FINAL PROGRAMME
&
ABSTRACTS OF LECTURES AND POSTERS

Rapid Methods Europe 2007

international conference
for biological and chemical contaminants
in food and feed

29-30 January 2007
Noordwijkerhout, the Netherlands

Rapid Methods Europe 2007

Organising Committee

Dr. Aart van Amerongen
Wageningen University and Reserach Centre, Agrotechnology & Food Sciences Group, the Netherlands

Dr. Daniel Barug
Ranks Meel, the Netherlands

Helena B. Bastiaanse
Bastiaanse Communication, the Netherlands

Margreet Lauwaars
Consultant, the Netherlands

Dr. Bert Popping
Eurofins Scientific Group, UK

Prof.dr. Sarah De Saeger
Ghent University, Department of Bio-analysis, Belgium

WELCOME AT RAPID METHODS EUROPE 2007!

Dear participant,

Rapid Methods Europe is a series of events dedicated to rapid methods and instrumentation of interest to the food and feed industry.

Rapid Methods Europe 2007 presents a firm base and structural framework for considering rapid methods for detecting biological and chemical contaminants in food and feed, featuring:

- invited lectures and contributed papers
- poster presentations
- interactive workshops & demonstrations
- spotlight presentations
- instrument & manufacturers exhibition
- matchmaking event

As a comprehensive overview **Rapid Methods Europe 2007** offers an excellent way to network and to share ideas, providing a reference source for anyone interested in the rapid determination of biological and chemical contaminants in food and feed.

You are cordially invited to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

On behalf of the Organising Committee,

Dr. Daniel Barug

Rapid Methods Europe

Secretariat

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- abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily program;
- the posters are grouped according to theme and then in an alphabetical order according to the first author.

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Monday
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CONFERENCE PROGRAMME

Monday 29 January 2007

08.45 **Opening of Rapid Methods Europe 2007**

Plenary meeting: What can be learned from others?

Chair: Dr. Aart van Amerongen, Wageningen University, Agrotechnology & Food Sciences Group, the Netherlands

09.00 *International perspectives for rapid methods in European research: opportunities in Eureka and the 7th Framework Programme*
Casper de Swarte, M.Sc., SenterNovem/EG-Liaison, the Netherlands

09.30 *High-throughput analysis: a pharma perspective*
Prof.dr. Wilfried M.A. Niessen, hyphen MassSpec and Vrije Universiteit, Department of Chemistry and Pharmaceutical Sciences, the Netherlands

10.00 *Field testing of a fully automated real-time PCR device designed for UK Armed Forces*
David Squirrell, Enigma Diagnostics, UK

10.30 **Networking break & exhibition**

Monday 29 January 2007

Parallel session 1: Microorganisms

Chair: Dr. Bert Popping, Eurofins Scientific Group, UK

11.00 *Sample preparation and enrichment for rapid detection*
Dr. Anthony N. Sharpe, Filtaflex Ltd., Canada

11.30 *Making molecular detection methods standard*
Prof.dr. Jeffrey Hoorfar, Technical University of Denmark, National Food Institute, Denmark

12.00 *Nanoparticles-based assays for detection of microorganisms: why, when and where?*
Dr. Aart van Amerongen, Wageningen University, Agrotechnology & Food Sciences Group, the Netherlands

12.30 **Lunch & exhibition**

Case studies

14.00 *Rapid detection methods for verocytotoxigenic Escherichia coli in the food chain*
Dr. Catherine M. Burgess, Teagasc, Ashtown Food Research Centre, Ireland

14.30 *New methods for Salmonella detection*
Dr. Mika Tuomola, Raisio Diagnostics, Finland

15.00 *Nucleic acid lateral flow immunoassay (NALFIA): rapid method for the simultaneous detection of Listeria monocytogenes and Listeria spp.*
Dr. Martina Blažková, Institute of Chemical Technology, Department of Biochemistry and Microbiology, Czech Republic

15.30 *Two simple technologies for screening herds on zoonotic agents such as Campylobacter, Salmonella and Trichinella*
Dr. Kitty Maassen, Wageningen University, Animal Sciences Group, the Netherlands

Contributed papers

16.00 *Electrical DNA arrays for rapid detection of bacterial pathogens*
Prof.dr. Sven-Olof Enfors, Royal Institute of Technology, Sweden

16.20 *Precise controls for rapid methods*
Dr. Richard Lilischkis, BTF Pty Ltd., Australia

Spotlight presentation

16.40 *Use of iQ-Check™ Legionella kits for efficient Legionella risk management in hot sanitary water and industrial water systems*
V. Todorova, Bio-Rad, Food Science Division, France

Monday 29 January 2007

Parallel session 2: Naturally occurring substances

Chair: Margreet Lauwaars, Consultant, the Netherlands

11.00 *Rapid detection of mycotoxins: an overview*
Prof.dr. Sarah De Saeger, Ghent University, Department of Bio-analysis, Belgium

11.30 *Rapid methods for bacterial toxins: potential and limitations*
Prof.dr. Erwin Märtlbauer, Ludwig Maximilian University, Department of Hygiene and Technology of Milk, Germany

12.00 *Functional assays for biotoxin detection*
Prof.dr. Gian Paolo Rossini, University of Modena and Reggio Emilia, Department of Biomedicine, Italy

12.30 **Lunch & exhibition**

Case studies

Chair: Prof.dr. Sarah De Saeger, Ghent University, Department of Bio-analysis, Belgium

14.00 *Screening of mycotoxins in strongly coloured food matrices*
Marieke Lobeau, Ghent University, Department of Bio-analysis, Belgium

14.30 *Innovative microarray tests for food allergen analysis*
Dr. Reinhard Hiller, Centre for Proteomic & Genomic Research, South Africa

15.00 *Rapid detection of aeroallergens*
Prof.dr. Dick Heederik, Utrecht University, Institute for Risk Assessment Sciences, the Netherlands

15.30 *Rapid ante mortem tests for TSEs: progress in testing blood for prions*
Dr. Alex Räber, Prionics AG, Switzerland

Contributed papers

16.00 *Rapid strip tests for the detection of deoxynivalenol in wheat and T2-toxin in wheat and oat*
Dr. Alexandra Molinelli, University of Natural Resources and Applied Life Sciences, Department for Agrobiotechnology, Austria

16.20 *EU-eBIOSENSE, electrical biosensor arrays for the analyses of Salmonella, mycotoxins and toxigenic fungi in food and feed*
Dr. Cees Waalwijk, Plant Research International, the Netherlands

Spotlight presentation

16.40 *A three-prong approach to ensuring confidence in sanitation and food quality*
Rob Langley, Neogen Europe Ltd., UK

Monday 29 January 2007

17.00 – 18.00 **Poster presentations at the Scientific Café**

17.00 – 18.00 **Matchmaking event**

Matchmaking
The best opportunity to meet companies and research institutes throughout Europe to identify possibilities for international R&D co-operation.

The development of new rapid detection techniques for food and feed diagnostics requires interaction between many stakeholders like the primary sector, food and feed producing companies, retailers, biotech companies, ICT companies, equipment manufacturers and government bodies. A prerequisite for the effective development of the required diagnostic systems meeting the criteria of all the stakeholders, is that companies from the different areas are able to find one another and identify interesting partners for co-operation.

Purpose
The purpose of this matchmaking event is to create a meeting point for technology co-operation. It will offer companies and research organisations a forum to effectively meet and discuss with the parties, which are of interest in the area of food and feed diagnostics. The event will be tailor made with a programme consisting of one-to-one meetings with pre-matched partners.

Theme
The matchmaking event will focus on R&D collaboration in food and feed diagnostics.


Rapid
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EUROPE 2007
29-30 JANUARY 2007

Tuesday 30 January 2007

Parallel session 3: Microorganisms

Chair: Dr. Aart van Amerongen, Wageningen University, Agrotechnology & Food Sciences Group, the Netherlands

08.30 *Bacterial pathogen detection using a novel light scattering technology*
Prof.dr. Arun K. Bhunia, Purdue University, Department of Food Science, USA

09.00 *Luminescent techniques for microbiological analysis of foods: future trends*
Dr. Mansel Griffiths, Canadian Research Institute for Food Safety, Canada

09.30 *Rapid methods for the detection of foodborne viruses*
Dr. David N. Lees, Centre for Environment, Fisheries & Aquaculture Science, Weymouth Laboratory, UK

10.00 *Rapid detection of toxigenic fungi in plants*
Dr. Antonio Moretti, National Research Council, Institute of Sciences of Food Production, Italy

10.30 **Networking break & exhibition**

Case studies

11.00 *Detection of Legionella with a microsieve and a LED-based photonic device*
Dr.ir. Frank H.A.G. Fey, Centre for Concepts in Mechatronics (CCM), the Netherlands

11.30 *Isolation of selected Shiga toxin-producing Escherichia coli (STEC) serotypes from artificially contaminated samples*
Björn Possé, University of Ghent, Department of Veterinary Public Health and Food Safety, Belgium

Contributed papers

12.00 *A PCR-DGGE approach to explore the predominant microbial spoilage flora associated with modified atmosphere packaged sliced cooked meat products*
Prof.dr. Kathy Messens, University College Ghent, Laboratory AgriFing, Belgium

12.20 *Electrical chips for the monitoring of biomarkers*
Dr. Britta Jürgen, Ernst-Moritz-Arndt University, Institute of Pharmacy, Germany

12.45 **Lunch & exhibition**

Tuesday 30 January 2007

Parallel session 4: Non-naturally occurring substances

Chair: Prof.dr. Erwin Märtlbauer, Ludwig Maximilian University, Department of Hygiene and Technology of Milk, Germany

08.30 *Acrylamide & Co - analysis of processing contaminants*
Dr. Thomas Wenzl, European Commission, Directorate-General Joint Research Centre, Institute for Reference Materials and Measurements, Belgium

09.00 *New developments in the rapid detection of pesticide residues*
Prof.dr. Sergei A. Eremin, Lomonosov Moscow State University, Department of Chemical Enzymology, Russia

09.30 *The rapid detection of antibiotic residues in food products*
Wim Reybroeck, Institute for Agricultural and Fisheries Research, Unit Technology & Food, Belgium

10.00 *Irradiation detection methods - and how to cheat the test*
Dr. Bert Popping, Eurofins Scientific Group, UK

10.30 **Networking break & exhibition**

Case studies

11.00 *DNA arrays for fast multiple GMO detection*
Prof.dr. José Remacle, University of Namur, Laboratory of Cellular Biochemistry and Biology / Eppendorf Array Technologies, Belgium

11.30 *Spice up spices - rapid detection and quantification of illegal dyes*
Silke Mass, Eurofins/Wiertz-Eggert-Jörissen, Germany

Contributed papers

12.00 *Luminex®-based multiplex immunoassays for drug residues in food*
Willem Haasnoot, RIKILT-Institute of Food Safety, the Netherlands

12.20 *Qualitative determination of β-lactams in milk in 3 minutes*
Willem Kokke, Charm Sciences/Kentron Microbiology, USA/the Netherlands

12.45 **Lunch & exhibition**

WORKSHOP PROGRAMME

Tuesday 30 January 2007

14.00-15.00 and 15.15-16.15
All workshops will run concurrently. Each workshop will last for one hour and will be given twice.

Workshop 1: Rapid tests for mycotoxins and allergens

Sponsored and presented by R-Biopharm AG.
R-Biopharm offers a wide range of analytical test kits for food and feed analysis, and clinical diagnostics.

Part I: New developments in rapid mycotoxin detection
- Introduction and technical background
- New developments:
  - semi-quantitative aflatoxin detection with a new lateral flow based test system
  - lateral flow methods for rapid *Fusarium* toxin (deoxynivalenol, fumonisin) screening
  - introducing: a new biosensor method for rapid mycotoxin detection
- Hands-on training of the aflatoxin detection method
- Discussion with the experts

Part II: Integrating rapid lateral flow test in a HACCP-based approach for allergen monitoring
- Introduction and technical background
- Lateral flow test systems for allergen detection
  - a R5 antibody based lateral flow device for gliadin detection
  - a new lateral flow based test for hazelnut detection
- Hands-on training of the methods
- Discussion with the experts

Workshop 2: Sample extraction / Fast detection of mycotoxins

Sponsored and presented by Euro-Diagnostica B.V.
Euro-Diagnostica is dedicated to testing the quality of food and to the diagnosis of disease.

Part I: Sample extraction / homogenisation
- Presentation of homogeniser and extraction apparatus
- Demonstration with real samples
- Discussion with the experts

Part II: Fast detection of mycotoxins
- Presentation of product news
- Demonstration of DON tube test and ochratoxin FTRT (Flow Through Rapid Test kit)
- Discussion with the experts

Tuesday 30 January 2007

Workshop 3: Practical aspects of manufacturing rapid tests

Sponsored and presented by Biodot, Inc.
Biodot will present a series of practical demonstrations, which will take the workshop through the processes involved in manufacturing rapid tests, from R&D, through batch manufacturing to full in-line systems.

Part I: Batch manufacturing
- Dispensing onto substrates
  - technologies (non-contact vs. contact; air-jet conjugates and blocking reagents)
  - applications (lateral flow; immunoblots; biosensors; biochips)
- Lamination
- Cutting

Part II: In-line manufacturing
- Reel to reel dispensing and drying (web handling considerations; non-contact vs. contact dispensing; air-jet of conjugate and blocking reagents; dipping; drying technologies; quality control)
- In-line lamination (web handling considerations; quality control)
- Cutting

Part III: Informal questions and answers
A chance to ask our team specific questions, and to inspect the various dispensing, web handling, lamination and cutting technologies.

Workshop 4: Rapid tests for microbiological screening

Sponsored and presented by FOSS A/S.
In the world of today efficiency has become inextricably linked to profitability and immediate control of bioburden factors as coliforms, total viable counts and moulds in food products becomes even more crucial to obtain fast and positive release of products. The key words are speed, flexibility, versatility and connectivity. FOSS presents a system, which offers rapid and reliable results, easy to use - all in a complete platform with a large selection of tests and network ability. Rapid results on the key spoilage and indicator organisms allow faster release of product through the production chain from raw material assessment to product release. The system has already been adapted by several big and small food companies and has demonstrated its usability and benefits.

Part I: Product introduction - a complete platform
- Technical background
- Examples of real life benefits

Part II: Hands-on training with meat and dairy products

Part III: Discussion with the experts

SEE YOU AT

RAPID METHODS EUROPE 2008

NOORDWIJKERHOUT, THE NETHERLANDS

28-29 JANUARY 2008

LECTURES

International perspectives in rapid methods research: opportunities in Eureka and the 7th Framework Programme

Casper de Swarte

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Food safety remains high on the public agenda. Although much of the public confidence in the food industry has been restored since the late nineties, we learned that trust in food safety is fragile. In a complex international food web where foodstuffs are continuously transported, processed, redistributed and reprocessed on it is essential to have fast and accurate information on the microbiological and chemical status of foodstuffs at any given point in the food chain. To achieve this both legislators and industry demand ever faster, easier and more accurate testing methods to monitor the quality of both bulk and individual food products. At the same time, technological development of these rapid tests is becoming increasingly complex and costly. It is not something, which can be done alone in a laboratory without any contacts with the outside world. Today's scientists and industry need to work together and they need access to advanced technical equipment.

Like the free movement of goods in Europe it is necessary to have a free movement of knowledge to cope with a more technology demanding society on the one hand and increasing costs to develop new technologies on the other hand. To realise this the European Union is building the European Research Area. The idea of a European Research Area grew out of the realisation that research in Europe suffers from three weaknesses: insufficient funding, lack of an environment to stimulate research and exploit results, and the fragmented nature of activities and the dispersal of resources. The European Research Area opens opportunities for research on rapid methods on an international scale: the 7th Framework Programme for fundamental and applied research in international consortia and Eureka for international market oriented development and innovation on rapid test methods.

The 7th Framework Programme is the principal means of the European Commission to fund European research. The total budget of the 7th Framework Programme is over $50x10^9$ Euro, which makes it the largest research programme in the world. Food safety, especially new detection methods of biological and chemical food hazards, as well as detection of unwanted genetically modified organisms (GMOs) is high on the agenda. Furthermore one of the new topics is development of cross-border technologies such as the application of nano-technology in food safety. Next to the 7th Framework Programme there is the EUREKA Programme. The objective of EUREKA is to raise, through closer co-operation among enterprises, and between enterprises and research institutes in the field of advanced technologies, the productivity and competitiveness of Europe's industries and national economies on the world market. This will be achieved by encouraging and facilitating increased industrial, technological and scientific international co-operation on projects directed at developing products, processes and services having a world-wide market potential and based on advanced technologies.

For the 7th Framework Programme as well as EUREKA industry and scientists, dedicated to bring rapid detection methods to the next level, need to set the European agenda aimed at future developments in rapid methods to fully exploit the opportunities European research presents.

High-throughput analysis: a pharma perspective

Wilfried M.A. Niessen

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and Pharmaceutical Sciences, the Netherlands
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Due to advances in legislation, the determination of residues of veterinary medicine, pesticides, and other components in food of either animal origin (eggs, meat, milk) or vegetable origin (fruits and vegetables) present an interesting and demanding challenge to analytical chemists. In these areas, the final confirmation of identity by means of mass spectrometric (MS) methods plays an important role. Therefore, combined chromatography-MS is used, involving both gas and liquid chromatography (GC and LC). Great progress has been made in recent years in the developments of especially LC-MS. However, LC-MS is widely used in other application areas. In this presentation, we will try to look at the challenges of residue analysis from other perspectives, from other application areas, and especially take lesson from high throughput quantitative bioanalysis, as performed within pharmaceutical industries. Special attention will be paid to matrix effects, which play an important role in quantitative analysis using LC-MS.

Field testing of a fully automated real-time PCR device designed for UK Armed Forces

David Squirrell

Enigma Diagnostics Ltd., UK
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A fully automated PCR device with integrated sample preparation has been developed for biodefence applications. The requirement was for equipment that could provide confirmation of detection events from immunoassay-based warning systems for bacterial or viral agents. A nucleic acid-based test is ideal for this purpose as it provides an independent ('orthogonal') means of verification and has greater sensitivity than the primary immunoassays. This paper describes the equipment which, as a confirmatory system, has been designed to test for a single agent at a time using assay cartridges that are pre-loaded with all the reagents needed for a particular test, including freeze-dried PCR reagents.

The machine is man portable, fully automated, and can be operated from a battery, a vehicle power supply or a mains supply. Tests are performed in less than 1 hour and can be as fast as 20 minutes, depending on the analyte and the PCR assay format employed. For simplicity of use, the output is given through a traffic light display: an embedded algorithm analyses the results cycle by cycle to operate the display, with red or green being positive or negative, respectively, whilst amber is used to indicate that a retest is needed (which is governed by results from internal controls in the PCR assay). With the machine connected to a computer, PCR runs can be displayed in real time, but it is intended that the machine should mainly be used as a stand-alone device. However, for evidential purposes, full data from the previous 256 runs can be downloaded and these are accompanied with a log of the machine's status during the test and GPS information, which confirms when and where the test was performed.

The training requirements are minimal with the user only having to add the sample (up to 1mL) to the assay cartridge, to load the cartridge into the machine, and to select the agent for test. Sample processing is carried out by a robotic system the program for which, along with the thermal cycling parameters, can be specific to each analyte. For example, a sonication step can be included in tests for spore-forming organisms.

As a means of practically testing operational issues, the equipment was evaluated in veterinary field trials where conditions were analogous to those that can be encountered in military use. Results from these trials are presented. A standard laboratory assay for an endemic viral disease was transferred to the portable equipment and then used for farmyard testing of blood samples from cattle. Lessons learned from these trials, which helped us to appreciate how the equipment can best be deployed, are discussed.

Sample preparation and enrichment for rapid detection

Anthony N. Sharpe

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One viable *Salmonella* or *Listeria* cell in 25 g sample represents an analytical limit of detection of around $1:10^{13-14}$ or $1:10^{17-18}$ for, say, a specific gene, arguably achieved by broth enrichments relying on noise-free microbial self-amplification. 'Rapid methods' (ELISA or DNA probe-based) have reduced times from 7-14 days to 48 hours (26-30 doublings) or even 24 hours and better for PCR-based detections. To do useful things like check shipments at loading bays, monitor critical control points, etc, we need to detect viable pathogens in more like thirty minutes.

We currently cannot detect one target cell directly in 25 g of food; we need to add liquid (225 mL for 25 g samples) and at time zero any aliquot less than the full 250 mL may not contain the target cell. Procedures such as ELISA or DNA probes needing 10^{4-5} cells/mL require 22-23 doublings (8-12 hours growth) to be reliable. Even PCR needs at least one target sequence; for a test aliquot of 100 µL we need at least 2,500 targets in the 250 mL (8 doublings or 3-6 hours growth). For really short analysis times we need alternative ways to 'enrich' the target.

In the limit microbial detectability depends not on their number, but on the signal:noise ratio (their concentration relative to interfering substances). Microscopy and cytometry which 'see' volumes of 10^{-12} L can (arguably) identify single cells within seconds if there is no noise. But food suspensions are almost 100 per cent noise; if we rely on microbial multiplication to improve signal:noise we are stuck with lengthy lead times. Physical, chemical or immunological methods can help by separating microbes from food components and minimising the volume in which they exist, rather than increasing their numbers, but they have a long way to go. Given powerful separation methods we could employ currently impractical rapid identification procedures. Two basic approaches need developing:

- Minimise getting noise (fats, proteins, gums, etc.) into suspensions when target microbes are removed from foods, i.e., prepare cleaner suspensions.
  - Pulsifier™. The action drives microbes into suspension as effectively as a Stomacher™ while (food dependent) suspending less food. For intact foods (carrot, liver, etc.) noise particles are dramatically excluded (membrane filtration rates 10-12 times better than from a Stomacher), for processed foods (ground beef, etc.) benefits are smaller.
  - Macrodialysis. If food samples are sealed in (nominal) 1 µm mesh pouches before stomaching or pulsifying, then food particles larger than the mesh size are excluded from the suspension.
  - Gas bubbles. Bubbles nucleated near surfaces dislodge bound materials, e.g., in electrolytic cleaning of metals 'gas scrubbing' by H_2 and O_2 at electrodes removes scale and dirt. Bubbles nucleated near microbes (e.g., sudden outgassing of CO_2 - saturated diluent, or by H_2O_2 at catalase (intrinsic or 'aimed' by conjugation to specific antibodies) might blast them from surfaces.
  - Microbe Trap. A smooth plastic film coated with antibodies or lectins can be placed in a test environment hours or days before a sample would normally be taken for analysis to trap and concentrate its target (microbes, toxins, etc.) until removed. Non-specifically bound material can be rinsed off and bound target released (scraping, change of ionic strength) for concentration and analysis. Microbe Traps could speed

detection by starting target purification earlier than normal. At low contamination levels where many subsamples do not contain target (high probability of accepting contaminated lots) they might “see” a greater proportion of the product.

- Better separate target microbes from noisy suspensions after they have been prepared. Though many separation techniques have been tried (few are mentioned here), none are perfect and there is great scope for research. The challenge is to find ways to cheaply reduce primary suspensions volumes of 250-1,000 mL to a mL or so, where options increase greatly.
  - Centrifugation. While suitable for, say, removing detritus from large suspension volumes prior to membrane filtration, centrifugation generally is inconvenient.
  - Membrane filtration. Filtration (after enzyme/surfactant) improves limits of microscope-counts of milk organisms by 10^{3-4} in the Direct Epifluorescent Filter Technique (DEFT). *Escherichia coli* O157:H7 at 10^3 cfu/mL was enumerated in 1 hour in milk and apple juice by an 'antibody-DEFT' method, and at 16 cfu/g in ground beef (but examination of 200 microscope fields was required).
  - Immunomagnetic separations: a powerful set of techniques with many commercial devices, discussed fully elsewhere in this conference. Until recently, handleable volumes were in the 1-2 mL range and magnetic particles were expensive, but treatments of 2-300 mL are now feasible.
  - Foam flotation. Biological molecules concentrate at the air-water interfaces of aerosols and foams (sea surf, rain splashes, sewage sprays, etc.); concentration gains up to 2,000-fold have been observed. Foam flotation has been used to separate microbes and microstructures from water; concentration factors depend on microbe, age of culture, etc. There is great potential for investigating specific flotation agents (e.g., antibodies conjugated to hydrophobic moieties).
  - Open-cell foams. Microbes have long been separated and purified by columns of antibody or lectin-coated beads; among problems are slow flow rates and relatively expensive, cumbersome columns. Flow rate is slow because even at the loosest packing (uniform bead size) interbead space is small (maximum void volume 26%). Open-cell polyester foam (physical 'negative' of a bead column) costs virtually nothing, has >97 per cent void volume, high flow rate and can be it’s own prefilter. It also can be compressed to 1/10th of its volume before eluting the target.

Making molecular detection methods standard

Jeffrey Hoorfar

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Recognising the need of standardisation, in 1999 the European Commission approved the 3-year research project FOOD-PCR (under the 5th Framework Programme), including 35 European institutes and companies, which aimed to validate and standardise the use of diagnostic PCR for detection of bacterial pathogens in foods. Standard PCRs were devised for 5 pathogens: *Salmonella enterica*, thermotolerant *Campylobacter* spp., enterohemorrhagic *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica.* The methodology was focused on 3 sample types from primary food production: chicken-rinse, pig-swab, and milk. The 3-year project comprised 6 work packages and 20 tasks. The tasks resulted in certified DNA material, preparation of a thermocycler-proficiency testing, strategies for sample preparation and performance of ring trials. The work on automated detection resulted in several real-time PCRs. The sample pre-treatment methods developed were based on current ISO pre-enrichment procedures. These procedures were adopted to allow PCR to replace conventional detection. The entire methods were subjected to inter-laboratory trials. The project resulted in more than 40 publications. The most important outcome of the project has been the production of standardised PCR publications, a biochemical kit for validation of different types and brands of thermocyclers and certified reference DNA material.

FOOD-PCR2

The consortium continued as FOOD-PCR2 project under the 6th EU Framework Programme, as part of the Network of Excellence MedVetNet (www.medvetnet.net). The overall objective was to facilitate implementation of PCR-based methods, such as real-time PCR or microarray-PCR, for both detection and verification of food-borne pathogens, through international, multicentre harmonisation and standardisation of methods. The target microorganisms were the aforementioned foodborne, zoonotic bacteria and foodborne viruses. The work involved establishment of five European working groups, a PCR database, preparation of European draft standards, hands-on workshops for harmonisation of methodologies, standard operating procedures (SOPs) for collaborative trials, and exchange of scientists and students. The five European working groups (WG) focused on various aspects of the overall objective:

- WG1 - Microarray standardisation
- WG2 - Real-time PCR ring-trials and reference materials
- WG3 - PCR testing on animal faeces
- WG4 - Requirements for quantitative real-time PCR standardisation
- WG5 - Sample-specific *Norovirus* protocols and reference materials

The work of Food-PCR2 and its WGs has substantially contributed to the development of guidelines and standards related to PCR and microarray testing. In particular, the CEN draft document CEN/TC 275/WG 6/TAG 3 N 0103 on real-time PCR has been achieved by active participation of several members of the Food-PCR2 project. The support has been provided through scientific discussions during the three project meetings held in Copenhagen (September 2004), Weybridge (March 2005) and Berlin (September 2005). In addition, the publications produced during FOOD-PCR 2 provided part of the scientific basis of the draft documents. Most members of the Food-PCR2 were also members of the CEN subcommittees of WG6: TAG3 and TAG4.

Real-time PCR

A simple search in the 'Title/abstract' of publications in the Entrez database for the words 'real-time PCR' results in more than 6500 hits, including more than 310 in Journal of Clinical Microbiology, only. The explosive use of real-time PCR technology for detection and quantification of pathogens, or study of gene expression, demonstrates the strength of this technique, but also the need to come some consensus on the performance criteria and data expression. The quality of publications reporting new tests varies and inter-laboratory validation data are often missing. The lack of standards may be more urgent for diagnostic and reference laboratories dealing with routine detection of pathogens (Hoorfar and Cook, 2002). Although many commercial kits are available, it can be difficult to assess their performance due to the lack of consensus criteria for strain selectivity, diagnostic accuracy, fluorescence readings, cut-off for cycle threshold (Ct), detection probability, and software incompatibilities (Josefsen et al., 2004). For example, detection of foodborne thermotolerant campylobacters in the same PCR-based method with the identical chemistry can result in marked difference results in two different platforms (Josefsen et al., 2004). Thus, the FOOD-PCR consortium has established a working group to harmonise similar international efforts. Large reference laboratories could use such an open-formula draft guideline, but also to evaluate to which degree the scientific reports comply with a minimum set of criteria.

Food-borne viruses

Although there are several published methods, none are as yet applicable for routine use. No method for virus detection in produce has as yet been validated. Amongst several barriers to standardisation are the lack of established criteria for appropriate performance characteristics, lack of reference material, and lack of consensus on which primer sets should be used for each virus type. Also, it is not clear whether methods should be specific to the type of produce or type of virus. The consortium established a working group to look into the possibilities to harmonise similar international efforts and draft a report on the requisites for standard method guidelines, detailing what is currently lacking, and making recommendations for activities in support of standardisation, e.g., collaborative trials of methods (Van der Poel et al., 2005).

Microarray standardisation

Efforts continue to assess the genomic variation among environmental, veterinary, and clinical isolates of *Salmonella* in order to trace the infections and support the risk assessments and intervention strategies. Microarray technology is an emerging diagnostic tool, which could be useful for rationalised characterisation of several thousand isolates at a time. Although microarrays have been reported for the expression of *Salmonella* genes at the RNA level, their potential use in diagnostic laboratories has not received sufficient attention. In addition, several international working groups have identified standardisation of DNA microarrays as one of the urgent bottleneck, before it can be used as a consensus method across boundaries.

The FOOD-PCR2 consortium established a working group to look into the possibilities to harmonise similar international efforts and draft a standard guideline for a simple, single-dye approach to routinely screen *Salmonella* isolates for the presence of genes encoding for serogroup, fimbrial antigens, pathogenic properties, antibiotic resistance determinants, phage susceptibility, etc. (Malorny et al., 2004). Such an open-formula draft guideline could be used, in the first instance, by large reference laboratories to set up uncomplicated and fail-proof microarray tests for a comprehensive characterisation of *Salmonella* isolates. This could be a straightforward approach for the early identification of new outbreak strains and to study the epidemiology of *Salmonella* on genotypic level. Due to the necessity of transparency in international standards, all information used in draft guidelines will be open

to the public. Diagnostic companies could then exploit any commercial potential of the guideline by providing smaller laboratories with ready-to-use kits based on this validated standard approach.

Nordic efforts

A Nordic project co-ordinated by Food-DTU is currently looking into the possibility of ring-trial assessment of the platform (www.foodpcr.com/salmonella_array/microarrya.htm). The overall aim is to provide risk managers with an additional tool for *Salmonella* control. The specific goal is to study the usefulness of three microarray platforms for a comprehensive development of a diagnostic DNA microarray for the characterisation of *Salmonella* isolates. The aspects of discriminatory power, robustness, reproducibility, cost-effectiveness, ease-of-use, etc. are under evaluation. The standard protocol will facilitate comparison of a large number of *Salmonella* isolates using markers for approx. 200 important genes. Experimental work is planed on bioinformatics and statistical data analysis. This will provide a novel and rationalised tool in risk assessment and risk management, which is needed for intervention strategies in risk management. The International collaboration includes MedVetNet, Public Health Agency of Canada in Guelph (PHAC-LFZ), and Sidney Kimmel Cancer Center, San Diego, California. The work will supplement similar European activities at the partners' laboratories. A training workshop for technology transfer to mail EU reference laboratories is planned. The work is intended to produce a draft protocol for NMKL and CEN standards.

References

Barkham, T. and Hoorfar, J., 2004. Internal amplification control for PCR should not be mandatory in the clinical medical environment. Journal of Clinical Microbiology 42:3379-3380.

Hoorfar, J., 2005. The need for standardization of real-time PCR methods. Journal of AOAC International 88:45A.

Hoorfar, J., and Cook, N., 2002. Critical aspects of standardization of PCR. Methods in Molecular Biology 216:51-54.

Hoorfar, J., Cook, C., Malorny, B., Wagner, M., De Medici, D., Abdulmawjood, M. and Fach, P., 2003. Making internal amplification control mandatory for diagnostic PCR. Journal of Clinical Microbiology 41:5835.

Hoorfar, J., Cook, C., Malorny, B., Wagner, M., De Medici, D., Abdulmawjood, M. and Fach, P., 2004. Diagnostic PCR: Making internal amplification control mandatory. Journal of Applied Microbiology 96:221-222.

Hoorfar, J., Malorny, B., Abdulmawjood, A., Cook, N., Wagner, M. and Fach, P., 2004. Practical considerations in design of internal amplification control for diagnostic PCR assays. Journal of Clinical Microbiology 42:1863-1868.

Hoorfar, J., Wolffs, P. and Rådström, P., 2004. Diagnostic PCR: validation and sample preparation are two sides of the same coin. APMIS 112:808-814.

Josefsen, M.H., Cook, N., D'Agostino, M., Hansen, F., Wagner, M., Demnerova, K., Heuvelink, A.E., Tassios, P.T., Lindmark, H., Kmet, V., Barbanera, M., Fach, P., Loncarevic, S. and Hoorfar, J., 2004. Validation of a PCR-based method for detection of food-borne thermotolerant campylobacters in a multicenter collaborative trial. Applied and Environmental Microbiology 70:4379-4383

Krause, M., Josefsen, M.H., Lund, M., Saadbye, P., Jacobsen, N.R., Brorsen, L., Moos, M. and Hoorfar, J., 2006. Collaborative and on-site validation of a real-time PCR method as a tool for the certified production of fresh, *Campylobacter*-free chickens. Applied Environmental Microbiology 72:5463-5468.

Malorny, B., Helmuth, R., Aarts, H. and Hoorfar, J., 2004. The need for standardization of *Salmonella* microarray-based methods. ASM News 70:501.

Malorny, B. and Hoorfar, J., 2005. Toward standardization of diagnostic PCR testing of fecal samples: lessons from the detection of salmonellae in pigs. Journal of Clinical Microbiology 43:3033-3037.

Van der Poel, W.H.M., Cook, N. and Hoorfar, J., 2005. The need for standardization of methods to detect enteric viruses on produce. ASM News 71: 1-2.

Nanoparticles-based assays for detection of microorganisms: why, when and where?

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Rapid, sensitive and accurate detection of microorganisms is needed for many applications, from bio-warfare and human diagnostics to the detection of feed and food pathogens. A large number of detection principles have been evaluated to meet this requirement by comparing a wide range of diagnostic methods. Unfortunately, none of these methods comply with the ultimate goal in microbiological detection, i.e., the real-time detection of a single cell. The actual situation is quite different, since in a lot of cases bacterial detection is still done by culture plate methods. These methods involve various phases, such as sampling, resuscitation of injured cells, pre-enrichment, selective enrichment, detection and identification. The total assay time may be from two to seven days. Moreover, due to the very diverse matrices (blood, faeces, food products, soil, etc.) it is not possible to standardise sample pre-treatment. Pre-treatment protocols have to be designed such that desired target compounds are optimally purified and enriched. Whether this target compound is phenotypic (e.g., a bacterial protein and a compound like ATP) or genotypic (DNA, RNA) will have a profound consequence on the steps to be taken in sample pre-treatment. Over the last two decades all kinds of technological solutions have been introduced to enable rapid and sensitive detection of microorganisms. One of these solutions is the use of nanoparticles and in this presentation the focus will be on these nanoparticles in microbial diagnostics.

Nanoparticles have been applied for different reasons such as for enhancing the sensitivity of the method (agglutinating bacterial cells into large, measurable complexes), for concentration (magnetic nanoparticles, or nanoparticles in combination with specific filters), for providing a chemiluminescent or fluorescent colour, or for a visible colour in lateral flow devices (compare to the pregnancy hormone test kit). In recent years the number of methods employing these characteristics of nanoparticles has increased substantially, not only for the simple and rapid one-step lateral flow methods, but also for highly sophisticated biosensors and lab-on-a-chip devices.

The question why these nanoparticles are being applied will be discussed, in combination with issues of time and place where the nanoparticles-based methods may be applied. Examples will be given of lateral flow immunoassays (LFIAs) detecting microorganism-specific proteins (*Escherichia coli* O157), of nucleic acid LFIAs (NALFIAs; *Salmonella* antibiotic resistance genes, *Bacillus cereus*) and of microarrays that detect amplified genetic material of, e.g., *Listeria monocytogenes*. In these microarrays the specifically bound nanoparticles are detected by conventional flatbed scanning followed by image analysis. These simple assay formats will be compared to biosensor methods in which nanoparticles are applied for various reasons. From these comparisons it will be clear that a complex mixture of sample-, location- and required-data-related aspects is guiding in the choice of the right method to rapidly, sensitively and accurately detect a particular microorganism in a sample of interest.

Rapid detection methods for verocytotoxigenic *Escherichia coli* in the food chain

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Verocytotoxigenic *Escherichia coli* (VTEC) are pathogens of significant public health concern worldwide. Their pathogenicity is related to their ability to adhere to and colonise the human large intestinal epithelial tissue destroying the microvilli by forming attaching and effacing lesions and the production of verocytotoxins. The clinical manifestations of VTEC infection can range from symptom-free carriage, to non-bloody diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome (HUS), thrombocytopenic purpura and death. From a public health point of view their relative significance lies not in the number of cases but in the severity of the illness they can cause, combined with their low infectious dose. *E. coli* O157 was the first serogroup to be associated with this illness but in recent years several other verotoxin-producing serogroups have been reported, including O26, O103, O111 and O145. Sources of VTEC infection include direct contact with animal faeces, foods of animal origin, vegetables, salads and water. The ubiquitous nature of this group of organisms, coupled with the severity of the illness caused, emphasises the necessity for sensitive, specific and rapid detection methods for VTEC. A major challenge in the detection of VTEC from food samples is that the numbers of the pathogen can be very low and they occur in a diverse number of matrices which may contain inhibitors to the detection method, e.g. competitive microflora, PCR inhibitors, etc.

Detection methods for VTEC generally fall into three categories, cultural methods, immunological methods and molecular methods. There is an international standard cultural method for the detection of *E. coli* O157:H7 in foods (ISO, 2001). This method incorporates the use of immunomagnetic beads coated with antisera to *E. coli* O157. The cell/bead complex is then plated on sorbitol MacConkey agar containing cefixime and tellurite. This exploits the fact that, unlike most *E. coli* strains, *E. coli* O157:H7 does not ferment sorbitol, resulting in a colourless colony on sorbitol indicator media. However, in recent years sorbitol-fermenting *E. coli* O157 strains have been isolated from both clinical samples and cattle (Karch and Bielaszewska, 2001; Lee and Choi, 2006). Such strains are likely to be missed by the standard O157 detection method and therefore their public health impact underestimated. Non-O157 strains display a heterogeneous range of genotypic properties. At present there is no internationally recognised standard method for the detection of non-O157 VTEC and the lack of such methods has most likely resulted in underestimation of the importance and prevalence of these pathogens. Nonetheless, progress has been made in the detection and surveillance of a selected number of clinically significant non-O157 serogroups. Enrichment and plating media have been reported for *E. coli* O26, O103, O111 and O145 (Catarame et al., 2003; Fukushima et al., 2000; Hiramatsu et al., 2002). Immunomagnetic beads are now also commercially available for these serogroups. A UK national reference method makes use of such beads to isolate *E. coli* O26, O103, O111 and O145 from faeces and environmental samples (SGDIA, 2006). As long as an internationally accepted standard is lacking for these emerging serogroups the true significance of these serogroups will not be ascertained.

A number of immunological techniques for VTEC have been developed based on the reaction between an antibody and an antigen specific to VTEC. These can be part of enzyme linked immunosorbent assays (ELISA), immunoprecipitate assays, impedance spectroscopy, colony blots or passive agglutination assays. Some of these assays target surface antigens

and therefore detect specific serogroups, while others focus on the detection of the verocytotoxins. The latter provides a means to detect all types of VTEC. Bettelheim and Beutin provide a review of a number of these assays (2003). A large number of immunological assays are available as commercial kits which are easy to use and are therefore used in routine testing for VTEC in many countries. These assays generally require prior enrichment of the target cells to reach detectable levels.

In recent years molecular methods have come to the fore in the detection of VTEC in a range of sample matrices. Although techniques such as DNA hybridisation and the use of reporter bacteriophage have been described, molecular assays are generally based on PCR, which amplifies a specific gene target in VTEC. Many methods focus on the detection of characteristic virulence factors such as the VT genes or the intimin gene. This, however, does not determine the serogroup of VTEC present. Closely related *E. coli* strains encode different O antigens and this can be exploited by designing primers to target specific serogroups. Such assays exist for the differentiation of serogroups including O157, O26, O103, O111 and O145 (DebRoy et al., 2004; Feng et al., 2005; Hsu et al., 2005; Perelle et al., 2005; Wang et al., 1998). An advantage of such techniques is that as well as identifying the presence of VTEC these assays can also be combined to identify the serogroup present. Many of these assays have been adapted to a real time PCR format allowing for increased sensitivity and speed of the assays. A point to note is that regardless of the type of rapid assay developed for the detection of VTEC it is always necessary to validate it against the currently accepted international standard method, where such a method exists, before it can be implemented as routine practice.

References

Bettelheim, K.A. and Beutin, L., 2003. Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). Journal of Applied Microbiology 95:205-217.

Catarame, T.M., O'Hanlon, K.A., Duffy, G., Sheridan, J.J., Blair, I.S. and McDowell, D.A., 2003. Optimization of enrichment and plating procedures for the recovery of *Escherichia coli* O111 and O26 from minced beef. Journal of Applied Microbiology 95:949-957.

DebRoy, C., Roberts, E., Kundrat, J., Davis, M.A., Briggs, C.E. and Fratamico, P.M., 2004. Detection of *Escherichia coli* serogroups O26 and O113 by PCR amplification of the *wzx* and *wzy* genes. Applied and Environmental Microbiology 70:1830-1832.

Feng, L., Senchenkova, S.N., Tao, J., Shashkov, A.S., Liu, B., Shevelev, S.D., Reeves, P.R., Xu, J., Knirel, Y.A. and Wang, L., 2005. Structural and genetic characterization of enterohemorrhagic *Escherichia coli* O145 O antigen and development of an O145 serogroup-specific PCR assay. Journal of Bacteriology 187:758-764.

Fukushima, H., Hoshina, K. and Gomyoda, M., 2000. Selective isolation of *eae*-positive strains of Shiga toxin-producing *Escherichia coli.* Journal of Clinical Microbiology 38:1684-1687.

Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M. and Miyazaki, Y., 2002. Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. Journal of Clinical Microbiology 40:922-925.

Hsu, C.F., Tsai, T.Y. and Pan, T.M., 2005. Use of the duplex TaqMan PCR system for detection of Shiga-like toxin-producing *Escherichia coli* O157. Journal of Clinical Microbiology 43:2668-2673.

ISO (International Organisation for Standardisation), 2001. Microbiology of food and animal feed stuffs - Horizontal method for the detection of *Escherichia coli* O157. No. 16654: 2001.

Karch, H. and Bielaszewska, M., 2001. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. Journal of Clinical Microbiology 39:2043-2049.

Lee, J.H. and Choi, S.J., 2006. Isolation and characteristics of sorbitol-fermenting *Escherichia coli* O157 strains from cattle. Microbes and Infection 8:2021-2026.

Perelle, S., Dilasser, F., Grout, J. and Fach, P., 2005. Detection of *Escherichia coli* serogroup O103 by real-time polymerase chain reaction. Journal of Applied Microbiology 98:1162-1168.

SGDIA, 2006. The Veterinary and Public Health Test Standardisation Group on behalf of the UK Surveillance Group for Diseases and Infections of Animals (SGDIA). Ref no: NRM006-Ecoli-isolation-O26etc [Jan06]. Standard Operating Procedure-for the isolation and presumptive identification of *E. coli* O26, O103, O111 and O145

Wang, L., Curd, H., Qu, W. and Reeves, P.R., 1998. Sequencing of *Escherichia coli* O111 O-antigen gene cluster and identification of O111-specific genes. Journal of Clinical Microbiology 36:3182-3187.

New methods for *Salmonella* detection

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Because of its low infective dose, the analytical methods used for *Salmonella* screening in food products have to be very sensitive and able to detect down to 1 bacterial cell per 25 g of sample. The standard methods used to detect *Salmonella* are based on culture enrichment and plating techniques, and take four to seven days to confirm. Rapid results are of key importance when testing for this pathogen and in order to fulfil this need Raisio Diagnostics has introduced new methods for 24 hours detection of *Salmonella*.

MAGDA Salmonella is a next-day screening method based on enhanced sample enrichment, unique immunocapture technology and inexpensive selective plating as a detection method. This novel test offers high sensitivity with low capital investment in instrumentation and allows the user to use their favourite plating medium for detection.

TAG24 is a new enrichment method for Transia Salmonella Gold ELISA method. This immunoassay is using antibodies targeted against unique bacterial lipopolysaccharides and the method is based on a simplified two-step enrichment procedure where no M-broth is needed to stimulate flagella growth. The users have now an option for obtaining *Salmonella* screening results in 24 hours. The method is fully compatible with our automated analysers, including our new immunoassay system, Transia 4U, which can automatically process up to 372 samples per run without user intervention.

Nucleic acid lateral flow immunoassay (NALFIA): rapid method for the simultaneous detection of *Listeria monocytogenes* and *Listeria* spp.

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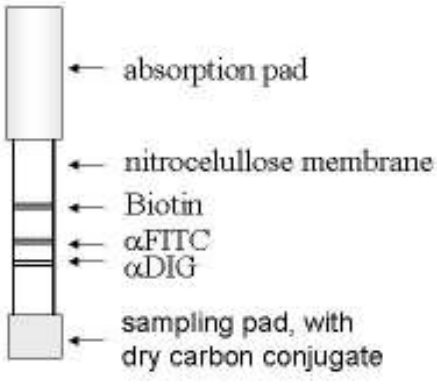
The genus *Listeria* consists of a group of gram-positive, facultative anaerobic rodshaped bacteria. All of the six recognised *Listeria* species can be isolated from a diversity of environmental sources, including soil, water, effluents, a large variety of foods, and from faeces of humans and animals. Although occurrence of *Listeria* strains in food may indicate errors in good hygienic and manufacturing practice, only *L. monocytogenes* is a significant human and animal pathogen responsible for the serious illness listeriosis. The standard isolation method for *L. monocytogenes* from food samples is described in ISO 11290-1 and 11290-2: Horizontal method for the detection and enumeration of *Listeria monocytogenes* (ISO, 1996). This reference method allows the recovery of *L. monocytogenes* from a variety of foods. However, the detection is laborious and takes several days to achieve a confirmed identification. Therefore, the procedure is not suitable for testing foods with short shelf lives and a rapid test for detection of *L. monocytogenes* is urgently needed by the food industry. As a result many rapid tests (Gasanov et al., 2005) have been developed based on chromogenic media, antibodies or nucleic acid based techniques.

The aim of this study was to develop a rapid, specific and user-friendly test for simultaneous detection of *L. monocytogenes* in particular and the genus *Listeria* in general. We used a one-step lateral flow immunoassay adapted to detect amplified genetic material in a so-called nucleic acid lateral flow immunoassay (NALFIA) (Van Amerongen and Koets, 2005). In this method a small volume of the final PCR solution is directly added to a one-step assay device and the appearance of a grey/black line is indicative of the presence of the specific amplicon. The whole procedure of the developed method consists of four different steps. Firstly the tested sample is cultivated and the genomic DNA is isolated from cultured sample. The DNA is used as a template for a duplex PCR. The duplex PCR was based on published results (Herman et al., 1995; D'Agostino et al., 2004), although the original protocol was slightly adjusted for NALFIA requirements. One of each set of primers was 5'-labelled with biotin and the other 5'-labelled with digoxigenin (DIG) or fluorescein-isothiocyanate (FITC), respectively. Duplex PCR product, containing the generic FITC-biotin labelled amplicons resulting from any *Listeria* strain and the DIG-biotin labelled *L. monocytogenes* amplicons, was run in NALFIA. The NALFIA comprised a nitrocellulose membrane with immobilised polyclonal antibodies against the digoxigenin- (αDIG) or fluorescein-isothiokyanate-tags (αFITC) and colloidal carbon nanoparticles with neutravidin immobilised onto the surface. As a control line a biotinylated goat anti-mouse IgG (biotin) was used. Double-labelled amplicons were sandwiched between the immobilised antibodies and the carbon-neutravidin conjugate. The appearance and the position of a grey/black line were indicative of the presence of the specific *L. monocytogenes* and *Listeria* spp. amplicons, respectively. Running the products from the *L. monocytogenes* culture resulted in the appearance of three lines; the products from the non-pathogenic *Listeria* (spp.) resulted in the appearance of only two lines (see Figure).



Several parameters of the developed method were assessed. The specificity was studied by testing a range of *Listeria* strains and other food relevant microorganisms. All tested strains were determined correctly. With the optimised NALFIA the presence of 0.01 ng of FITC amplicons (*Listeria* spp.) and of 0.313 ng of DIG amplicons (*L. monocytogenes*) could be detected. The lowest amount of genomic DNA detectable with the one step assay was 0.05 ng *L. monocytogenes* DNA and 0.25 ng *L. innocua* DNA. The method was finally verified by testing samples of sterilised milk that had been artificially contaminated with different subspecies of the *Listeria* genus. Additional experiments were focused on more complicated bacterial mixtures. Our method was able to reveal cells of *L. monocytogenes* in the presence of a hundred times higher concentration of other *Listeria* species and even a tenthousand times higher concentration of other bacteria. The method has already been used for testing some real food samples and has shown very good results for *Listeria* detection in frozen vegetable, Pablum, sundry salads and sprouted seeds. However, we have not obtained satisfactory results for cakes of cheese yet.

In conclusion, using the combination of nucleic acid amplification and an immunochemical based detection principle offers distinctive advantages in terms of sensitivity, specificity, and speed. Furthermore, since the *Listeria*-NALFIA simultaneously detects *Listeria* spp. and *L. monocytogenes* this method may be instrumental in improving the microbiological standards of foodstuffs. The method can be used to assess hygienically errors in food production, indicated by the presence of *Listeria* spp., whereas it also reveals contamination of food products by the human pathogen *L. monocytogenes*.

References

D'Agostino, M., Wagner, M., Vazquez-Boland, J.A., Kuchta, T., Karpiskova, R., Hoorfar, J., Novella, A., Scortii, M., Ellison, J., Murray, A., Fernandes, I., Kuhn, M., Pazlarova, J., Heuvelink, A. and Cook, N., 2004. A validated PCR-based method to detect *Listeria monocytogenes* using raw milk as a food model – towards an international standard. Journal of Food Protection 67:1646-1655.

Gasanov, U., Hughes, D. and Hansbro, P.M., 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. FEMS Microbiology Reviews 29:851-875.

Herman, L.M.F., De Ridder, H.F.M., Vlaemynck, G.M.M., 1995. A multiplex PCR method for the identification of *Listeria* spp. and *Listeria monocytogenes* in dairy samples. Journal of Food Protection 58:867-872.

Van Amerongen, A. and Koets, M., 2005. Simple and rapid bacterial protein and DNA diagnostic methods based on signal generation with colloidal carbon particles. In: Rapid methods for biological and chemical contaminants in food and feed, edited by A. van Amerongen, D. Barug and M. Lauwaars, Wageningen Academic Publishers, Wageningen, the Netherlands, pp.105-126.

Two simple technologies for screening herds on zoonotic agents such as *Campylobacter*, *Salmonella* and *Trichinella*

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In the Netherlands and also other European countries, new or changed programs for the monitoring of zoonotic agents have started recently or will start in the near future, mostly due to new European Union (EU) legislation. This is only possible when appropriate assays are available. At the Animal Sciences Group in Lelystad, in collaboration with other institutes/companies several assays were developed especially with the purpose to be used in surveillance systems. Important factors are that those assays are simple, quick, and robust. In this presentation an example of a lateral flow test for the detection of *Campylobacter* in chickens will be shown and a surface plasmon resonance technology (SPR) will be used for the detection of antibodies against *Salmonella* and *Trichinella* in pigs.

Campylobacter

Campylobacter is considered the most important food pathogen in the Netherlands and Europe (approximately 5 times as important as for instance *Salmonella*). It may cause campylobacteriosis with symptoms such as fever, diarrhoea and vomiting. *Campylobacter* is also mentioned in relation to Guillain-Barré syndrome. Despite many efforts the contamination of poultry products hardly decreases and stays above 15%. Currently, there are no good intervention methods in place for *Campylobacter*. From a large risk-assessment project it has become clear that logistic slaughter of chickens contaminated with *Campylobacter* can attribute to the reduction of human campylobacteriosis because such measurement will contribute to a reduction of contaminated meat that ends up as fresh meat at the consumer. The efficacy of the measurement will strongly depend on the reliability with which positive herds will be determined. Currently, the detection of *Campylobacter* in chicken manure is done by culturing the bacteria. In addition to the fact that this is a time consuming method, it also needs the bacteria to be viable. This is a problem with *Campylobacter* because of it rapid decrease in numbers during transport from the farm to the laboratory. Another practical problem is that the samples are taken approximately 2 weeks before slaughter, where herds still can become positive in the last 2 weeks. Therefore alternative methods are developed. The goal is to develop a simple, quick and robust assay for the detection of *Campylobacter* in chicken manure. The assay should be in a format that can be performed at the farm by somebody without laboratory experience and without any equipment. It was chosen to develop a lateral flow assay for the direct detection of *Campylobacter*. Antibodies against *Campylobacter* are spotted on the nitrocellulose strip. *Campylobacter* present in the manure can bind to the antibodies on the strip. This is followed by antibodies with conjugated carbon. When *Campylobacter* is present, this shows as a black line. Next to the production of the right antibodies, it was very challenging to find out how to treat the manure in a manner that no equipment is necessary. Currently, the assay is validated with field samples from Dutch farms.

Salmonella

Salmonella is a major cause of bacterial, food-born infections. The latest report from the

National Institute for Public Health and the Environment estimates the incidence of *Salmonella*-derived infections in the Netherlands to be approximately 50,000 cases per year, from which about 25% can be associated with the consumption of pork. In the Netherlands, a monitoring program started in 2005 to identify infected pig herds. This is done by testing serum for the presence of antibodies against *Salmonella* serogroups B, C and D, the most common serogroups in Dutch pigs. In addition to sampling blood at the farm, it is also permitted to sample at the slaughterhouse, reducing costs. Serological assays are much cheaper and faster than traditional microbiological methods, which can take up to three days. Monitoring using serological assays provides information that can be used to give farms a status reflecting a certain level of prevalence of *Salmonella*. Highly infected farms have to take (hygienic) measurements. Denmark has shown that these kinds of programs can help reduce the amount of *Salmonella* contaminated pork, and thus reduce the number of human *Salmonella* infections.

A test has been developed that is based on the surface plasmon resonance phenomenon. Surface plasmon resonance (SPR) occurs at the surface of a gold film that is adhered to a glass plate (the sensor chip). Electrons on the surface of the gold film ripple around in waves (surface plasmons). When incident polarised light is directed to the gold film, energy is transferred to the surface plasmons and this causes a dip in intensity of the reflected light. A prerequisite for this to occur is that the incident light reaches the gold film at a certain angle (the resonance or SPR angle). The value of the reflected resonance angle changes when the refractive index at the sensor surface changes. Since the relation between the two is linear, the change in resonance angle can be used to measure changes in refractive index caused by a liquid (containing the analyte of interest) that is guided along the sensor chip surface. For instance, an antigen can be immobilised to the gold film of the sensor chip. A sample is injected into a flow channel that transports the sample to the sensor chip. The antigen captures the antibody (if present) from the liquid and this causes a mass change at the sensor chip surface resulting in a different refractive index at the sensor surface. In turn the value of the resonance angle of the reflected light is changed and measured. Events are displayed by a sensorgram on the screen of the computer that instructs the Biacore Q/Q100 apparatus. An advantage of this technology is that the surface can be regenerated and reused many times.

Although Biacore technology is routinely used in many fields, it has not been used for the detection of antibodies directed against pathogenic microorganisms in animals in a routine setting. Currently, an assay to detect antibodies against *Salmonella* in pig serum is being validated. The assay is based on lipopolysaccharides (LPS) immobilized on sensor chip CM5. For an assay to be used in a monitoring program, it is a prerequisite that the total testing time per sample is short (6 minutes in our assay). The Biacore assay was compared with the HerdCheck Swine *Salmonella* ELISA from Idexx Laboratories. The overall agreement between the assays is approximately 92%. The results obtained with field sera indicate that it is feasible to screen pig herds for *Salmonella* antibodies using this Biacore assay.

Trichinella

Trichinella are nematodes (round worms) which live as intracellular parasites. The diseases they cause are collectively referred to as trichinellosis. The most prevalent human infections are caused by *Trichinella spiralis*. Domestic pigs are the dominant reservoir host for *T. spiralis*, which is now considered endemic in Japan and China. *Trichinella* infect nearly all orders of mammals, making it one of the world's most widely distributed parasite groups. Infection occurs by ingesting contaminated raw or undercooked meat, what might cause severe problems, sometimes even death. The Dutch pig population is practically free of *T. spiralis*. However, at slaughter every pig is tested for presence of larvae using the digestion

method for export certification. One gram of meat is taken from the diaphragm and 100 samples are pooled before testing. This is a labour intensive and costly method. Since 2006, the EU approved new legislation on *Trichinella* control where serological tests can be used as an alternative monitoring tool on *Trichinella* free herds. Therefore, a serological assay for the detection of antibodies against *Trichinella* was developed for the Q and the Q100 system. The latter is 8 channel system, facilitating a much higher through put. Because *Trichinella* is a notifiable disease it has enormous consequences when a farm is (falsely) accused of a *Trichinella* infection. The sample, which turned out positive, can not be confirmed with the digestion method because the pig already has gone into retail. All positive farms have to go back to monitoring with the digestion method. A very high specificity is therefore a prerequisite for this assay. Therefore a highly sensitive screening assay was developed. Samples that show a response above a certain threshold are retested in another SPR assay. This assay has an additional step resulting in highly specific and highly sensitive results.

Several serum collections were tested including approximately 1,800 digestion negative Dutch field sera; sera from pigs that were experimentally infected with different doses *T. spiralis;* samples from an experimental *S. panama* infection (10^8 CFU) and field sera from herds infected with *S. panama*. Also almost 1,000 sera from endemic area's in Argentina were collected. These sera were tested in both Biacore SPR assays and compared to the results from different ELISAs. Overall prevalence in the endemic area, sensitivity and specificity of all assays were estimated in the absence of a gold standard test using a Bayesian approach including a Gibbs sampler as implemented in WinBUGS. The test parameters were estimated using a linear regression for individual animal test data correcting for interdependence between tests and missing values.

Electrical DNA arrays for rapid detection of bacterial pathogens*

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Analysis of pathogenic bacteria is traditionally based on phenotypic properties, often in specific cultivation media. Improvements of molecular-based nucleic acid detection methodologies have introduced a new era of rapid, miniaturised and automated biochip techniques for genotypic characterisation. Commercially available DNA chips are usually high-density arrays based on optical detection. However, there are several advantages with the use of low-density silicon based electrical DNA chips. These chips have the potential for custom fabrication and the detection of most genetically determined features. Furthermore, the use of established silicon technology offers potential for low production costs.

Confirmative analysis of pathogenic *Bacillus cereus* strains is a typical case of the applicability of these low-density electrical DNA arrays. *B. cereus* is one of the most frequent food poisoning microorganism causing both intoxications and infections. Three enterotoxins, i.e. hemolysin BL, non-hemolytic enterotoxin, and cytotoxin K are known to elicit diarrhoea, while the toxin called cereulide is recognised as an emesis-causing toxin. However, only about 50% of those strains that are phenotypically classified as *B. cereus* are pathogenic. In addition, many other *Bacillus* species can produce some of the toxins. Thus, a genetically based analysis showing the capacity of an organism to produce the toxins would be superior to the traditional classification of microbial species.

Consequently, our efforts were dedicated to develop a DNA based electrical platform for simultaneous detection of all presently known toxin-encoding genes of pathogenic *B. cereus*. Using a piezo spotting device, a 16-position silicon chip array was functionalised with capturing oligonucleotide molecules specific to eight *B. cereus* genes involved in the synthesis of the four toxins. Parallel, a protocol for DNA analyte preparation was developed. Bacteria from enrichment cultures suspected to be pathogenic *Bacillus* were ultrasonicated for release of the DNA. The further fragmentation of the DNA by ultrasonication was optimised to increase the diffusion and hybridisation rate of the analyte. Flow cytometry measurements and semi-quantitative early endpoint amplification analyses were used, respectively, to monitor bacterial cell disintegration and to characterise the DNA break-up. Based on these results, the DNA analyte preparation procedure comprising first 5 min of ultrasonic treatment, DNA extraction, and afterwards an additional 10 minutes sonication, was established as the most effective way of sample processing. No DNA amplification step prior to the analysis was included.

The assay was carried out with the functionalised silicon chip array placed in a reaction chamber of the automated array analyser 'eMicroLISA' (eBiochip System GmbH, Itzehoe, Germany). Built-in miniaturised pumps and valves transport the sample and all reagents to the flow cell on the chip array. The assay is fully automated to reduce hands-on manipulations, which makes this method suitable for confirmative analysis of suspected pathogens. Only specific hybridisation (temperature controlled) between capture probes, DNA analyte, and detection probes labelled with an enzyme alkaline phosphatase, resulted in production of the electrochemical active compound p-aminophenol from an electro-

* Contributed paper

chemical inactive enzyme substrate p-aminophenyl phosphate. p-Aminophenol is redox cycled at the chip electrodes, thereby producing an electrical current in the nanoampere range. Measurement of the nA signal increase is made under stop-flow conditions.

The electrical analyser was able simultaneously to measure the enzyme product generated at each individual array position. The confirmative assay of one diarrhoeal and one emetic strain, carrying different combinations of the toxin coding genes, was carried out within 30 min, once the DNA analyte from 10^8 bacterial cells, corresponding to one agar colony, was subjected to the assay.

Precise controls for rapid methods*

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A major limitation on the uptake of rapid methods is the concern that the test is reliable and that the results are comparable to traditional test methods. The use of precise reference materials alleviates these concerns and can significantly improve the acceptance and uptake of a rapid method. We have developed a suite of technologies that enables the production of freeze dried pills that contain precise amounts of biological material. Exact numbers of genes, cells, particles or molecules can be produced. The viability of cells and the activity of biological material can be maintained throughout the process and is stable over a period of at least one year.

The variability between different pills is far lower than can be achieved with traditional methodologies. For example, for materials such as specific DNA sequences the coefficient of variance (CV) is typically less than 1% even with numbers as low as 5 copies per pill. For viable cells such as bacteria and fungi the CV is typically less than 5%.

This technology allows the production of precise reference materials for many testing applications including rapid methods such as nucleic acid and immunological based methods. The high level of precision means that reliable reference materials can be produced which are close to the limit of sensitivity of the test.

* Contributed paper

Use of iQ-Check™ *Legionella* kits for efficient *Legionella* risk management in hot sanitary water and industrial water systems*

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Water safety is an important part of Bio-Rad's investment and commitment for industrial control. *Legionella* bacteria are widely spread in natural water environment and proliferate in man made water systems, such as hot sanitary water, fountains, cooling towers. Regular testing for the presence of *Legionella* in water supply systems is the first necessary action for preventing Legionellosis (Legionnaires' disease). Conventional culture methods for *Legionella* and *L. pneumophila* in environmental samples show poor sensitivity and results take as long as 13 days. Therefore, these methods are not satisfactory for routine laboratory applications and faster methods are needed.

Bio-Rad has developed a complete and integrated solution for *Legionella* risk management based on iQ-Check real-time PCR technology. iQ-Check™ *Legionella* PCR kits allow detection and quantification of *Legionella* spp. and *L. pneumophila* in water, biofilms and air. Bacteria lysis and DNA extraction are performed using Aquadien™ kit, based on innovative technology for waterborne pathogens DNA extraction. Results are delivered in 5 hours, allowing real-time monitoring of water systems.

iQ-Check *Legionella* method has been routinely used for water, biofilm and air control of *Legionella* proliferation. This presentation shows several examples for biofilm *Legionella* analysis and biocide treatment monitoring using iQ-Check *Legionella* method.

* Spotlight presentation

Rapid detection of mycotoxins: an overview

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Mycotoxins are toxic secondary fungal metabolites with deleterious effects for humans and animals. Some mycotoxins are carcinogenic while others are nephrotoxic, hepatotoxic, immunosuppressive or teratogenic. Food and feed can be contaminated with mycotoxins since they can be formed in commodities before as well as after harvest. Commodity circulation around the world, irregular storage and a lot of (other) uncontrolled reasons causes mycotoxin contaminations. Therefore many countries have set regulations with maximum levels for the major mycotoxins in different commodities.

In the last decade there has been a continuous growth in development of rapid methods for mycotoxin analysis. Indeed, results are expected immediately, so that commodities can be further processed without delay. Food and feed industry are forced to reduce costs, employ cheaper labour, but deliver in time safe goods that comply with US and/or EU regulations.

Chromatographic techniques such as liquid chromatography – mass spectrometry (LC-MS) are laborious methods, but give quantitative results with high sensitivity and precision. Innovations in these techniques and instruments led to the development of methods in which even up to 39 mycotoxins can be simultaneously determined using a single extraction step without any clean-up. So, this results in a lot of information in a relative limited time period. Of course, expensive equipment, highly qualified personnel and a laboratory environment are prerequisites for LC-MS analysis.

Rapid screening techniques, which can be used outside the laboratory environment, at the place of sampling, are becoming more and more important. Ideally, such rapid tests should be used at different points in the pre- and post- harvest supply chain. So, they could represent key tools in a HACCP type approach to mycotoxin control. In general there are two basic requirements for rapid testing in a non-laboratory environment. Firstly, test kits should be simple to use, and secondly, results should be easy to interpret. It means consisting of a simple sample extraction, minimal manipulations, little assay steps, short assay time, and no or minimal use of toxic solvents. Also, it should be easy to see the difference between positive and negative results. Ideal non-laboratory rapid tests are non-instrumental, making use of visual evaluations (e.g., lateral flow and flow-through tests). However, if quantitative or semi-quantitative results are necessary, instrumental methods are more advisable. Current advancements have led to simple, low-cost, and handheld equipment (e.g., portable immunobiosensors). Time for analysis is in terms of minutes. Test kits should be portable, containing stable prepacked reagents, and delivered with easy to use instructions. Low levels (ppb and ppt range) of mycotoxins should be detected and results should be accurate and reproducible. Therefore, to provide confidence to the end-users a well-performed validation of these tests is of outmost importance.

In this presentation an overview will be given of commercially available test kits for mycotoxin detection. These are immunochemistry-based and include ELISA, fluorometric assays, and membrane-based tests (flow-through, lateral flow). Advantages and limitations of the different techniques will be compared. A second part of the presentation will deal with emerging technologies of potential application in the rapid analysis of mycotoxins. Examples are immunosensors, fluorescence polarisation immunoassays, capillary electrophoretic immunoassays, molecular imprinted polymers, aptamers, array biosensors and near-infrared

spectroscopy.

Currently there are two major tendencies, one being improvement of speed and user-friendliness, and the other being the simultaneous determination of multiple mycotoxins. Until now, only few attempts have been made in multi-mycotoxin rapid on-site testing. A new challenge is the efficient simultaneous extraction of the different mycotoxins from different food matrices.

References

Aldred, D. and Magan, N., 2004. The use of HACCP in the control of mycotoxins: the case of cereals. In: Mycotoxins in food. Detection and Control, edited by N. Magan and M. Olsen, Woodhead Publishing Ltd., Cambridge, UK, pp.139-173.

Goryacheva, I.Y., De Saeger, S., Eremin, S.A. and Van Peteghem, C. Immunochemical methods for rapid mycotoxin detection: evolution from single to multiple analyte screening. A review. Submitted to Food Additives and Contaminants.

Sulyok, M., Berthiller, F., Krska, R. and Schuhmacher, R., 2006. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Communications in Mass Spectrometry 20:2649-2659.

Rapid methods for bacterial toxins: potential and limitations

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For more than 100 years bacterial exoproteins have been recognised as primary virulence factors. The mode of action of these toxins includes different categories such as inhibition of protein synthesis, damage of cell membranes, activation of second messenger pathways, and others, which all result in deleterious effects on the host cell. The bacterial toxins, which play a role in foodborne intoxication of man and animal, may be divided into two categories. The first group consists of toxins, which are formed directly in the food, e.g., *Staphylococcus aureus* enterotoxins, *Bacillus cereus* emetic toxin and *Clostridium botulinum* toxins. These toxins may be present in the food even after heating or processing steps and demand direct detection methods. On the other hand there are toxins, which are usually produced in the intestine after digestion of bacteria or spores, e.g., *B. cereus* enterotoxins, *Escherichia coli* toxins and *Vibrio cholerae* toxins and which are analysed in order to gain information about the virulence (quantification, spectrum of toxins) of the bacterial strains involved. Analysis of the latter group usually involves isolation of the strains, an enrichment step and a detection method. In general, most of these toxins are well characterised, but there are still several open questions either on the mode of action and possible receptors, e.g., *S. aureus* and *B. cereus* enterotoxins, or on the three dimensional structure, e.g., *B. cereus* enterotoxins. Answers on these questions will also help to improve the analytical techniques.

For several decades the detection of bacterial toxins was based mainly on biological assays, which are time consuming, costly and show only low specificity. The introduction of antibody based techniques to detect these toxins was the essential step to develop rapid and reliable methods, which have gained a broad range of applications in biomedical research and microbiology. Most of the techniques available today use polyclonal and monoclonal antibodies, whereas the manipulation of antibody genes using recombinant DNA techniques does not play a major role in this field so far. Although numerous such antibodies have been described for analytical purposes, there is still a lack of antibodies against the emetic toxin of *B. cereus* and the 'new' *S. aureus* enterotoxins as well as of immunoassays for *C. botulinum* toxins, which reach sufficient sensitivity. Rapid detection methods for these bacterial toxins are almost exclusively based on immunochemical principles, such as marker-free and labelled reagent techniques used in competitive and non-competitive methods. The most prevalent assay formats are either 'classical' tests, such as microtiter plate formats, immunofiltration assays and capillary migration assays, or a slowly increasing number of biosensor based methods. Although these assays offer detection times of some hours down to a few minutes and in most cases sufficient sensitivity, a further reduction of assay time and improved sensitivity would be desirable for several purposes.

Functional assays for biotoxin detection

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The relevance of functional assays in biotoxin detection stems from their capacity to provide measurements of the overall content of biologically active compounds sharing the same common mechanism of action, by a specific response they induce in sensitive systems. The discrimination between active and inactive components present in a complex mixture is accomplished by a cellular molecule, which selectively recognises the structure of the chemicals, and then operationally behaves like their receptor. Based on this feature, the proximal target molecules of individual biotoxins represent ligand-modulated effectors, and the characteristics of the events triggered in cellular systems depend on the functional properties of the ligand-effector complex.

Functional assays exploit the high selectivity of receptorial systems in the recognition of structural properties of their ligands. Furthermore, functional assays rely on the capacity of receptors to integrate the relative potencies of the biologically active analogues of the chemical class present in a single sample and trigger an overall response in the sensitive system. In functional assays the analytical challenge posed by the extreme complexity of toxin profiles that may characterise natural samples is handled by the biological system itself.

Functional assays for several distinct biotoxin classes exist, but the potential of this type of methods is far from being fully exploited. The concept of toxin-selective molecular markers for the detection of individual classes of bioactive components in sensitive systems, in conjunction with the specificity inherent in the functioning of ligand-modulated effectors, can support more advanced formats of functional assays aimed at the detection of multiple biotoxin classes in the same sample by a single analytical step. Multitoxin detection should be accomplished once the toxin-selective molecular markers are identified for the different classes of compounds, and their effector systems are expressed and functional in the same responsive cell.

Screening of mycotoxins in strongly coloured food matrices

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Mycotoxins are presenting a big problem concerning the safety of food matrices. Beer, spices, wine, etc. are all included in the daily diet and in this way they contribute to the intake of mycotoxins such as ochratoxin A, deoxynivalenol and aflatoxin B1. Therefore screening of these mycotoxins is necessary and rapid tests are desirable. Once such a rapid immunochemistry-based assay is developed for a particular food matrix, this test is not automatically suitable for another one. The matrix can interfere on a peculiar way with membranes, antibodies, enzymes, etc. of the immunoassay because of certain ingredients (colouring agents like flavonoids, melanoidins, etc.). Even within a certain food matrix, different types can give other assay results: a French and Spanish red wine sample can differently react with the clean-up layer (e.g., aminopropyl-derived silica). Dark beer can give more interferences than blond beer. Therefore, a good clean-up is necessary to eliminate interfering compounds.

In this presentation, some simple and rapid clean-up procedures for different strongly coloured food matrices will be discussed. First, a gel-based clean-up tandem assay column was developed for the simultaneous detection of ochratoxin A and aflatoxin B1 in spices. In case of multi-mycotoxin detection, care should be taken in the development of an extraction method capable of efficiently extracting all mycotoxins from the food matrix. In another approach, membranes were applied in a column for the visual detection of ochratoxin A and deoxynivalenol in beer after a suitable clean-up. Both tests are immunochemistry-based. So, there is competition between the mycotoxin in the sample and the mycotoxin-enzyme-conjugate for the specific antibodies bound on the gel or the membrane. After the addition of the substrate for the enzyme, a colour is developing for a negative sample. In case of a positive sample (equal or above the legal limit), no colour appears because of the lack of bound enzyme-conjugate. The tests are developed by adapting the dilutions of the antibodies, conjugates, volume of the sample, wash solution. For the assay of ochratoxin A and deoxynivalenol in beer two different enzyme-conjugates were compared: ochratoxin A-horse radish peroxidase and ochratoxin A-alkaline phophatase. Our goal is to get a blue coloured spot appearing for a sample negative for deoxynivalenol and simultaneously a purple one for the same sample being also negative for ochratoxin A.

References

Goryacheva, I.Y., De Saeger, S., Delmulle, B., Lobeau M., Eremin, S.A., Barna-Vetro, I. and Van Peteghem, C. Simultaneous non-instrumental detection of aflatoxin B1 and ochratoxin A using a clean-up tandem immunoassay column. Submitted to Analytica Chimica Acta.

Innovative microarray tests for food allergen analysis

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For both the systematic as well as for the global analysis of protein function, interaction and abundance, protein microarrays represent one of the pillars underlying modern high-throughput proteomics. In principle, protein biochips are the counterparts of DNA biochip technology, using spatially separated and individually addressable microspots of antibodies, proteins, small molecules or cell extracts contained in a microarray to monitor the function, interaction or expression of (protein) analytes of interest. Apart from the power of analysing protein function on the proteome level, the prospect of developing diagnostic applications has become an attractive goal of researchers in the protein microarray field.

For the development of novel diagnostic applications, protein microarray technology has been employed to immobilise purified (natural or recombinant) antigens or antibodies as capturing agents for the screening of analytes (e.g., IgEs or IgGs) in the serum of diseased patients. Examples include the monitoring of autoantibody responses in autoimmune diseases, alopecia areata, diabetes, systemic lupus erythematosus, allergy, cancer, rheumatoid arthritis, and the profiling of linear allergen epitopes.

Type-1 allergic diseases

Type 1 IgE-related allergy represents a major health burden of industrialised nations (USA, Europe and Japan) where an estimated 25% of the population suffers from IgE-mediated atopic diseases (e.g., asthma, rhinitis, atopic dermatitis, conjunctivitis, or sinusitis). Usually, type 1 allergy is initiated by the generation of IgE antibodies in response to the primary contact of an allergen (e.g., pollen) with the immune system of a potential patient. Following subsequent allergen exposure, allergen-immune complexes formed on mast cells or basophils induce the pathomechanism of allergy, culminating in the release of biological mediators (e.g., histamines or leukotrienes) and the generation of the well-known symptoms of allergy (e.g., rhinitis, asthma, anaphylaxis).

More than 40 years ago, IgE antibodies were identified as central mediators of allergic diseases, and the first concomitant in vitro diagnostic tests for allergy were designed based on the detection of IgE in the serum of allergic patients. For the development of these tests, allergenic material is typically immobilised onto a solid phase, such as the well of a micro-plate, a nitrocellulose membrane, or the surface of a glass slide. Subsequently, the patient's serum is incubated in the presence of immobilised allergen(s), and specific IgE is adsorbed and retained at the surface. Bound IgE is then detected by a specific anti-IgE antibody, bearing a label (e.g., fluorescence or HRP) to generate quantitative results.

Protein microarrays for the profiling of allergen-specific antibodies

The idea of employing protein microarrays to study the presence of disease-related antibodies in blood samples is attractive. Recently, the principle of microarray technology has been adopted into the diagnosis of allergen-specific IgE antibodies. For several decades, diagnosis of type I allergic diseases was performed by assays based on the principle of the radioallergosorbent test (RAST). RAST was originally introduced in 1967, shortly after the discovery of IgE antibodies. Advances in the field of molecular allergology originating in the 1990s have lead to the identification of many common disease-eliciting allergens in

molecular form. These allergens have been characterised on the DNA and protein levels and many have been produced in recombinant form. Eventually, panels of recombinant allergens have been created that comprise the epitope complexity of the corresponding allergen sources, for diagnostic and therapeutic purposes. Based on the availability of purified recombinant allergens, the concept of component resolved diagnosis (CRD) has been introduced in the treatment of type I allergy. Based on CRD, specific immunotherapy (SIT) can be performed using exactly those allergens that have been identified as the disease-eliciting source.

An important advantage of such a component-resolved immunotherapy (CRIT) approach is the fact that allergic patients can be treated according to their unique reactivity profile. In a number of studies, it has been shown that the combination of recombinant allergens with protein microarray technology adds significant benefits to the microarray-based diagnostic approach: (i) the IgE reactivity profile of individual patients to a large number of allergen components can be obtained reliably in a miniaturised format; (ii) microarray-based IgE testing correlates well with state-of-the-art techniques like ELISA, immunoblot or RAST; and (iii) allergen panels can be extended or modified easily to develop population-, region- or disease-specific arrays.

The benefits and future of microarray-based allergy testing

In comparison with other *in vitro* IgE analytical procedures (e.g., ELISA), the fabrication of allergen microarrays consumes only minute amounts of purified or recombinant proteins and can be done in fairly automated batch procedures. Therefore, the resulting biochip-based applications turn out to be cost-effective and can be assessed in accordance with state-of-the-art requirements for laboratory and in vitro diagnostic testing (e.g. reproducibility or accuracy). In addition to the low amounts of biological material required for manufacturing, allergen microarrays typically employ only ml-amounts of clinical samples (such as venous or capillary serum) for the generation of a comprehensive allergen-specific IgE reactivity profile. The latter is especially beneficial in clinical settings where infants and small children have to be analysed in a routine manner.

Above that, the use of different fluorescence labels in combination with individual specific detection antibodies permits the measurement of several analytical parameters (e.g., allergen-specific IgEs and IgGs) in a single assay. Differential quantitative monitoring of IgE and IgG antibodies against a large number of allergen molecules will potentially improve the monitoring of immunotherapy when assessing component-based therapeutic applications or potential side effects (e.g., novel sensitisation acquired du ring immunotherapy). In addition to that, for the development of CRD-based tests, the expandability of the microarray format permits the employment of allergen panels that comprehensively represent the epitope repertoire of the respective natural sources.

Conclusion

In summary, the benefits of microarray-based antibody profiling in allergic patients lie in its potential (i) to individually arrange panels of allergen molecules that reflect the epitope repertoire of a variety of biological sources, (ii) to accurately identify the disease-eliciting agents on a molecular level, (iii) to reveal cross-reactivities to homologous proteins in a single analytical step, (iv) to determine disease-, population-, or regional-specific antibody patterns on a comprehensive scale, and (v) to identify or validate proteins with therapeutic potential.

Rapid detection of aeroallergens

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Labelling practices in food processing are such that information about constituents is usually not sufficient to judge potential health risks for those with occupational exposures. For instance, specific enzymes, which are added to dough improver mixes for the baking industry, are not mentioned on labels. Enzyme use is high in the baking and flour processing industries where a range of enzymes is added to flour as dough improver. Many workers are sensitised against one or several enzymes. Assessment of exposure to enzymes is difficult because this requires airborne dust sampling at the worksite followed by dust extraction and enzyme immunoassay (EIA) analysis at the laboratory. Immunoassays for enzymes are only available in a limited number of highly specialised research laboratories. Use of semi-quantitative lateral flow immunoassays (LFIAs) may allow a more rapid detection procedure with direct on-site demonstration of a bioallergen exposure hazard.

In a field study, we evaluated a recently developed LFIA for fungal alpha-amylase, an important bakery allergen. Airborne and surface dust (wipe) samples and samples from flours and baking additives used at the workplace were collected in 5 industrial bakeries and tested in the LFIA for fungal amylase. For comparison, amylase was measured in sample eluates with the reference EIA method. Sensitivity of the LFIA was 1 to 10 ng/mL, and of EIA, approximately 25 pg/mL. In LFIA, most flour samples, 84% of wipe samples, 26% of personal airborne dust, and none of the 26 ambient air dust samples produced a visible reaction. Wipe samples from dough-making areas and flour samples gave the strongest reactions. All extracts with >5 ng allergen per mL showed a positive LFIA reaction.

The LFIA for fungal amylase is an easy and rapid method to demonstrate the allergen directly at the worksite in less than 10 to 20 minutes. Similar LFIA methods may be used for other occupational allergens in other work environments. Lateral flow immunoassays for occupational allergens may be of great value in occupational hygiene surveys to demonstrate directly to workers and supervisors the hazards of work-related bio-allergen exposure. They may also be helpful in intervention and allergen avoidance strategies for workers with specific allergen sensitisation.

Rapid *ante mortem* tests for TSEs: progress in testing blood for prions

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Bovine spongiform encephalopathy (BSE) is a fatal acquired neurodegenerative disease in cattle, belonging to the group of transmissible spongiform encephalopathies (TSEs) or prion diseases. Since its first recognition in the UK in 1986, BSE has raised great public health concerns because the BSE agent has been shown to cause variant Creutzfeldt-Jakob disease (vCJD) in humans. With the introduction of mandatory active surveillance programs in the European Union the need to develop rapid tests to diagnose BSE has become a high priority. Up to now, the European Union has approved thirteen rapid tests for BSE monitoring in cattle. These rapid screening tests have been used in active surveillance of BSE and have greatly improved the detection of infected cattle before their entry into the human food chain.

At present, no diagnostic test exists for the detection of prions in live animals or humans. Blood of prion infected individuals can contain TSE infectivity. BSE and scrapie can be transmitted between sheep via transfusion of blood, and blood donations from three pre-clinical vCJD patients almost certainly were responsible for infecting three blood transfusion recipients. Detection of the TSE agent in blood has been hampered by the low amounts of PrP^{Sc}, which are estimated to be 10,000-100,000 times lower than those in brain homogenate. Currently used *post mortem* rapid TSE tests are not sufficiently sensitive to detect the low levels of PrP^{Sc} in blood. Most procedures are based on the presence of PrP^{Sc} as an indicator for disease; however some tests are directed at detecting non-prion biomarkers. One such method uses mid-infrared spectroscopy, where subtle changes in infrared spectra in blood of TSE affected animals are used to diagnose TSEs.

Many of the approaches to *ante mortem* tests use concentration steps to enrich PrP^{Sc} from blood. This is achieved through precipitation with phosphotungstic acid or by increasing PrP^{Sc} fibril complex size by addition of streptomycin followed by ultra centrifugation and detection with PrP specific antibodies. These methods, however, involve Proteinase K digestion to distinguish PrP^{Sc} from PrP^{C} and may have serious disadvantages as PrP^{Sc} in blood might be less protease resistant than its counterpart in the brain. Approaches that seem very promising at the moment are directed at specific capture of PrP^{Sc} from the infected blood. In this approach fluorescently labelled palindromic peptides of the prion protein or sulphated poly anions (e.g., heparin sulphate) on magnetic beads bind ß-sheet-rich PrP, obliviating the need for Proteinase K digestion. Detection then follows with anti-PrP antibodies on ELISA or Western Blot or by fluorescence measurements.

A very elegant method, that is also Proteinase K independent, uses the prion specific antibody 15B3 that only binds PrP^{Sc}, but not the normal form of the prion protein for detection of the TSE agent in blood. Two different assay formats have been developed, ELISA and fluorescence activated cell sorting (FACS) based on a capture and enrichment of PrP^{TSE} from serum or plasma using the prion specific antibody 15B3 and a subsequent detection step with a secondary anti-PrP specific antibody. With this antibody sandwich we are able to detect brain derived PrP^{Sc} spiked into serum in the femtomolar range which is about 2-3 orders of magnitude more sensitive than currently approved TSE *post mortem* tests.

Rapid strip tests for the detection of deoxynivalenol in wheat and T-2 toxin in wheat and oat*

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Mycotoxins are toxic natural secondary metabolites produced by several species of fungi on agricultural commodities in the field or during storage. Deoxynivalenol (DON) and T-2 toxin are prevalent representatives of the B- and A-type trichothecenes, respectively, produced by *Fusarium* spp. Rapid strip tests were developed to test DON in wheat at a cut-off level of 1.25 ppm, and T-2 toxin in wheat and oat at a cut-off level of 100 ppb. These are one-step lateral flow immunochromatographic tests based on a competitive immunoassay format. An antibody-colloidal gold particle complex is dissolved in assay diluent and mixed with 50 µL sample extract in a microwell. The strip test is inserted into the well and the mixed content then migrates onto a membrane, which contains a test zone and a control zone. Mycotoxin-protein conjugate coated on the test zone captures free antibody-colloidal gold particle complex, allowing colour particles to concentrate and form a visible line. A positive sample with a contamination greater than the cut-off level will result in no visual line in the test zone. Alternatively, a negative sample containing less than the cut-off level will form a visible line in the test zone. One line will always be visible in the control zone regardless of the presence of DON or T-2 toxin confirming correct test development.

The tests are rapid qualitative methods with assay results to be obtained within 2 minutes. A semi-quantitative T-2 lateral flow device (LFD) was furthermore developed using a photometric reader for read-out in the range of 0-500 ppb. The accuracy of the LFDs for the detection of DON and T-2 toxin was determined using spiked and naturally contaminated wheat and oat samples. Results indicated that the tests are accurate and effective for qualitative and/or semi-quantitative measurement of DON and T-2 toxin, respectively, greater or less than the cut-off level in agricultural commodities such as wheat and oat.

* Contributed paper

EU-eBIOSENSE, electrical biosensor arrays for the analyses of *Salmonella,* mycotoxins and toxigenic fungi in food and feed*

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Detection tools for the detection of contaminants in food and feed are constantly evolving. In the EU consortium eBIOSENSE, electrical chip technology, molecular biology and advanced immunology are integrated for fast, cheap and robust detection of bacterial contaminants, mycotoxins as well as their corresponding fungi. The platform is based on conductivity of the electrochemical mediator amino-phenol, which is released from electrically inactive p-amino-phenol-phosphate by alkaline phosphatase. Conductivity is measured by interdigitating electrodes, which facilitate redox-cycling and micro-diffusion, leading to a 100-fold signal amplification.

Two different platforms are being developed: a protein-based chip for the detection of ochratoxin A, fumonisin and DON and a DNA based chip for the detection of fumonisin producing species, e.g., *Fusarium verticillioides* and *F. proliferatum* as well as *Salmonella enterica* species.

To detect mycotoxins we raised highly specific monoclonal antibodies in Llama, which have the unique feature that they consist of only heavy chain moieties. Characterisation of the antibodies is currently in progress.

DNA based chips have been designed on the basis of sequence data obtained from the polyketide synthase gene (*fum1*) of *F. verticillioides*, *F. proliferatum*, *F. nygamai* and *F. globosum*, all *Fusarium* species known to produce fumonisin. Through the selection of proper regions, capture and detection oligonucleotides have been designed that allow the discrimination between *F. verticillioides* that occurs mainly on maize and *F. proliferatum* that is much more polyphagous. Currently the detection of *Fusarium* DNA using the electric chip is being optimised to improve sensitivity and robustness. This electric chip approach might in time lead to extensive automation of early warning systems for *Fusarium* spp. in cereals and other commodities.

In parallel we target the *invA* gene of *S. enterica* to detect its presence in foodstuffs including fresh vegetables.

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* Contributed paper

A three-prong approach to ensuring confidence in sanitation and food quality*

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With consumer awareness of food safety issues at a high, the need for producers to effectively monitor and react to sanitation and food quality issues has never been more important. Recent research showed that food safety alerts might result in a long lasting impact on purchasing decisions by consumers; up to 15% state they stop eating a product entirely after a food safety incident.

Final product quality assurance starts with careful supply chain and process analysis and Neogen is in the unique position to be able to offer a complete tailor-made testing solution for three of today's most important quality issues: rapid sanitation monitoring, labelling compliancy for allergenic proteins and rapid microbiology testing. Neogen's wide range of diagnostic products, coupled with its experience and expertise allows us to be able to offer complete and practical testing solutions for the modern food and beverage industry regardless of the size of operation and level of technical competency.

* Spotlight presentation

Bacterial pathogen detection using a novel light scattering technology

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The advances in biophysical methods in diagnostics have created an increasing interest in light scattering technologies such as spectroscopy, surface plasmon resonance, and optical forward scattering technologies for label-free detection of pathogens from food, clinical specimens or environmental samples. The light scatter signature of an illuminated object is dependent on the refractive index, composition, size, and shape. It is expected that bacterial cell morphology and composition are reproducible for each culture under a similar growth environment. Differential polarised light scattering had been used for detection of bacterial cells in suspension, but with limited success.

Our team has developed an optical forward scattering sensor for label-free detection of bacterial colonies growing on the solid agar surface. The sensor consists of a diode laser source, a Petri-plate holder, and digital image acquisition system and image analysis software for identification of the culture. A bacterial colony results from an exponential multiplication of presumably a single cell on a nutrient agar surface and the colonies grow radially on the surface, forming disc- or dome-shaped structures and each bacterium produces colonies of characteristic size, shape and composition depending on its metabolic and genetic make up. We employed light scattering sensor to generate light scatter signature of colonies of different bacterial species belonging to genera *Listeria, Salmonella, Escherichia, Vibrio*, and *Staphylococcus*, which were very unique and each could be differentiated with 90-100% accuracy. Extracellular materials produced by each culture in a colony along with cellular arrangements are thought to provide the unique scatter signatures. Application of this technology in foodborne pathogen detection and identification from various spiked food samples is very encouraging which will be discussed in detail.

In summary, the light scattering technology is a non-invasive, non-destructive and virtually reagentless simple method, which takes only few seconds to identify a bacterial colony on the agar plate. Since this is a non-destructive method, if needed, the same bacterial colony can be used for further confirmation by standard biochemical, serological or nucleic acid based methods.

Luminescent techniques for microbiological analysis of foods: future trends

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There are many naturally bioluminescent organisms existing in nature and the mechanisms whereby these creatures emit light has been fully characterised. These include the luciferin-luciferase system of bacteria, insects (fireflies and click-beetles) and the jellyfish *Aequorea victoria*. In essence, bioluminescence involves the conversion of chemical energy into light energy by an enzyme, commonly termed luciferase. The luciferases from different organisms catalyse different reactions but all require oxygen. A major advantage of using bioluminescent systems as analytical tools is that extremely low levels of enzyme activity can be detected by measuring the emitted light. Modern instruments are capable of detecting single photons with both temporal and spatial distribution; thus providing accurate information on the location and intensity of the light source. Another feature of bioluminescent systems that makes them an excellent investigative tool is an almost absolute specificity for their substrates. For example, for firefly luciferase even minor changes in the structure of ATP and firefly luciferin result in a total loss of enzyme activity and this consequently results in a loss of light emission. This specificity for substrates allows the real-time measurement of luciferase activity *in situ* in very complex samples without the need for any pre-treatment.

The best known among bioluminescent methods is the ATP-assay, based on use of firefly luciferase. This assay has been used to determine biomass and is based on the principle that all living cells contain ATP and the ATP levels are proportional to the number of cells present. Thus, the amount of light emitted by the luciferase/luciferin reaction following extraction of ATP from cells provides an estimate of cell populations. Arguably the most widely used application of this technology is 'ATP hygiene monitoring'. This involves assessment of ATP levels on environmental swabs, which directly relates to the cleanliness of the surface. Several commercial systems are available. In a twist to hygiene monitoring, it has been proposed that these tests can also be used to assess the potential for the presence of allergens on food contact surfaces.

Using methods for differential extraction of ATP from prokaryotic and eukaryotic cells it has been possible to develop assays for microbial cells that can be performed in minutes. However, the detection limit for these assays is high ($>10^6$ cfu/mL) and methods to improve their sensitivity have been a preoccupation of many microbiologists over the last two or three decades. To improve sensitivity, techniques involving filtration or centrifugation have been investigated and these have made it possible to detect approximately 10^4 cfu/mL in foods. This sensitivity can be further improved through assays of adenylate kinase, an enzyme present in cells that can be made to produce ATP by the addition of ADP in excess.

Another drawback of ATP bioluminescence assays is their inability to distinguish between the types of organisms present either in food or on food contact surfaces. This has been addressed in a variety of ways. Recently there has been interest in coupling ATP bioluminescence assays with immunomagnetic separation, but the main disadvantage of this is the possibility of non-specific binding of microorganisms to the paramagnetic beads used in the technique. A commercial system has appeared that utilises host-specific bacteriophage to lyse target bacteria and the adenylate kinase that is released can be assayed using a bioluminescent method.

Bacteriophages have been used in other ways to detect pathogens using a bioluminescent platform. Several researchers have used bacteriophage modified to carry luminescent of fluorescent reporter genes. When the host cell is infected by the phage, the reporter genes are expressed and light is emitted. It has been shown that this method can be used for direct detection of bacteria on food surfaces. As well as the structural genes encoding the enzymes involves in the bioluminescence reaction, researchers have constructed phage containing the *luxI* gene which encodes a signalling compound that then induces several other genes, *lux*CDABE, whose activation leads to bioluminescence in a reporter bacterium present on a biosensor. We are currently investigating methods for the immobilisation of phage that will make it possible to concentrate and detect bacteria in a single step. It has also been proposed that biotinylated phage coupled with quantum-dot nanocomplexes can be used to detect bacteria.

An interesting application of bioluminescence is the 'CANARY' biosensor. This involves a macrophage cell that exhibits pathogen-specific antibodies on its surface. When the target cell binds to the antibody natural signalling pathways are triggered in the cell, which can be detected by aequorin that is expressed by the macrophage. This method has been applied to the detection of *Escherichia coli* O157:H7 in ground beef.

Bioluminescent assays have also been developed for the detection of toxicants, antibiotics and cellular metabolites.

Much recent research has been focused on *in vivo* imaging of bacterial pathogens that have been genetically modified to carry luciferase genes. The efficacy of treatments to prevent foodborne infections can be determined using this technique.

As well as building on current applications, there are several new and exciting opportunities offered to microbiologists by bioluminescence.

Rapid methods for the detection of foodborne viruses

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Epidemiological evidence shows that the viruses primarily associated with foodborne illness are *Norovirus*, causing gastroenteritis, and hepatitis A virus. Recent years have seen a proliferation of publications on methods for detection of these viruses in foodstuffs using PCR. However, thus far no standard harmonised procedures have been elaborated and formalised quality assurance procedures are generally lacking. This laboratory runs a worldwide external proficiency testing programme for detection of viruses in molluscan shellfish. The results illustrate the current diversity of methods in use, their variable performance, and the urgent need for standardisation and formal quality assurance. This is particularly necessary before virus methods can be considered for adoption within a regulatory framework. This presentation highlights data from the Cefas proficiency testing programme and focuses on European progress towards standardisation and validation of methods through the European Committee for Standardisation (CEN) working group on detection of viruses in foodstuffs.

Rapid detection of toxigenic fungi in plants

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Mycotoxins are fungal metabolites with toxic activity toward humans and animals. They are natural contaminants of agrofood products, food and feed. Cereals and grapes are food commodities showing a high level of mycotoxin contamination. Data from the Food and Agriculture Organization of the United Nations (FAO) estimate that every year 10-30% of the amount of cereals can be contaminated with mycotoxins. Mycotoxins also show a high level of resistance to the most common chemical, physical and microbiological techniques used in cereal processing. Among cereals, especially maize can be contaminated with mycotoxins mainly produced by fungal species belonging to the *Fusarium* genus, each with a specific mycotoxin profile. Several mycotoxins produced by *Fusarium* occur in maize in the field, among which fumonisins are the most frequent and dangerous. Fumonisins cause several animal diseases such as pulmonary oedema in swine and leukoencephalo-malacia in horses, and they have also been related to human oesophageal cancer. Moreover, trichothecenes such as deoxynivalenol and nivalenol, potent protein synthesis inhibitors, and zearalenone, capable of estrogenic capability, can occur in maize kernels. The European Union set maximum limits (aflatoxins and ochratoxin A) for maize-based products to reduce the risks for consumers. However, also fumonisins, deoxynivalenol and nivalenol, and zearalenone are and/or will be soon regulated.

On the other hand, since a decade the presence of ochratoxin A (OTA) in wine and grape juices has become a relevant mycotoxicological problem mostly in Southern-Europe, where the concentration of OTA particularly in red wines is often high. These findings roused a big alarm among consumers, wine producers, and national and European authorities. OTA is produced mainly by species of the genera *Aspergillus* and *Penicillium*. However, several recent studies have shown that the presence of OTA in wine is due to contamination by black aspergilli, primarily strains of *A. carbonarius* and others belonging to the *A. niger* species aggregate causing a disease called 'black rot' disease. Each species usually possess a specific mycotoxin profile, which determines the potential toxicological risks. *A. carbonarius* appears to be the most important fungal source of OTA contamination, because of the frequency of its occurrence on grapes and the highest proportion of toxigenic strains among its field population.

It is important for agrofood companies involved in cereal storage and processing, and grape processing for wine production to have rapid, reliable and highly sensitive analytical methods to detect toxigenic fungi in their products and to evaluate their occurrence and potential risks in order to establish prevention strategies. Traditional methods of fungal identification, still mainly based on physiological and morphological features, are laborious and time-consuming. Therefore, they are not efficacious for screening on a commercial scale. Innovative systems based on DNA identification could present an interesting alternative for a real-time screening of bio-safety of field products and food. The sensitivity of such techniques could provide the identification of single molecules of a given nucleotidic sequence belonging to a specific fungal species. This could provide a tool for early diagnosis of mycotoxigenic fungi on food products, also in the absence of typical symptoms of contamination.

Different PCR assays have been developed for the detection of mycotoxigenic species, some of which were adapted for the detection of the fungal pathogens directly from food and

plant matrices. We have developed two different rapid assays using sequence analysis of some variable and conserved DNA regions of the calmodulin gene useful to design molecular markers:

- A TaqMan real-time PCR assay for identification and quantification of genomic DNA of *A. carbonarius, F. verticillioides, F. proliferatum* and *F. subglutinans* occurring on naturally contaminated grapes and maize samples, respectively, to predict the potential risk of toxin production in the above mentioned food products.
- A new type of low complexity oligonucleotide microarray (OLISA™, OLIgo Sorbent Arrays - APIBIO) based on DNA oligonucleotide probe specificity at species level to set up a rapid molecular method for simultaneous identification of *A. carbonarius, A. japonicus / A. aculeatus* and *A. ibericus* from grapes.

Detection of *Legionella* with a microsieve and a LED-based photonic device

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The presentation will discuss (i) the fluXXscan method, (ii) the application of this method for detection of microorganisms in general and *Legionella* bacteria in particular, and (iii) possibilities for future developments.

Before focusing on applications the method developed by Centre for Concepts in Mechatronics and fluXXion will be characterised. As brief summary is given below.
The fluXXscan method consists of the following three steps:

- Filtration of the fluid to be investigated using a silicon wafer based micro-sieve; the remaining residue will contain the microorganisms to be detected.
- *In situ* marking of the microorganisms with fluorescent markers.
- Scanning of the sieve surface using a fluorescence microscopy system; analysis of the separate fluorescence images enables the counting of the separate microorganisms.

As described above it is clear that the fluXXscan method does not require a multiplication/ amplification step. As a consequence this method can be considered to be a rapid method for detection. A complete cycle time from start of filtration until the finish of the data analysis can be well below 30 minutes.

During the presentation we will zoom in to several relevant aspects of the process like:

- The special properties of the silicon wafer based microsieves and the effects on the process; especially the pore size distribution, the flatness of the sieve and porosity of the sieves will be discussed.
- Usage of different types of markers - the type of marker used will define the type of analysis.
- Surface scanning microscope - important optical properties like numerical aperture, working distance and focusing to the surface and scan speed

A prototype model for demonstrating the method has been developed and applied to detect *Legionella pneumophila.* The experiments were performed under the following conditions:

- microsieve pore diameter 0.45 µm;
- colouring - fluorescent in situ hybridisation (FISH); and
- detection using prototype set-up of fluXXscan.

Results will be shown and discussed in detail during the presentation.

The presentation will conclude with a discussion on future developments:

- complete automation of the from sample delivery to results; and
- an in-line version for fab automation.

Isolation of selected Shiga toxin-producing *Escherichia coli* (STEC) serotypes from artificially contaminated samples

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A novel range of serotype-specific Shiga toxin-producing *Escherichia coli* (STEC) isolation methods was used for isolation of O26, O103, O111, O145, and both sorbitol positive and negative O157 strains from various artificially contaminated sample matrices. Sample matrices evaluated were raw milk, scald milk and raw milk cheeses, minced beef, salami sausage and cattle faeces. Samples were contaminated at different levels ranging from < 1 to 500 cfu per gram of sample. A detection limit of <1 cfu was obtained for all matrices, except for cattle faeces, for which a detection limit of 15 cfu per gram was obtained. Inclusion of immunomagnetic separation (IMS) is needed to improve the sensitivity of the isolation method for faeces.

A PCR-DGGE approach to explore the predominant microbial spoilage flora associated with modified atmosphere packaged sliced cooked meat products*

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The consumption of cooked meat products such as ham, turkey and chicken breast is steadily increasing in Belgium. A large part of these are sold in supermarkets as sliced products packaged under vacuum or modified gas atmosphere. Accordingly, the portion of pre-packaged cooked meat, mostly modified atmosphere packaged (MAP), to the total amount of cooked meat sold in Belgian retail, increased from 40% in 2003 to 52% in 2004. The combination of microaerobic conditions, the presence of NaCl and $NaNO_2$ and a reduced a_w, favours growth of psychrotrophic lactic acid bacteria (LAB) and inhibits growth of Gram-negative spoilage flora (Borch et al., 1996; Korkeala and Bjorkroth, 1997). The presence of LAB *in se* is not a danger for public health, but they can cause undesired defects such as off-flavours, discoloration, gas production, exudates formation, pH decreases and slime production, resulting in premature spoilage of the end-product and a reduction of shelf-life (Borch et al., 1996; Korkeala and Bjorkroth, 1997; Samelis et al., 1998).

In production plants, general plate count methods are routinely used throughout the production process in microbial quality assessments of MAP meat products. However, these assays only result in divergent information on the microbial quantity and do not provide any qualitative information on the composition nor the origin of the bacterial community. In recent years, denaturing gradient gel electrophoresis (DGGE) of community amplicons obtained from ubiquitous genes such as the 16S rRNA gene has proven to be a versatile method to assess the biodiversity and population dynamics of microbial communities occurring in various ecosystems including terrestrial, aquatic, gastrointestinal and food environments (Muyzer et al., 1993). So far, the use of PCR-DGGE in food microbiology mainly focussed on the study of bacterial successions in fermented foods such as sausages, whisky, sourdoughs, and cheese (Ercolini, 2004) or on the compositional characterisation of probiotics (Masco et al., 2005; Temmerman et al., 2003). Recently, PCR-DGGE was used to investigate the occurrence of undesired bacterial flora in food matrices such as cheese (Cocolin et al., 2004), wine (Bae et al., 2006), gelatine (De Clerck et al., 2004) and meat (Takahashi et al., 2004) as well.

In the present study, a PCR-DGGE protocol was optimised to analyse the flora associated with spoilage of MAP sliced cooked meat products. The cultivable fraction of three different batches of three cooked meat product types (turkey, chicken breast and ham) stored at 4°C during shelf life were analysed at sell-by date on MRS and PCA medium at 20°C, 28°C and 37°C under aerobic and anaerobic conditions in order to recover a broad range of bacterial groups associated with the sampled MAP products. Subsequently, the bulk of bacterial colonies was harvested from the respective media and used for DNA extraction using the method described by Pitcher and co-workers (Pitcher et al., 1989). In parallel, DNA was also directly extracted from matrix material to validate the relevance of plating assays. The based

* Contributed paper

amplification of the V3 region (approximately 200 bp) of the 16S rRNA gene (Yu and Morrison, 2004) from community DNA extracts. Amplicons were separated by DGGE using a 30-70% gradient. Based on relative dominance, specific DGGE bands were further analysed by band excision and subsequent sequencing. Irrespective of aerobic or anaerobic incubation conditions, DGGE fingerprints originating from cultivable fractions from MRS (lactic acid bacteria, LAB) and PCA (total heterotrophs) displayed a high level of similarity indicating that LAB represent a predominant group in the cultivable bacterial community. In addition, the high frequency of three DGGE bands in different batches of each matrix type pointed to a 'core' spoilage flora. The involvement of a 'core' microbial flora in spoilage of cooked meat products was confirmed when comparing fingerprints of two poultry products (turkey and chicken) and one pork product (ham): three bands appeared to be relatively common among the three matrix types. In addition, some other bands tended to be matrix-specific and/or batch-specific. In order to validate the PCR-DGGE analysis protocol of cultivable fractions, cooked meat products were also subjected to culture-independent DGGE analysis by direct DNA extraction from a matrix suspension. Overall, the same dominant bands were observed in both approaches although some weak bands detected in the matrix DNA extracts were not observed during analysis of any of the cultivable fractions. Based on band sequencing and positioning, the three major DGGE bands that were relatively common in all cooked meat products could be assigned to bacterial taxa most closely related to the spoilage organisms *Lactobacillus sakei, Carnobacterium divergens* and *Leuconostoc carnosum,* respectively. Along with these bacteria, other LAB such as *L. curvatus* and *Pediococus pentosaceus* were rather batch and/or matrix specific.

In conclusion, spoilage of MAP cooked meat products is mainly associated with LAB. PCR-DGGE analysis of cultivable fractions associated with MAP sliced cooked meat products shows good potential to explore the diversity and persistence of predominant spoilage LAB.

References

Bae, S., Fleet, G.H. and Heard, G.M., 2006. Lactic acid bacteria associated with wine grapes from several Australian vineyards. Journal of Applied Microbiology 100:712-727.

Borch, E., KantMuermans, M.L. and Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. International Journal of Food Microbiology 33:103-120.

Cocolin, L., Innocente, N., Biasutti, M. and Comi, G., 2004. The late blowing in cheese: a new molecular approach based on PCR and DGGE to study the microbial ecology of the alteration process. International Journal of Food Microbiology 90:83-91.

De Clerck, E., Devos, J. and De Vos, P., 2004. Molecular characterisation of bacterial contamination in semi-final gelatine extracts, using denaturing gradient gel electrophoresis. Systematic and Applied Microbiology 27:612-619.

Ercolini, D., 2004. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. Journal of Microbiological Methods 56:297-314.

Korkeala, H.J. and Bjorkroth, K.J., 1997. Microbiological spoilage and contamination of vacuum-packaged cooked sausages. Journal of Food Protection 60:724-731.

Masco, L., Huys, G., De Brandt, E., Temmerman, R. and Swings, J., 2005. Culture-dependent and culture-independent qualitative analysis of probiotic products claiming to contain bifidobacteria. International Journal of Food Microbiology 102:221-230.

Muyzer, G., Dewaal, E.C. and Uitterlinden, A.G., 1993. Profiling of complex microbial
populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59:695-700.

Pitcher, D.G., Saunders, N.A. and Owen, R.J., 1989. Rapid extraction of bacterial genomic DNA with guanidine thiocyanate. Letters in Applied Microbiology 8:151-156.

Samelis, J., Kakouri, A., Georgiadou, K.G. and Metaxopoulos, J., 1998. Evaluation of the extent and type of bacterial contamination at different stages of processing of cooked ham. Journal of Applied Microbiology 84:649-660.

Takahashi, H., B. Kimura, Yoshikawa, M., Gotou, S., Watanabe, I. and Fujii, T., 2004. Direct detection and identification of lactic acid bacteria in a food processing plant and in meat products using denaturing gradient gel electrophoresis. Journal of Food Protection 67:2515-2520.

Temmerman, R., Scheirlinck, I., Huys, G. and Swings, J., 2003. Culture-independent analysis of probiotic products using denaturing gradient gel electrophoresis (DGGE). Applied and Environmental Microbiology 69:220-226.

Yu, Z.T. and Morrison. M., 2004. Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-denaturing gradient electrophoresis. Applied and Environmental Microbiology 70:4800-4806.

Electrical chips for the monitoring of biomarkers*

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The electrical chip is a small and easy to handle device for the detection and monitoring of biomarkers like RNA, DNA and proteins. The detection of the appropriate analyte is based on the technology of interdigitated gold ultramicroelectrode arrays manufactured in silicon technology by eBiochip Systems combined with a sandwich hybridisation assay. The detection principle is an electrical readout of the red/ox-electrodes. Two different detection technologies, based on the sandwich hybridisation, are possible. The first one is the array-based sandwich hybridisation. In this case, a biochip with multiple electrode arrays is used where the different positions of the array are immobilised with the appropriate capture molecules. The capture molecules allow the specific binding of the analyte of interest on the defined array position. The second, the bead-based technology, is based on the use of paramagnetic beads as a solid support and carrier for the capture molecules. The detection of the analytes is in both technologies achieved by a labelled detection molecule that binds to the analyte. The labelled detection molecule allows the binding of a reporter enzyme. The enzyme hydrolyses the substrate and generates an electrochemically active product that is measured via 'redox-recycling' at the electrodes which leads to an electrical signal that corresponds to the amount of detected analyte.

Different applications of the electrical chip technique will be addressed and discussed in this contribution. For example, data will be presented which demonstrate that the electrical chip technology allows a fast and reproducible expression analysis of critical and significantly expressed marker genes during biotechnological industrial production processes. Furthermore, it will be revealed that this technology combined with an automated sample preparation can establish a basis for continuous at-line monitoring of the host cell physiology, by measuring the significantly expressed marker genes during fermentation processes. Finally, first results will be shown that electrical DNA- and protein-chips might also potential tools for the food industry, and medical and pharmacological diagnostic purposes, like the detection of pathogens, e.g., staphylococci , or the detection of autoimmune diseases.

* Contributed paper

Acrylamide & Co - analysis of processing contaminants in food

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Heat induced food contaminants, such as acrylamide, furan, or chloropropanols have recently gained a lot of attention on all levels of society. The finding of substances classified by the International Agency on Research on Cancer as probably carcinogenic to humans (acrylamide) or potentially carcinogenic to humans (furan) in staple food caused concern on the global scale. The European Commission has set measures to either restrict their content in food to a certain level, or is initiating perennial monitoring campaigns on the EU level to evaluate the effectiveness of voluntary mitigation measures of industry.

The mentioned substances have different precursors and different formation mechanisms in food. Common to them is that they are small molecules and that their chemical and physical properties need special consideration in chemical analysis. A number of approaches have been developed in recent years for their determination in different food matrices. Most often mass spectrometric methods are applied for the detection of the analytes. These include gas chromatographic methods with single stage mass analysers, but also tandem mass spectrometry and high-resolution mass spectrometry. Especially for acrylamide a number of liquid chromatographic tandem mass spectrometric methods have been established. Alternative methods employing capillary zone electrophoresis, or sensor techniques have been described only very recently.

By reason of topicality, the presentation aims to give an overview of mainstream methods that are applied for the determination of acrylamide, furan, and chloropropanols in food, but will also highlight recent developments and new approaches.

New developments in the rapid detection of pesticide residues

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It is clear that global scale environmental monitoring must be carried out with high-throughput screening (HTS). The number of environmental contaminants and their toxic degradation co-products can be present in real samples in a wide range of concentrations: from negligible or 'zero' to very high levels up to µg per mL. Moreover, the distribution of environmental contaminants in time and different types of samples may also be very variable. These factors mean that high numbers of sample must be measured for different contaminants for correct quantitative estimation and statistical interpretation of chemical contamination results.

The current assay methods like chromatography techniques have several limitations for HTS. The analytical methods applied for detection of environmental contaminants must ideally be quick, cheap, and simple with high throughput in a wide operating range. These requirements are best met by immunoassays methods.

At present, the enzyme-linked immunosorbent assay (ELISA) is dominating as developed and applied immunoassay method for detection of environmental contaminants. Fluorescence polarisation immunoassay (FPIA) is a useful alternative approach to ELISA, because it is a homogeneous method without any separation and washing steps as in common ELISA. FPIA is a direct competitive immunoassay method. The method is based on the measurement of fluorescence polarisation (FP) values for reaction mixtures of samples and immunoreagents: specific antibodies and fluorescent-labelled antigen (tracer). The FP value for free tracer is significantly low in comparison with FP for tracer bonded to specific antibody. To perform the assay only the tracer and the antibody solutions must be added to an aliquot of liquid sample (typically 10-50 µL), mixed and measured. The analytical signal FP could be measured in a few seconds. The total time required for an assay is a few seconds or minutes, depending of pipettes and instrument used.

Recent results for the development of FPIA for pesticides like pirimifos-methyl, imazalil, acetochlor, butachlor and others will be presented and discussed.

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The rapid detection of antibiotic residues in food products

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Due to intensification in modern farming systems, the use of antimicrobials in the production of food-producing animals for the treatment or the prevention of specific diseases has become a well-established practise, certainly in the developed countries. Each administration of a veterinary drug can result in residues in edible products. Residues of antibacterial substances in foodstuffs from animal origin could raise technological problems for the food industry in the production of fermented products or could represent a risk for the consumer of these products. Hence the possible presence of residues of antimicrobials in primary food-stuffs of animal origin as milk, meat, honey is a critical control point in the HACCP system of food producers which requires (rapid) test methods.

For screening purposes microbiological inhibitor tests are generally used. The test principle of microbial inhibition assays is usually based on the detection of growth inhibition, noticed visually by interpreting the colour change of a pH-indicator in the test medium or by measuring the diameter of a clear inhibition zone around a with sample impregnated filter disk of a small piece of sample. The following bacteria are widely used in microbial inhibitor tests: *Geobacillus stearothermophilus* var. *calidolactis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli and Micrococcus luteus*. However, due to the long incubation period ranging from 3 up to 16 hours to allow growth of the test organism, microbial inhibitor tests are too slow for entrance control. So more rapid tests were developed for the control of milk, meat, fish and honey. These fast enzymatic, receptor or immunological tests are more substance/ group specific.

In dairy production the main cause of the presence of inhibitors is related to the treatment of clinical mastitis therapy and secondly to drying off therapy. The most occurring residues in raw milk belong to the group of β-lactam antibiotics (penicillins and cephalosporins). So many rapid tests (test result within 10 minutes) for the detection of ß-lactams in milk, such as the receptor tests SNAP, Charm MRL Beta-lactam Test (ROSA), Beta s.t.a.r. and the immunoassay Parallux, are commercially available. In many countries such tests are used in routine at dairy plants to screen the incoming milk on the presence of antimicrobials (β-lactam antibiotics) on MRL (maximum residue limits) or tolerance level. More than 90% of all loads with antimicrobial residues can be identified and rejected in this way and so the most dominant cause of failures in the production of cheese and yoghurt can be prevented.
More recently also rapid tests became available for the detection of other groups of antimicrobials like tetracyclines (TetraSensor Milk, SNAP Tetracyclines and Charm Tetracyclines Test [ROSA]) or for the simultaneous detection of β-lactams and tetracyclines (TwinSensor Milk and Charm ROSA BL/TET). In order to allow control before the collection of the milk at the farm, tests giving a result within 3 minutes (Charm ROSA MRL-3 and Beta-s.t.a.r. [2 minutes procedure]) were recently developed.

Most of these rapid tests were validated at the Institute for Agricultural and Fisheries Research (Unit Technology & Food) or validation studies are still going on (Charm MRL-3 test and Beta-s.t.a.r. [2 minutes procedure]). In Table 1 an overview is given of the test sensitivities found in the validation studies of some rapid tests for the most important β-lactam compounds.

Table 1. Test sensitivities of the rapid tests for the most important β-lactam compounds (penicillins and cephalosporins). MRLs (Council Regulation No. 2377/90 and amendments, situation 01/2007) and test sensitivities are expressed in µg/kg (ppb).

Substance	MRL	Parallux (5 min)	New SNAP (9 min)	Beta-s.t.a.r. (5 min)	Charm MRL Test (8 min)	TwinSensor Milk (6 min)
benzylpenicillin	4	4	3	3	2	2
ampicillin	4	8	5	4	3	3
amoxicillin	4	6	7	4	3	4
cloxacillin	30	8	35	6	25	6
nafcillin	30	15*	80	14	45	50
ceftiofur	100	35	3	110	6	10
cefquinome	20	250*	20	10	14	30
cefazolin	50	-	15	60	10	18
cefapirin	60	16	9	12	5	6
cefacetril	125	-	10	40	6	35
cefoperazone	50	-	12	6	4	3
cefalexine	100	-	25	>1000	15	>200
cefalonium	20	-	4	4	3	4

- = not detected; * = cross-reactivity

The main occurring antimicrobial residues in meat are belonging to the group of tetracyclines, β-lactams and (fluoro)quinolones (poultry). The rapid milk assays SNAP (β-lactams, tetracyclines) and Parallux can also be used for testing in this matrix after a special sample preparation. The Parallux can also be used for the control of kidneys. Meat and fish can be screened in 10 minutes after a simple sample preparation on the presence of tetracyclines by means of the TetraSensor Tissue. For the detection of (fluoro)quinolones no real rapid test is existing, the performance of an ELISA after sample preparation takes 90 minutes. About the same total test time is required to check the presence of residues of an antibiotic group in animal tissues with a Charm II test; reagents of Charm II tests are available for the most important antibiotic families. Broad-spectrum inhibitor tests for the control of meat or kidney take at least 3 hours.

Antibiotics and chemotherapeutics are sometimes used in apiculture to prevent or to treat bacterial brood diseases as American foulbrood (*Paenibacillus larvae* subsp. *larvae*) or other bee diseases as nosemosis. This practice, causing residues in the honey is illegal in Europe since no MRLs for anti-infectious agents are fixed for honey (Council Regulation No. 2377/90). The main problems of residues in imported honey or honey from professional beekeepers are related to streptomycins, tetracyclines, sulfonamides, (fluoro)quinolones, macrolides and chloramphenicol (Chinese honey). A receptor-based assay using dipsticks, Tetrasensor Honey, is available for a rather rapid (30 minutes) and simple screening of honey on the presence of tetracyclines. Since no special equipment is needed, even beekeepers or honey packers can easily perform the test. Depending on the type of tetracycline, a detection capability between 6 and 12 µg/kg was obtained in a validation study at T&V-ILVO. When the dipstick becomes dry, the detection capability further improves. When reading dry dipsticks, detection capabilities between 4 and 7 µg/kg were obtained. The test time of Charm II tests for honey for the other compounds or groups is about 30 minutes after a simple (streptomycin, tetracyclines) or a long and work intensive (macrolides, sulfonamides) sample preparation. Besides different ELISA assays for the detection of chloramphenicol, recently also a single one step test device (Chloramphenicol Residue Rapid Inspection Device) became available, giving results in about 40 minutes (sample preparation plus assay).

For a high throughput rapid multi-analyte screening of veterinary drug residues in foodstuffs like milk, meat and honey, the use of a commercial biosensor (BIAcore^{TM}) looks very promising. The system is based on surface plasmon resonance (SPR). Milk can be directly applied, honey mostly needs a simple sample pre-treatment (dilution step [streptomycin]; dilution, SPE and concentration step]tylosin]; dilution, liquid-liquid extraction and concentration step in buffer [chloramphenicol]; hydrolysation, pH adjustment and dilution step [sulfonamides]), while kidney and muscle samples need to be homogenised in an aqueous extraction buffer and clarified by centrifugation. Analysis is fully automated and a whole analysis cycle including regeneration only takes around 5 minutes. The range of available kits for the detection of antibiotics is increasing year by year.

For the control of food of animal origin, rapid and simple tests are already on the market for the detection of certain groups of antibiotics. For the control of milk on β-lactam antibiotics these tests are already frequently used in routine. However, for the control of other matrices there is still a need for rapid tests for certain important antibiotic families.

Irradiation detection methods – and how to cheat the test

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Irradiation is a physical treatment of food with high-energy, ionising radiation. It can be used to prolong the shelf life of food products and/or to reduce health hazards associated with certain products due to the presence of pathogenic microorganisms. The treatment may be applied for different purposes, such as:

- prevention of germination and sprouting of potatoes, onions and garlic;
- disinfestation by killing or sterilising insects which infest grains, dried fruit, vegetables or nuts;
- retardation of ripening and ageing of fruit and vegetables;
- prolongation of the shelf life and prevention of food-borne diseases by reducing the number of viable micro-organisms in meat, poultry and seafood; and
- reduction of microorganisms in spices and herbs.

Food irradiation can be achieved by using either a radioactive source like ^{60}Co or by using high-energy beta beams. The food itself will not be radioactive if a radioactive source has been used. Irradiation treatment of foodstuffs is regulated by two European Union (EU) directives, 2/1999/EC and 3/1999/EC. Directive 2/1999/EC covers general and technical aspects for carrying out the process, labelling of irradiated foods and conditions for authorising food irradiation. Directive 3/1999/EC lists products authorised for irradiation within the whole EU. It presently contains only a single food category: 'dried aromatic herbs, spices and vegetable seasonings'. The marketing of any product not complying with the Directives has been prohibited since 20 March 2001. The framework Directive sets out that

- The treatment with ionising radiation of a specific food item may only be authorised if: (i) there is a reasonable technological need, (ii) it presents no health hazard, (iii) it is of benefit to the consumers, and (iv) it is not used as a substitute for hygiene and health practices or for good manufacturing or agricultural practice.
- Any food irradiated as such or containing irradiated food ingredients has to be labelled.
- A favourable opinion of the Scientific Committee on Food (SCF) is needed to place a specific food item on the EU-wide list of products authorised for irradiation.
- National authorisations allowing the irradiation of certain foods within Member States can be maintained until the completed EU-wide list of products authorised for irradiation enters into force.
- Member States shall ensure that the analytical methods used to detect irradiated foods are validated or standardised. The European Committee for Standardisation (CEN) has standardised a number of analytical methods developed with the financial support of the European Commission.
- Foodstuffs may only be irradiated in approved irradiation facilities in the Member State or in irradiation facilities in third countries which have been approved by the Community.

In the early 1990's, the European Commission funded a research programme for the development and validation of detection methods for foods treated by ionising radiation. In the course of this programme, a number of efficient methods were developed. In 1993, the European Commission gave a mandate to the European Committee for Standardisation (CEN) to standardise these methods. CEN created within its Technical Committee 275 'Food analysis - Horizontal Methods' (CEN/TC 275) the working group 8 'Irradiated Foodstuffs' (CEN/TC275/WG8).

The principal techniques developed and standardised for irradiation detection are:

- gas chromatographic analysis (GC);
- gas chromatographic analysis/spectrometric analysis (GC-MS);
- electron spin resonance (ESR) spectroscopy;
- thermoluminescence (TL) detection;
- photostimulated luminescence detection (PSL);
- direct epifluorescent filter technique/aerobic plate count (DEFT/APC) - screening;
- DNA comet assay - screening; and
- microbiological screening.

The techniques, which are by far most frequently used, are ESR, PSL and TL. Although a fast technique, ESR can only be used for high-cellulose or apatite containing materials like pharmaceutical products, bone materials and nutshells. The most versatile techniques used are TL and PSL as they are based on the detection of high energy levels in mineral materials, which are almost, found everywhere, including in dust and dirt. While PSL is the fastest technique (measurement takes place within 1 minute) it is a screening technique only and can easily be tricked into showing false negative results. We have shown that a sample, treated by a very simple procedure and without the need for sophisticated equipment can produce false-negative results if measured by PSL (Popping et al., 2006). TL, although more laborious, is not producing false-negative results if the sample is measure having undergone the same treatment as described for PSL.

References

Popping, B., Schneede, K., Baden, M. and Toerber, C., 2006. Photostimulated luminescence testing (PSL): how to cheat irradiating testing. Poster presented at AOAC International Annual Meeting, 17-21 September 2006, Minneapolis, MN, USA.

DNA arrays for fast multiple GMO detection

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The DualChip GMO microarray is a screening tool for the simultaneous detection and identification of multiple genetic elements potentially present in genetically modified (GM) events within a single experiment. It gives an orientation and identification of GMO events present in the sample based on a matrix approach of the different elements, which constitutes the GMO event. The DualChip GMO microarray contains 3 different detection parts:

- the GMO screening elements;
- the plant reference elements; and
- different controls like the CaMV element.

The assay consists of PCR followed by hybridisation of the different amplicons directly on the GMO Chip. The detection is obtained by colorimetry using the Silverquant labelling and scanning followed by the DualChip evaluation software for appropriate quantification and data analysis. We will present the design of the chip and the results obtained in terms of sensitivity and reproducibility, and the results on different samples.

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Spice up spices - rapid detection and quantification of illegal dyes

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Synthetic azo dyes like Sudan dyes, Dimethyl Yellow, Para Red, Orange II and other forbidden dyes like Rhodamine B are frequently found in spices and spice products. They are used to improve the visual appearance of spices but they are also found to be present as a result of cross contamination, for example from inks used for the inscription of sacks in which spices are transported and stored. Several notifications appeared in the European Union rapid alert system for food and feed (RASFF). More and more dyes came into the focus of interest: Metanil Yellow, Oil Orange SS and Auramin, furthermore Acid Red 73, Congo Red, Naphthol Yellow, Ponceau 3R and Ponceau MX. All of these dyes are considered to be carcinogenic and they are not part of the list of permitted food colours (European Parliament and Council Directive 94/36/EC). Also forbidden for the use in spices, but frequently found, are the legal dyes Bixin (E 160b) and Azorubine (E122). Commission Decision 2005/402/EC is requiring as a condition of import that all hot chilis, hot chili products, turmeric and palm oil have to be tested for Sudan I-IV.

A rapid method has been developed to detect and quantify all mentioned dyes using one simple sample preparation and different detection modules that is 3 different LC-MS/MS runs. Quantification limits from 10-100 ppb are achievable for the different dyes depending on sample type.

Luminex®-based multiplex immunoassays for drug residues in food*

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In monitoring programs, in which different drug residues need to be tested, the general comment about immunoassays is that they are too specific. For each compound or group of compounds, a different immunoassay has to be performed. A fairly new system for multiplex detection uses the combination of the Luminex 100 flow analyser and the MultiAnalyte Profiling (xMAP™) technology. This microsphere-based flow cytometric system is developed by Luminex (Austin, Texas, USA) and involves covalent coupling of the antigen (drug-protein conjugates) on carboxylated polystyrene microspheres (5.6 µm beads) internally dyed with a red and orange fluorophore. By varying the ratio of the two fluorophores, up to 100 different bead sets can be distinguished and each bead set can be coupled to a different biological probe. A dual laser instrumentation system allows both the identification of the bead set by its characteristic colour and the quantification of the amount of fluorescent dye corresponding with the amount of reporter molecules (antibodies) bound to the beads. Thus, this combination makes it possible to simultaneously measure up to 100 different biomolecular reactions in a single well.

Results will be presented about (i) application of this technology for the detection of residues of animal drugs (sulfonamides, aminoglycosides and beta-agonists) in food and related products (e.g., urine) and (ii) comparison of the Luminex results with those obtained with other rapid technologies such as enzyme-linked immunosorbent assays (ELISAs) and biosensor immunoassays (BIAs).

* Contributed paper

Qualitative determination of β-lactams in milk in 3 minutes*

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The Charm ROSA MRL-3 β-lactam Test was developed to screen milk for β-lactam antibiotics at European Union/Codex maximum residue limits (MRL) in record time. The MRL-3 test combines speed and sensitivity in a simple one-step lateral flow immunoassay. The test uses a strip incubator for consistent temperature control and a Charm ROSA reader for interpretation of results.

The MRL-3 test utilises a three-line format to determine whether a raw milk sample is negative or positive for β-lactams. The 'T' line is beta-lactam specific, the 'X' line is cloxacillin specific, and the 'C' line is a control line. Any β-lactam antibiotic present in a milk sample will bind to the red-coloured gold beads on the test strip, thereby preventing some of the beads from binding to the T or X lines on the strip. If either T or X line is lighter than C line, the test is positive for beta-lactam at MRL. Visual test strip inspection indicates if the β-lactam is likely cloxacillin when the X line is lighter than the C line. A ROSA reader measures the binding intensity of each line, compares the values, and then produces a numerical result along with a qualitative interpretation of 'negative' or 'positive'.

The 90% positive with 95% confidence detection levels of the MRL-3 test have been determined in-house. Commingled raw milk was spiked with each β-lactam antibiotic at four concentrations. Thirty replicates of each drug concentration were tested along with 100 negative samples. The 90/95% detection levels were calculated using probit analysis. The corresponding levels are 2-3 ppb for penicillin G, 4-5 ppb for amoxicillin and ampicillin, 6-10 ppb for cephapirin, 20-30 ppb for cloxacillin, and 20-60 ppb for ceftiofur metabolite. A total of fourteen β-lactam drugs were detected at or below MRL.

A similar lateral flow test, the MRLBLTET-3 Test, is under development. It is a three minute, three-line test which detects both β-lactam and tetracycline antibiotics on the same strip. In this version the BL line is β-lactam specific and the TE line is tetracycline specific. The test uses the same ROSA equipment but the test strip is analysed on a reader channel that allows for an interpretation identifying the likely positive-drug family.

* Contributed paper


Rapid
Methods
EUROPE 2007
29-30 JANUARY 2007

POSTERS

Microorganisms
P1 – P15

P1 *Rapid and simple detection of amplified mRNA/DNA using antibody micro-arrays and colloidal carbon nanoparticles*
A. van Amerongen[1], M. Koets[1], G. Besselink[2], L. Berendsen[1], B. Beelen[1], M. Blažková[1] and J. Wichers[1]
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P2 *Rapid quantification methods of Escherichia coli in bathing waters*
P. Bergeron[1], H. Oujati[2] and S. Courtois[3]
[1]Lyonnaise des eaux, Centre Technique Littoral, France, [2]CAMBREX Bio Science Clermont Ferrand, Biopôle Clermont-Limagne, France and [3]SUEZ Environnement, CIRSEE, France

P3 *FastScan® - rapid test systems for an RNA based identification of microorganisms and cell activity measurements*
A. Breitenstein, H. Zinecker, K. Schewe and S. Bau
Scanbec GmbH, Germany

P4 *Development of a rapid nucleic acid based method for total viable counts (TVCs) on fresh meat using quantitative real-time PCR*
A. Dolan[1], C. Burgess[1], T. Barry[2], S. Fanning[3] and G. Duffy[1]
[1]Teagasc, Ashtown Food Research Centre, Ireland, [2]National University of Ireland, Department of Microbiology, Ireland and [3]University College Dublin, School of Agriculture, Food Science and Veterinary Medicine, Ireland

P5 *Quantitative multiplex detection of plant pathogens using PRI-lock probes and universal, ultra-high-throughput real-time PCR on OpenArrays™*
R. van Doorn, M. Szemes, P. Bonants and C.D. Schoen
Plant Research International, the Netherlands

P6 *Rapid detection of Enterobacteriaceae including identification of E. sakazakii in one test*
M. Kiehne, C. Grönewald and K. Berghof-Jäger
BIOTECON Diagnostics GmbH, Germany

P7 *An automated immunomagnetic separation enzyme linked immunoassay for the detection of Salmonella in poultry environmental samples*
C.G. Leon-Velarde, L. Zosherafatein and J.A. Odumeru
University of Guelph, Laboratory Services Division, Canada

P8 *A real-time assay for the sensitive and specific detection of Salmonella spp. on fresh meat*
E. Mc Cabe[1], C. Burgess[1], S. Fanning[3], T. Barry[2] and G. Duffy[1]
[1]Teagasc, Ashtown Food Research Centre, Ireland, [2]National University of Ireland, Department of Microbiology, Ireland and [3]University College Dublin, School of Agriculture, Food Science and Veterinary Medicine, Ireland

P9 *redprot®: Rapid staining of biological particles and analysis of their physiological state*
G. Meinardus-Hager[1], N. Sinclair La Rosa[1] and G. Lewandovski[2]
[1]A.E.R.O. medi B.V., the Netherlands and [2]HistoService, Germany

P10 *Detection of Salmonella in foods using real-time PCR*
B. Mercanoglu[1] and M.W. Griffiths[2]
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P11 *The use of precise bacterial reference standards to compare selective and non-selective culture media*
C. Morgan[1], N. Herman[1], P.A. White[2] and G. Vesey[1]
[1]BTF Pty Ltd., Australia and [2]University of New South Wales, Australia

P12 *BeviStat: a new ATP-based method for the rapid detection of yeasts and moulds in liquid samples*
M. Pressel, F. Olivieri, S. Rouillon, M. Hohnadel and S. Ribault
Millipore S.A.S., France

P13 *New immunological rapid tests for detection of Listeria monocytogenes and Bacillus cereus*
J. Slaghuis[1], M. Goll[2], H. Kono[3], R. Dietrich[3], M. Bülte[2] and A. Bubert[1]
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P14 *Magnetic separation of bacterial contaminants from yeast slurries*
S. Taskila and P. Neubauer
University of Oulu, Department of Process and Environmental Engineering, Finland

P15 *Rapid enumeration of microorganisms in milk powders*
K. Walker, S. Flint, M. Dwyer, B. Waters, S. Scott and S. Holroyd
Fonterra Co-operative Group Ltd., New Zealand

Naturally occurring substances
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P16 *The detection of aflatoxins by optical biosensor analysis*
S.A. Haughey[1], G.A. Baxter[1], C.E.N. Jones[1], R. O'Kennedy[2], L.P. Dunne[2] and S.C. Stapleton[2]
[1]Xenosense Ltd., UK and [2]Dublin City University, School of Biotechnology and National Centre for Sensors Research, Ireland

P17 *Fluorometric test for rapid and quantitative determination of deoxynivalenol*
Z. Kubus[1*], M. Hafner[1], M. Freudenschuss[2], E.M. Binder[3] and R. Krska[1]
[1]University of Natural Resources and Applied Life Sciences, Department for grobiotechnology (IFA-Tulln), Austria, [2]Biopure Referenzsubstanzen GmbH, Austria and [3]Romer Labs Diagnostic GmbH, Austria

P18 *Detection of staphylococcal enterotoxins A and B using VIDAS Staphylococcal Enterotoxins II (SET 2)*
J.G. Rola and W. Korpysa-Dzirba
National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Poland

P19 *ATP test, with ppm food sensitivity, used as a rapid cleaning indicator to reduce food cross contact*
R. Salter
Charm Sciences, Inc., USA

P20 *Quantitative lateral assay for the detection of zearalenone in grains*
S.J. Saul and M. Tess
Charm Sciences, USA

Non-naturally occurring substances
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P21 *Alternative real-time PCR chemistries in quantitative GMO detection*
M. Buh Gasparic, K. Cankar, K. Gruden and J. Zel
National Institute of Biology, Department of Plant Physiology and Biotechnology, Slovenia

P22 *Group specific ELISA for the detection of sulfonamides in liver using simplified sample preparation*
I. Cernoch, I. Diblikova and M. Franek
Veterinary Research Institute, Czech Republic

P23 *AgraStrip® GMO (genetically modified organism): RR (CP4 EPSPS) and YG+ (Bt-Cry3Bb1 and Bt-Cry1Ab) test kits*
D. Houchins[1], M. Prinster[1], H. Binder[2] and G. Gutscher[2]
[1]Romer Labs, Inc., USA and [2]Romer Labs, Inc., Austria

P24 *Pork and cow kidney screening using the kidney inhibition swab test and the fast antimicrobial swab test in slaughter facilities*
R.S. Salter
Charm Sciences, Inc., USA

P1

Rapid and simple detection of amplified mRNA/DNA using antibody micro-arrays and colloidal carbon nanoparticles

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Biosensor analysis set-ups usually comprise components such as a sampling device, an interface with interactive layer, a detector to record signals and a processing unit to analyse data. The signal is often based on optical or electrochemical principles and in many cases this requires a rather sophisticated module, which often make the apparatus and/or the running costs quite expensive. We aim to develop a micro-array system that is rapid, simple to perform and cheap. Therefore, the format of a previously developed one-step nucleic acid lateral flow immunoassay (NALFIA) was translated into a micro-array system. Several microorganisms were chosen as targets to assess the applicability of the method. Methods. The previously described NALFIA approach employs differently tagged primers, one labelled with biotin and the other one with a tag such as digoxigenin or fluorescein. Microarrays were prepared by spotting (TopSpot/E apparatus, 1 nL per spot; diameter ± 200 µm) of different amounts of antibodies onto several types of commercially obtained glass slides. Neutravidin was physically adsorbed onto colloidal carbon nanoparticles. After the sandwich assay, the arrays were analysed by conventional flatbed scanning and image analysis (TotalLab, non-linear dynamics) and the pixel grey volumes were used for quantifying the binding yield of amplicon conjugated colloidal carbon. Results. A comparison was made between the final binding yields obtained with UltraStick slides (amino-propyl-tri-ethoxy silane surface), XanTec slides (preactivated hydrogel coating) and Whatman FAST slides (coated with nitrocellulose). Physical adsorption of capture antibodies onto FAST slides gave the best results. Spots were scanned and the corresponding spot grey volume clearly increased as a function of antibody spotting concentration. Serial dilutions of PCR material were incubated on the arrays in the presence of various volumes of 0.2% (w/v) colloidal carbon nanoparticles - neutravidin conjugate in a total volume of 70 µL and incubated at room temperature for 7.5 minutes to several hours. Initial results indicated that approximately 0.5 to 2 µL of PCR material, 1 µL carbon conjugate and incubation for 15 minutes to 1 hour is sufficient to obtain significant results by flatbed scanning and image analysis. Several application examples will be shown such as for *Escherichia coli* and *Listeria (monocytogenes)*. Discussion. As compared to conventional quantification on DNA micro-arrays, for example based on fluorescence, the present approach is sufficiently rapid and does not require expensive apparatus. In addition to a PCR set-up, the necessary equipment consists of a micro-arrayer and a flatbed scanner. The disposable part consists of slides and reagents. In addition to PCR an adapted protocol is being studied to use the colloidal nanoparticle based micro-array in combination with isothermal NASBA (i.e., RNA) amplification, which would further simplify the total procedure.

P2

Rapid quantification methods of *Escherichia coli* in bathing waters

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The quality of waters in recreational areas is a subject of special interest during the summer season. The protection of bathers from possible health risks requires fast, sensitive simple and quantitative methods for the real-time monitoring of faecal pollution. Although faecal coliforms are used universally as microbiological indicator of water quality and are commonly used to determine the quality of bathing waters, *Escherichia coli* has been found to be a better indicator and has been chosen for the new guidelines fixed by Directive 2006/7/EC of the European Parliament and of the Council. The current culture-based standard methods (the USEPA 1603 method using m-TEC agar, as well as the ISO 9308-3 based on microplate determination of the most probable number) take about 24-48 hours to provide a result on *E. coli* numbers. Although culture-based tests to enumerate *E. coli* were improved in terms of specificity and rapidity by incorporating chromogenic and fluorogenic substrates, they still require 18-24 hours to complete. Thus, there is always a minimum one-day delay between sample collection and analytical results. Several rapid assays have been developed for enumerating *E. coli*. None of them uses any cultivation step. We have selected two different approaches: (i) the first method was a fluorimetric measure of the ß-D-glucuronidase activity based on the hydrolysis of the substrate 4 methylumbelliferyl-ß-D-glucuronide; (ii) the second approach was a molecular test based on real-time reverse transcription - polymerase chain reaction (quantitative RT-PCR) for specific quantification of *E. coli* 16S rRNA molecules. Special effort was made to standardise and automate the quantitative RT-PCR analysis, especially for the RNA extraction and purification steps. The aim of this study was to evaluate the capacity of these both methods to be used for the rapid assessment of bathing water samples. A total of 499 samples and 220 samples collected from two different coastal sites (French Atlantic coast) were respectively analysed by the fluorimetric and RT-PCR assays. Results show that both methods are rapid: 1 hour for 6 samples analysed by one fluorescence spectrophotometer, or 3 hours for the simultaneous analysis of up to 15 samples by using a KingFisher mL (Thermo Electron) robotic processor and real-time PCR apparatus. With regard to the microbiological criteria of the current regulation, the results obtained with the fluorimetric and RT-PCR analysis compared with the standard method (ISO9308-3) are congruent in more than 81% of analysed samples. The potential ability of these alternative methods to be more protective than the reference method will also be discussed.

P3

FastScan® - rapid test systems for an RNA based identification of microorganisms and cell activity measurements

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Nucleic acid based rapid test systems offer new and innovative perspectives for the detection and identification of microorganisms as well as for the determination of the metabolic activity of prokaryotic and eukaryotic cells. FastScan® is a new molecular test system for the detection of RNA molecules by means of a so-called sandwich hybridisation. The FastScan-Technology is based on the detection of hybridisation events of target molecules like rRNA and mRNA by specific capture and detection probes. The capture probe is used for immobilisation of the sandwich on a solid support (microtiter plates [Wegener et al., 2006], magnetic beads [Leskela et al., 2005], biochip surfaces [Gabig-Ciminska et al., 2004], affinity columns), whereas the detection probe is used for an enzyme linked signal read out. Dependent on the enzyme and corresponding substrate used either an optical (Wegener et al., 2006), fluorometrical (Leskela et al., 2005) or electrical (Gabig-Ciminska et al., 2004) signal can be measured. Using the above described technology attomoles of the respective target RNA molecules can be detected. Besides an inexpensive read-out device, the high flexibility and the enormous saving of time the FastScan-Technology opens new and better alternatives regarding the analytical quality of RNA monitoring in comparison to conventional technologies. The fields of application of the FastScan-Technology are microbial routine diagnostics in food industry and medicine as well as the monitoring of the metabolic activity of prokaryotic and eukaryotic cells in bioprocesses, e.g., recombinant protein production and drug screening. Applying specific probes, a 16S rRNA based FastScan-Test enables a group-specific as well as a species-specific detection of microbial pathogens. Thus, the spectrum of microbes to be analysed can be easily and flexibly varied. Especially for monitoring of transcriptional changes the FastScan-Technology offers great opportunities for a rapid, sensitive and cost-efficient detection and quantification of mRNA compared to other methods like microarray technology, Northern hybridisation and RT-PCR. A description of the FastScan-Technology and its application for the quantification and identification of pathogenic microorganisms in food and medical diagnostics and the mRNA-targeted monitoring of the metabolic activity of eukaryotic cells (rat liver hepatocytes, yeast cells) will be presented.

References

Gabig-Ciminska, M., Holmgren, A., Andresen, H., Bundvig Barken, K., Wumpelmann, M., Albers, J., Hintsche, R., Breitenstein, A., Neubauer, P., Los, M., Czyz, A., Wegrzyn, G., Silfversparre, G., Jurgen, B., Schweder, T. and Enfors, S.-O., 2004. Electric chips for rapid detection and quantification of nucleic acids. Biosensors & Bioelectronics 19:537-546.

Leskela, T., Tilsala-Timisjarvi, A., Kusnetsov, J., Neubauer, P. and Breitenstein, A., 2005. Sensitive genus-specific detection of *Legionella* by a 16S rRNA based sandwich hybridization assay. Journal of Microbiological Methods 62:167-179.

Wegener, S., Mau, M., Balcke, G.U., Breitenstein, A., Zinecker, H. and Schlömann, M., 2006. Sandwich hybridization assay for the detection of prokaryotic mRNA (submitted).

P4

Development of a rapid nucleic acid based method for total viable counts (TVCs) on fresh meat using quantitative real-time PCR

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The total viable count (TVC) is used to determine the microbiological quality of foods. A TVC can be performed by a cultural method (ISO 4833 which takes 3 days) or by an alternative rapid method if shown to be equivalent (ISO 16140:2003). The aim of this project is to develop and apply highly specific and user friendly nucleic acid based (DNA and RNA) rapid methods based on real-time PCR for the determination of total viable counts on fresh meat carcasses. As it is essential that only viable organisms be detected, reverse transcriptase PCR which detects specific RNA sequences (only expressed by viable cells) will be employed. It will be applied in a real-time (LightCycler) automated PCR format, which uses fluorescence to detect the presence and quantity of a particular gene in real time thus making it make more rapid and user friendly than conventional PCR (Yaron and Matthews, 2002). In order to establish the microflora of the beef carcass, 90 slaughtered steers were sampled over a period of 5 weeks in an Irish abattoir. Each carcass was sampled using the sponge swab technique of four sites (neck, flank, brisket and rump) before chilling as outlined in Commission Decision 2001/471/EC. The samples were stored at 4°C until further processing on the same day. Processing of samples involved plating all samples on Plate Count Agar to establish the total viable count and on a range of different agar bases in order to identify particular species that were typical or dominant. Following sampling, 40 bacterial colonies were selected at random for identification by 16S rDNA sequencing. The principal components of the microbial flora at this stage of the slaughter process have been shown to be lactic acid bacteria and *Enterobacteriaceae*. In addition, a broad range of Gram-positive and Gram-negative bacteria were detected, including *Acinetobacter* spp., staphylococcae and *Pseudomonas* spp. Using the information obtained from the sequencing, pure bacterial cultures typical of those occurring on beef carcasses were selected. Following a literature search, a novel target was selected for which two sets of primers were designed: one for Gram-negative species and one for Gram positive species. Both sets have been shown to select for the groups, which they were designed for with no cross over, indicating high specificity. At present work is on-going to show that both sets of primers are amenable to quantitative reverse transcriptase RT-PCR. Using cDNA from two model organisms, *Escherichia coli* and *Lactococcus lactis,* it has been shown that in both assays a standard curve can be generated for quantification of viable cells. Based on these results it may be concluded that this method seems to be adaptable to a real-time PCR assay to quantify total bacteria on fresh meat carcasses. An alternative rapid method, which gives results within a few hours, would be of immense benefit to the meat industry, allowing real time assessment of product quality and reduced holding time of product.

References

Yaron, S. and Matthews, K.R., 2002. A reverse transcriptase-polymerase chain reaction assay for detection of viable *Escherichia coli* O157:H7: investigation of specific target genes. Journal of Applied Microbiology 92:633-640.

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P5

Quantitative multiplex detection of plant pathogens using PRI-lock probes and universal, ultra-high-throughput real-time PCR on OpenArrays™

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Current technologies for multiplex, quantitative analyses frequently suffer from compromises between the level of multiplexing, throughput and accuracy of quantification. In general, for the detection of nucleic acids, microarrays provide very high levels of multiplexing, but less accurate quantification and usually low throughput. Real-time PCR provides the most reliable means of target quantification and is suitable for the analysis of a relatively high number of samples. However the achievable level of multiplexing is low. Nanoscale technology provides high-density, low-volume microchambers, which could accommodate very high number of reactions, performed under standard conditions. Yet many of these systems are still experimental and are incapable of real time fluorescent monitoring required for quantification. However recently, a conceptually new, ultra-high-throughput platform has become available for real-time PCR; capable of accommodating more than 3,000 reactions per array. The OpenArray™ has 48 subarrays, which allows parallel testing of up to 48 samples, with each subarray containing 64 microscopic (33 nL) through-holes. The primers are pre-loaded into the holes, while the sample and reagents are auto-loaded due to surface tension. Plant Research International (PRI) has developed PRI-Lock probes for multiplex detection; providing flexibility and bridging the gap between target-specific recognition and high-throughput amplification. These probes are long oligonucleotides (similar in structure to Padlock probes) and contain artificially selected primer sites and a TaqMan probe region, flanked by target complementary regions. In this paper, we report the development of a high-throughput, quantitative multiplex diagnostic assay based on above technologies.

P6

Rapid detection of *Enterobacteriaceae* including identification of *E. sakazakii* in one test

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In Commission Regulation (EC) No. 2073/2005, in force since 1 January 2006, the European Commission states that *Salmonella* and *Enterobacter sakazakii* are the microorganisms of greatest concern in infant formulae, formulae for special medical purposes and follow-on formulae. The presence of these pathogens constitutes a considerable risk if conditions after reconstitution permit multiplication. *Enterobacteriaceae*, which are more often present, could be used as indicator for risk. BIOTECON Diagnostics' new *Enterobacteriaceae* plus *E. sakazakii d*etection system allows to rapidly detect these both parameters in one reaction using real-time PCR. In a multiplex set-up, all members of the *Enterobacteriaceae* group are detected and the identification of *E. sakazakii* is carried out in parallel in a separate channel. The time to run the analysis is about 1 hour, the preparation of the samples needs approximately 30 minutes for 30 samples. The system can be fully automated. Including a short enrichment the time to result is less than 24 hours, i.e., a time saving up to 4 days. False positive results (e.g., *Aeromonas*) and false negative results (e.g., non-pigmented *E. sakazakii*) are prevented. Especially the existing official methods for the detection of *E. sakazakii* (ISO/TS 22964) are not sufficient for producers of such sensitive products like infant formula, since the obtained results are known not to be too reliable. The time to result for both parameters, using microbiological methods, is far too long to be helpful in in-process control or hygiene monitoring during production. This worldwide unique test has been validated intensively using isolates of the very heterogeneous *E. sakazakii* group from different companies around the globe. Different relevant matrices such as finished products or environmental samples have been used to check the applicability of the method in the routine lab of a production plant.

P7

An automated immunomagnetic separation enzyme linked immunoassay for the detection of *Salmonella* in poultry environmental samples

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An automated visual immunomagnetic separation enzyme linked immunoassay was developed and evaluated for the detection of *Salmonella* in poultry environmental samples. No false positives were observed when challenged with 33 cultures of microorganisms other than salmonellae, and positive results were obtained with pure cultures of 53 *Salmonella* isolates tested. From post-enrichment *Salmonella* cultures, the limit of detection of the assay was estimated at 10^4 to 10^6 cfu/mL. Application of the assay on 711 naturally contaminated poultry environmental samples achieved a 100% sensitivity and 96.2% specificity. The assay results were compared with those of a culture reference method performed concurrently on the same samples. The assay allows for the identification of positive samples within 1 hour of testing of post-enrichment broths.

P8

A real-time assay for the sensitive and specific detection of *Salmonella* spp. on fresh meat

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The safety of food must be assured by a preventative approach based on the application of a food safety management system such as hazard analysis critical control point (HACCP). The setting of microbiological criteria and testing of food samples against the criteria can be used to support food safety management systems and to provide assurance that microbial criteria have been met. The EU Commission document 'Microbiological criteria for foodstuffs' includes a requirement to examine fresh meat for the presence of *Salmonella*. Testing may be carried out by a cultural method (ISO 6579) which takes 3-4 days for positive clearance of samples, or by an equivalent rapid method in EN/ ISO 16140:2003. The requirement to test for *Salmonella* means that industry has an immediate need for an alternative rapid method as the delays incurred in waiting for test results may otherwise involve a considerable sized product holding area and delayed product release and thus having a significant economic impact. This research sought to develop a rapid, real-time PCR technique for detecting *Salmonella* spp. on fresh meat. Potential DNA/RNA targets for *Salmonella* spp. were identified from the literature and were assessed for suitability in a real time PCR assay. A target was chosen and DNA real time PCR for the gene target was carried out on twelve typed *Salmonella* strains. The products were sequenced and aligned and new primers designed for the target. RNA and DNA extractions were carried out on twenty-three typed *Salmonella* strains and twelve non-*Salmonella* strains. RNA was reverse transcribed into cDNA, real time PCR was carried out on both the DNA and the cDNA for all of the twenty three strains with the newly designed primer set. The method was found to be both sensitive and specific for *Salmonella* spp. and there was no cross reactivity with the primers and any of the twelve non-*Salmonella* strains. The newly developed real time protocol using a SYBR Green detection format was assessed using inocula as low as 10 cfu/mL in pure liquid culture. Fluorescently-labelled hybridisation probes were designed to anneal to the target PCR product. The assay incorporated an internal amplification control (IAC) co-amplified with the target gene of *Salmonella* to monitor potential PCR inhibitors and ensure successful amplifications. The newly designed target used in a real time system with fluorescent probe technology, as well as incorporation of an internal amplification control (IAC) ensures specificity, sensitivity and efficiency of the method.

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P9

redprot®: Rapid staining of biological particles and analysis of their physiological state

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redprot® is a rapid *in vitro* assay for visualisation and assessment of biological organisms and structures. The staining solution redprot stains biological material, like mites, small worms and insects, pollen, mould spores, yeast cells, and bacteria. The staining pattern differs with a certain morphological and physiological state of the particles and indicates its biological activity, e.g., growth, viability, maturation and in consequence may be pathogenic capacity. Procedure. Staining is based on the interaction of the dye with certain residues of aminoacids. redprot selectively stains proteins, glycoproteins and protein containing structures - preferentially in the cytoplasmatic compartment. The intensity of colour formation depends on the amount of protein and its current charge status. The highly specific affinity of the dye to the biological structures results in background-free staining in colour ranges from blue to violet and pink within few minutes. The concentration of dye in the non-toxic redprot-solution is standardised to give reliable stains, which permit rapid identification and classification of the particles. Stained material can be stored for long time in the dark. Two alternative staining procedures are rapid and easy to perform:

- A sample of the material to analyse is placed on a slide, a membrane or a glasfiber filter, preferentially dry or in a small drop of liquid which is allowed to dry. The sample on the support is embedded in redprot-solution and after approximately two minutes, the result can be examined. A global examination of colour change or detailed analysis of structures by microscopic inspection is possible.
- A liquid sample is mixed with an appropriate amount of redprot-solution (5 to 20% of total volume) and after about 15 minutes the colour change is read in a spectrophotometer. This method combines easy handling, high sensitivity and broad range of measurable protein concentration.

Application. The use of the redprot-solution enables the detection of particles in a broad spectrum of applications, like monitoring and control of hygiene and biological processes. Samples from air, liquids or surfaces can be scanned easily for biogenic material according to their protein moiety. Additionally to a 'rough' quantitative estimation of biological contamination, a detailed discrimination of samples for kind and physiological state of the particles, e.g., pollen, spores, yeast cells, bacteria or others by microscopy is possible. The development of automated analysis of differential staining of organisms at microscopic level is in progress.

P10

Detection of *Salmonella* in foods using real-time PCR

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A rapid, specific, and sensitive method for detecting *Salmonella* spp. in pasteurised milk, ground beef, and alfalfa sprouts was developed. The method combined immunomagnetic separation with a real-time PCR assay based on the double-stranded DNA binding dye SYBR Green I. The primers used produced a product with a melting temperature of 87±0.5ºC during the PCR assay by amplifying a 284 bp sequence from the invasive gene (*invA*) of *Salmonella*. The method was successful in detecting 20 *Salmonella* strains, but the expected PCR product was not formed by any 11 other bacterial strains. To test this combined method for the monitoring of *Salmonella*, *S. enterica* serotype Newport was inoculated into 52 samples each of pasteurised milk, ground beef, and alfalfa sprouts. Following a 10 hours non-selective enrichment step in buffered peptone water, cells were removed by immunomagnetic separation and DNA extracted using the High Pure PCR Template Preparation Kit. The DNA produced was used as a template in the real-time PCR assay. When spiked pasteurised milk, ground beef, and alfalfa sprout samples were analysed by this protocol, an initial inoculum of 1 cfu/mL, 25 cfu/25 g, and 1.5 cfu/25 g, respectively, was detectable within 13 hours. These results indicate that the combination of immunomagnetic separation and real-time PCR assay was a highly specific and sensitive method for the rapid detection of *Salmonella*.

P11

The use of precise bacterial reference standards to compare selective and non-selective culture media

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The use of selective culture medium is widely used in microbiology to detect target organisms from background microbes in environmental, food and clinical samples. Selective media contain constituents such as salts, dyes and antibiotics to inhibit the growth of certain microbes and enables selected organisms to grow and be enumerated. The effects of these added constituents on cells that have sustained sub-lethal injury is unknown and can lead to false negative results, or a lower recovery of target cells. BioBall is a new technology that uses laser cytometry to accurately count cells into a freeze-dried stable pill. This pill can be tipped directly onto an agar plate without loss of cells. Using a precise bacterial reference standard (BioBall) with a mean of 30 cells ± 3 standard deviations (SD) enables precise recovery data to be generated when comparing selective to non-selective media. Previous studies have relied on using microbial dilutions to prepare spikes for media testing, a precise standard can show media recovery differences down to a single colony rather than 10s-100s of colonies, which is the possible variation within a liquid dilution spike. With accreditation bodies requiring data on the measurement of uncertainty of test results, the use of precise bacterial standards to detect the possible cell losses on selective media will be required to enable industries to detect the sources of uncertainty within their methods. This study aims to look at cell recovery of a wide range of bacteria and fungi, including *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Aspergillus niger*, comparing the recovery of selective to non-selective agar with an accurate and low number of spike microorganisms. Results have shown significant losses of cells on selective agar compared to selective media, indicating that sublethally injured cells are unlikely to grow, if directly plated onto selective media.

P12

BeviStat: a new ATP-based method for the rapid detection of yeasts and moulds in liquid samples

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The detection of yeasts and moulds in liquid samples is a long process since it takes about 5 to 7 days to obtain the final result. The use of classic microbiology methods requires some manipulations, time, material and space to store the control plates. Rapid methods such as molecular methods require highly trained and skilled technicians. They are known to be very sensitive to cross-contamination leading to false positive results. Moreover, cost per test is relatively important. Other rapid but non-molecular methods are usually not enough sensitive to meet user specifications going down to 1 cfu. A specific ATP-based method was designed to detect as low as 1 cell per sample. Methods. Liquid samples were filtered on 47 mm membranes using Milliflex funnel. Two different methods were used: (i) classic methods (agar plates) were performed to control the number of cfu per sample, and (ii) the rapid protocol, based on the use of bioluminescence reagent coupled to an extremely sensitive photomultiplier, was used to determine the presence or absence of contaminants. Briefly, after a pre-treatment step and microwave lysis of the microorganisms, the membrane was exposed to a bioluminescent reagent and the emitted light was recorded using a specific sensor. Results. The pre-treatment step used in this protocol removed any interference coming from the matrix to focus on ATP coming from contaminants. *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus niger* were detected down to 1 cell in the different liquid tested including water, alcoholic beverages and energetic drinks. From sampling to result, only 15 minutes were required. Conclusion. The test was sensitive down to 1 cfu in all the samples tested. No manipulation was required as the protocol was fully automated. This new method allows rapid monitoring of liquids with detection in less than 15 minutes in samples going up to 500 mL.

P13

New immunological rapid tests for detection of *Listeria monocytogenes* and *Bacillus cereus*

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We have developed two new immunochromatographic (lateral flow) assays for the detection of *Listeria monocytogenes* and *Bacillus cereus* in various food and environmental samples. Up to now the identification of both bacteria with rapid methods is still difficult if the user wants to have results quickly, while avoiding a high level of automation. The new Singlepath L'mono test allows specific identification of *L. monocytogenes* in a large range of food samples. It also facilitates the very rapid confirmation of the bacteria from different agars such as Chromoplate, PALCAM, ALOA, bLEB, UVM II and others. Singlepath L'mono was evaluated with diverse natively and artificially contaminated food samples and showed 100% and 95% positive results, respectively. The latter yielded 100% positive results after additional 24 hours enrichment. Our other new test, Duopath Cereus Enterotoxins allows the specific identification of *B. cereus* via their enterotoxins Hbl and Nhe. This new method makes it possible to have a rapid screening of foods within 24 hours with a detection limit of 100 cfu/g food (rapid method). A detection limit of $\geq$1 cfu/g food is obtained after overnight enrichment and 6 hours post-enrichment (sensitive method). Duopath Cereus Enterotoxins could also be used as confirmation assay for suspect colonies on agar. Preliminary evaluation results with artificially contaminated food samples (1,000 cfu/g food) showed a 100% detection with the rapid method in different foods including semolina pudding, baby milk powder, pre-prepared baby food jars, coffee whitener and different rice varieties. Naturally contaminated follow-on milk and different rice varieties were detected with the sensitive method from a level of 0.36 cfu/g food with a rate of 100%. In conclusion, both tests provide an easy-to-use reliable method for the specific detection of *L. monocytogenes* and *B. cereus*, respectively, in food.

P14

Magnetic separation of bacterial contaminants from yeast slurries

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The detection of bacteria in brewer's yeast slurries is usually limited by the low amount of cells in the sample and the high concentration of yeast cells and trub. Recently, we demonstrated that yeast slurry decreases the sensitivity of 16S rRNA based fast detection of lactobacilli with a sandwich hybridisation method (Huhtamella et al., 2006; Rautio et al., 2003), and also PCR is inhibited by compounds present in the yeast slurries (Koivula et al., 2006). Therefore, there is a need for new efficient methods, which separate target bacteria from the sample matrix. Separation by paramagnetic particles has been earlier proposed as one promising strategy (Deponte et al., 2004). Here we provide evidence that magnetic separation of beer spoiling lactobacilli from brewer's yeast slurries is a functioning approach. Therefore, we used superparamagnetic beads coated with an inexpensive anion-exchange ligand for separation of *Lactobacillus plantarum* from spiked brewer's yeast suspension and real brewery slurries. The separation is based on the selective binding of the negatively charged surface of the bacterial cells to the anion exchange material. The separation works well with tenfold-diluted brewer's yeast slurries collected from the fermentation tanks. With the method, which was optimised for the pH and salt concentration of the buffer, 1000 *Lactobacillus* cells could be separated from yeast suspension or actual yeast slurry with recovery rates of almost 100% and 70%, respectively, as was shown by plating analysis. The whole separation protocol is performed in only 30 minutes with the KingFisher (Thermo Electron) automated magnetic separation device.

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References

Deponte et al., 2004. Biomagnetic separation of *E. coli* by use of anion-exchange beads: measurement and modeling of the kinetics of cell-bead interactions. Analytical and Bioanalytical Chemistry 379:419-426.

Huhtamella, S. et al., 2006. RNA-based sandwich hybridisation method for detection of lactic acid bacteria in brewery samples. Journal of Microbiological Methods, in press.

Koivula et al., 2006. Characterization of the brewery spoilage bacterium *Obesumbacterium proteus* by automated ribotyping and development of PCR methods for its biotype 1. Journal of Applied Microbiology 100:398-406.

Rautio et al., 2003. Sandwich hybridisation assay for quantitative detection of yeast RNAs in crude cell lysates. Microbial Cell Factories 2:1-9.

P15

Rapid enumeration of microorganisms in milk powders

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Flow cytometry has been selected as the technology with the best potential for enumerating microorganisms in dairy samples. The technique is capable of producing results within hours compared with days for traditional plating methods. The rapid results possible with this method have the potential to assist in managing the manufacturing processes, provide confidence in the early release of product, and problem solving. Using a Chemunex flow cytometer, a protocol for milk powder was developed for total viable bacteria based on the esterase activity within the cells. This protocol has been used to monitor manufacturing processes over a 2-year period. More recently we have developed a protocol for enumerating thermophilic bacteria in milk powders. Flow cytometry is a successful way of rapidly enumerating viable bacteria in milk powders and samples taken throughout the milk powder process. Future work will extend into enumerating other groups of organisms including moulds, mesophilic bacteria, bacterial spores, and *Enterobacteriaceae*.

P16

The detection of aflatoxins by optical biosensor analysis

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Aflatoxins are a group of closely related toxic secondary metabolites produced by certain strains of the fungi *Aspergillus flavus* or *A. parasiticus*. They have been shown to be highly carcinogenic to laboratory animals, primarily targeting the liver, and have acute toxicological effects on humans. There has also been great interest on the effects of long-term exposure to low levels of aflatoxins due to the positive association between dietary aflatoxins and human hepatocellular carcinoma. Many countries, therefore, have attempted to limit exposure by imposing regulatory limits on aflatoxins in a wide range of foodstuffs and animal feeds. Traditional techniques for the detection and/or quantification of aflatoxins include TLC, HPLC or GC/MS, or immunoanalytical methods such as ELISAs. These methods, however, are either laborious, inconsistent and require skilled laboratory workers, or are not sufficiently sensitive to detect levels of aflatoxins to the regulatory limits. An assay has been developed to detect and quantify aflatoxin B1 using a Biacore biosensor, utilising the phenomenon of surface plasmon resonance (SPR). It is anticipated that this assay will overcome the problems associated with other techniques and provide a high throughput, automated system with detection levels consistent with aflatoxin regulatory requirements.

P17

Fluorometric test for rapid and quantitative determination of deoxynivalenol

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Fusarium graminearum is the main deoxynivalenol (DON) producing fungus. Corn and small grains such as wheat, oats, and barley are the major crops affected. It was shown that deoxynivalenol affects nutrient absorption in human intestinal epithelial cells (Maresca et al., 2002) and influences human lymphocyte proliferation and cytokine production (Meky et al., 2001). Livestock affected by this toxin exhibits reduced feed intake, weight loss, vomiting, and diarrhoea. The availability of reliable testing techniques is therefore a crucial issue to prevent distribution of DON in the food chain. The test system for quantitative detection of deoxynivalenol developed in our laboratory is based on fluorometric detection of a metalorganic derivative of the analyte. The novelty is the clean-up/drying column, which provides a one step removal of matrix components and water residues from sample extract. The testing method is designed as single-sample test and can be applied at the early stages of grain control, for elevators, export terminals, mills, breweries, but is also appropriate for analytical laboratories. The complete procedure of the test takes approximately 10 minutes including extraction, purification, derivatisation and end determination. The advantage of this system is its speed and simple procedure, furthermore the test components do not require storage in the fridge. The developed quantitative method has been validated in the working range of 0.25 mg/kg to 5 mg/kg DON in wheat. The limit of detection (LOD) obtained for wheat, barley and maize is 0.1 mg/kg. The recoveries and RSD values obtained during internal validation fulfil European Union (EC, 2006) and GIPSA (Grain Inspection, Packers & Stockyards Administration) criteria (USDA/GIPSA, 2006).

References

EC, 2006. Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union L70, pp.12-34.

Maresca, M., Mahfoud, R., Garmy, N. and Fantini, J., 2002. The mycotoxin deoxynivalenol affects nutrient absorption in human intestinal epithelial cells. Journal of Nutrition 132:2723-2731.

Meky, F.A., Hardie, L.J., Evans, S.W. and Wild, C.P. 2001. Deoxynivalenol-induced immunomodulation of human lymphocyte proliferation and cytokine production. Food and Chemical Toxicology 39:827-836.

USDA/GIPSA, 2006. Design criteria and test performance specifications for quantitative DON test kits, 2006/03/6.

P18

Detection of staphylococcal enterotoxins A and B using VIDAS Staphylococcal Enterotoxins II (SET 2)

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Strains of *Staphylococcus aureus*, *S. intermedius* and *S. hyicus* have the ability to produce enterotoxins. There are about 20 kinds of staphylococcal enterotoxins (SE) but toxins A, B, C, D and E are the most common. These proteins are responsible for the gastrointestinal symptoms, which may occur after eating food containing one or few kinds of staphylococcal enterotoxins. Food often associated with this type of foodborne illness is meat, poultry, dairy products, eggs, mayonnaise and canned mushrooms. Most commonly contamination of these products occurs during production, preparation or processing by humans handling the products. The aim of this study was to compare the sensitivity of Vidas Staphylococcal Enterotoxins II (SET 2) depending on toxin type. In our study we used 25 g samples of Roquefort cheese, artificially contaminated by staphylococcal enterotoxin A on four levels of detection: 0.1ng/g; 0.25 ng/g; 0.5 ng/g and 1.0 ng/g. Staphylococcal enterotoxins were detected using Vidas SET 2. Before that, extraction and concentration of samples was done according to the procedure developed in the Community Reference Laboratory for Milk and Milk Products (AFSSA, France). The same protocol was used with reference to staphylococcal enterotoxin B. For this toxin 20 samples on each level of contamination were tested except for level of contamination 0,5 ng/g - 19 samples.

Level of contamination (ng/g)	Sensitivity							
	Toxin A		Toxin B		Toxin A		Toxin B	
	Number of positive results	% of positive results	Number of positive results	% of positive results	Number of negative results	% of negative results	Number of negative results	% of negative results
0.1	21	84	6	30	4	16	14	70
0.25	19	95	17	85	1	5	3	15
0.5	10	100	17	90	0	0	2	10
1.0	20	100	20	100	0	0	0	0

P19

ATP test, with ppm food sensitivity, used as a rapid cleaning indicator to reduce food cross contact

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Cleaning to prevent cross contact of allergenic and non-allergenic foods is of increasing industry concern with new food regulations on allergens and labelling. Cleaning can be verified with protein-specific ELISA methods, which detect foods to the parts per million (ppm) concern level; however, these methods require technical competence and take more than 30 minutes to complete. In contrast, cleaning verification with adenosine triphosphate (ATP) swabs is simple and rapid, but conventional ATP tests lack ppm sensitivity. The need for a fast and simple test that detects food to the ppm level has recently been addressed by a new sensitive ATP detection system called AllerGiene®. This work summarises studies that compare the surface sensitivity of AllerGiene to the surface sensitivity of ELISA methods. In independent research laboratories, food-standards of milk, peanut, and egg in ppm solutions were dried on stainless steel plates. The plates were simultaneously swabbed for qualitative result by AllerGiene and ELISA: the AllerGiene swabs were analysed in NovaLum™ that converts ATP into relative light units (RLU) and were positive when they exceeded a 'clean' RLU threshold, while ELISA swabs were positive when the swab-extract contained protein that exceeded supplied controls. These data show AllerGiene detected ATP in these foods at levels similar to ELISA: 5-10µg NIST powdered milk (5-10 ppm dried); 0.5-2.5 µg EU peanut standard (0.5-2.5 ppm dried); 50-250 µg NIST egg (50-250 ppm dried). In a separate food-plant evaluation, an almond-packaging company implemented an AllerGiene cleaning assessment before ELISA testing. In their integrated cleaning regimen, if a 'clean' RLU threshold was not met by AllerGiene, the equipment was re-cleaned. Once the “clean” RLU threshold was met, the cleaned surface was tested with ELISA. Cleaning assessment with ELISA alone yielded a positive-almond (> 5ppm) rate of 15%. Use of AllerGiene with re-cleaning if necessary before ELISA yielded a 1% ELISA positive rate. Because each positive ELISA result must be followed by re-cleaning and another 30 minutes ELISA verification, use of the 30 seconds AllerGiene to decrease the positive ELISA rate significantly reduced cleaning-verification time. These studies indicate ATP detection by AllerGiene is a useful and rapid cleaning assessment tool to reduce food cross contact. Although ATP levels vary in different food types and with processing, and although ATP detection lacks specificity to allergens, these studies show that AllerGiene quickly detects ATP in dried food residues of milk, peanut, egg and almond similar to levels detected by ELISA methods. Integrating the 30 seconds AllerGiene test with allergen-specific tools can significantly improve the cost, efficiency and time of cleaning verification after allergenic food-products.

P20

Quantitative lateral assay for the detection of zearalenone in grains

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A quantitative lateral flow test was developed for the detection of zearalenone in grains. The test uses the Charm ROSA test format which includes a test strip, an incubator and a reflectance reader. The general procedure is as follows: (i) ground sample is extracted with solvent; (ii) 100 µl portion of extract is added to 1 ml dilution buffer; (iii) 300 µl portion of diluted extract is added to a lateral flow test strip; (iv) test strip is incubated for 10 minutes at 45°C; and (v) reflectance reader provides a numerical result from 0 to 1000 ppb by comparing the binding intensities of 2 test lines and control line. The Charm ROSA Zearalenone (Quantitative) test has received USDA/GIPSA (US Department of Agriculture/Grain Inspection, Packers & Stockyards Administration) approval as a quantitative test method by meeting the necessary accuracy and precision requirements as evaluated by an independent laboratory. HPLC certified incurred corn samples at 105 ppb, 265 ppb and 1,028 ppb were each run 21 times on the assay. The 105 ppb incurred sample averaged 121 ppb with a 10% CV, the 265 ppb sample averaged 252 ppb with a 9 % CV and the 1028 ppb sample averaged 950 ppb with a 12 % CV. The LOD of the kit is less than 10 ppb making it capable of zearalenone detection at all of the European Union levels of concern.

P21

Alternative real-time PCR chemistries in quantitative GMO detection

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Real-time polymerase chain reaction (PCR) is currently the most effective and widely used method for quantification of genetically modified (GM) components in food and feed. The method uses fluorescent reporters to detect the amount of PCR product in each cycle of the reaction. Some fluorogenic molecules can incorporate in double stranded DNA non-specifically, while others are attached to different types of oligonucleotides, used as primers or probes for amplification. Although more than thirty different chemistries have been developed, vast majority of laboratories uses TaqMan and/or SYBR Green I only. We have tested four alternative chemistries that have not yet been used for detection of GM organisms: Lux™ flourogenic primers (Invitrogen), Plexor™ (Promega), Cycling Probe Technology™ (TaKaRa) and LNA Probes (Sigma Proligo). Their performance characteristics were compared to TaqMan chemistry to check their appropriateness for research and routine analysis. The Cycling Probe Technology (CPT) and Locked Nucleic Acid (LNA) probes are like TaqMan both hydrolysis probes while Lux and Plexor technologies do not employ a probe. Instead they use one fluorogenic primer in primer pair and dissociation curves are plotted to monitor the specificity of the product. We have established detection systems including the endogene reference and the transgene amplicons for all alternative technologies mentioned. While they were all very comparable in efficiency there were differences in other characteristics. Lux and Plexor were the least expensive, but at the same time quite difficult to establish due to primer dimer formation in many design trials. LNA chemistry's performance was most similar to TaqMan. Because LNA and CPT probes are much shorter, they might be especially appropriate for SNP detection and for the sequences where designing a common TaqMan probe is difficult or even impossible.

P22

Group specific ELISA for the detection of sulfonamides in liver using simplified sample preparation

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Broad-specificity immunoassays for sulfonamide drugs were reported recently in our paper (Franek et al., 2006). The ELISA based on rabbit antibody no. 43 was used for the rapid detection of sulfonamides in porcine liver. The procedure enabled the detection of at least 15 sulfonamide targets in a matrix supernatant, after homogenisation and buffer extraction from liver samples at the level of the declared MRL (100 µg/kg). A negligible cross-reaction with N4 metabolites allowed the measurement of responses to parent sulfonamides in the presence of their metabolised forms. The analytical recovery of individual sulfonamides added to blank liver samples at a concentration of 100 µg/kg varied from 36% to 113%. Inhibition value determined for sulfamethazine (in liver blank at 100 µg/kg) was established as a reference value, to decide whether a sample was negative or positive. The strongest response was found for sulfapyridine, sulfamethoxypyridazine, sulfamethoxazole, sulfamethoxydiazine, sulfadimethoxine, sulfaquinoxaline and sulfathiazole. Only sulfacetamide, sulfisoxazole and sulfaphenazole showed lower inhibition characteristics compared with sulfamethazine. Sulfamethizole was borderline. Because the ELISA under discussion is intended to be a qualitative screening tool for the group of sulfonamide drugs, any positive finding must be confirmed using a standard instrumental technique. Thirty different liver samples were purchased from retail outlets within the Brno area. Samples were analysed using the group specific ELISA, as described above, the highly specific and sensitive ELISA for sulfamethazine (Franek et al., 1999) and LC-MS/MS methods. A good correlation of results was obtained by the ELISA methods and LC-MS/MS in both real and fortified liver samples.

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References

Franek, M., Kolar, V., Deng, A.P. and Crooks, S., 1999. Determination of sulfadimidine (sulphamethazine) residues in milk, plasma, urine and edible tissues by sensitive ELISA Food and Agricultural Immunology 11:339-349.

Franek, M., Diblikova, I., Cernoch, I., Vass, M. and Hruska, K., 2006. Broad-specificity immunoassays for sulfonamide detection: immunochemical strategy for generic antibodies and competitors. Analytical Chemistry 78:1559-1567.

P23

AgraStrip® GMO (genetically modified organism): RR (CP4 EPSPS) and YG+ (Bt-Cry3Bb1 and Bt-Cry1Ab) test kits

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The AgraStrip® GMO (genetically modified organism) assays are sandwich ELISA lateral flow tests for the determination of the presence of GMO traits in corn and soybeans based on a single-step method. Samples to be tested for the CP4 EPSPS protein may be extracted in water or SEB4 buffer. Samples to be tested for the Bt-Cry3Bb1 and Bt-Cry1Ab proteins are extracted in SEB4 buffer. Portions of the resulting extracts are placed in microcentrifuge tubes. The AgraStrips are then placed in the tubes and allowed to develop. A control line will be present on each sample analysed to indicate the test is performing properly. Test lines are present if the sample is positive for the trait. The RR test is a single trait test and will have one control line and one test line if positive. The YG+ test is a dual trait test and shows separate test lines for the Bt-Cry3Bb1 protein and Bt-Cry1Ab protein in addition to the control line. Results obtained from internal studies assessing the performance of the strips showed that the AgraStrip GMO: RR (CP4 EPSPS) and YG+ (Cry-Bt3Bb1 and Bt-Cry1Ab) showed the strips were capable of detecting the CP4 EPSPS protein at levels of 1 seed in 1000 or greater, and the Bt-Cry3Bb1 and Bt-Cry1Ab proteins at levels of 1 seed in 100 or greater at the 95% confidence level. The sampling plan may also be adjusted in order to use the strips for testing at other confidence levels or % GMO. The test is simple enough for field use in grain, feed and food handling facilities, but is also appropriate for use in analytical laboratories.

P24

Pork and cow kidney screening using the kidney inhibition swab test and the fast antimicrobial swab test in slaughter facilities

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Antimicrobial residues in animal tissue are screened by the US Department of Agriculture (USDA) using animal history, pathologies and the Fast Antimicrobial Swab Test (FAST). The FAST test is a *Bacillus megaterium* inhibition assay of a kidney swab-sample that takes 6 hours and requires microbiological preparation. The Kidney Inhibition Swab (KIS) test is a new ready to use, pre-assembled, *Bacillus stearothermophilus* based inhibition swab assay that takes about 3 hours. The KIS test has enhanced sulfa drug sensitivity compared to FAST. This work describes on-going comparisons of KIS and FAST test in pig and dairy cattle slaughter facilities. Kidneys from slaughtered animals were simultaneously swab sampled for KIS and FAST tests at the processor facility. In the cow facility 200 kidney samples were evaluated. Three samples were FAST and KIS positive, two samples were FAST negative and KIS positive, and 195 samples were FAST and KIS negative. The 3 FAST/KIS positive samples were analysed by Charm II receptor assay. The Charm II assay detected beta-lactam, tetracycline and sulfa drug residues. Detected in the 2 FAST negative KIS positive samples were sulfa drugs and tetracyclines. None of the FAST/KIS positive samples had violative tissue levels as determined by a reference laboratory using official HPLC methods for antibiotics. In the hog slaughter facility 50 kidney samples were evaluated. Six samples were from beta-lactam incurred animals and were positive by FAST and KIS test. The other 44 random surveillance samples were negative by both FAST and KIS test. Data collected to date indicate that the KIS result agrees with the FAST result with a low false positive rate. The two additional KIS positives in the cow facility may be due to enhanced detection of sulfa drug or tetracycline residues below the US safe levels or due to mixed drug combinations. The KIS test was very simple to use, developed twice as quickly as the FAST test and received favourable comments for convenience and user-friendliness.