v/v) in order to determine the minimum inhibitory concentration (MIC) for *Enterococcus faecalis* using the Bioscreen C device which is based on optical density measurements.

Results: The results indicated that garlic (*Allium sativum*) and Mint (*Menth spicata*) extracts showed MIC of 12.5% (v/v), and 1.58% (v/v) for *Enterococcus faecalis*. The antimicrobial activity was enhanced in response to extract mixture than individual extracts of each species, as no growth was observed at all concentrations from 50% to 0.39% (v/v) of the mixture extract.

Significance: Edible plants can be a potential source for inhibitory substances for some forborne pathogens. Both extracts studied in this research were effective on *Enterococcus faecalis* and the combination of them showed synergistic effect on inhibition of the growth. So the potential of plant extracts when combined with each other can be used as a more effective barrier for food preservation.