Detection of Salmonella in Meat: Comparative and Interlaboratory Validation of a Noncomplex and Cost-Effective Pre-PCR Protocol

Cost-effective and rapid monitoring of Salmonella in the meat production chain can contribute to food safety. The objective of this study was to validate an easy-to-use pre-PCR sample preparation method based on a simple boiling protocol for screening of Salmonella in meat and carcass swab samples using a real-time PCR method. The protocol included incubation in buffered peptone water, centrifugation of an aliquot, and a boiling procedure. The validation study included comparative and interlaboratory trials recommended by the Nordic Organization for Validation of Alternative Microbiological Methods (NordVal). The comparative trial was performed against a reference method (NMKL 187, 2007) and a PCR method previously approved by NordVal with a semiautomated magnetic bead-based DNA extraction step using 122 artificially contaminated samples. The LOD was found to be 3.0, 3.2, and 3.4 CFU/sample for the boiling, magnetic bead-based, and NMKL 187 methods, respectively. When comparing the boiling method with the magnetic beads, the relative accuracy (AC), relative sensitivity (SE), and relative specificity (SP) were 98, 102, and 98%, respectively (Cohen's kappa index 0.95). When comparing results obtained by the boiling to the culture-based method, the AC, SE, and SP were found to be 98, 102, and 98%, respectively (kappa index 0.93). In the interlaboratory trial including valid results from 11 laboratories, apart from two false-positive samples by the boiling method combined with PCR, no deviating results were obtained (SP, SE, and AC were 100, 95, and 97%, respectively). This test is under implementation by the Danish meat industry, and can be useful for screening of large number of samples in the meat production, especially for fast release of minced meat with a short shelf life.

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Lipid determination by the Smedes method was tested in an interlaboratory trial performed by 9 laboratories from 7 countries belonging to the West European Fish Technologists Association Analytical Methods Working Group. 5 samples of fish and fishery products with different lipid contents, including 2 blind duplicates, were distributed among the participants. All laboratories applied a slightly modified Smedes method, which included extraction of lipids by cyclohexane and isopropanol, transfer of lipids to the cyclohexane phase by addition of water, phase separation by centrifugation and gravimetric lipid determination. The relative s.d. for reproducibility (RSDR) was between 4.11 and 6.31% for samples with moderate (7%) and high (14%) lipid contents, depending on the sample. Larger SDs among the laboratories were obtained for a cod sample with low lipid content of 0.5%. The method is judged to be suitable as a routine method for lipid determination in fish and fishery products.
Determination of Ergot Alkaloids: Purity and Stability Assessment of Standards and Optimization of Extraction Conditions for Cereal Samples

Results obtained from a purity study on standards of the 6 major ergot alkaloids ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine and their corresponding epimers are discussed. The 6 ergot alkaloids studied have been defined by the European Food Safety Authority as those that are the most common and physiologically active. The purity of the standards was investigated by means of liquid chromatography with diode array detection, electrospray ionization, and time-of-flight mass spectrometry (LC-DAD-ESI-TOF-MS). All of the standards assessed showed purity levels considerably above 98% apart from ergocristinine (94%), ergosine (96%), and ergosinine (95%). Also discussed is the optimization of extraction conditions presented in a recently published method for the quantitation of ergot alkaloids in food samples using solid-phase extraction with primary secondary amine (PSA) before LC/MS/MS. Based on the results obtained from these optimization studies, a mixture of acetonitrile with ammonium carbonate buffer was used as extraction solvent, as recoveries for all analyzed ergot alkaloids were significantly higher than those with the other solvents. Different sample-solvent ratios and extraction times showed just minor influences in extraction efficacy. Finally, the stability of the ergot alkaloids in both raw cereals and cereal-based processed food extracts was studied. According to these studies, extracts should be prepared and analyzed the same day or stored below ambient temperatures. Barley and rye extracts, which were stored at 4 and 15 degrees C after PSA cleanup, proved to be stable overnight. However, storage over a period of 14 days at 4 degrees C resulted in significant epimerization, which was most pronounced in rye and particularly for ergocornine, ergocryptine, and ergocristine.

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The need for standardization of real-time PCR methods
Development, validation, and standardization of polymerase chain reaction-based detection of E. coli O157

A diagnostic polymerase chain reaction assay was developed for the detection of E. coli O157 as the first part of a multicenter validation and standardization project. The assay is based on amplification of sequences of the rfbE O157 gene and includes an internal amplification control. The selectivity of the assay was evaluated against 155 strains, including 32 E. coli O157, 38 E. coli non-O157, and 85 non-E. coli. It was shown to be highly inclusive (100%) and exclusive (100%). The assay has a 100% detection probability of approximately 2 x 10(3) cells per reaction.

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Multicenter validation of PCR-based method for detection of Salmonella in chicken and pig samples

As part of a standardization project, an interlaboratory trial including 15 laboratories from 13 European countries was conducted to evaluate the performance of a nonproprietary polymerase chain reaction (PCR)-based method for the detection of Salmonella on artificially contaminated chicken rinse and pig swab samples. The 3 levels were 1-10, 10-100, and 100-1000 colony-forming units (CFU)/100 mL. Sample preparations, including inoculation and pre-enrichment in buffered peptone water (BPW), were performed centrally in a German laboratory; the pre-PCR sample preparation (by a resin-based method) and PCR assay (gel electrophoresis detection) were performed by the receiving laboratories. Aliquots of BPW enrichment cultures were sent to the participants, who analyzed them using a thermal lysis procedure followed by a validated Salmonella-specific PCR assay. The results were reported as negative or positive. Outlier results caused, for example, by gross departures from the experimental protocol, were omitted from the analysis. For both the chicken rinse and the pig swab samples, the diagnostic sensitivity was 100%, with 100% accordance (repeatability) and concordance (reproducibility). The diagnostic specificity was 80.1% (with 85.7% accordance and 67.5% concordance) for chicken rinse, and 91.7% (with 100% accordance and 83.3% concordance) for pig swab. Thus, the interlaboratory variation due to personnel, reagents, thermal cyclers, etc., did not affect the performance of the method, which will be proposed as part of a developing international PCR standard.

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Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies

This study investigated the speciation and bioavailability of selenium in yeast-based intervention agents from multiple manufacturers from several time points. Sources of selenized yeast included Nutrition 21 (San Diego, CA), which supplied the Nutritional Prevention of Cancer (NPC) Trial from 1981-1996; Cypress Systems (Fresno, CA; 1997-1999); and Pharma Nord (Vejle, Denmark; 1999-2000), which supplied the Prevention of Cancer by Intervention by Selenium (PRECISE) Trial pilot studies. The low-molecular-selenium species were liberated from the samples by proteolytic hydrolysis followed by separation by ion exchange liquid chromatography and detection by inductively coupled plasma-mass spectrometry. The results for the NPC tablets showed that selenomethionine, together with 3 unidentified selenium compounds, were predominant in the sample hydrolysates. The relative amounts of the 4 selenium species varied (p <0.05) among several of the 7 tablet batches used during the course of the NPC Trial. In comparison, 5 batches of more recently produced selenized yeasts, which were used as a source of selenium in the PRECISE and other trials, contained less of the unknown compounds and more selenomethionine at 54-60% of the total selenium in the yeasts. One batch of yeast, however (from 1985), which originated from the same producer as the yeast used in the NPC tablets, contained only 27% of selenium in the sample as selenomethionine. Human subjects receiving 200 mug selenium/day in the UK PRECISE Pilot Trial showed a higher concentration (p <0.01) and higher increase from baseline -in plasma selenium than did the same dosage used in the NPC Trial. Differences in intake, speciation, or bioavailability of selenium from the yeast-based supplements in the population groups studied may explain this. Furthermore, the selenium concentration in whole blood from the Danish PRECISE Pilot Trial was higher (p <0.001) than that obtained with synthetic L-selenomethionine in a comparable group of Danes, both groups having been treated with 300 mug selenium/day.

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Toward an international standard for PCR-based detection of foodborne Escherichia coli O157: Validation of the PCR-based method in a multicenter interlaboratory trial

The performance of a polymerase chain reaction (PCR) method for detection of Escherichia coli O157, previously validated on DNA extracted from pure cultures, was evaluated on spiked cattle swabs through an interlaboratory trial, including 12 participating laboratories from 11 European countries. Twelve cattle swab samples, spiked at 4 levels (0, 1-10, 10-100, and 100-1000 colony-forming units, in triplicate) with E. coli O157 were prepared centrally in the originating laboratory; the receiving laboratories performed pre-PCR treatment followed by PCR. The results were reported as positive when the correct amplicons were present after gel electrophoresis. The statistical analysis, performed on 10 sets of reported results, determined the diagnostic sensitivity to be 92.2%. The diagnostic specificity was 100%. The accordance (repeatability) was 90.0%, calculated from all positive inoculation levels. The concordance (reproducibility) was 85.0%, calculated from all positive inoculation levels. The concordance odds ratio (degree of interlaboratory variation calculated from all positive inoculation levels) was 1.58, indicating the robustness of the PCR method. Thus, the interlaboratory variation due to personnel, reagents, minor temperature or pH fluctuations and, not least, thermal cyclers, did not affect the performance of the method, which is currently being considered as part of an international PCR standard.