P1 Validation of a Method for Molecular Typing of *Vibrio* Strains in Seafood

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Introduction: The members of the genus *Vibrio* have the capacity to cause foodborne infections associated with the consumption of raw or undercooked contaminated fish or shellfish. For these reasons, the rapid detection of *Vibrio* species, serogroups, biotypes and virulence factors is an important issue for the protection of consumer health and the seafood industry.

Purpose: To validate a robust method readily adaptable in routine molecular diagnostic laboratories, that allows the simultaneous detection of *Vibrio* species, serogroups, biotypes and virulence factors, reducing the cost and time required by other analytical methods.

Methods: The strains used in the present study were obtained from CECT (Spanish Type Culture Collection) and CCUG (Culture Collection, University of Göteborg). End point multiplex PCR was used to detect five *Vibrio* strains: *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus and V. mimicus.* PCR was carried out using specific primers and particular amplifying conditions for each case. Set of primers used: 16SFa, 16SFb, 16SR, (Harris and Hartley, 2003) ctxA94F, ctxA614R (Fields et al., 1992), L-tl, R-tl (Taniguchi et al., 1985; Taniguchi et al., 1986) and VM-F, VV-Rmm,V.al2-MmR, VM-Rmm (Nhung et al., 2007). The detection of serogroups (O1 and O139 of *V. cholerae*) biotypes (El Tor and Classical of *V. cholerae* O1) and virulence factors of *V. parahaemolyticus* (tdh and trh) was carried out. Moreover, Fragment analysis to differentiate viable and dead *Vibrio* bacteria by relative quantification of DNA using a genetic analyzer was performed. The fragment analysis was used to estimate the relative amount of DNA amplified at different incubation times. The viable bacteria grow in an exponential rate and there is an increase of the genome copies parallely to the cells growth. This fact can be observed as an increase in the peak area of the electrochromatogram.

Results: All the samples contaminated with some *Vibrio* strands were analyzed with the proposed method and a positive result was obtained. The positive samples were confirmed in TCBS plate. Neither false negative nor false positive Results were found.

Significance: In terms of sensitivity, specificity, and analysis time, selective enrichment followed by PCR is clearly a powerful, rapid, and robust methodology for detecting the *Vibrio* strains. The proposed detection system by means of multiplex PCR allows to determine if a food sample is negative for the presence of the studied *Vibrio* bacteria much more quickly than by following the complete microbiological identification scheme.

P2 Technological Processing Affecting Organotin Compounds Levels in Canned Mussels

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Organotins such as butyltins are toxic compounds used mainly as biocides in antifouling paints for protection of vessels. Despite ongoing restrictions, butyltins concentration in marine organisms as mussels remain considerably high, therefore the study of the levels of butyltins in raw mussels as well as the influence of technological processes involved in the elaboration of canned mussels on those levels, are important issues from the point of view of the food safety. Regarding to this, the aim of this work was to study the effects of technological processes on butyltins levels (MBT, DBT and TBT) in manufacturing of canned mussels.

Raw material and different samples taken in those points considered critical into the technological process (washing, cooking and sterilization), were analyzed by high resolution gas chromatography-mass spectrometry (HRGC/MS) previous extraction with tetramethylammonium hydroxide, derivatization with sodium tetraethylborate and further purification with alumina. The results obtained showed that washing of mussels previous

cooking step, produces the total elimination of MBT. On the other hand, cooking causes an increase in the DBT and TBT levels due to the dehydration of the mussels. After sterilization it is observed a variation on the levels, however, thermal treatment does not seem to be the only cause, since the observed profile is different depending on the sauce (natural, pickling and American sauce). While the use of American sauce increases the TBT concentration in the drained product, pickling sauce causes a light decrease on that levels.

The knowledge of the factors affecting organotin levels during technological process is of great interest for canning industry since it could allow optimizing the process with the aim to reduce the organotin levels in canned mussels.

P3 Mouse Bioassay as the Reference Method for Lipophilic Toxins Detection in the EU: Searching for Alternative Techniques

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Introduction: Lipophilic marine toxins presented in molluscs and other invertebrates are mostly analyzed by mouse bioassay (MBA), the European reference method. The use of other techniques is allowed by the EU, if they provide an equivalent level of protection and if they are validated according to an international protocol, although so far none of the alternative techniques gives similar levels of consumer protection as MBA. Big efforts are done searching for alternative techniques because the demand to not use animals in experiments is legally increasing.

Purpose: Compare MBA of different species of shellfish with LC-MS/MS analysis to determine the possibilities of employing it as an alternative technique to the EU reference method.

Methods: Mussels, oysters, cockles, clams, small clams, small scallops and edible whelks in fresh, frozen, boiled and canned presentations were analyzed through MBA, performed according the European Harmonized Protocol based on that of Yasumoto, and also using liquid chromatography-mass spectrometry (LC-MS/MS) analysis based on McNabb's protocol.

Results: 78 samples were analyzed with MBA and LC-MS/MS and results were compared. The toxins analyzed with LC-MS/MS were OA, DTX1, DTX2, YTX, PTX2, AZA1 and SPX1. 54 samples showed the same *Results* with both *Methods*, 20 samples were positive with MBA but none of the toxins analyzed were detected with LC-MS/MS and 4 samples cannot be assigned to any group.

Significance: MBA, employing the European harmonized protocol, was compared for the first time with LC-MS/MS methodology. These studies are required to make progress in the implementation of analytical methods in marine biotoxins monitoring.

P4 Supplementing Soy-Seasoning with Pectin, Nisin and a *Pediococcus Fermentate*, Reduces Processing Waste and Inhibits Growth of *Listeria monocytogenes* in Seasoned Roe without Affecting Palatability

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Introduction: Post-processing growth of *Listeria monocytogenes* in seasoned roe is an important food safety issue. Roe however, has poor tolerance for strong processing parameters and additives. Listeria-safe yet palatable seasoned roe products are desirable.

Purpose: The objective of this study was to evaluate the post-processing anti-listerial activity and acceptability of roe prepared with soy seasoning containing pectin, *Pediococcus fermentate* and nisin.

Methods: Soy used to season roe was supplemented with 2% pectin (Wako), nisin powder (0.05g/ml) (Sigma), and freeze-dried MRS broth fermentate of *Pediococcus pentosaceus* Iz3.13 (0.1g/ml). As a positive control, samples of seasoned roe were prepared according to standard commercial procedures. After inoculation with 5 og CFU/g of a *L. monocytogenes* cocktail (IID 578, IID581, ATCC 7644) samples were stored at 12°C for 12 days and analyzed.

Results: Total viable and *L. monocytogenes* counts in roe seasoned with supplemented soy were 3 and 2 log CFU/g respectively lower (no growth observed) than counts in roe prepared according to standard commercial procedures (growth observed) after 6 days. In addition, total acid content, chloride concentration, and organoleptic acceptability of roe seasoned with supplemented soy and roe seasoned according to standard commercial procedures were not significantly different ($P < 0.05$). Since pectin increased viscosity and therefore retention of seasoning, only one third of volume of soy seasoning was needed to prepare an acceptable seasoned roe product.

Significance: Supplementing soy seasoning with pectin, *Pediococcus* sp. fermentate and nisin, can improve safety of seasoned roe products without significantly affecting acceptability. Furthermore, processing waste (used seasoning) is greatly reduced.

P5 Reductions of *Salmonella* and *Campylobacter* on Broiler Carcasses Using SonoSteam® – A Combination of Ultrasound and Steam

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Introduction: Contaminated food as well as poor cooking and kitchen hygiene are the typical causes of food borne bacterial infections. These infections are an increasing health problem and since poultry is the most common source, intervention programmes have been initiated to help eliminate the occurrence of *Campylobacter* and *Salmonella* on fresh poultry products. SonoSteam® is a new technology for decontamination of surfaces by the use of steam and ultrasound combined.

Purpose: The overall aim of these studies was to evaluate the potential for SonoSteam® to reduce on *Salmonella and Campylobacter* on freshly slaughtered broilers.

Methods: Naturally contaminated broilers were treated with SonoSteam® for 1.6 second. *Salmonella* and *Campylobacter* from 10 gram samples were counted using NMKL 119 for *Campylobacter* and ISO 6579 modified for quantification for *Salmonella*. The impact of treatment on bacterial numbers was evaluated and calculated by statistical analyses of variance.

Results: After 1.6 second SonoSteam® treatment on freshly slaughtered broilers *Salmonella* were significantly reduced from 3.47 ± 0.09 to 1.70 ± 0.09 $\log_{10}$ units, corresponding to an absolute $\log_{10}$ reduction of 1.77 or 51% reduction on a logarithmic scale. In addition *Campylobacter* were significantly reduced from 2.11 ± 0.20 to 0.82 ± 0.05 $\log_{10}$ units, corresponding to an absolute $\log_{10}$ reduction of 1.29 or 61% reduction on a logarithmic scale.

Significance: It has been revealed that SonoSteam® has potential in reducing the numbers of *Salmonella and Campylobacter* on freshly slaughtered broilers. In general the SonoSteam® technology can be applied on several food products and has the potential in reducing the prevalence of foodborne infections in humans.

P6 *Campylobacter* spp. Isolates from Poultry and Human: Typing and Antimicrobial Resistance

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Introduction: Campylobacter is a leading cause of diarrhoea and foodborne gastroenteritidis in humans in EU. This microorganism is zoonotic and poultry is considered a major reservoir for transmission to humans.

Purpose: Over the last decade, the emergence of antimicrobial resistance in *Campylobacter* strains isolated from poultry and human in EU has increased dramatically. In the Czech Republic, the prevalence of human campylobacteriosis is the highest in the EU, but there are no available data about the cause, particularly about typing and resistance to the antibiotics.

Methods: Cultivation was made according to ISO10272; samples were directly planted onto mCCDA and Karmali. For species differentiation mPCR was used for regions in the *16S rRNA*, *map*A and *ceu*E genes. Antimicrobial resistance to seven antibiotics were tested by agar dilution method according to international standard guideline recommended by NCCLS and by molecular methods.

Results: Broilers (n = 1083) were colonized by *C. jejuni* (80.21%) and *C. coli* (19.79%). Similar proportion of species was found in human patients (n = 423) with campylobacteriosis: 79.67% were infected by *C. jejuni*, 11.58% by *C. coli* and 8.75% was infected by both species. Broiler and human isolates were sensitive to gentamicine, chloramphenicol. Human *Campylobacter* isolates were resistant particularly to ciprofloxacine (36,20%), nalidixic acid (40,77%) and tetracycline (10,77%). Isolates from poultry were resistant first of all to nalidixic acid (54,14%), ciprofloxacine (41,60%), ampicillin (37.84%) and erythromycin (9.02%). Resistant isolates with different levels of MIC were positive for the presence of genes (*tet*O) and specific mutations of genes (*gyr*A, *23S rRNA*) associated with the resistance to quinolones, macrolides and tetracyclines.

Significance: Monitoring of prevalence and resistance in poultry and human isolates is important in adoption of the elimination programs of *Campylobacter* in broiler production and for the antimicrobial policy in human treatment.

P7 Analytic Control of Cleaning Operations in Food Catering: Classic and Rapid Methods Comparison

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One of the most important considerations in food safety, is the hygiene of the surfaces that food comes in contact with. The efficacy of cleaning practices must be evaluated and validated to avoid bacteria growth and biofilm formation. This study aim to compare the results from different evaluation methods among different kitchen surfaces: inox surface, marble surface and chopping boards.
The methods selected for comparison were classic methods for microbial quantification – Aerobic mesofils and *Pseudomonas* spp. – and rapids tests – ATP quantification (luminometry) and organic residues (multi-enzymatic reaction).The results from aerobic mesofils and luminometry prove to be correspondent (P = 0,000). However mesofils does not seem to be correspondent with rapid test
for organic residues (P = 0,15). There is no relation between the two rapid testes (luminometry and organic residues). We found a strong relation between the two classic methods (aerobic mesofils and *Pseudomonas*), and established a correlation of 0,749 (P =0,01).

P8 Inhibition Test for Detecting Tetracyclines in Milk at the MRL

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Introduction: Current inhibition tests to detect beta-lactam and other antibiotic contamination in milk are sensitive to 150-300ppb tetracycline which is above the maximum residue limit, MRL, of 100 ppb.

Purpose: Present data on a next generation inhibition test that improves detection to 100 ppb tetracycline.

Method: The Charm Blue Yellow and Cowside test are Bacillus Stearothermophilus based inhibition tests that detect the absence of inhibitor by turning yellow as the bacteria produce acid during growth. With agar/media modifications the tests can be made more sensitive to the presence of tetracyclines.

Results: Raw commingled cow milk fortified with tetracycline drug reference standards were detected at the following concentrations: Blue-Yellow II test-Tetracycline: 75-100 ppb, oxytetracycline 75-100 ppb. Cowside II test- Tetracycline: 50-75 ppb, oxytetracycline 75-100 ppb. Other drug family
sensitivity, e.g. beta-lactam, sulfa drugs, macrolides, aminoglycoside, were also adjusted to more closely match the ECMRL.

Significance: Tetracyclines are commonly used in dairy practice and may cause inhibition and economic loss in manufactured dairy products. The new formulation addresses lack of tetracycline sensitivity in older inhibition methods.

P9 Practical Application of Sensitive ATP Test to Allergen Cross-Contact Prevention in Food and Beverage Facilities

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Introduction: A sensitive ATP test has been shown to detect foods containing allergenic ingredients derived from milk, eggs, peanuts and soy. These foods dried on surfaces are detected at similar µg/cm^2 levels as protein-specific ELISA.

Purpose: Demonstrate application of sensitive ATP test in allergen-cross-contamination avoidance.

Methods: Sensitive ATP, AllerGiene, is performed after cleaning verification by conventional ATP. The method uses a luminometer channel for sensitive ATP. At 20 seconds, the relative light units, RLU, are compared to a limit and if exceeded surfaces are re-cleaned. Surfaces that pass are verified clean using protein specific ELISA. The limit is established based on food ATP levels and proportion of allergenic ingredient.

Results: Filling equipment, used for milk and juice, was tested using ATP and a milk-protein ELISA. ATP levels less than 70000 tested <5 ppm (µg/cm^2) by ELISA. 70000 limit successfully predicted negative results by ELISA of surfaces and juice. An egg nog producer detected egg-yolk at 50 ppm with ATP, and egg nog at 20 ppm. A limit of 30,000 was able to predict ELISA negative results < 10 ppm. A soy milk processor found neither ATP (500ppm) nor ELISA (> 1000 ppm) detected ingredient at 20 ppm. The food containing 6% ingredient was detected by ATP at 100 ppm. A limit of 0 verified cleaning to ~6 ppm soy ingredient using the food ATP to amplify the risk of ingredient presence.

Significance: Sensitive ATP, AllerGiene, is a rapid cleaning verification tool that is useful in avoiding foods cross contaminating their potentially allergenic ingredients. The low level ATP in foods can be used to effectively develop allergen cross-contact prevention strategy that is inexpensive, easy and predictive of allergen specific tests.

P10 Prevalence of *Listeria monocytogenes* in Minced Meat

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Introduction: *Listeria monocytogenes* is widely distributed in nature, which inevitably results in contamination of numerous food products. The detection of *Listeria monocytogenes* in meat is of particular interest to consumer safety and its incidence has been investigated in several countries, but little has been reported about the prevalence of this organism in raw meat products in Portugal.

Purpose: The purpose of this study was to investigate the incidence of *Listeria monocytogenes* in minced meat in Portugal.

Methods: A total number of 676 fresh minced meat samples were analysed for the presence of *Listeria monocytogenes*. Samples were collected at different Portuguese shops by specially trained technicians and stored at 4°C until arrive our lab. The direct and specific screening of *Listeria monocytogenes* was performed using the automated VIDAS® LMO2 system (bioMérieux) according to manufacturer's instructions to all types of food. Only the positive samples confirmed by the standardized methods described on the EN ISO 11290 were considered positive.

Results and *Discussion*: Of a total of 676 minced meat samples analysed, 147 (21,7%) were positive for the presence of *Listeria monocytogenes*. These results are in agreement with those obtained in similar studies performed in Spain. The high incidence of this pathogen on this products can be attributed either to faecal contamination during evisceration, or to cross contamination during the mincing process. If no good methods of sterilization are adopted to keep the mincing machines clean, once a contaminated meat is processed, all subsequent meat minced with the same machine will be contaminated.

Significance: Human listeriosis is mainly attributable to the food borne transmission of *Listeria monocytogenes*. Our results suggests the presence of a significant public health hazard related to the consumption of minced meat contaminated with *Listeria monocytogenes*, especially if undercooked meat is consumed.

P11 Growth of *Enterobacter* spp. in Infant Formula Milk

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Introduction: Cronobacter (*Enterobacter sakazakii*) have been established as opportunistic pathogens associated with ingestion of contaminated reconstituted powdered infant formula (PIF). *Enterobacter* spp., such as *E. aerogenes*, *E. cloacae* and *E. hormaechei*, have also been implicated as causal organisms in outbreaks within neonatal intensive care units (NICUs).

Purpose: This study aims to investigate the likelihood that PIF can be a vehicle for *Enterobacter* proliferation, ingestion and infection.

Methods: The growth rates of the type strains of *Enterobacter* spp. and related organisms were determined in infant formula using the indirect impedance technique. Additionally the ability of strains to ferment lactose, producing acid and gas, was determined.

Results: The mean doubling time for Enterobacter spp. was 30 minutes. In comparison to this, the mean doubling time for *Cronobacter* spp. was 25 minutes, whereas for *Klebsiella pneumoniae*, *Salmonella* Typhimurium and *Citrobacter* freundii the doubling times were 22 minutes, 26 minutes and 37 minutes respectively. The ability to ferment lactose did not correspond directly to the growth rates obtained for individual species.

Significance: This work indicates that contamination of IFM, either from endogenous organisms or poor hygiene during preparation, can lead to proliferation of potential pathogens increasing the risk of infection in neonates.

P12 Internal and Independent Laboratory Validation of a Reverse-Transcriptase PCR Assay for Detection of Genus *Listeria* from Stainless Steel Surfaces

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Introduction: *Listeria* spp. detection and monitoring is important in food processing plant safety management because it can be an indicator of possible *L. monocytogenes* contamination. Sensitive methods that facilitate faster time to result are needed so that interventions strategies may be implemented more rapidly.

Purpose: We hypothesized that we could develop a more sensitive/rapid assay for genus *Listeria* detection using Reverse Transcriptase PCR (RT-PCR). The goal was to develop an assay using RT-PCR that would detect genus *Listeria* from environmental samples in < 8 h.

Methods: Spiked surface studies were conducted comparing an RT-PCR assay for genus Listeria against the USDA-FSIS reference culture method. *L. ivanovii*, *L. monocytogenes*, and *L. innocua* were used as test organisms for the internal studies,and *L. ivanovii* was used for the external lab studies. Competitive flora was also applied for some studies. Twenty spiked replicates and five unspiked controls were tested by both the test and reference method. In addition, 58 *Listeria* and 52 non-*Listeria* were tested for inclusivity and exclusivity.

Results: The results of the internal studies showed that the RT-PCR method, across all three surfaces and organisms, detected 100/100 samples in < 8 h compared to 51/100 for the reference method, which required ~5 days for final result. Ninety-eight of the 100 samples positive by RT-PCR were culture-confirmed as *Listeria*. The results of the external studies showed that the RT-PCR method detected 20/20 versus 5/20 for the reference method. Of the 20 RT-PCR positives, 12 were culture-confirmed as *Listeria*. All unspiked samples, for both the internal and external evaluations, were negative by RT-PCR and culture. The RT-PCR method detected 58/58 *Listeria* strains and did not detect any of 52 non-*Listeria* sp

Significance: The aggregate data indicates that the RT-PCR method was statistically superior (X2 = 56.7) to the reference method. In addition to superior performance, results were obtained in < 8 h.

P13 Comparison of the TEMPO® System with Conventional Plate Counts for the Enumeration of Coagulase-Positive Staphylococci in Food Products

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Enumeration of coagulase-positive staphylococci in food samples remains critical to monitor manufacturing processes and release finished goods. A new reagent (TEMPO STA) was developed on the TEMPO system to provide an alternative to the labor intensive and time consuming ISO reference method. TEMPO Results were obtained in 24 h instead of 2 days for the ISO reference method without any need of confirmation.

In this study, TEMPO STA was compared to the ISO 6888-2 method. A total of 559 different food samples were tested including raw and processed meat, dairy food, seafood, vegetables and Ready-to-Eat meals. The samples for the ISO method were serially diluted up to 10-4 and enumerated by inclusion in BP+RPF incubated 48 h at 37°C. An aliquot of the 10-1 dilution was used for the TEMPO STA inoculation. After filling, the TEMPO STA cards were incubated 24 h at 37°C. Reading was performed with the TEMPO Reader without any confirmation test.

After log-transformation, statistical tests were done to compare the performances using a combination of regression analysis, difference $\log_{10}$ distributions and T-tests at the 5% confidence level. The results obtained by TEMPO STA are comparable to the results obtained by the ISO 6888-2 method. The percentage agreement between both methods was 98%. The study also demonstrates the limitations of the reference method in case of high background

flora. Interestingly, TEMPO was able to provide a precise count by an efficient growth inhibition of non SCP cells on the medium.

The TEMPO STA method provides a rapid, standardized and cost efficient alternative method to the ISO 6888-2 for enumeration of coagulase-positive staphylococci in a large variety of food products.

P14 Comparison of a New TEMPO® Method for the Enumeration of Lactic Acid Bacteria in Food Products with the ISO 15214 Method

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Lactic Acid Bacteria (LAB) are widely distributed through out nature. They are very important in both production and spoilage of food products. The enumeration of these microorganims is widely used in food industry. The traditional method is based on the De Man Rogosa Sharpe agar incubated 72 h at 30°C (ISO 15214). The purpose of this study is to evaluate a new LAB enumeration method adapted to the TEMPO® system, known for the enumeration of Quality Indicators in Food and Environmental samples.

The comparison between both methods was performed on three different sites with more than 1000 food products naturally contaminated. The samples consisted of different categories such as raw and cooked meat and poultry products, fish and seafood products, vegetables, dairy products, bakery, delicatessen, pet food, Ready-to-Eat products. From the primary dilution, both methods were performed in parallel. After filling, the TEMPO card were incubated 40–48h at 30°C aerobically whereas the MRS plates inoculated by inclusion were incubated 72 h at 30°C aerobically. After reading, results were log transformed for statistical analysis.

Our results indicate that the new TEMPO LAB method performs as well as the reference method. On the whole data, the global rate of agreement was calculated on all tested categories and all the dilutions tested. In term of enumeration, the comparison does not show any significant bias on the data compared using a paired t-test.

With a correlation coefficient of 0.94, a the intercept on the y axis and b the slope are respectively close to 0 and 1.With a high degree of automation and standardisation for food laboratories, this new method performs as well as the ISO 15214 reference method reducing the time to results from 72 h to 40–48 h.

P15 Survey of OTA in Wheat Bread from Lisbon and Alentejo Regions, Portugal: Winter of 2007

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Ochratoxin A (OTA) is a nephrotoxic and possible carcinogenic mycotoxin, that can enter the feed and food chains. It is extensively documented as a global contaminant of a wide variety of human foodstuffs.

It is therefore essential the pursuit for further information concerning the public health significance of OTA occurrence and the correspondent exposure of the populations to this mycotoxin. The objective of this work was to study the occurrence of OTA in wheat bread samples from Lisbon (n = 35) and Alentejo (n = 15) regions, collected in assorted local stores during the winter of 2007. The methodology included PBS:CH3OH extraction, immunoafinifity clean-up and HPLC-FD determination. Recoveries oscillated between 77% and 103% for fortification levels between 2 and 0.1ng/g. The intra-day RSDs were between 4% and 14%, and the inter-day ones between 7% and 13%. The limit of quantification (LOQ) was 0.1ng/g.

OTA was detected in 60% of the samples from Alentejo, although in levels below the LOQ. In opposition, OTA was detected in 54.3% of the Lisbon samples, with an average contamination level of 0.21 ± 0.09 ng/g, contributing to an estimated daily intake (EDI) of 0.21 ng/kg.bw/day, which is nearly a quarter of the total OTA dietary intake for the Portuguese population. The latter fact corroborates the indication that bread is the most important contributor to the daily intake of OTA. Although under the EU established legal limit, the results and the high incidence found in Lisbon wheat bread samples, one of the most consumed foodstuffs, should be perceived as an economic and public health threat justifying the need for further studies. Conversely, for the populations of Alentejo, the EDI can be ascribed as insignificant. Therefore, this mycotoxin does not represent a public health hazard in this region trough bread consumption.

P16 Ochratoxin A in Urine from Lisbonian Population: Winter of 2007

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Ochratoxin A (OTA) is a mycotoxin produced by several Aspergillus and Penicillium species. OTA is recognized as teratogenic, immunotoxic, genotoxic, as well as nephrotoxic. The OTA present in urine is almost exclusively related to the ingestion of several contaminated foods.

OTA levels of urine were evaluated in 43 individuals living in Lisbon. The evaluation of OTA levels was performed in 21 females and 22 males. Urine samples were collected between January and February, 2008.

A sensitive and accurate analytical method for the determination of OTA in human urine, based on extraction with a solution of 5% $NaHCO_3$ in distilled water, immunoaffinity column for clean-up and HPLC with fluorescence detection, was used.

Linearity was evaluated in the range from 0.05 ng to 0.5 ng, and the correlation coefficient was 0.999. The obtained recoveries were higher than 91% for all fortification levels and the precision values were lower than 15.4%. The limit of quantification of the proposed method was 0.008 µg/L.

This study revealed that the frequency of detection was 72%. The overall concentration ranged from 0.01 to 0.07µg/L. The mean concentration and standard deviation was 0.02 ± 0.02 µg/L. For both genders, the mean concentration and standard deviation were 0.02 ± 0.02 µg/L. Comparing these results with those from other studies of different populations, the values obtained are lower than those found in the Coimbra population 0.027 ± 0.004µg/L and in the Setúbal population 0.022 ± 0.008 µg/L in 2005.

Frequency of OTA in the Portuguese population is lower than that of other European populations, such as those of Spain, Italy, Hungary, UK, and Bulgaria.

P17 Salmonella Newport as Reported by the Animal Arm of the National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS)1997–2007

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Introduction: Since the early 1990s there has been increasing awareness and concern regarding the development of antimicrobial resistance among bacteria of public health significance. Reports targeting zoonotic bacteria, and in particular Salmonella species, suggest that resistance is trending upward. Of particular concern starting in 2000, was the emergence of multiple drug resistant (MDR) Salmonella Newport.

Purpose: The purpose of this study was to determine the trend of *S.* Newport submitted to the animal arm of NARMS for genotypic and phenotypic characterization from 1997 through 2007.

Methods: Isolates submitted to NARMS were tested for susceptibility to 16 antimicrobials using a custom made panel and a semi-automated broth microdilution system (Sensititre, Trek Diagnostics, Cleveland, OH). Isolates were further characterized by pulsed field gel electrophoresis (PFGE)

Results: Of the 48,238 *Salmonella* isolates tested from 1997 through 2005, the percent identified as *S*. Newport increased from 0.75% (n = 18) in 1997 to a high of 9.0% (n = 483) in 2003, declining to 2.6% (n = 50) in 2007. The majority (n = 1768) originated from diagnostic submissions followed by slaughter (n = 740) and on-farm (n = 100) sources. Slaughter isolates originated most often from cattle. The percent of isolates resistant to 2 or more antimicrobials averaged 74.6% from 2000 through 2006, declining to 52 % in 2007. PFGE analysis indicated a high degree of heterogeneity among the isolates. Interestingly, isolates which were indistinguishable by PFGE were not necessarily indistinguishable by phenotypic analysis.

Significance: These data indicate that the percent of *S*. Newport originating from animals and submitted to the animal arm of NARMS appears to be declining. However, the MDR phenotype is persisting among a high percentage of the isolates. Continued monitoring and further characterization of isolates is warranted.

P18 Effect of Commercial Broiler Processing on Prevalence and Antimicrobial Resistance of Salmonellae

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Introduction: Broiler processing includes interventions specifically designed to lessen bacterial contamination of carcasses.

Purpose: This study was undertaken to determine the prevalence, serotype and antimicrobial resistance profiles of *Salmonella* on broiler carcasses collected from commercial processing plants.

Methods: Twenty commercial processing plants in the U.S. representing eight integrators in thirteen states were included in the survey. In each of four replications, ten carcasses from one flock were collected at re-hang and ten more at post-chill; each carcass was sampled by whole carcass rinse. *Salmonella* were isolated from carcass rinses using standard cultural techniques, serotype was determined and the resistance to 15 antimicrobials was measured.

Results: Overall, *Salmonella* was detected on 72% of carcasses at re-hang (ranging from 35 to 97 %) and 20% post-chill (ranging from 2.5 to 60 %). In every instance, a significant ($P < 0.05$) decrease in *Salmonella* prevalence was noted between re-hang and post chill which can be attributed to processing interventions. The four most common serotypes accounting for 64% of all *Salmonella* isolates were: Kentucky, Heidelberg, Typhimurium and Typhimurium var 5-; most isolates of Kentucky (52 %), Heidelberg (79%) and Typhimurium (54%) were susceptible to all antimicrobial drugs tested. However, only 15% of the Typhimurium var 5- isolates were pan-susceptible; more than half of the isolates of this serotype were resistant to 3 or more drugs. No isolate of any serotype exhibited resistance to amikacin, ceftriaxone, ciprofloxacin or the combination of rimethoprim/sulfamethoxozole.

Significance: These data demonstrate that although processing lessens carcass contamination with Salmonella, antimicrobial resistant isolates may still be present.

P19 Detection of *Enterobacter sakazakii* in Powdered Infant Formulas: Implementation of the Method ISO/TS 22964:2006

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Studies developed in the 90 years in powder milk formulas, were able to identify *Enterobacter sakazakii* of the family *Enterobacteriaceae* as an etiologic agent involved in foodborne infections, affecting specially infants and so considered an emergent pathogenic. This microorganism is now considered a severe hazard to a restricted population, and can cause serious or chronic sequelae, morbid states of long duration or death. Due to the fact that in Portugal the knowledge
regarding the occurrence of this microorganism in this type of food is scarce, it is of extreme importance to implement accurate detection methods. This study was conducted in the National Institute of Health Dr. Ricardo Jorge (INSA), in Lisbon and the *Purpose*s were the implementation of a detection method for E. sakazakii in powder milk formulas, according to the standard ISO/TS 22964:2006, and the comparison of the efficiency and recovery of three different chromogenic media ESIA (Laboratoire AES), DFI (Oxoid) and ChromoCult (Merck). We used a reference strain *Enterobacter sakazakii* ATCC 51329 and as food matrix a sample of an infant formula for suckles subdivided in 60 portions, which was free of *Enterobacteriaceae*.

The DFI and ChromoCult were the media that gave the most reliable results, when compared with the ESIA medium. It was verified, in the performed assays, that with an inoculation level of 10 CFU/plate, the ESIA medium presented a recovery percentage of 56%, which was a lower value when compared with both DFI and ChromoCult media (86%). With inoculation levels above 10 CFU/plate, the DFI and
ChromoCult media presented a recovery percentage of 100%, whereas the ESIA medium showed recovery percentages of 77% and 63%, with inoculation levels of 10-99 CFU/plate and higher than 100 CFU/plate, respectively. In these assays it was also verified that in the ESIA medium, whenever it has occurred growing of the *E. sakazakii*, the same was very reduced or scarce when compared with the growing observed in the other two media.

Since INSA is the National Reference Laboratory for health, it is of extreme importance as a matter of fact to develop new methodologies for emergent pathogens in order to promptly answer to eventual occurrences and/or solicitations concerning suspected foodborne illnesses.

P20 Aflatoxin M1 Contamination in Cheese Commercialized in Portugal

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Introduction: Aflatoxin M1 (AFM1) a potential genotoxic carcinogen hazard is a hydroxylated derivative of aflatoxin B1 (AFB1). It's presence in cheese can be due to the contamination of AFM1 in raw milk.

Purpose: This study was carried out in order to assess the presence of aflatoxin M1 in the cheeses that are more distributed and consumed in Portugal.

Methods: One hundred and twenty eight samples of several types of hard cheese from five different European countries were randomly acquired from supermarkets. Each 10g sample was analysed using a method that involves immunoaffinity as a purification step followed by high-performance liquid chromatography with post-column derivatisation and fluorescence detection. The calibration curve was determined using a range of standards from 0.1ng/ml to 0.8ng/ml. The detection limit was 0.05 ug/Kg. The recovery was determined spiking an AFM1-free cheese at 20-80 ug/Kg, for a total of 24 samples.The method accuracy was examined based on the Horwitz equation, which had provided a statistical evaluation of the results.

Results: Aflatoxin M1 was detected in eight samples (6.3%). Positive samples contained levels near the maximum admitted level (0.05 g/Kg).

Significance: Considering the fact that the concentration of this toxin is much higher in many soft cheeses and even more in hard cheeses than that in milk from which these cheeses were made, these results indicate a very low occurrence of AFM1 in these dairy products, which might be attributed to a good monitorization and control in raw milk used for cheese production

P21 Isolation and Characterization of Cronobacter (*Enterobacter sakazakii*) from Dried Milk and Related Products

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Introduction: Cronobacter (*E. sakazakii*) are considered emerging opportunistic pathogens and have previously been associated with outbreaks of infections amongst infants, particularly neonates. Symptoms include bacteremia, necrotizing enterocolitis and meningitis with case fatality rates as high as 80% reported. More recently, infections amongst immunocompromised adults have also been reported.

Purpose: The primary reservoir of Cronobacter remains unknown due to its ubiquitous nature and while powdered infant formula has been epidemiologically linked to infections in infants, less is known regarding the prevalence of Cronobacter in other dairy food products. The aim of this work was to determine if Cronobacter could be isolated from a collection of dried milk and related products and characterize any isolates obtained.

Methods: Biochemical profiles, antimicrobial susceptibility, pulsed-field gel electrophoresis (PFGE), rep-PCR and recN gene sequence analysis was generated for all the isolates obtained.

Results: Antimicrobial susceptibility testing indicated that all isolates were susceptible to ampicillin, furazolidone, gentamicin, compound sulphonamides, spectinomycin and streptomycin, however, three isolates were resistant to neomycin and one was resistant to trimethoprim. PFGE identified 8 pulse-types amongst the collection of isolates and rep-PCR analysis identified 3 rep-PCR types amongst the collection. Analysis of the collection using recN gene sequencing identified that all 16 ronobacter strains obtained in this study comprised of *C. sakazakii* or *C. malonaticus* species.

Significance: This study identified and characterized Cronobacter isolated from dried milk and related products and although the majority of the strains were susceptible to the antibiotics tested, the resistance observed in three isolates may indicate increasing risks associated with the presence of Cronobacter in foods. Phenotypic and genotypic analysis should be applied to further monitor and characterize the presence of Cronobacter in food production environments and prevent its transmission into end food products.

P22 Tracing the Source of *Listeria monocytogenes* Contamination Using Serotyping and PFGE Genotying in Pork Cutting Plants in Korea

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Introduction: Since a heating process is not included for inactivating the microorganisms, the contamination with *Listeria monocytogenes* is one of the major concerns in pork cutting plants.

Purpose: The purpose of this study was to determine the contamination profile of *L. monocytogenes* in pork cutting plants and to find the control points to prevent or reduce the contamination in the final meat products.

Methods: Samples were collected from carcasses, equipments (knife, table, glove, transport belt, boning and skinning machine), environment (wall and floor), and processing meats in the process of cutting. Serotyping and pulsed-field gel electrophoresis (PFGE) were used to characterize the *L. monocytogenes* isolates.

Results: Among 402 samples, 73 isolates (18.2%) were identified as *L. monocytogenes*. Four serotypes were detected among the isolates. The major serotype identified were 1/2c (86.3%), and 1/2b (6.9%), 4e (5.5%), 1/2a (1.4%) were followed. Although, the isolation rates of pathogenic serotype 1/2a and 1/2b was low, their detection from carcasses, equipments, and environment showed a possible cross-contamination between the final meats. PFGE yielded 8 different genotypes. The predominant genotype Lm8 (48 isolates) was present on the carcasses, equipments, environment, and the final meats. It suggested that incoming carcasses, which were contaminated during transportation from slaughterhouses, were a major source of spreading *L. monocytogenes* throughout the process. Also, the consistent cross-contamination due to improper cleaning and disinfection operation was another possibility.

Significance: Incoming carcasses and cleaning procedures were considered as critical points, and the strict control measures should be taken in this two points to produce the safe meats in pork cutting plants.

P23 Cronobacter spp. (*Enterobacter sakazakii*) and Implications for Infant Health

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Introduction: *Enterobacter sakazakii* is an opportunistic pathogen associated with meningitis, necrotising enterocolitis and bacteraemia in neonates; cases of infection have been linked to ingestion of contaminated infant formula. Polyphasic investigation has led to the alternative classification of these organisms as a new genus, Cronobacter, comprising several novel species. There has existed some confusion over the taxonomy and identification of these organisms and clarification is necessary to enable more accurate estimation of the risk they pose to infant health.

Purpose: To apply a polyphasic approach to better understand the classification and identification of Cronobacter species. Also to investigate the risk of infection in relation to the ability of these microorganisms to attach and invade a human epithelial cell line.

Methods: Polyphasic taxonomic analysis comprised full length 16S rRNA sequence comparison, automated ribotyping, f-AFLP analysis and DNA-DNA hybridization. Biolog GN2, BBL Crystal, RapID ONE, API 20E, ID 32E and rapid 32E galleries were inoculated and incubated according to the manufacturer's instructions. Selected strains were assessed for the ability to attach to and invade CaCo-2 human epithelial cells. A review of available documented cases of Cronobacter *(E. sakazakii*) infections in children was undertaken.

Results: Cronobacter strains are divided into six separate genomospecies. Although the ability to attach and invade human epithelial strains varies, there are clinical isolates from normally sterile sites representative of all named species. Therefore all Cronobacter strains should be considered potentially dangerous to infant health. Of the identification kits tested, Biolog GN2 plates provided the best identification of the collection of strains.

Significance: The Cronobacter genus comprises multiple species and microbiologists should be aware of the limitations of some biochemical based identification systems for confirmation. In vitro studies indicate that Cronobacter have the potential to invade human epithelial cells, posing a serious risk to infants, particularly neonates.

P24 Monitoring of Pathogenic *Vibrio* species in Ready-to-Eat Raw Seafood

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Every year, many people have been suffering from foodborne illness caused by the ingestion of seafood containing pathogenic *Vibrio* species in the world. We have monitored pathogenic *Vibrio* species in various seafood and water samples.

Samples were analyzed by a method of cultivation and multiplex PCR. The multiplex PCR assay was performed to detect *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* species. Presumptive positive colonies formed on a TCBS plate were confirmed by biochemical test of VitekII system. The biochemical identification with VitekII system detected *Vibrio* species in 19.3% seafood and 60.5% water samples. *V. alginolyticus*(10.2 and 28.9%), *V. parahaemolyticus* (6.1 and 28.9%) and *V. vulnificus* (3.0 and 0 %) were identified by biochemical testing in seafood and water samples. The multiplex PCR performed on enrichment broth from each sample gave positive *Results* for pathogenic *Vibrio* species in 13.2% seafood and 39.4% water sample. *V. parahaemolyticus* was the predominant *Vibrio* species identified by multiplex PCR in total samples.

The aim of this study is to examine the distribution of pathogenic Vibrio species in seafoods. The results of this study help the need for seafoods inspection plan to detect the presence of *Vibrio* species in order to eliminate public health risks associated with seafood consumption.

P25 Presence of *Campylobacter* spp. in Slaughtered Goats in Northern Greece

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The present research was carried out to address the lack of data regarding the prevalence of *Campylobacter* spp. in goats in Northern Greece. Goat meat and offals are basic components in a variety of traditional Greek dishes, while *Campylobacter* spp. are major food pathogens.

The objective of this study, still in progress, was to determine the degree of carriage of *Campylobacter* spp. in goats and the incidence of contamination of their meat and offals at the abattoir.

Between November 2007 and September 2008, 149 animals (100 kids, 49 goats) originating from 22 different farms were randomly sampled after slaughter in two E.U.-approved abattoirs situated in Northern Greece. A total of 398 samples consisting of 149 intestinal contents, 149 carcass swabs and 100 kid liver swabs were collected and examined for the presence of *Campylobacter* spp. The isolation and identification was performed according to ISO 10272-1 International Standard.

60 out of 149 intestinal contents (40.3%) examined revealed the presence of campylobacters with isolation rates being 44% for kids and 32.7% for goats, respectively.107 out of 149 carcass swabs (71.8%) and 86 out of 100 liver swabs (86%) were positive for *Campylobacter* spp. The contamination rates were 79% for kids and 57.1% for goats regarding carcass samples and 86% for kids regarding liver samples. *C. jejuni* was the predominant species in all-positive samples followed by *C. coli* and *C. lari*, while a few samples yielded more than one *Campylobacter* species.

These results, although preliminary, indicate that the goat population investigated in the study is frequently colonized by campylobacters. In addition, the high contamination rates of the carcass and liver surfaces with *Campylobacter* spp. can be attributed to weak points in slaughter hygiene.

P26 Cronobacter (*Enterobacter sakazakii*) in Baby Food

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Cronobacter spp. (*E. sakazakii*) a member of the *Enterobacteriaceae* family is an opportunistic pathogen identified as the etiological agent in rare cases of sepsis, bacteraemia, meningitis and necrotizing enterocolitis in newborn babies. Powdered infant formula (PIF) has been recognised as one of the infection vehicles. Previous work showed the great tolerance of *Cronobacter spp.* to low water activity and the survival of the microorganism in such adverse conditions.

The purpose of this study was to investigate the presence of Cronobacter spp. and total viable counts (TVC) in baby food for infants aged from 4-6 months.

Thirty different commercially available products, in the form of powder (28 samples) and liquid (two samples) were investigated twice over a period of 6 months. The presence of Cronobacter spp*.* was investigated in 25 g (or 25mL in the case of liquids). A pre-enrichment step of the inoculated sample was carried out at 37°C during 24 hours, followed by enrichment in E.E. broth during 24 hours at 37°C. Inoculation of the enrichment of every sample was performed on DFI agar. Typical colonies were further identified in ID32E galleries. TVC were calculated in 1 g of each baby food product, using Plate Count Agar (PCA). The first investigation took place in April and the second in September. In both occasions, of the 30 samples studied, 13,3% were found to be positive for Cronobacter spp*.*, while total viable counts varied from < 1 CFU/g to 8,33 $\times 10^2$ CFU/g (or mL).

The presence of *Cronobacter* spp. in this type of baby food may raise questions on the safety of these products bearing in mind the possibility of abuses once the food has been reconstituted. The observance of preparation recommendations is imperative to avoid the possibility of the pathogen growth.

P27 Cold Food Storage – The 70+ Need for Information

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Older people (70 years plus) represent a risk category concerning complications with food-borne infections. As part of the project CHANCE, taking place in Austria, Germany, Latvia, Romania, Sweden and United Kingdom (Lifelong Learning Programme of European Union 2007–2009) a pilot study was performed in the urban area Eriksberg, Uppsala municipality, Sweden. The aim was to understand this target group's need for information about cold food storage and food handling within the context of understanding and perception of health related messages.

Methods: Nine individuals 72–93 years were individually asked to purchase certain food items(soft cheese; vacuum-packed, smoked salmon; vacuum-packed, sliced ham) and store them in their own refrigerator using their normal food practices. Subsequently, qualitative interviews were performed. The temperature was then measured in these food items after storage for one night. Data were qualitatively processed.

Results: The study group were neither aware of the temperature in their refrigerator nor did they know about temperature differences on different shelves, although they did consider themselves to have a sound knowledge of how to handle and store foods. They expressed confidence in the grocery store and as such did not see the need for information. None of the informants were afraid of food-borne infections and yet a common habit was to taste raw minced meat, thus indicating a risk related optimism. The recorded temperatures of the various foodstuffs also suggested need for extra information.

Significance: This group seemed to overestimate their own skills concerning cold food storage. Education about food handling was taught in childhood but arguably a need for information about how to handle food today exists. The trust given to their grocery store might

contribute to a decrease in their own responsibility, which might be an obstacle concerning accessing further information.

P28 Comparison of Rapid Culture Based Methods for Detection of Salmonella

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Traditional culture methods for detecting *Salmonella* are time consuming, taking up to 4-5 days to complete. This study compared the detection of *Salmonella* from foods spiked with pure cultures of appropriate *Salmonella* serotypes using commercial methods from: AES Chemunex (SMS® with confirmation SALSA plate), Bio-Rad (RAPID'*Salmonella* 7 h and 24 h *Methods*) and Salmonella Precis™ from Oxoid Ltd. methods were compared to ISO 6579:2002 with method specificity evaluated using unspiked samples. Six commercial *Salmonella* media: *Brilliance*™ Salmonella (Oxoid), RAPID'Salmonella (Bio-Rad), ASAP (AES Chemunex), ChromID™Salmonella (bioMérieux), Harlequin ABC (Lab M) and CHROMagar™ Salmonella Plus (CHROMagar) were also compared for use as a second plate of choice alongside XLD in ISO 6579:2002.

Six spiked (4 at 1–10 CFU/100 µl and 2 at 10–50 CFU/100 µl) and 24 unspiked samples from each of five foods (minced beef, lettuce, chicken, unpasteurised cheese and milk powder) and 12 spiked (8 at 1–10 CFU/100 µl and 4 at 10-50 CFU/100 µl) and 24 unspiked samples of egg white were analysed by each method. Presumptive positive *Results* were confirmed by PCR (BAX® system PCR assay for Salmonella). The sensitivity of the commercial and ISO reference *Methods* varied from 47.6% (RAPID'*Salmonella* 7 h) to 61.9% (Salmonella Precis) and specificity from 92.2% (ISO 6579:2002) to 100 % (RAPID'*Salmonella* 7 h and 24 h and Salmonella Precis). In addition, Salmonella Precis was the only method able to detect the presence of *Salmonella* in egg white.

Of the chromogenic media, Brilliance™ Salmonella Agar (Oxoid), ASAP, ChromID Salmonella and RAPID' Salmonella performed better than mBGA as second choice media. Salmonella Precis is an accurate, reliable and user friendly rapid method that has been shown to accurately detect *Salmonella* from food samples with excellent sensitivity and specificity. Brilliance Salmonella Agar is a suitable alternative medium for ISO 6579:2002.

P29 Oxoid Salmonella Precis Method for *Salmonella* spp. Detection in Food, Feed and Environmental Samples: ISO 16140 Performance Validation

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Salmonellosis continues to be significant in global public health. Traditional culture methods for the detection of *Salmonella* are time-consuming, taking up to 4–5 days to complete. The Oxoid Salmonella Precis™ method is a new method making it possible to confirm the presence/absence of *Salmonella* spp. in less than 48 h. The method comprises incubating the sample in a selective enrichment broth (ONE Broth-Salmonella) for 16–20 h; followed by plating onto a single Brilliance™ Salmonella plate, incubated for 22–26 h. Suspect colonies can be rapidly confirmed by latex agglutination. The purpose of this study was to independently validate the performance of the new Salmonella Precis method against EN ISO 6579:2002 according to the specifications in EN ISO 16140:2003. 424 samples from 6 different food categories (meat products, milk products, seafood products, egg products, animal feed and environmental samples) were analysed for relative accuracy, sensitivity and specificity. Inclusivity and exclusivity of the Salmonella Precis method were conducted using pure cultures of 40 non-*Salmonella* strains and 53 *Salmonella* strains from 38 different serovars. In addition, a collaborative study was performed by 13 laboratories to test the repeatability and reproducibility of the new method. The values of relative accuracy, sensitivity, and specificity for the Salmonella Precis method were statistically equivalent to the reference method. Characteristic colonies were detected on Brilliance Salmonella from all 38

different *Salmonella* serovars. Exclusivity testing showed 38 out of the 40 non-target strains gave non-*Salmonella* characteristics on Brilliance Salmonella. The collaborative study showed a relative accuracy, sensitivity and specificity of 99.7%, 100% and 100% respectively, and demonstrated equivalence with the reference method. The Salmonella Precis method has been successfully, independently AFNOR validated according to EN ISO 16140 and is proven to be a fast, reliable, and convenient method for the detection of *Salmonella* in food and environmental samples.

P30 Evaluation of Chromogenic Salmonella Media for Use in ISO6579:2002

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XLD Agar is the recommended first choice culture medium for use in the detection of Salmonella in ISO6579:2002. The standard leaves open the choice of formulation for the second medium. This study evaluated six chromogenic media as potential second choice formulations for use in this ISO standard. 186 food samples, including 42 artificially contaminated samples, comprising raw minced beef, raw chicken meet, unpasteurised soft cheese, lettuce, liquid egg and milk powder, were analysed according to ISO6579. Artificially contaminated foods were spiked with *Salmonella* serotypes typically associated with each food, to produce samples with 1–10 CFU of *Salmonella*/25 g and samples with 10-50 CFU of *Salmonella*/25 g. Milk powder samples were artificially contaminated at < 5 CFU of *Salmonella*/25 g using reference material capsules of *S*. Typhimurium.

In parallel to inoculating XLD Agar and modified Brilliant Green Agar, 10 µl of each secondary enrichment broth was inoculated onto each chromogenic medium; Brilliance™ Salmonella Agar (Oxoid), ASAP (AES Chemunex), SM® ID2 (bioMérieux), Harlequin™ Salmonella ABC (Lab M), CHROMagar™ Salmonella Plus (CHROMagar) and RAPID' Salmonella (Bio-Rad). Presumptive positive colonies were confirmed by PCR. All media successfully isolated *Salmonella*, however, there were important differences in specificity and selectivity. Harlequin Salmonella ABC gave rise to false positive results. Brilliance Salmonella Agar, showed the lowest level of background non-target growth, followed by RAPID' Salmonella Medium and CHROMagar Salmonella Plus. Conversely, XLD, SM ID2 and Harlequin Salmonella ABC media had the highest growth of non-target flora, suggesting that these media would be less suited for samples with high levels of background flora.

P31 Subtyping of *Campylobacter* spp. Isolates from Poultry and Game Birds

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Introduction: Infections caused by *Campylobacter* spp. in man are quite common and are one of the most frequent causes of diseases originating from food in the Czech Republic. The most important source of Campylobacter spp. is poultry. Other farm birds reared for food processing may also be dangerous for human health.

Purpose: Genotyping of *Campylobacter* spp. from broiler chicken, guinea fowls and pheasants kept on farms was done in order to determine genome instability in flocks which could result in genotype variability and thus in problems in the interpretation of epidemiological studies. Genotype diversity was analysed by pulsed-field gel electrophoresis (*Sma*I-PFGE) and PCR/RFLP (*fla*-RFLP).

Methods: Sub-typing of *C. jejuni* and *C. coli* strains was performed by PFGE analysis of the whole chromosome digested with *Sma*I. For fla-RFLP sub-typing (*C. jejuni*) the part of the flagellinA gene was amplified and digested with AfaI, MboI, HaeIII.

Results: 343 *Campylobacter* spp. isolates from poultry (19 flocks), 252 isolates from pheasants and 19 isolates from guinea fowls were subjected to PFGE and *C. jejuni* strains

(319 poultry, 132 pheasant and 19 from guinea fowl) were classified by fla-RFLP. The findings of poultry isolates was that PFGE genotypes within some flock with the same PCR subtype were identical and considerably different between istinct PCR subtypes. In pheasant flock one PFGE and fla-RFLP subtype occured with the highest frequency (32% and 15%, respectively). In guniea fowl flock 12 isolates represent one clone with the same PFGE and fla-RFLP subtype.

Significance: Flocks in farms are mostly infected with a single clone, during subsequent colonization their genotypes altered. Some subtypes were found in all three bird species which may support the hypothesis of association between different subtypes of *Campylobacter* spp. and certain species or guilds of hosts.

P32 Incidence of Cronobacter (*Enterobacter sakazakii*) in Follow-on of Formulae and Infant Drinks

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Introduction: Cronobacter (*E. sakazakii*) is associated with the development of serious illness in infants on consumption of contaminated powdered infant formula. Infants less than 1 month old appear to be the group most at risk and a WHO risk analysis has been implemented to address this problem. The risk to infants from consumption of follow-on infant formula intended for infants over 6 months of age is now being considered.

Purpose: A survey of the incidence of Cronobacter in infant formulae and infant drinks commercially available in Europe and intended for consumption by infants older than 6 months was undertaken.

Methods: Samples (30 g) were diluted 1/10 in Buffered Peptone Water and incubated 18–24h at 37°C. From each of the overnight cultures 100 µl was added to Cronobacter Screening Broth and incubated overnight at 41.5°C. 10µl volumes were streaked aseptically onto Tryptone Soya agar and Druggan-Forsythe-Iversen agar. Presumptive positive Cronobacter isolates from DFI were confirmed by real-time PCR. All isolates were identified using ID 32E test strips.

Results: A total of 16 products were found to contain low levels of mesophilic facultative anaerobic/aerobic Gram-negative organisms (7 Enterobacter spp., 3 *Klebsiella* spp., 2 Cronobacter spp., 1 Pantoea sp., 1 Acinetobacter sp., and 4 unidentified spp.); two samples contained multiple organisms. The levels of contamination ranged from 0.0008 – 0.01 MPN/g. Cronobacter was found in two brands of cereal-based infant drinks, from separate manufacturers, at 0.003 MPN/g and 0.013 MPN/g; this represents 5/60 (8.3%) cereal-based infant drink samples.

Significance: Considering the diversity of food and drink that infants begin to consume from 4 months of age, and the apparent relatively low risk of infection in infants greater than 3 months, the health risk posed by the incidence of Cronobacter in follow-on infant drinks may be minimal.

P33 Pathatrix Re-Circulating Immuno-Magnetic Separation – a Unique and Versatile System for the Rapid Detection of Foodborne Pathogens in Leafy Produce, Herbs and Spices

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Fresh produce has been implicated in a significant number of foodborne disease outbreaks during the past decade. Herbs and Spices are used ubiquitously as seasoning in a variety of foods, some of which have been linked to cases of Salmonellosis and listeriosis.

This study describes the development of re-circulating immuno magnetic separation (RIMS) methods that allow: post growth sample pooling; analysis of large (1875 g) spice samples for *Salmonella* or *Listeria*; and simultaneous same-day isolation of *E. coli* O157:H7 and *Salmonella* from fresh produce.

Fresh produce samples (25 g) were diluted 1:9 with pre-warmed Brain Heart Infusion broth. Spice samples (375 g) were diluted 1:9 with Half-Fraser broth or Buffered Peptone Water supplemented as appropriate with 0.5% potassium sulfite with respect to the target pathogen and sample type. One sample in each set of 5 received an appropriate inoculum of either: *E. coli* O157 and *Salmonella* (fresh produce); *Listeria* or *Salmonella* (spices) to mimic low-level contamination (1–10 CFU/sample). After pre-enrichment samples were pooled to create single samples and analysed by RIMS. *E. coli* O157:H7 and Salmonella were isolated simultaneously. Detection of the target pathogens was achieved using both real time PCR and selective agar plating.

The isolation and detection of *E. coli* O157:H7 and Salmonella in pooled (125 g) fresh produce samples was comfortably achieved within a working day (< 8 h). The data also shows that the method can be successfully used to detect Listeria spp. and Salmonella in post growth pooled spice samples. Recovery of the target pathogens on selective agar plates confirmed the PCR *Results*.

The RIMS pooling methods offers high sample throughput, significant cost savings and enhanced pathogen detection, without loss of sensitivity as sample pooling is carried out post pre-enrichment but prior to PCR. Full traceability of the original sample is maintained until result*s* are confirmed.

P34 High Throughput *Salmonella* Testing Using a 10 Sample (Post Pre-enrichment) Pooling Strategy Linked to Re-circulating IMS and Real Time PCR

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Introduction: A Re-circulating Immunomagnetic Separation technique (RIMS) has previously been AOAC-RI validated for the rapid isolation of low level *Salmonella* contamination (1–10 CFU per sample), from pooled samples consisting of a combination of five different individual samples.

Purpose: The purpose of this study was to evaluate the feasibility of using the RIMS system for the analysis of pooled samples; consisting of a combination of ten individual post pre-enriched samples. A variety of food matrices were investigated.

Methods: Food matrices (up to 75g) (including chocolate, cocoa products, milk powders and dried soups) were weighed and diluted as appropriate to sample type. A single sample from each sample type was inoculated with low levels of Salmonella (1–10 CFU/sample) while all other samples remained uninoculated. All samples were incubated for 18 h at 37°C after which aliquots from 10 individual samples were combined (1 inoculated: 9 uninoculated) creating a 10-pooled sample. A 30 minute RIMS capture selectively concentrated the target bacteria; detection of Salmonella was achieved using real time PCR and selective agar plating techniques.

Results: The data showed that RIMS can successfully isolate *Salmonella* serovars from 10-pooled samples in a variety of different food matrices. Detection of the low level inoculums was achieved using either Real Time PCR or selective agar plates.

Significance: The RIMS 10-pooling method enables the detection of *Salmonella* from up to 10 × 75 g of sample using only one Pathatrix consumable and PCR reagent. This is an extremely cost effective and labour efficient method of pathogen testing allowing laboratories greater sample throughput together with significant cost savings.

P35 Detection of Low Level *Salmonella* Contamination in Cocoa Products, Chocolate and Peanut Butter Using Re-circulating Immuno-magnetic Separation and PCR

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Introduction: *Salmonella* cannot grow in peanut butter and chocolate due to low water activity (a_w) but can survive in these commodities for prolonged periods. Several outbreaks of Salmonella food poisoning have been linked to peanut butter and chocolate. This study describes the development of a re-circulating IMS (RIMS) real time PCR method that allows rapid isolation and detection of *Salmonella* contamination in large samplings of pooled pre-enriched peanut butter and cocoa products.

Methods: Cocoa products were weighed and diluted 1:9 with brilliant green skim milk broth, peanut butter (375 g) was diluted in BPW (1 L). One sample in each set of 5 received a *Salmonella* serovar inoculum (1–10 CFU per sample) to mimic low-level contamination. After pre-enrichment (18 h at 37°C) five aliquots from sets of 5 individual samples were pooled to create single 250 ml samples. Re-circulating immuno-magnetic separation linked to Real Time PCR was used to detect *Salmonella* in the RIMS eluates.

Results: Detection of initial low level *Salmonella* contamination in post growth pooled peanut butter and cocoa products was achieved using the RIMS RT-PCR method. Large pooled samples could be analysed as follows; peanut butter (1857 g), chocolate crumb (500 g), finished chocolate (250 g) and cocoa butter and cocoa liquor (125 g). Isolation of Salmonella colonies on agar plates confirmed the 40 minute real-time PCR results in all cases.

Discussion: The use of the RIMS method allows detection of *Salmonella* contamination in < 24 hours. The use of “post growth” pooling maintains the integrity and traceability of the original samples. The pooling method allows large samples to be tested by PCR and offers significant cost savings related to PCR testing for *Salmonella*. The method is suitable both to enhance routine *Salmonella* testing in food laboratories or pathogen surveillance and epidemiological investigation of outbreaks by regulatory agencies.

P36 The Effect of Lactic Acid Bacteria and Glucono-Delta-Lactone in the Behavior of *S. aureus* and *Salmonella* app*.* in Alheira

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Introduction: Alheira is a sausage made from cooked meat. It is difficult to classify in the classes used for meat products once the meat is very manipulated after boiling, and the heat eliminates its natural microflora, and no starter cultures are added.

Purpose: Evaluate the effect of Lactic Acid Bacteria (BAL) (*L. sake* or *L. plantarum*) as starter culture and glucono-delta-lactone (GDL) in the behavior o*f S. aureus* and *Salmonella* spp*.* in alheira.

Methods: Alheira batter was minced and sterilized (115°C, 15’). Batches were inoculated (6 log CFU/g) with LAB isolated from fermented sausages, or treated with 0.75% (v/w) of GDL and contaminated with ± 2 log ufc/g of pathogens (1 reference and 3 indigenous strains). One control (C) without LAB or GDL was used. Samples were challenged at 8°C and 20°C. At 2h, 24 h, 48 h and 5 days pathogens and LAB were enumerated by plating in Baird Parker agar (Biokar 055), Brilliant Green Agar (Biokar 071) and deMann Rogosa & Sharp agar (Biokar 089), respectively. Similar samples were prepared without pathogens, and analyzed by a selected-trained sensorial panel.

Results: At 8ºC the effect of LAB or GDL was not significative ($P > 0.05$) for any pathogen. At 20°C LAB and GDL avoided the overgrowth of these pathogens at the end of 5 days: *Salmonella* spp. (results expressed as log CFU/g) C - 4.94 ± 0.08, LP -1.60 ± 1,47, LS - 1.37

± 1.21, GDL - 2.07 ± 0.31; *S. aureus*: C - 4.36±0.30, PL - 2.24 ± 0.37, LS - 2.65 ± 0.86; GDL - 1.17 ± 0.0.82;. In both cases the differences between the control and the others were highly significative ($P < 0.001$). Nonetheless this inhibition of pathogens, the sensory impact of LAB was evident in several aroma and flavor attributes, and the acceptability was lower ($P < 0.001$) in the samples inoculated with starter cultures. GDL was not detected under the tested conditions

Significance: These findings allows the possibility of using LAB as protective cultures, or GDL, particularly important if temperature abuse during storage is previewed, even considering the sensorial limitations.

P37 The Effect of Fat Level on The Thermal Inactivation *S. aureus* In Alheira

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Introduction: The use of heat to inactivate pathogens is usually used as a CCP in cooked meat products. The knowledge of the parameters of thermal inactivation (D- and z- values) is an important strategy to increase the confidence of its use.

Purpose: Evaluate the effect of fat level in D- and z- values of *S. aureus* in alheira.

Methods: Alheira batter (before stuffing) was minced and fat level was adjusted to 10% and 15% with pork lard; samples were sterilized (115°C, 15'). Inactivation studies were performed in a thin layer of 3 g of sample in plastic bags, contaminated with a mixture of *S. aureus* ATCC 25923 and 3 indigenous strains. Heat sealed bags were immersed in a water bath at (55°C to 67,5°C; 2,5°C increases). At defined intervals, 3 bags were removed, cooled and the survivors enumerated by plating in MSA (Difco 0306).D-values were calculated from the straight portion of the survival counts vs their corresponding heating times (y = a+bx); D-value was obtained throughout D = |1/b|. The z-values were calculated using the same strategy, but plotting D-values vs heating temperature.

Results. D-values for *S. aureus* ranged from 13.0 min at 55°C to few seconds (0.03 min) at 67.5°C (10% of fat) and 13.5 min at 55ºC to 0.06 min at 67.5°C (15% of fat). Nonetheless the higher D-values observed in samples with 15% of fat, these differences only had statistical significance ($P < 0.001$) at the highest temperature challenged. The level of fat influenced significantly ($P < 0.05$) the z-values, 5.1ºC and 5.5°C, respectively for the lower and higher fat level.

Significance: These findings are important to industry in order to certificate that thermal treatment recommended after stuffing is correctly designed.

P38 Validation of a New TaqMan® Real-Time PCR Method for the Detection of *Salmonella enterica* in a Variety of Food Samples Using a Single 18-Hour Enrichment Protocol

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Salmonella is one of the largest causes of food-related illnesses worldwide and is associated with a wide variety of matrices. Numerous methods are available for the detection of Salmonella in foods, but often take up to four days to confirm a negative sample. As a result, a reliable rapid method which is sensitive, specific and robust is in demand. The TaqMan® *Salmonella enterica* detection kit from Applied Biosystems is a genetic-based detection kit using TaqMan® chemistry, offering results after just 18 hours enrichment in Buffered Peptone Water (BPW). The method was certified by AFAQ AFNOR validation according to the ISO 16140 standard, analysing naturally and artificially contaminated raw poultry, raw meat, raw fish, milk, cheese, frozen vegetables, raw egg and pet food amongst others comparing TaqMan® Salmonelle enterica detection method with the reference method ISO 6579.

Positive results were confirmed by performing the ISO 6579 standard. A total of 333 samples were analyzed, 38.1% of which were contaminated naturally. Statistical analysis of the data showed that the relative accuracy of the alternative method was 98.5%, the relative specificity 99.4% and the relative sensitivity 97.4%. During the specificity study all 58 Salmonella target strains gave positive results, and all 36 non-target strains gave negative results. Non-target strains are commonly found in food and show no cross reactivity with our detection method. Ten laboratories from 7 countries in Europe participated in the inter-laboratory study, giving comparable results which illustrates that the method is reproducible. The practicability of the method was found to be better than the reference method, requiring less than one day of training for technicians with no experience. Negative results were obtained in less time than the reference method and the software gives permanent traceability. The validation demonstrated that the TaqMan® *Salmonella enterica* detection kit from Applied Biosystems is not only rapid and easy to use, but also selective and specific, offering the food industry high accuracy and sensitivity.

P39 Highly Specific and Sensitive Detection of *Escherichia coli* O157:H7 Using Real-Time PCR

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Introduction: *Escherichia coli* O157:H7 contamination of food is a major public health concern because of the potential for widespread outbreaks and the severity of enterohemorrhagic illnesses it can cause. Accurate differentiation of *E. coli* O157:H7 from *E. coli* of other serotypes is critical for identifying O157:H7-contaminated foods.

Purpose: The aim of this study was to design a sensitive, easy-to-use, and highly specific real-time PCR assay to detect *E. coli* O157:H7 while avoiding detection of other *E. coli* serotypes, including the closely related strain O55:H7.

Methods: Using a whole genome comparison approach that involved the publicly available genomes of *E. coli* O157:H7, non-O157:H7 *E. coli*, and the genome sequence of an *E. coli* O55:H7 strain generated with Applied Biosystem's SOLID (TM) sequencing platform, we identified putative *E. coli* O157:H7-specific sequences that were used for real-time PCR assay designs. The specificity of the assay was determined using an inclusivity panel of *E. coli* O157:H7 strains and an exclusivity panel consisting of *E. coli* strains of various serotypes and closely related pathogens such as *Shigella*. The lyophilized assay, in conjunction with novel sample reparation procedures, was evaluated for sensitivity using various spiked food matrices, including ground beef.

Results: The assay was specific for *E. coli* O157:H7. Sensitivity was estimated at 10 copies using purified DNA and 1–3 CFU in 25 g of cultured ground beef within an 8 h sample-to-result workflow or after overnight enrichment for all food matrices tested.

Significance: Our *E. coli* O157:H7 real-time PCR assay is highly specific and is able to distinguish *E. coli* O157:H7 from other *E. coli* serotypes, including O55:H7 which has typically been difficult to exclude detection due to its close phylogenetic relationship with O157:H7. The assay is also simple to use in the lyophilized format and demonstrates reliable performance, which is essential in detecting *E. coli* O157:H7 contamination in food.

P40 Control of Pathogenic and Spoilage Microorganisms from Cheese Surface by Whey Protein Films Containing Malic acid, Nisin and Natamycin

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Introduction: Cheese is a ready-to-eat food easily contaminated on the surface by undesirable microorganisms, including foodborne pathogens. The use of antimicrobial films could be an option to resolve this problem.

Purpose: Study the inhibitory effect of nisin (50 IU/ml), natamycin (0.002 g/ml) and malic acid (3%) incorporated in whey protein isolate (WPI) plasticized films with pH 3 alone or in addition with EDTA (0.1%), Tween80 (0.15%) or sucrose esters (0.075%), against *Listeria monocytogenes*, *Pseudomonas aeruginosa, Penicillium* spp. and *Yarrowia lipolytica* isolated from Castelo Branco Cheese rind samples.

Methods: Was used an antimicrobial disc-diffusion assay. The film discs (6 mm of diameter) with antimicrobial agents were aseptically transferred to Tryptone Soy Yeast Extract Glucose Agar (TSYEGA) or Glucose Yeast Peptone Agar (GYPA) plates, previously seeded with a swab of a 18 h to 24 h culture, and after the incubation the width of the inhibition zone around each film disc was measured.

Results: The sucrose esters reduced significantly ($P < 0.05$) the inhibitory effect for *Yarrowia lipolytica* and *Penicillium* spp. EDTA and Tween 80 did not significantly influence the inhibitory activity of films against *Ps. aeruginosa* and *Y. lipolytica* in contrast with the improved effect against *L*. m*onocytogenes* and *Penicillium* spp. The effect of the agents tested on inhibition of *Ps. aeruginosa* strains shown that no one was able to cause an increase of inhibition.

Significance: The present work provides an edible film formulation with antimicrobial activity to protect cheese surface against a wide range of undesirable microorganisms.

P41 Study on the Quality of Market Dahi Compared to Laboratory Made Dahi

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`Dahi is yoghurt like fermented dairy products available in Bangladesh made by traditional ways from raw milk. The aim of the present study was to check and compare the present quality status of the market dahi in reference to laboratory made dahi. For this, dahi was prepared in the laboratory (A) and collected from Kishoregonj district (B), Gazipur district (C), Bogra district (D) and Dhaka district (E and F). Consumer acceptance quality was judged on 100 point by a judge panel and chemical and microbiological quality test were done by established methods. 'A' type dahi was superior followed by E, D, F, B and C considering the consumer acceptance quality parameter. From chemical test, it appears that, B possess the lowest pH (3.75 ± 0.05) and highest was in C (4.46 ± 0.15). Total solids content of F type dahi (318.40 ± 4.44 g/kg) was highest and fat (52.00 ± 2.00 g/kg), protein (44.33 ± 2.00 g/kg) and ash (10.76 ± 0.31 g/kg) content were also higher in F type dahi. E and A type dahi were also with considerable figure in chemical quality parameter. Dahi B was inferior in respect of microbiological quality- TVC ($\times 10^5$ CFU/ml) content was 94.00 ± 4.58, yeast (CFU/ml) content was 183.33 ± 15.28 and mold (CFU/ml) content was 53.33 ± 15.28. The TVC ($\times 10^5$ CFU/ml), yeast (CFU/ml) and mold (CFU/ml) content of A type dahi were 39.67 ± 4.51, 50.00 ± 10.00 and 20.00 ± 10.00, respectively. Considering all the three major quality aspects dahi A was superior than others followed by F, D, E, C and B.

P42 Quality Determination of Different Wholesale Cuts of Sheep Carcass

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The research was conducted to determine the nutritive value and microbial status of different wholesale cuts of sheep carcass. The meat sample was obtained from the age of 1 year, 2 years and 3 years of sheep. The wholesale cuts were shoulder, rack, loin and leg of each sheep carcass. The color, odor, pH, juiciness, proximate composition, total bacteria, coliform bacteria, yeast and mould were studied to assess the quality of meat. The value of juiciness

was decreased with the age. The ranges of juiciness of all samples were from 34.80 to 41.25%. Proximate composition, such as dry matter, ash, ether extract and calcium of all the samples increased gradually with the age, except crude protein. The phosphorus content of the sample did not show any specific trend due to age and cuts. The value of dry matter, ash, crude protein, ether extract, calcium and phosphorus content of all samples were ranged from 23.32–30.40%, 0.88–1.027%, 20.50–24.88%, 8.10–12.13%, 0.012-0.038% and 0.048–0.185% respectively. The range of pH value was 5.077–5.927. Statistical analysis indicated that the value of pH, juiciness, dry matter, crude protein, ether extract, calcium, phosphorus and yeast (log value) was highly significant ($P < 0.01$) due to age and cut. The value of ash content was highly significant ($P < 0.01$) due to age. Different meat cuts and ages did not show significant difference on total bacteria and coliform bacteria. The range of total bacteria and coliform bacteria was 4.21–4.79 and 2.38–3.64 log CFU/g of sample respectively. The range of yeast was 1.30–2.78 CFU/g of sample. Nutrient content of the sheep carcass varies due to different cuts as well as age.

P43 Effect of Freezing on the Quality of Cattle and Goat Meat

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The experiment was conducted to find out the effect of freezing on the quality of cattle and goat meat. For this purpose eight meat samples were taken as washed fresh cattle meat (M1), washed salted cattle meat (M2), washed fresh goat meat (M3), washed salted goat meat (M4), non washed fresh cattle meat (M5), non washed salted cattle meat (M6), non washed fresh goat meat (M7) and
non washed salted goat meat (M8). These samples were stored at -10°C temperature in the freezer for 120 days and analyzed on first day, 30th day, 60th day and 120th day. To assess the quality of frozen meat samples organoleptic ssessment and chemical assessment were studied. All the samples were acceptable organoleptically up to the end of the storage time. Crude protein, fat and ash of all the samples decreased but dry matter gradually increased with advance of storage time. Statistical analysis indicated that with the advance of storage time quality parameters of meat samples degraded significantly ($P < 0.01$). Quality parameters also varied among the samples. Cattle and goat meat can be refrigerated for 120 days with more or less change in the quality. Dry matter increased but ash, protein and fat decreased in both species (cattle and goat meat). Highly significant difference exists in species when considering crude protein and fat. Meat can be kept non-washed in the freezer because there was less loss of nutrients in non washed meat than that of washed meat.

P44 Microbiological Monitoring of Cheese Made from Milk of Goat Algarvian Breed

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A traditional cheese from Algarve region (Portugal) is produced with milk from algarvian breed goats and is coagulated by aqueous extracts from cardoon flowers (*Cynara cardunculus*).

These cheeses are usually produced under artisanal conditions, and during the processing procedures several contaminations may occur. The contaminant microbiota could influence the quality during storage. The main goal of this work was to monitor the cheese microbial quality along forty five days, both under frozen and maturation conditons.Total heterotrophic microbiota was enumerated in Plate Count Agar (30 ± 1°C, 48 h), by incorporation technique; Lactic Acid Bacteria (LAB) in Rogosa Agar (36 ± 1°C, 48 h), by incorporation technique, and moulds and yeasts on Cook Rose Bengal (25 ± 0.5°C, 5 days), by spread technique. Total and faecal coliforms had been cultivated in anaerobic conditions in Violet Red Bile Agar (30 ± 1 °C , 48 h and 44.5 ± 0.5°C, 48 h, respectively), by incorporation technique. All assays were done in triplicate. The organoleptic characteristics of goat cheeses changes along the ripening process due to microbial activity among other factors. After forty five days, samples under frozen conditions showed lower concentrations of total microorganisms when compared to the ripened ones (7.5 and 9.0 log CFU/g). Nevertheless, coliforms, which may

cause safety problems, were lower for samples submitted to ripening (3.0 and 2.0 log CFU/g). These results should be due to the competition with LAB, which increased during the first period of the ripening process (from 6.5 to 8.5 log CFU/g), becoming the dominant species. In samples under ripening, coliforms showed a decrease of one to two orders of magnitude and a tendency to decrease. However, the ripening period could not be extended due to the excessively hardness acquired by this type of cheese.These results suggested that the quality of the milk should be controlled before processing.

P45 Conventional PCR Analysis for Detection of the Major Hazelnut Allergen COR A 1 on Food Products

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Introduction: Undeclared allergenic foods as contaminants in food products pose a major risk for sensitized persons. The detection of the main hazelnut (Corylus avellana) allergen Cor a 1 represents the tool of regular food labelling authentication.

Purpose: The *Purpose* of this study was to develop a conventional PCR method for the detection of major hazelnut allergen Cor a 1 in food matrixes. Declared and nondeclared food products were investigated for the presence of hazelnut residues.

Methods: A set of primers for the amplification of the partial genomic sequence of the Cor a 1 gene (397 bp) was designed and applied to the investigation of 68 commercial food samples with or without hazelnut declaration. The specificity of primer pairs was tested on a broad range of food ingredients. Universal plant primers were used for the plant matrixes confirmation in food (123 bp).

Results: Thirty samples of food products with hazelnut and thirty eight samples without hazelnut labelling were analyzed using developed PCR method. In non-declared samples no hazelnuts were detected. The detection limit of the PCR method was assessed 0,01%.

Significance: The developed PCR method is specific enough to detect hazelnut allergen Cor a 1 in food products and could therefore prevent the occurence of allergic reaction in context of early hazelnut residues detection.

P46 Detection of Pigskin Emulsions (Alpha I Collagen) in Meat Products by ELISA

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Introduction: The skin emulsions and gels are often used as the texture modifying agents and water binders in reduced fat meat products. The high percentage gel addition decreases acceptable sensory attributes and nutritious quality of the product and moreover can be economically very profitable.

Purpose: The purpose of this study was to develop the indirect competitive ELISA method to detect the addition of pork skin emulsions in meat products to reveal the commercial fraud and adulteration.

Methods: The indirect competitive ELISA for the detection of pork skin emulsions (alpha I collagen as a marker) in meat products has been developed. The polyclonal antibodies using New Zealand White rabbits have been prepared. The detection limit of the method was 1.5% of the addition of pork skin emulsion. The specificity testing did not show any cross reactivity with common used bovine and chicken meat (muscles) extracts.

Results: The first thirty two model samples and twenty two retail meat products with declared composition from the food chains were tested by this method. In two cases meat products contained pork skin emulsions in spite of the Czech legislation where it is forbidden.

Significance: This ELISA method is enough sensitive and specific to detect the addition of pigskin emulsion to the commercial meat products. So the monitoring of possible pork skin emulsion adulteration of selected retail meat products is needed. Financially supported by grants MZe 00027 16201 and 1B53004).

P47 Polycyclic Aromatic Hydrocarbons Profile in a Portuguese Traditional Meat Product

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Smoking is still widely used in many Portuguese traditional dry-fermented meat products, not only to achieve particular sensorial characteristics but also for preserving purposes. The smoke generated from wood burning under low oxygen environment contains considerable amounts of polycyclic aromatic hydrocarbons (PAHs), some of them characterized for carcinogenic and mutagenic properties. The present study was undertaken to investigate the prevalence of 16 PAHs in 24 samples of a Portuguese traditional meat product manufactured in a traditional smoking house under discontinuous smoking operation, during about 18 days. PAHs were analysed from the superficial and inner portions and the whole product by HPLC according to Simko *et al.* (1993). The PAHs content was expressed as $mg.kg^{-1}$ DM of sample. From 16 PAH analyzed naphthalene, acenaphthylene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene were not detected in any sample analyzed. Among those PAHs existing in products immediately after processing, the light compounds (up to 4 aromatic rings) prevailed, with phenanthrene being the most concentrated (532.6 $\mu g.kg^{-1}$) followed by fluorene, acenaphthene, fluoranthene, and anthracene (367.1 $\mu g.kg^{-1}$; 206.3 $\mu g.kg^{-1}$; 143.4 $\mu g.kg^{-1}$ and 131.9 $\mu g.kg^{-1}$, respectively). In relation to the heavy compounds only benzo[b]fluorantene, benzo[k]fluorantene and benzo[a]pyrene (BaP) were present in products, reaching the BaP a level (1.22 $\mu g.kg^{-1}$) always below the concentration limit established by the legislation (Commission Regulation (EC) Nº 1881/2006 of 19 December 2006). In general, PAH contents increased with the smoking process duration, up to day 14 (2768.1 $\mu g.kg^{-1}$). After this processing stage, the total PAH content decreased up to the end of the drying phase (1477.9 $\mu g.kg^{-1}$). During smoking process, PAHs sediment on the surface of product, reaching higher concentrations in the outer layer (3014.8 $\mu g.kg^{-1}$).

P48 An Innovative E-Learning Training Programme in Food Safety — From Farm to Fork Food Safety Specialists Training Programme (F4ST) and the Evaluation of First Session Results

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EU authorities address the “farm-to-fork” approach by giving priority to consumer demand and the right for high quality and safe food. To achieve this, an efficient and effective food control system along the food chain should be established and implemented.

F4ST is an innovative 90 hours e-learning program on food safety which is going to be piloted across all European countries in 2 sessions, April and September 2008. F4ST aims to fill the gaps for each profession working as food safety specialists with a common “from farm to fork” approach; to cover all food safety
measures, EU regulations and quality management principles. In addition, the project also aims to satisfy the needs of EU countries related to identified new qualification needs; to enhance the skills of food safety specialists from each profession; easily be accessible using e-learning facilities.

One candidate and 7 European member countries participated in the project to share their knowledge and experiences on food safety issues and e-learning technology. To reward their endeavor the EU decided to fund the project through the European Program “Leonardo da Vinci” (TR/06/B/F/PP/178050).

The training program has been developed by a pan European committee of top professionals as to bring about a state of the art curriculum which consists of 9 modules; *Introduction* to Food Safety, Food Microbiology, Food Chemistry, Physical and Chemical Hazards, Primary Production-Agriculture, Primary Production-Animal, Food Safety Technologies, Inspection & Audit and HACCP. 1.700 Food Safety Specialists have being trained by F4ST free of charge. 842 participants were selected for the first session, and 858 participants have being trained in the second session of the F4ST. The effects of the program were measured by satisfaction surveys. 1st session ended in September 2008, and its
Results will be presented in IAFP's Fourth European Symposium on Food Safety.

P49 Portuguese Ready-to-Eat Salads: Are They Safe?

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Minimally processed salads consumption has increased during the last few years. Probably the main reason for that is the major convenience although nutritional and taste reasons can contribute as well. Ready-to-Eat products have been commonly associated to outbreaks caused by *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and more recently *Aeromonas hydrophyla*. A high lethality has been associated to risk groups, such as children, pregnant women, elderly and people with underling illness. Consequently all possible knowledge about microorganisms present in this kind of food is of extreme importance. The purpose of this work was to estimate the microbiological flora, both indicators and pathogens, of Minimal Processed Salads, and to compare different brands available in Portuguese market.

During this study, both presence and concentration of important pathogens and indicator microorganisms in Ready-to-Eat salads were determined by simple microbiological analysis, according to ISO standards, and ELFA (Enzyme-linked Fluorescent Assay) method.

The indicator microorganisms of 134 samples analysed showed a high level of hygiene contamination. *L. monocytogenes*, *E. coli* VTEC and *A. hydrophila* were found in 15 samples. In spite of the low occurrence of pathogenic organisms, some samples could represent a potential hazard for human health. Despite the fact that there is no legislation in EU related to these foods, measures to increase hygiene standards in the production process are important in order to obtain safer salads. Although the pathogenic values in this study were very low, an increase in numbers can occur, if these salads were temperature abused during improper conditions of transport (high temperatures) or afterwards, if not stored at 4°C.

P50 The Enterotoxin Gene Cluster of *Staphylococcus aureus* – A Possible Origin of Further Variant Enterotoxins in Staphylococcal Food Poisoning

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Introduction: Staphylococcal food poisoning (SFP) is caused by secreted proteins known as staphylococcal enterotoxins (SEs). To date, 21 SE's and staphylococcal enterotoxin-like proteins (SEls, not tested for emesis in monkeys) have been described. Either five or six of the genes coding for these proteins are arranged in an operon termed the enterotoxin gene cluster (*egc*) containing the *selo, selm, sei, seln* and *seg* genes and either two pseudogenes *ψent1* and *ψent2* or the *selu* gene between the *sei* and *seln* genes.

Purpose: Variants of these genes have been described, displaying approximately 95% sequence identity with the 'original' genes. An investigation was conducted on variations within *egc* loci from a range of isolates using the genomic data of fully sequenced *egc*$^+$ strains and a selection of isolates from man and animals.

Results: As standard practice, reports in the literature have divided *egc* loci into *egc1* (those harbouring pseudogenes *ψent1* and *ψent2*), *egc2* (those harbouring the *selu* gene) and *egc3* (those harbouring so-called variants of all the genes, including the $selu_v$ gene). Further variations of the locus have been identified in strain RF122, a bovine strain, and in strain FRI137, a human thigh abscess isolate, that indicate a need for further extension of this *egc* locus classification. Four alleles of the *sei* gene have been characterised – sei_1 in strain FRI445 (the original *sei*), sei_2 in strain FRI137, sei_3 in strain 382F (the original sei_v), and sei_{bovine} in strain RF122.

Significance: These data indicate the potential for further allelic variation of genes of the *egc* loci of *S. aureus* which may confer different biological properties on these enterotoxins including emetic potency as effectors of staphylococcal food poisoning and TCR Vβ specificity repertoire as superantigens. More extensive sequencing of *egc* loci of strains of different animal and clonal origins is merited.

P51 Detection of *Salmonella* spp. in Feed and Food Samples by Cultivation and Real-Time PCR Techniques

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Salmonella spp. represent a very important pathogen with 55.245 reported infections of humans in 2005 in Germany (RKI, Berlin). Consequently, the EC regulation 2073/2005 demands for testing the absence of *Salmonella* spp. in a 25 g sample of e.g. Ready-to-Eat products. In addition, feed is tested to prevent the entry of *Salmonella* spp. into the food chain.

The detection of *Salmonella* spp. or their absence, respectively, is still done by cultivation techniques by most of the service laboratories. These Methods are less expensive in comparison to PCR methods. However, the cultivation approach is much more time consuming (3 up to 5 days) in comparison to PCR based detection systems (approx. 1 day).

The aim of this study was to compare the sensitivity of both detection methods. Identical samples were analysed and it was found that PCR based analytics is significantly more sensitive than standard cultivation techniques. The possibility of obtaining false positive results e.g. due to remaining DNA of dead cells, has been excluded for the investigation of egg products.

Our results suggest a broader application of PCR based detection systems in order to improve food safety.

P52 Aflatoxin in Milk: Seasonal Crisis, Sampling Schemes and Risk Assessment

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Introduction: Risk assessment for aflatoxin in milk should be based on life-long consumption data; it should take into account sampling uncertainty and effects of HACCP corrective actions and veterinary inspections, as well as the sensitivity of population (i.e. prevalence of Hepatitis B infection). Aflatoxin crises can arise in consequence of unfavorable conditions or circumstances, endangering the supply of safe feed to lactating cows.

*Purpose***:** The risk assessment was aiming at evaluating the consequences of aflatoxin crisis that affected Italy in 2003-2004.

*Methods***:** 8874 bulk milk samples milk samples were taken from tank trucks at eight dairy plants located in five regions (north, centre and south of Italy) in the period 2001-04. The monitoring plan design was aiming at analyzing the milk produced by each group of neighbor farms every two weeks and was not biased by specific surveillance on farms with restrictions.

Aflatoxin M was analyzed with a commercial ELISA test, but HPLC was also used to confirm results above 100 $ng \cdot kg^{-1}$. A model to simulate mixing at the dairies was used to estimate the aflatoxin concentration in pasteurized milk.

Results: The prevalence of samples showing aflatoxin concentration above an action level fixed at 40 $ng \cdot kg^{-1}$ increased from 15% to 19% in consequence of a widespread crisis that affected Italy in the fall 2003. However, the average aflatoxin concentration in pasteurized milk was always below 50 $ng \cdot kg^{-1}$ (95th percentile < 90 $ng \cdot kg^{-1}$). Increment of primary hepatocarcinoma cases was irrelevant (less than 0.005 estimated cases per year/ 100 million people)

Significance: this study shows that seasonal crisis can be controlled by contingency surveillance plans if monitoring is carried out regularly. Their consequences are actually mitigated by vaccination against Hepatitis B virus in children and adolescents that reduced the number of Hepatitis B carriers among milk consumers.

P53 Development of Sakacin A-Containing Pullulan Films for Active Antimicrobial Packaging

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Introduction: Consumers demand safe and high quality food products and request packaging materials to be sustainable, recyclable or degradable. The use of bio-polymer films as antimicrobial delivery systems to reduce undesirable bacteria in foodstuffs may satisfy consumers' demands, while enhancing food safety and quality.

Purpose: The aim of this study was to optimize mechanical and optical properties of pullulan films containing sakacin A, a natural antimicrobial peptide, and evaluate its effectiveness to control *Listeria monocytogenes* growth in Ready-to-Eat (RTE) foods.

Methods: A Central Composite Rotatable Design was used to find the optimal components combination used in the development of pullulan films. The effect of pullulan (P), glycerin (Gly), xanthan gum (Xa) and locust bean (Lb) concentrations on thickness, transparency, tensile strength, elastic modulus, elongation and puncture force were investigated. Responses were fitted by
Multiple Linear Regression, employing SAS Software. Sakacin A, a bacteriocin produced by Lactobacillus sakei, was added to the optimal formulation. Turkey deli meat slices, experimentally inoculated with L. monocytogenes, were treated with the resulting sakacin A-containing pullulan films, vacuum packaged and remaining bacterial populations was evaluated for up to 3 weeks at 4°C.

Results: Pullulan and glycerin significantly influenced mechanical and optical films properties. Using predictive models, contour plots and the characteristics of commercial LDPE films as criteria (thickness < 0.8 mm, transparency > 10 T600, tensile strength > 40 Mpa, elastic modulus > 200 Mpa, 100% < elongation < 640% and puncture force > 13 N), the following optimal formulation was found: 10% w/v P, 1% w/v Gly, 0.1% w/v Xa and 0.1% w/v Lb. The active films showed significant activity against *L. monocytogenes*, reducing the population of one strain by 3 log10 CFU/g when applied to experimentally inoculated turkey slices.

Significance: *Results* demonstrate that sakacin A-containing pullulan films are an active packaging system that can be used to inhibit *L. monocytogenes* in RTE foods.

P54 Salt Consumption Risk Evaluation in a Public School for Food Production

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Introduction: Food safety issues can be assessed from the nutritional point of view. Excess salt intake is a major public health problem worldwide. There are several studies that relate

high salt intake with cardiovascular diseases, including hypertension, which is the major risk factor for stroke. Several studies have also shown that a reduction in salt consumption would reduce the burden of hypertension and related diseases. Portugal presents high prevalence of hypertension as well as high salt intake among the population, and cardiovascular disease is the major cause of death.

Purpose: The *Purpose* of this study was to analyse the amount of salt in meals served at a public school of restaurant, food production, tourism and hotels studies. It was also our purpose to describe the students' salt perception of the consumed meals.

Methods: Salt was analysed through chemical analyses, performed by a certified laboratory and salt perception was evaluated using a questionnaire. We analyse 105 different meal components, including bread, soup and main meal.

Results: The average salt amount of the whole meal was 5,85 g, which is very close to the daily recommended amount. Bread and soup have an average of 0,85g and 2 g of salt. 78% of the students consider the salt flavour as normal.

Conclusion: Considering public health concerns, and that the school where the study was performed is related to food production and restaurant studies, this represents a major environment to take action, not only because of the students health, but mostly because these students will become future chefs in food production for all society, and will, most probably take their taste perceptions and salt use to their daily professional practice, hence influencing population salt consumption.

P55 Food Safety in Catering for Events: Critical Factors in Portugal

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Introduction: The Tourism for Events should be considered as an emergent market and a current opportunity for Portugal to emerge as an international destiny, to host great international events. The Catering of Events represents one of the pillars of this kind of Tourism and is performed by restaurants, hotels or caterings companies whose activity can be regarded as commercial and traditional, associated to mass catering. Some critical factors for Food Safety (FS), that represent potential hazards to the consumers' health, have been associated to Catering of Events.

Purpose: The general aim of this work consisted in studying the frequency of those critical factors in Catering of Events establishments.

Methods: Fifty three establishments (7 Restaurants, 28 Event Catering establishments (EC), 6 four star Hotels and 12 five star Hotels), with Event catering activities, were selected. Questionnaires were applied to those in charge of the events and activities and Food Safety procedures were assessed.

Results: The following conclusions were drawn: 58% of cold meals and 79% of desserts and pastry, served in buffets, had no cooling or protection from crossed contaminations of the surrounding environment; 70% of the establishments' displayed warm food buffets with temperature below 60 degrees; only 35,8% of the establishments controlled food temperature (either warm or cold); only 35,8% of the establishments controlled food time exposure (either warm or cold); 72% of the establishments displayed their warm buffets for more than two hours; 43,4% of the establishments didn't use pasteurized eggs in their food confection; 59% of the establishments didn't use pasteurized eggs in their dessert confection; 45% of the establishments allowed their clients to take their meals leftovers home; 90% of the establishments employed seasonal workers with no medical control; 80% of the off- premise Catering weren't licensed as Catering activities and 67,9% of the establishments had no Food Safety systems.

Significance: Every Food Safety critical factor, described and identified in the study can cause Food Born Diseases. The identification of these critical factors is essential for the implementation of adequate prevention measures to avoid diseases caused by food in Event Catering. The control and eradication of identified critical factors and its inclusion in the elaboration of Food Safety Management Systems (based on HACCP principles) represent better quality and safety in the food production and distribution on Event Catering.

P56 Monitoring of *Listeria monocytogenes* in Slicer Rooms – Processing Injected and Non-injected Salmon Fillets

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Introduction: Cold smoked salmon is categorized as a ready-to-eat-food that has to be stored under cooled conditions. Unfortunately, it is well-known that the presence of *L. monocytogenes* represents one of the most important hazards of this product. Although regular processing does not inactivate *Listeria*, considerable emphasis has to be taken on hygiene aspects of the production as well as on maintaining chilled storage.

Purpose: The *Purpose* of this study was to investigate local niches for *L. monocytogenes* in two different slicing divisions by applying environmental sampling with sponges and swabs, in combination with an Enzyme Linked Immuno Fluorescent Assay (VIDAS®, bioMérieux).

Methods: The target points were determined by zoning into food contact surfaces: direct contact, indirect contact and no contact to food. The collected samples from presumptive areas during production have been analysed according to the VIDAS® LDUO protocol-screening for both *Listeria* genus and presence or absence of *L. monocytogenes*-after a two-step enrichment. Typical *Listeria* colonies were isolated in all enrichment steps to ensure that *L. monocytogenes* is not masked by other species, and positive VIDAS® *Results* were confirmed according to the protocol of the assay.

Results: In one slicer room where no preserved salmon is processed, 6 confirmed *L. monocytogenes* and 5 confirmed *L.* spp. were detected (out of 50 samples) and 2 of these were directly associated with food. Injected salmon as produced in the second slicer room are characterised by a higher hygiene level, and the prevalence was 4,4% *L. monocytogenes* among 46 samples tested (no confirmation of other *L.* spp). However, the risk of recontamination does not correlate with the type of product (injected or non-injected).

Conclusion: Screening for *L. monocytogenes* should be tailored and focused on areas of production (e.g., slicing) where the risk of recontamination is high. VIDAS® LDUO can be applied as an internal alert system to get just-in-time results and to determine problematic niches in such neuralgic zones.