Rapid Methods Europe 2005
international conference and marketplace
for food and feed quality determination

24-25 May 2005
Noordwijk aan Zee, the Netherlands

Organising Committee

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Agrotechnology & Food Innovations (A&F), the Netherlands

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Key to the abstracts of lectures and posters:
• abstracts of lectures, spotlight presentations and posters are grouped separately;
• the lectures and spotlight presentations are grouped according to the daily programme; and
• the posters are grouped according to theme.

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Welcome at Rapid Methods Europe 2005!

Dear participant,

Rapid Methods Europe is the leading conference & exhibit series dedicated to rapid methods and instrumentation of interest to the food and feed industry. There is an ever-increasing need for rapid methods and instrumentation in the field of food and feed quality. Developments in analytical techniques have led to the emergence of a wide range of rapid methods to complement the traditional methods. Faster results, higher productivity, lower costs and increased sensitivity are key words for all those professionally involved. How to keep up with the developments?

Rapid Methods Europe 2005 is aimed at the latest developments in rapid methods and instrumentation for food and feed quality determination, presenting:

- an information packed conference programme offering the very latest news;
- a marketplace/exhibit providing information on rapid methods and instrumental methods in use or becoming commercially available;
- a spotlight presentations theatre covering a wide range of topics.

As a comprehensive overview Rapid Methods Europe 2005 offers an excellent way to network and to share ideas, providing a reference source for anyone interested in the rapid determination of food and feed quality.

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
PROGRAMME
TUESDAY 24 MAY 2005

08.30 Opening of Rapid Methods Europe 2005

Plenary meeting
New rapid technologies

There is an ever-increasing need for rapid technologies in the field of food and feed quality determination. An overview of still-developing techniques and emerging technologies, which will change your world, will be presented.

Chair: Dr. Bert Popping
Eurofins Scientific Group, UK

08.45 Lab-on-a-chip systems
Dr. Han J.G.E. Gardeniers
University of Twente, MESA+ Institute for Nanotechnology, the Netherlands

09.15 Nanosensors identifying food quality
Terje Berg
Cientifica, Norway

09.45 Electronic sensing: food and feed applications
Prof.dr. Naresh Magan
Cranfield University, Institute of BioScience and Technology, UK

10.15 Coffee/tea break – exhibition and poster presentations

10.45 Magnetic biosensors
Dr. Menno W.J. Prins
Philips Research, Integrated Device Technologies, the Netherlands

11.15 Genomics in microbial food quality: rapid access to the essence
Dr. Jos M.B.M. van der Vossen
TNO Quality of Life, the Netherlands

11.45 Proteome and metabolome analyses for rapid authentication of food
Dr. Seetharaman Vaidyanathan
University of Manchester, School of Chemistry, UK

12.15 General discussion

12.30 Buffet lunch – exhibition and poster presentations
TUESDAY 24 MAY 2005

Session 1
Rapid testing of food and nutritional quality

With the increasing need to monitor processes at many stages and to show due diligence in the control of food and nutritional quality, there is also a need to generate fast and accurate analysis results. This session will focus on modern techniques covering their application and limitations.

Chair: Margreet Lauwaars
EC-DG JRC Institute for Reference Materials and Measurements (IRMM), Belgium

14.00 Application of spectral analysis to food and feed quality monitoring
Dr. Pierre Dardenne
Centre wallon de Recherches agronomiques (CRA-W), Quality Department, Belgium

14.30 New developments in analytical tools to measure nutritional quality
Prof.dr. Colin H. Self
University of Newcastle upon Tyne, School of Clinical and Laboratory Sciences, UK

15.00 Rapid identification of plant and animal species in foods
Dr. Hermann Broll
Federal Institute for Risk Assessment (BfR), Germany

15.30 Coffee/tea break – exhibition and poster presentations

16.00 Rapid and reliable in vitro method for assessment of the nutritional quality of proteins (and carbohydrates)
Dr. Robert Havenaar
TNO Quality of Life, the Netherlands

16.30 Rapid quality monitoring of packaging materials
Dr. Alexander Gurlo
University of Tübingen, Institute of Physical and Theoretical Chemistry, Germany

Spotlight presentations

17.00 Rapid and reliable determination of food safety and quality
Dr. John Butler
Biacore, Sweden

17.20 Rapid methods for the detection of food adulteration: the R-Biopharm solution
Dr. Ronald Niemeijer
R-Biopharm, Germany

17.40 CarboDeep™ – NutriCognia’s technology platform
Dr. Ofer Markman
NutriCognia, Israel
TUESDAY 24 MAY 2005

Rapid Test Workshop

17.00-18.30

Whatman/Schleicher & Schuell and BioDot are offering a practical 90-minute workshop on the development and manufacture of Membrane Rapid Tests. Whatman/Schleicher & Schuell is a major supplier of high quality nitrocellulose membranes and papers used in lateral flow, flow-through and dipstick immunoassay formats. BioDot is a market leader in the supply of equipment and technology for the manufacture of rapid tests and biosensors.

Flow-through and lateral flow immunoassays have gained a major role in medical, veterinary and environmental testing and have much to offer for rapid QC monitoring of food and feedstuffs. The workshop will present an immunodiagnostic device platform giving consistent, fast and accurate results with emphasis on the particular challenges of food sample presentation. We will explain the step by step building of a rapid assay combining the right components, reagents and equipment and provide a hands-on demonstration of the production process from raw materials to finished test stick. For highly specific tests a simple and fast DNA preparation technology will be presented together with biosensors and other emerging rapid test formats.

20.30 Conference dinner
WEDNESDAY 25 MAY 2005

Session 2
Rapid testing of quality deterioration and spoilage

Food and feed are subject to varying degrees of (micro)biological, chemical and physical deterioration during storage. A range of technologies with potential use to achieve rapid results for food quality determination, will be presented.

Chair: Dr. Aart van Amerongen
Agrotechnology & Food Innovations (A&F), the Netherlands

08.45 Rapid detection method for microbial food spoilage using FT-IR spectroscopy and machine learning
Dr. David I. Ellis
University of Manchester, School of Chemistry, UK

09.15 Flow cytometry as a rapid tool for microbiological analysis in the food industry: potentials and restrictions
Prof.dr. Geertrui Vlaemynck
Hogeschool Gent, Department of Biotechnological Sciences, Belgium

09.45 PCR for the detection and identification of live spoilage organisms
Dr. Karin M.D. Pawlowsky
Brewing Research International (BRI), UK

10.15 Coffee/tea break – exhibition and poster presentations

10.45 Electronic nose technology for detecting taints and off-flavours
Dr. John-Erik Haugen
Norwegian Food Research Institute (MATFORSK), Norway

11.15 Application of a rapid analytical technique for the determination of oxidation stability in food development
Dr. Paola Maiocchi
Velp Scientifica, Italy

Spotlight presentations

11.45 Microscreen – total solutions on microbiological diagnostics using FISH and qPCR
Ron Wolbert
Microscreen, the Netherlands

12.05 A multi-antibiotic enzyme-linked immunosorbent assay for screening five banned antimicrobial growth promoters in animal feedingstuffs
Dr. Ron Verheijen
Euro-Diagnostica, the Netherlands

12.25 AK-Phage™assay: a fully automatic method for the detection of very low levels of pathogens in food within a working day
Malcolm Walpole
Alaska Food Diagnostics, UK

12.45 Buffet lunch – exhibition and poster presentations
WEDNESDAY 25 MAY 2005

Session 3
Rapid testing of authenticity and adulteration

The authenticity of foods is an important and an essential attribute. Many foods traded in Europe are classified according to their region of production, traditional method of production or the unique ingredients they contain. But how to detect rapidly and reliably authenticity and/or adulteration?

Chair: Dr. Michèle Lees
Eurofins Scientific Analytics, France

08.45 DNA-based techniques used for authenticity and adulteration testing
Dr. Bert Popping
Eurofins Scientific Group, UK

09.15 Immunoassay techniques to detect adulteration
Prof.dr. Christopher Smith
University College Chester, Department of Biology, UK

09.45 Rapid detection of species adulteration in milk and dairy products
Dr. Francesco Addeo
National Research Council, Institute of Food Science and Technology, Italy

10.15 Coffee/tea break

10.45 Rapid testing for authenticity of (olive) oils
Dr. Ramón Aparicio
Spanish Council for Scientific Research (CSIC), Instituto de la Grasa, Spain

11.15 Real-time PCR for meat quantification
Dr. Hez Hird
Central Science Laboratory, UK

11.45 DNA-IQ: development of molecular genetic methods for the identification and quantification of fish and seafood
Dr. Bert Popping
Eurofins/GeneScan, Germany

12.15 Enforcement of food standards legislation using DNA-based techniques
Dr. Mark Woolfe
Food Standards Agency, Enforcement Division, UK

12.45 Buffet lunch – exhibition and poster presentations
WEDNESDAY 25 MAY 2005

Final plenary meeting
Rapid testing and quality tracking & tracing

Quality tracking & tracing does not only establish the identity and location of products, but also their quality and history in relation to parameters such as temperature, relative humidity, ingredients, production circumstances. The quality of products followed in this manner, can be predicted along in the chain at any given moment.

Chair: Prof.dr. Brigitte Petersen
       University of Bonn, Department Preventive Health Management, Germany

14.00 Quality tracking & tracing: an overview
       Dr. Robert Madge
       European Association for Sure & Secure Identification (IDtrack), Spain

14.30 Quality tracking & tracing: technology
       Dr. Floor Verdenius
       Agrotechnology & Food Innovations (A&F), the Netherlands

15.00 Quality tracking & tracing: application
       Dr. Claude Mabilat
       bioMérieux, France

15.30 Industrial vision on tracking & tracing and rapid methods
       Dr. Rodolphe de Borchgrave
       Arcadia International, Belgium

16.00 Scientific vision on tracking & tracing and rapid methods in food supply chain networks
       Prof.dr. Jack G.A.J. van der Vorst
       Wageningen University, Department of Logistics and Operations Research, the Netherlands

16.30 Closing Rapid Methods Europe 2005

16.45 End of conference
LECTURES

Lab-on-a-chip systems

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During the last decades, an increasing number of pocketsize chemistry equipment based on the so-called 'lab-on-a-chip' approach has become available. Well-known examples are the miniature DNA analysers based on electrophoresis, which speeded up the identification of the human genome. In a similar fashion they are now being used in other areas of the life sciences, like proteomics. The core of the lab-on-a-chip system is a microfluidic channel structure, through which fluid sample plugs with less than a nanoliter volume are propelled by hydraulic or electrokinetic forces. Besides the popular applications of the microfluidic chips for the analysis of biological macromolecules, such chips, in combination with portable electronic equipment, are suitable for applications in many other fields as well. Examples are the ‘point-of-care’ ion analysis in body fluids, forensics, identification of explosives, tracking of pollution in environmental or waste waters, monitoring nutrients in agricultural or horticultural water, controlling quality in food production, or general process control in chemical industry.

This contribution will provide an overview of recent achievements in the field of bioprocess technology and food quality testing. Three cases will be discussed in detail, viz. miniaturised biofermentors for high throughput optimisation of industrial fermentations, on-line monitoring of bioprocesses (or chemical processes) with a lab-on-a-chip, and lab-on-a-chip formats for single-cell studies.

On-line monitoring of bioprocesses and chemical processes

In order to obtain real-time data from industrial fermentation (or chemical) processes, sensors that can be inserted directly into the fermentation broth (or reaction vessel) would be the best option. At the moment such sensors exist and are routinely applied for a limited number of simple measurands, such as pH, temperature and dissolved oxygen. However, more on-line information about the (fermentation) process in the form of the chemical composition of the fermentation or the reaction medium (or the contents of the micro-organisms) is often desirable, and it is hardly possible to find or develop a good sensor for each measurand, let alone that all these sensors could be interfaced properly and or that their output could be handled efficiently. Therefore, more generic analysis methods are desired, e.g. methods based on the separation and subsequent detection of constituents of samples extracted from the fermentor or reactor. Such methods have routinely been used off-line, in research environments but also in quality control. This contribution will show that nowadays such separation methods, based on e.g. electrophoretic and/or chromatographic concepts, are also available in a miniaturised format and therefore may be used directly on-line- or in-line with the process. The rationale for miniaturisation is obvious: reduced sample and reagent/solvent consumption, and improved analytical speed by shorter analysis times or by running several analyses in parallel.
**Miniaturised biofermentors**

The development of a new technological platform for fed batch fermentations in very small volume (10-300 μl) reactors in an array format will be described, which in the end will allow to improve production properties of micro organisms by rapid screening under industrially relevant conditions. On-line monitoring will be performed with the aid of integrated sensing elements for pH, dissolved oxygen, biomass and temperature, which are fabricated with state-of-the-art semiconductor technology, adapted in order to fulfil the specific requirements for operation in a fed-batch medium. Downscaling, sterilisation and long-term stability of the different sensor elements was studied and will be discussed in detail.

**Single-cell studies in a lab-on-a-chip**

Ultimately, and mainly for research purposes, studies on a single cell under normal culturing conditions offers advantages with respect to obtaining fundamental knowledge about the metabolism and life cycle of cells in response to certain additives, e.g. drugs. Investigations on cell ensembles always lead to average information, which in some cases is not accurate enough to evaluate the cell-to-additive response. Recent developments in the lab-on-a-chip field allow such single cell studies, and when used in parallel, will also lead to the desired stochastic distribution of cell responses. In this contribution a number of aspects of single cell studies in a lab-on-a-chip will be discussed, like cell transport and trapping, and cell content sampling and analysis.
Electronic sensing: food and feed applications

Naresh Magan
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Today food and feed quality and management in the food chain has become of primary importance. With the application of HACCP systems there is a requirement for the effective monitoring of Critical Control Points (CCPs) in such food chains. Rapid methods for monitoring CCPs are thus essential for effective management systems to be employed and the microbiological quality of food and feed safeguarded for consumers. It is well known that raw materials and processed products for food and feed can be contaminated by a wide range of micro-organisms. These often produce characteristic volatile fingerprints depending on environment and nutritional status of the food matrix. The volatiles produced include a range of alcohols, ketones and aldehydes. There has been significant interest in utilising these volatile fingerprints for early detection and diagnosis of quality. Rapid developments in sensor technology and sensor arrays have facilitated the production of devices, known as electronic noses, that can detect and discriminate in situ microbial contamination. These sensor arrays, e.g. conducting polymer, metal oxide, metal ions, optical sensors, which respond to these volatile fingerprints in different ways. These can then be compared using artificial intelligence for determining the presence of specific species or groups of species and can be used as part of a QA system. Studies have been carried out to examine the early detection of contamination of raw materials such as grain and flour with micro-organisms, especially moulds, milk contamination by bacteria and yeasts and more recently bakery products. Recent studies also suggest the potential of monitoring feed ingredients with this approach. Attempts have also been made to detect toxin contamination in cereals. The advent of sensor arrays, which can be used as an electronic tongue, may also provide opportunities for examining food and feed samples for quality determination against required standards. The potential for using these approaches as at line and on line devices will be discussed.
Magnetic biosensors

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Philips Research, Integrated Device Technologies, the Netherlands

We are investigating biosensors based on magneto-resistive sensors, with magnetic nanoparticles as detection labels. The sensors are fabricated using silicon technology and are packaged in a plastic cartridge. We expect the technology to be well-suited for rapid and sensitive detection in complex biological samples. In this talk I will describe the motivation, the biosensor device, the detection method, and the assay.
Genomics in microbial food quality: rapid access to the essence

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TNO Quality of Life, the Netherlands

The food industry is helped extensively when a clear insight into the presence and behaviour of micro-organisms present in ingredients, process line and final product could be given. A clear insight regarding the microbiology of food products and production is essential for prediction and management of food quality. The insight into microbial issues was to date depending on culturing, genetic typing and PCR detection. The information collected with these methods was rather restricted. Additionally, the time needed for collecting the limited amount of information was enormous and not matching with an adequate response for quality management.

This presentation will show how genomics tools can assist to get access to the potential of micro-organisms and their behaviour in food processing and shelf life. Similarly, the tools can also be used for beneficial micro-organisms such as probiotics and starter cultures for food fermentation. Genomes of many of the micro-organisms of concern to food quality and human health are known today and become more easily addressable.

A powerful genomics tool is genomotyping. This tool is equipped with a unique resolution and allows efficient characterisation of large sets of bacterial strains belonging to one species. Based on a several thousand different parts of the species genome, represented as individual spots on the genomotyping array, individual differences of strains can be visualised. From each strain a passport photograph is made that is unique for the strain. The features of the strains such as temperature resistance, preservative resistance, off-flavour production, dry resistance etc., can be linked to a group of individual spots (biomarkers) that can be used for detection-probe definition or further strain selection for starter cultures or probiotics. Traditional testing of strains with respect to the physiological properties on the contrary is extremely laborious for large sets of strains. By using the above described genomotyping tool a smaller representative set of strains with known physiological performance can be used for learning the genomotyping system (identifying markers) to discriminate strains with potential physiological features from the others lacking such feature, thus simplifying and shortening selection of detection probes and/or strains. For food spoilage- or pathogenic-strains, relevant biomarkers can be found that can form the basis for rapid detection tools such as dedicated DNA-chips or PCR detection systems. These systems are not based on species names but on the essential properties of the bacterial strains. Selection of starter strains or probiotics, proving their industrial property or authenticity, assessing their process resistance and capacity to survive GI tract passage as well as detection in complex micro-floras such as faeces from clinical trials, are the items that can be universally addressed by using genomotyping.

By addressing the DNA, the strain potential can be predicted. The behaviour of a strain can be assessed via transcriptomics, which is in essence also a genomics tool. Transcriptomics allows expression profiling. By using this tool, key transcripts can be identified that are linked to the physiological condition of the cell. Based on the detection of key transcripts the behaviour of an organism can be described and predicted. Here you can think of the survival of a bacterium at higher temperatures or in the presence of increasing concentrations of food...
preservatives. Taking advantage of this tool in to account, we can design optimised preservation processes as well as challenge tests. Another example will be given for the optimal formulation of probiotics in yoghurt drinks, which can be predicted based on genomics.

We will show of course how to exploit this genomics information in an effective way. Furthermore, we will focus on the progress that has been made on sample handling and preparation. This part of the analysis is most difficult to realise. We show how genomics can be used in high-resolution identification as well as tracking and tracing. Finally, we discuss ways in which the industry could exploit genomics tools in the generation of robust predictive microbial survival / growth models for quality assurance and quality control purposes.
Proteome and metabolome analyses for rapid authentication of food

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Proteomics and metabolomics are progressively being recognised as key tools for characterising biological systems. Several strategies have been developed for profiling proteomes and metabolomes and these can be used for monitoring food and environment safety, food authentication and food processing.

Reliable and rapid authentication of species of origin, especially with meat and meat products, authentication of geographical origin of plant products, monitoring adulteration/contamination, authentication of organic produce and genetically modified products are some of the issues concerning food authentication. A common objective in all these is the identification of suitable markers or group(s) of markers to characterise the authenticity of foods or their potential adulterants/contaminants and to use these to resolve authenticity issues. The issue of genetically modified crops has been a subject of intense debate ever since field trials were introduced in the 1980s, and is a major issue in food traceability.

Food, processed or packaged, is generally of plant or animal origin. Proteins and metabolites form a substantial fraction of organic matter, usually with significant functional import in most foods. Several macro- and micronutrients, anti-nutrients, plant toxins, secondary metabolites and allergens are proteins or metabolites. They are thus suitable candidates as (bio) markers and have the potential to be ideal candidates as indicators of the functional status of food and for characterising it.

Post-genomic technologies are typified by simultaneous high-throughput measurement of several analytes at the level of gene products, i.e., transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics). The underlying theme in these analyses is the parallel large scale detection of several of these analytes (transcripts, proteins, metabolites) simultaneously or within an analysis, so that a ‘holistic’ picture of the biological system under study emerges, more than would be afforded when analysing for specific components individually.

In a food authentication context, rapid methods that have the potential to give information on proteomes and metabolomes, albeit partially, are invaluable in complementing or preceding elaborate DNA based methodologies for characterising food. In particular, short and simple protocols that can capture prominent changes in a reproducible manner will be useful in a food authentication context. In such approaches it may not be required to identify and quantify the relevant proteins or metabolites, but to obtain a consistent fingerprint pattern for monitoring a given food, be it packaged or processed. Any deviations from the fingerprint outside the statistically relevant confidence thresholds would be indicative of safety concerns or loss of authenticity. Rapid strategies using techniques such as FT-IR, Raman spectroscopies, NMR and Mass spectrometry can be informational in this context. A general overview on current proteomic and metabolomic technologies with emphasis on rapid methods will be discussed, followed by examples of analysis in a food authentication context.
Application of spectral analysis to food and feed quality monitoring

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Centre wallon de Recherches agronomiques (CRA-W), Quality Department, Belgium

In recent years, spectroscopic techniques have come to be regarded as attractive and promising analytical tools for analyses carried out in research, control or industrial laboratories. These techniques are increasingly considered by analysts as an obvious solution. This trend stems from instrumental developments, the intensive use of computers and the development of appropriate chemometrics procedures. Every day, new applications of spectroscopic techniques are demonstrated and published in the fields of chemistry, pharmaceutical industry, agrofood sector, life sciences and environmental analysis. The objective should be not only to extend the field of application of spectroscopic techniques, but also to use them as a matter of course in control and industrial laboratories and to develop (according to internationally accepted guidelines) fully validated analytical methods.

CRA-W is involved in the NIR application developments for 25 years. The animal nutrition (forage, cereals, feed ingredients and complete feed) constituted the first applications of NIRS. During the last two decades, the applications moved on to the food sector with spectral data bases developed on milk, cheese, meat, honey, fruit juice, fruits, fertilisers, pesticides, biofuels, …. NIRS is mainly used in the laboratory, but several applications have been developed for on-line measurements like fermentation monitoring and precision agriculture.

Recently, thanks to its experience in calibration transfer, CRA-W joined its resources with a laboratory in UK (Central Laboratories, http://www.central-labs.co.uk) in order to bring to the fore the spectroscopic methods for the analysis of forage and feed products. Combining their databases, universal global calibrations are now proposed and can be installed directly on several instrument brands (Foss, Bruker, Nicolet, Buchi, Perten, Unity, Zeiss). INGOT™ calibrations are available across most instrument types for feed and feed ingredients, flour and milling, forage, petfood, and animal proteins. Robustness is synonymous with INGOT due to decades of seasonal, geographical, variety and product variations. These vast datasets (> 60,000 reference spectra) are supported with accredited reference chemistry, which cover the major parameters analysed within the different business sectors.

On the other hand, recent developments in spectroscopy have also led to the use of spectroscopic imaging instruments for the control and monitoring of agricultural, biological and environmental food and feed products. Spectral imaging techniques offer the possibility of collecting thousands of spectra of one sample or process. This can be done non-destructively and without interfering with the composition of the sample or the process. For the first time in the scientific world, an analytical tool is available that allows researchers to acquire spatially resolved chemical information about biological and agricultural materials. With imaging systems, researchers are able to obtain information not only about composition, but also about its distribution. They are also able to obtain chemical information at the microscopic level as well as at the macroscopic level. For the research community, this is an important revolution.
New developments in analytical tools to measure nutritional quality

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Immunoassays are set to have major impact on the monitoring of nutritional quality within the food and drink industries bringing high performance with convenience and being adaptable to provide excellent results from high-throughput laboratory-based systems to point-of-need devices. Their potential is, however, best seen when they are employed in non-competitive format systems for large molecular weight analytes where sandwich, non-competitive format assays may be employed. Unfortunately, a great many substances of key importance to the food and drink industries are simply too small to allow the simultaneous binding of two antibodies required by such classical sandwich assays. Inferior competitive-format systems have had to be used. We have developed three non-competitive format systems to overcome the limitations of sensitivity, speed, robustness and precision of competitive systems such that the advantages already clearly seen with large molecular weight analytes can be extended to the large array of small molecular weight analytes, from nutrients to toxins and other unwanted compounds, of direct importance to the food and drink industries.

The first non-competitive system is the Anti-Complex System. A secondary antibody is raised which binds the immune-complex of analyte and a primary antibody against it but not to either component alone. Binding of the primary antibody is thus analyte-dependent and can be used to determine directly analyte in a two-site reagent-excess system. This is applicable to all immunoassay labels and formats from specialised laboratory systems to point-of-need devices.

Our second system is the Selective Antibody System. Secondary antibodies are raised against a primary anti-analyte antibody and selected on their ability to bind primary antibody in the presence of its small analyte but not in its absence when a specific blocking reagent (such as an analyte-analogue or an anti-idiotypic antibody) can specifically bind the analyte-binding site and prevent secondary (selective) antibody binding. Thus binding of the selective antibody with the primary antibody is analyte dependent, providing a generic system for direct analyte measurement.

We have now developed the Apposition System as a way of dramatically speeding up the development of non-competitive systems. For this system we synthetically position a bindable reporter moiety close to the analyte binding site of, for example, a monoclonal antibody against the analyte, such that a secondary detector antibody may bind the moiety in the presence of analyte while in the absence of analyte unbound receptor sites are bound by the blocker, preventing binding of the detector antibody. Binding of detector antibody to receptor is thus again analyte dependent. Thus the Apposition System is a generic non-competitive immunoassay system for small molecules that, by optionally employing a universal reporter moiety and thus universal secondary antibody, can be rapidly configured. Again, it is directly applicable to all formats from high throughput systems to point-of-need systems such as dipsticks where it has been demonstrated to have particular advantages.
Rapid identification of plant and animal species in foods

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Investigations demonstrated that fraudulent replacement of food components as well as adverse reaction to unexpected food ingredients are quite common problems causing a significant economical damage. Therefore tools are needed to identify and control mislabelling of food products in all European member states. Up to now official methods for the detection of plant and animal species declared as ingredients in foods are exclusively based on protein analysis. In 2001 a European Commission 3-years funded project (MolSpec-ID, QLK1-2001-02373) started to develop DNA-analytical methods for qualitative and quantitative identification of plant and animal species in foods to monitor product safety and traceability.

Fourteen partners from 11 European countries took part in the project. In total ten qualitative PCR based methods for the identification of animal species and five PCR systems for the detection of plant species were developed and validated at least in-house. Specific emphasise has been put on the development of methods to identify hidden potential allergenic compounds. In addition, four collaborative studies were carried out towards the end of the project. Quantitative real time PCR based methods were established with respect to threshold values for supporting the surveillance of legal requirements. Furthermore, an important research aspect was the enhancing throughput by introducing multiplex-PCR, PCR-ELISA and chip technology.

All information has been included into a publicly available database including those to carry out the analysis (http://www.molspec.org). The added value of the database is the validated and transparent description of the methods developed through the project duration. The methods developed can be used without any restriction around whole Europe for control and traceability purposes. All necessary reagents can be purchased from different independent suppliers. Many laboratories in the European member states are already equipped with the machines necessary to conduct the PCR analysis. Due to the potential for an extended degree of automation, PCR methods are highly efficient for the analysis of many samples in parallel.
Rapid and reliable in vitro method for assessment of the nutritional quality of proteins (and carbohydrates)

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Food and feed industries continuously want to control and optimise the nutritional quality of their products. To do this, the industry urgently needs cheap, fast, reproducible and validated tools to determine the quality of the proteins in their products. The nutritional quality of protein sources depends on their amino acid composition and the digestibility of the proteins; especially the essential amino acids which cannot be produced by the body. The nutritional quality may also be affected by processing steps during manufacturing. A protein of high biological quality contains all the essential amino acids in bioavailable proportions that correspond to the needs of the consumer, such as infants, pets, and production animals. The essential amino acid(s) that is (are) in shortest supply in relation to the need is (are) referred to as the limiting amino acid(s).

Limitations and drawbacks of present methods

Traditionally the protein quality is evaluated by determining the Protein Efficiency Ratio (PER). This reflects the amino acid requirements of young animals such as broiler chickens and young rats, as determined by feeding these animals single protein sources and measuring growth. However, these experiments are quite slow and give no insight into the availability of the amino acid(s) that is (are) responsible for growth limitation. The test may also result in strong growth retardation due to amino acid deficiencies and therefore has strong ethical drawbacks. Moreover, this method determines the requirements of rats and broilers, not of humans or dogs.

The FAO/WHO has adopted the Protein Digestibility Corrected Amino Acid Score (PDCAAS) as the preferred method for determining protein quality. This method is based on a comparison of the amount of limiting amino acid in the test protein with the optimum requirement, corrected for the calculated ‘true’ digestibility of the protein. This does not necessarily correspond with the digestibility of the limiting amino acid(s). Rats are usually used in this method to calculate ‘true’ digestibility based on faecal apparent digestibility corrected for endogenous protein losses. This determination of digestibility on faecal level is not generally accepted as the best correction for digestibility. Beyond the ileum, protein is used by the colon microbiota and is therefore lost for body protein synthesis. For this reason, true ileal digestibility is regarded as the best parameter for correcting the amino acid score. Besides, this is also a time consuming method.

Does the ideal method exist?

The above mentioned methods have their limitations (e.g. limited information, indirect measurement) and strong drawbacks (time consuming, ethical constrains of animal experiments). The ideal method provides reliable insight into the true ileal digestibility of (single or mixed) proteins and availability for absorption of the essential amino acids in a fast and cheap way so that manufacturers could balance their products.
Gastrointestinal models

The digestibility of food and the availability for absorption of nutrients are related to:

- the type and processing of the food product (food factors), such as sources of protein and the disclosure of amino acids; and

- conditions in the gastrointestinal tract after the intake of the product (host factors), such as pH values, the secretions of digestive enzymes and bile, and the transit time of the food through the gastrointestinal tract.

The host factors are dynamic processes within the GI tract such as the impact of food matrix on GI transit and the secretion of digestive juices. These processes cannot simply be simulated in static in vitro models (beaker experiments). The TNO in vitro model of the upper gastrointestinal tract closely simulates the successive dynamic processes in the stomach and small intestine (TIM-1 system). It is a unique tool for studying the digestibility and availability for absorption of nutrients in the gastro-intestinal tract and validation studies have proven the high predictive quality of the system for single ingredients and complex meal matrices. For fast screening of the digestibility of proteins and the availability for absorption of amino acids a dedicated system has been developed and validated: the Tiny-TIM system (Figure 1).

Tiny-TIM system

The Tiny-TIM system simulates the body temperature, gastric emptying, pH profiles, and the secretion of digestive fluids such as salivary-amylose, gastric acid, gastric-lipase and pepsin, pancreas enzymes, bile, electrolytes and bicarbonate. The set-points of the system are not only based on the physiological data of humans (infants, adults) and animals (dogs, pigs, pre-ruminant calves), but also on the type of meal (e.g. liquid or solid meals). A specific dialyses unit is hooked up to the compartment of the small intestine for the absorption of low-molecular weight digestive products and water.

Figure 1. Diagram of Tiny-TIM.

- a: gastric compartment
- b: small-intestinal compartment
- c: gastric secretion products
- d: pH electrodes
- e: duodenal secretion products
- f: pyloric sphincter
- g: dialysis fluid
- h: semi-permeable membrane device.

Rapid and reliable assessment of protein quality

In Tiny-TIM experiments the test proteins (from single raw material to processed food) are mixed with saliva and drinking water (and if necessary with a meal matrix) and introduced into the gastric compartment. The food is gradually transported from the stomach into of the small intestine via the simulated pyloric sphincter. The gastric pH curve and the speed of gastric emptying are depending on the type of meal and the animal species that is mimicked. A blank experiment is performed to determine the contribution of secreted (endogenous) proteins.

Samples from the Tiny-TIM system can be collected from the lumen and the dialysate during
the digestion experiments (e.g. collected per hour). Analyses of these samples on nitrogen and amino acids will give a kinetic profile of the digestion and availability for absorptions of the nutrients. Nitrogen analyses give reliable information about the true ileal digestibility of the protein. The data can be used in combination with the amino acid analyses of the test product in order to calculate the PDCAAS. Amino acid analysis of dialysate samples gives reliable information about the availability for absorption of the (first limiting) amino acids.

Validation of this rapid Tiny-TIM method in comparison to in vivo studies showed a very high correlation between the bioavailability for absorption of the first limiting amino acids and the growth rate of the animals.

**Advantages of this method**

First of all it is a rapid method in comparison to animal trial. Within two days (one day for Tiny-TIM experiments, one day for analyses) you will have reliable data on the protein quality (in animal trials it takes a couple of weeks).

Another strong advantage of the Tiny-TIM method is that information is obtained on which essential amino acid is limiting and to what extent (in contrast to standard animal trials). Petfood companies have a strong advertising argument that scientific information of their products is obtained without animal experiments.

By using the Tiny-TIM system, a rapid and complete assessment of the nutritional quality of your proteins is obtained. This method supports improvements of production processes and the development of innovative products in a time and cost efficient way.
Rapid quality monitoring of packaging materials

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The quality of packaging materials in food industry is regularly controlled in order to avoid negative effects on the product caused by emissions. In this work a fast and economic system based on a sensor array is presented. To eliminate cross interferences a separation unit in the sampling system was added. As shown for paperboard samples it is possible to separate the main interferent, water vapour, and organic solvents into two peaks with a short packed polar column. With the same approach other packaging materials can also be analysed. For example the emission of acetaldehyde from PET (polyethylene terephthalate) bottles for drinking water, which causes an undesirable off-flavour, is easily detectable.

Providing healthy, safe, and high quality food to the consumer is a big challenge for the manufacturers involved in the complete food chain. To prevent food from degradation through environmental factors, today nearly every product is packaged. Modern packaging materials ensure a long shelf life stability of foodstuffs and protect them from environmental influences. However, the packaging material itself can have a negative impact on the content; for example, it can release solvents and various off-odour components. Sources of these undesirable substances (i.e. off-odours or off-tastes) are usually residual solvents from printing inks, over lacquers, adhesives and varnishes used in manufacturing processes (i.e. roto gravure, offset, hot foil stamping). To protect the consumer, for safety reasons and for customer satisfactory there is a need to monitor off-odours and residual solvents during the production of packaging materials at an early stage. Currently, the amount of residual solvents is determined quantitative with instrumental analysis like HS-GC-MS (headspace - gas chromatography - mass spectrometry) to rate if the threshold value is not exceeded. The total amount of volatile compounds is usually expressed as mass of the odour component per unit surface area of packaging (in mg/m²) [1].

The migration of acetaldehyde from PET in bottles for drinking water has a negative effect on the taste and can be recognised with mineral waters and soda when they are exposed to higher temperatures. This is the reason why in other areas of the packaging materials similar analyses as above described are necessary. One of the most important drawbacks is the time consumption and the individual cost. HS-GC requires a rather expensive instrumentation and skilled employees to provide a reliable interpretation of the data obtained.

For these reasons it is desired to develop another technique, which permits a fast and easy quality control. Using a sensor array, we have as the ultimate goal to develop a rapid monitoring tool that allows at-line tests and can replace spot checks [2-3]. Thus, the processes in the production chain can be controlled at early stage to save time, manpower and resources. The improvement of the applicability of the sensor system, consisting of micromachined metal oxide sensors, is the focus of the actual study. This was achieved by adding a dispersive element with the role of eliminating the interfering compound and developing an easy to handle independent system.
Escape Quality Control System

Experimental set-up

The ESCAPE Quality Control System (EQCS) has been developed for rapid quality control of packaging materials, i.e. for the detection of the total amount of the residual solvents. The EQCS consists of a headspace sampler, a dispersive element for separating water from organic compounds, the sensor system for detection and software solutions for data evaluation. All components are designed in a way to keep the overall system simple and reliable and therefore cost effective. Base of the EQCS is the sampling unit consisting of a new valve-free headspace sampler [4] combined with a packed chromatographic column. This allows a robust design suitable for use in a factory environment. In an actual version of the factory floor system (Figure 1A), vapours of water and organic solvents are separated by a packed polar column (flow rate 20 ml/min, temperature 120°C) and detected with 3 metal oxide sensors (Figure 1B).

Figure 1. A, ESCAPE Quality Control System (EQCS); B, Micromachined gas sensors based on semiconducting metal oxides were used as detectors (sensing elements) in sensor arrays due to their high sensitivity and long-term stability (http://www.appliedsensor.com).

Performance: separation of cross interferences

As known, paper and board packaging is not resistive to water, taking up moisture from ambient air during production and storage. It is also known that water (i.e. humidity in the gas phase) is a cross interference for the gas sensors based on semiconducting metal oxides. To achieve more water independent results, the first step was to use a packed column to ‘only’ separate the water from the solvent peak (all solvents in one peak). With the packed polar column, a separation of water from the organic solvents is easy (does not even need a temperature program) and fast (takes just 4 minutes for getting the integral solvent related signal) (Figure 2A).

One can see that the peaks corresponding to the organic solvents and water are fully separated and, accordingly, it is possible to evaluate the data with no influence from the main interferent (water). The next step is to carry forward this procedure on other packaging materials like for example PET bottles. On the one hand this problem is easier to handle because emissions from PET are caused by acetaldehyde which is the only analyte. On the other hand the acetaldehyde concentration in comparison with the paperboard samples is
much lower and a high sensitivity and fast response time of the sensors is necessary. First measurements (Figure 2B) are very encouraging and promise a good prediction of the emission even in this concentration range.

![Figure 2. Typical examples: A, Chromatograms of a board sample produced with roto gravure printing; B, Signal of the Pd 3% sensor (280°C) to the different concentrations of acetaldehyde. The acetaldehyde concentration of the PET-sample is in the range of 1.6 mg/kg.](image)

**Performance: prediction of the total solvent amount**

After the selection of the most appropriate parameters, the peak area of the corresponding sensor signals was used for the evaluation. The demonstration, if it is possible to predict the total amount of organic solvents, was carried out in two steps. The first approach was to correlate the area of sensor signals ('predicted solvent amount') with the 'true solvent amount' obtained from a FID, which detected the residual solvents of the headspace without separating each compound before. The correlation was obtained by using a PCR in which the inputs were the signals of the sensors in the array. The FID was calibrated using ethanol as a standard and the FID signals (i.e. 'true solvent amount') from the packaging samples were expressed in ethanol equivalents (under the assumption that ethanol is representing the residual solvents) (Figure 3).

![Figure 3. Prediction of the ethanol equivalents of residual solvents (measurements on the laboratory prototype of the EQCS with 4 sensors.](image)
Outlook

We developed a rapid procedure to quantitatively determine total amount of the residual solvents in the packaging materials using separation strategy (chromatographic column) and sensor array consisting of 3 micromachined metal oxide sensors. The next step is to devise an application specific calibration method that will function even without analytical instrumentation and will allow for transforming the proposed approach into an independent measurement system for applications in different areas for food and quality control.

References


Acknowledgements

This work has been performed in the frame of the ESCAPE Project (Electronic Sensor System for the Characterisation of Packaging Emissions, QLK1-CT-2001-02194, http://www.escape-project.org), supported by the European Commission, Quality of Life and Management of Living Resources Programme, Key Action 1 on Food, Nutrition and Health.
The requirement for real-time monitoring in the modern and highly automated food-processing environment has stimulated research into rapid microbiological testing. The conventional microbiological approach to food sampling has changed little over the last half century and it has been estimated that there are currently in excess of 40 methods to measure and detect bacterial spoilage in meats. The ideal method for the on-line microbiological analysis of meat would be rapid, non-invasive, reagentless and relatively inexpensive and these requirements can be met via the application of a spectroscopic approach, in combination with any appropriate data deconvolution strategy based on statistics or machine learning.

Fourier transform infrared (FT-IR) spectroscopy is a rapid, non-invasive technique with considerable potential for application in the food industry. We have shown that this technique can be used directly on the surface of food to produce biochemically interpretable ‘fingerprints’. Microbial spoilage in meats is caused by the growth and enzymatic activity of micro-organisms and FT-IR was exploited to measure changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage.

Quantitative interpretation of FT-IR spectra was possible using partial least squares regression and allowed accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s. Genetic programming was used to derive rules showing that at levels of $10^7$ CFU.cm$^2$ the main biochemical indicator of spoilage was the onset of proteolysis. Thus, using Fourier transform infrared spectroscopy (FT-IR) and machine learning we were able to acquire a metabolic snapshot and quantify, non-invasively, the microbial loads of food samples accurately and rapidly in 60 s, directly from the sample surface.
Flow cytometry as a rapid tool for microbiological analysis in the food industry: potentials and restrictions

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The food industry requires methods for the detection of bacteria, yeasts, moulds and viruses in food samples that are rapid, sensitive, accurate and cheap. In this presentation it will be discussed which requirements flow cytometry can meet best.

Flow cytometry was developed during the last half of the sixties. It was not until the seventies, microbiologists started studying the possibilities of flow cytometry for the research and analysis of bacteria. Still nowadays many microbiologists are not aware of the possibilities that flow cytometry can offer or acquiring knowledge about various aspects of micro-organisms. The potential in microbiological research is far from fully utilised.

Flow cytometry is a means of measuring (meter) certain physical and/or chemical characteristics of cells (cyto) or particles as they flow in a liquid. It is essentially comparable to a fluorescent microscope with cells flowing through the focus. The fluid column passes through a beam a few cell diameter across. Laminar flow permits laser interrogation of the cells, one cell at a time. The modern flowcytometer consists of a light source, collection optics, fluidics, detectors, electronics and a computer to translate signals to data. The light source is nowadays usually a laser, which emit coherent light at a specified wavelength. During the analysis, both scattered (right or orthogonal and forward) and emitted fluorescent light (for cells stained with fluorochromes) is collected. As such as well physical characteristics of one cell such as size, shape etc. as other aspects as cell density etc. can be studied. Various light sources and also various dyes are available each with their own possibilities of application.

Today flow cytometry has a wide range of microbiological applications, ranging from detection of the specific bacteria or the amount present, studying the viability, studying the cell cycle of an organism, studying the susceptibility to chemicals (antimicrobials) or physical treatments, monitoring bacteria in various environments e.g. in food fermentations, etc. However, for sensitive determination of micro-organisms directly in food matrices, discrimination against non-specific fluorescence due to particulate matter has to be overcome and too much noise has to be excluded. The possibilities under research in food will be discussed.

A short evaluation of some experiments and results obtained in food analysis by using a compact flowcytometer with a green laser carried out in our laboratory will also be discussed.
PCR for the detection and identification of live spoilage organisms

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Beer has a low pH (pH 4), contains typically about 3–4 % w/v ethanol and the hop compounds present have antimicrobial properties. Therefore it is a relatively poor medium for bacterial growth and the range of bacteria that commonly contaminate is small. However, spoilage of beer by micro-organisms such as bacteria or ‘wild’ yeast (unwanted yeast) is of great concern to the brewer. These infections can lead to turbidity of the beer and abnormal flavours and aromas.

Consequently the need for rapid and reliable microbiological detection methods is ever present. Whilst traditional microbiological methods for the detection of beer spoilage organisms are extremely accurate and sensitive they are laborious and slow. Methods based on the polymerase chain reaction (PCR) can be of great help here as they are highly specific and provide results much faster than traditional microbiology techniques. These methods are increasingly being adopted by the food and beverage industries for high throughput analysis and microbiological troubleshooting.

One of the drawbacks of PCR methods is the inability to differentiate between live and dead cells. This can be partially overcome by the use of a pre-enrichment step, where the sample is incubated in liquid medium for a short period. Whilst the pre-enrichment step increases the number of live cells, it adds 1-3 days to the time elapsed before detection and has the drawback that the initial cell concentration cannot be calculated.

Recently, a novel method for live/dead cell determination has been described. This method has been adapted for brewing samples and has shown promise in laboratory studies. Ethidium monoazide bromide (phenanthridium, 3-amino-8-azido-5-ethyl-6-phenyl bromide) or EMA is a chemical agent that can traverse dead cell membranes and bind irreversibly to DNA under exposure to light. The covalently bound DNA cannot be amplified by PCR, thus preventing the detection of dead cells.

This methodology has been tested using Saccharomyces cerevisiae and Lactobacillus brevis cells. A range of cell inactivation methods were investigated employing the EMA-PCR technique. For example, cultures of the organisms were pasteurised, EMA was then added and PCR carried out. No PCR product was detected, indicating that EMA did indeed inhibit the amplification of dead cell DNA. The EMA-PCR technique was tested using two different PCR methodologies, Standard PCR and Real Time PCR, with detection by gel electrophoresis and fluorescence respectively. In both cases the results were good. Real Time PCR was the more sensitive of the two methods and allowed discrimination between live and dead cells in mixed cultures. Clearly this technique should reduce the ‘false-positive’ signals from dead organisms, often obtained with current methods.

The evolution of live/dead assays for the quantitative detection of spoilage organisms by Real Time PCR represents an exciting development in microbiology. The EMA-PCR technique could provide a simple, cost effective method for the rapid detection and identification of viable beer spoilage micro-organisms.
Electronic nose technology for detecting taints and off-flavours

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Analysis of taints and off-flavours in food has traditionally been performed either by a trained sensory panel or by headspace gas chromatography mass spectrometry. These methods are time consuming and costly and there is a need in the food industry for rapid objective techniques that can detect taints and off-flavours in food. Taints represent unpleasant odours or flavours imparted to food through external sources, whereas off-flavours can represent unpleasant odours or flavours imparted to food by internal deteriorative changes (Kilcast, 1996). New methods for detecting taints and off-flavours should allow a high number of samples to be analysed within a short period of time with a sufficient reproducibility and accuracy.

Gas sensor array technology combined with multivariate data processing methods (electronic nose) has demonstrated to have a potential for rapid analysis of food quality. Detection of tainted food and off-flavours in food represent one of the application areas for this technology. The technique cannot completely replace reference methods like the use of more specific chemical analysis or sensory panels as the technique requires a frequent calibration against some valid reference method. The sensor array of an electronic nose has a very large information potential and will give a unique overall pattern of the major volatile compounds of the food that are being analysed. In principle, both the electronic and the human nose operate by sensing simultaneously a high number of components giving rise to a specific response pattern. However, there are two basic differences between the human and the electronic nose that should be kept in mind. The electronic nose has both large differences in sensitivity and selectivity from the human nose. The sensors of an electronic nose respond to both odorous and odourless volatile compounds. Taking these constraints into consideration in the choice of sensors used for these instruments it is in principle possible to design an electronic nose with a response similar to the human nose for specific compounds. Still, the sensing mechanism involved will be fundamentally different.

Taint and off-flavour compounds in food frequently occur in very low concentrations and may have extremely low threshold values, and require sophisticated sampling equipment. Accordingly, for a number of cases the taint and off-flavour compounds will be out of the sensitivity range of the chemical gas sensors applied in electronic noses, and will be masked by the other major volatile compounds. The most successful applications so far, have therefore, shown to be cases where the key taint/off-flavour compounds are represented by the major compounds in the headspace of the food sample, and which can be verified by simultaneous headspace GC/MS analysis. Typical cases are off-flavours related to lipid oxidation, polymer oxidation (packaging), spoilage (bacteria and fungi) and inks and solvents of packaging materials. Provided that the electronic nose instruments have been calibrated (trained) properly with sensory analysis or with GC/MS, and perform according to the required accuracy and reproducibility, they could be applied to partly replace a sensory panel or traditional chemical methods in the food industry on a routine basis for screening purposes.

Different taint and off-flavour food applications using gas-sensor array systems will be presented and discussed. Among these some have already been implemented in the food industry.
Application of a rapid analytical technique for the determination of oxidation stability in food development and control

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\textsuperscript{1}Velp Scientifica, Italy and \textsuperscript{2}Barilla G.&R.F.Ili, Italy

The evaluation of oxidation stability of foods can be performed in an accelerated way, at comparatively high temperatures, in the presence of a measurable oxygen pressure. Useful information is obtained by recording the decrease of oxygen pressure, being it partly used to cause oxidation of the fat contained in food. In particular a useful parameter is the Period of Induction (I.P.), that is obtained as showed in Figure 1. This technique, having the geometry detailed in ASTM D942 \textendash IP 142 method, proved to be very useful for this kind of evaluation; in previous work realised with the collaboration of TNO (the Netherlands) it was possible to study the relative stability in different formulas of pasta sauces.

![Figure 1. Evaluation of the Period of Induction.](image)

Unfortunately, this kind of study can be performed only on products having a high fat content (> 10%); more recent needs regard products and raw materials with low fat content, which cannot be analysed by the traditional geometry instrument. Barilla, some years ago, started a collaboration with Velp Scientifica, having the aim to solve this kind of request too. Taking into consideration the previous experiences, the original instrument was completely redesigned, exclusively with the aim to increase its sensitivity and hence the field of application. Particularly, in order to increase the signal of the instrument and to reach higher sensitivity levels, the importance of the following aspects was noticed:

- Increasing sample amount that could be introduced into the autoclave.
- Reducing oxygen volume, to make more evident the pressure drop.
- Increasing the sample exposure surface and reducing thickness, to make the oxidative process more homogeneous.
- In case of need, wetting the sample, to avoid burning.
- Providing the management software with a smoothing system for the signal, to counterbalance the small temperature variations of the thermostat, which cause pressure-oscillating variations.
With the new version of the Oxitest instrument it is now possible to load into the autoclave chamber up to 80-100 g of sample (depending on its density) and the available volume for the oxygen has been strongly reduced. These improvements enable us to measure oxidation stability of cereals having about a 3% fat content.

Oxitest’s fields of application can be summarised as follows:
- It is possible to make rapid comparisons among different formulas of a product, by performing oxidation at pre-fixed temperature (for instance 90°C).
- It is possible to verify the incidence of different lots of the same raw materials, directly on the final product.
- It is possible to associate other kinds of determinations, for example gas-chromatographic analyses, to evaluate oxidation products formation and disappearance of flavour compounds.
- It is possible to evaluate the performances of different packaging materials: the different packaged products are analysed by Oxitest after storage at defined temperatures for some months, making a direct comparison among their induction periods.
- It is possible to obtain a prediction of oxidation stability during a shelf-life study, measuring the product at defined time intervals and building an experimental curve (Table 1).
- In some cases it could replace the determination of the peroxides, avoiding the use of dangerous solvents, such as chloroform.

Table 1. Data obtained during the time on the same biscuit sample.

<table>
<thead>
<tr>
<th>Preservability – Biscuits (35°C)</th>
<th>Oxitest at 90 °C induction time (hour.min)</th>
<th>Peroxides U.V. (meq/kg fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time preservability (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.45</td>
<td>1.3</td>
</tr>
<tr>
<td>15</td>
<td>16.26</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>14.48</td>
<td>1.6</td>
</tr>
<tr>
<td>45</td>
<td>13.19</td>
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<td>60</td>
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<td>2.5</td>
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<td>75</td>
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<tr>
<td>90</td>
<td>11.45</td>
<td>2.4</td>
</tr>
<tr>
<td>120</td>
<td>11.06</td>
<td>2.6</td>
</tr>
<tr>
<td>135</td>
<td>10.35</td>
<td>2.7</td>
</tr>
<tr>
<td>160</td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>

The technique has been tested with satisfactory results; it resulted to be applicable to many food samples, both final products and raw materials, without the need of a preliminary separation of the fat to perform the test. Some examples could be: bread, crispbread, biscuits, sauces, minicakes, creams, meat, cheese, cereals, etc. The obtained results show the reliability of the technique, making us able to conclude that Oxitest is suitable for providing useful information in food development and control, otherwise obtainable only by long and expensive instrumental and organoleptic tests.
DNA-based techniques used for authenticity and adulteration testing

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Molecular biology has not long ago been a science considered as blue-sky research based in universities with no applications in real life. This view has radically changed in recent years and these techniques are implemented in more and more laboratories across the world, especially since they are very useful for detecting fraud and adulteration of food and non-food products.

The presentation will focus on food products and give an overview over the different molecular biological approaches taken to solve analytical problems, which could not be solved using standard chemical or physic-chemical techniques. Topics addressed, *inter alia*, will be authenticity testing of coffee, olive oil and wine; adulteration of expensive fish species like monkfish and yellofin tuna as well as testing for the presence of allergens and GMO in line with new European Regulations and Directives.
Immunoassay techniques to detect adulteration

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Food adulteration has been known for centuries. It is the process by which wholesome food is deliberately mixed with or replaced by lower quality or inappropriate materials. There is a variety of legislation across the world, which is intended to end the practice of adulteration, however the practice is believed to continue.

The route to the elimination of deliberate adulteration is the development of simple, reliable, routine assays, which can sensitively detect adulterants in complex food matrices. This paper attempts to give an understanding of the problems and to compare the different methods, which have been employed to solve these problems. Comparisons will be made between the different assay formats, their utility in routine application and the needs for development of novel assays for the future. It is intended that this overview should provide a guide to the development of appropriate new assays.
Rapid detection of species adulteration in milk and dairy products

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Some cheese varieties labelled with the protected denomination of origin from the European Union exclusively require non-bovine milk as milk components. Such strict requirement also interests European common cheeses labelled as pure ovine, caprine, and bufaline as the European legislation rules out that no additional components must be contained without appearing on the cheese label. The foods destined to the celiac patients must bee gluten free. However, a 20 ppm limit for a gluten content is tolerated (Codex Alimentarius). Cryptic casein in cheese is also detrimental for allergic humans. Traditionally people suffering for bovine protein components are compatible with non-bovine cheese. Vice versa, there is also an increasing number of people detected allergic to caprine or ovine cheese having tolerance for the homologous bovine products [1]. The optimal issue in cheese authenticity validation would require a two-step analytical procedure screening the collect milk and the final product for the target milk species alone (and the simultaneous absence of any contaminating milk from other species). The positive result of foreign milk detection from a quick immunological test is validated later by application of the conventional analytical confirmatory methods.

The current EU reference method for the detection of bovine casein in cheese made from milk of other species is judged positive when the signal of the marker $\gamma_2$- and $\gamma_3$-CN is lower than 0.5% [2]. Therefore, a pre-requisite for adopting any screening method would be a high sensitivity, involving little or no sample treatment, and a fast response for immediate decision-making, together with a non secondary low-cost characteristic.

Traceability in screening analysis

Therefore, a strategy for ensuring traceability of the non-bovine cheese products would require any screening procedure like a simple test using a paper strip for the approximate pH value measurement which change colour according to the pH value. Figure 1 shows the ideal schematic diagram of the screening and the confirmatory methods in detecting fraudulent addition of cheaper and common milks to higher price milks.

Sample preparation:

whey and casein by isoelectric precipitation from skimmed milk

<table>
<thead>
<tr>
<th>Screening method</th>
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<tbody>
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<td>Binary response: yes/no</td>
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<table>
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<tr>
<th>Confirmation methods</th>
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<tr>
<td>Any analytical procedure based on protein or peptide separation further integrated by the identification of the separated milk components:</td>
</tr>
<tr>
<td>1) Gel electrophoresis; 2) immunoblotting</td>
</tr>
<tr>
<td>1) HPLC; 2) electrospray mass spectrometry</td>
</tr>
<tr>
<td>1) MALDI-TOF; 2) MALDI-PSD</td>
</tr>
<tr>
<td>1) LC-ESI; 2) MS-MS</td>
</tr>
<tr>
<td>Quantitative procedure: internal standard or relative peak intensity measurement</td>
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Figure 1. Diagram of (1) screening and (2) confirmation methods for milk mixture evaluation.
Quick tests based on immunoglobulin detection

A simple and fast method for detecting the presence of bovine IgG at low levels in sheep, goat and water buffalo milk is now available [3]. This test is claimed able to detect bovine IgG in the water soluble milk phase at concentration as low as 1% (v/v) from either raw or variously heated milk (pasteurised to sterile milk). In a migration taking ~20 min the absence of a line in the test position indicates the lack of bovine IgG in the sample (or the presence in an amount less than 1%). An indirect competitive ELISA assay has been also developed using monoclonal antibodies for the detection of IgG in adulterated milk samples, and to quantify bovine milk amounts as low as 0.1% adulterating sheep, goat and buffalo milk [4]. At our knowledge no test kit is available to detect the presence in a sample of the four animal species simultaneously. Detection of caprine in ovine milk or bovine (ovine) in water buffalo milk represents the emerging problem in some European regions. Conformational epitopes in the tertiary structure of bovine IgG binding monoclonal antibodies are responsible for positive response. If these epitopes were destroyed by extensive denaturation or partial hydrolysis, decreased or negative response is obtained. In this case, polyclonal antibodies against sequential IgG epitopes might more useful to detect intensively treated milk samples than the monoclonal ones. Overlapping peptide micro-array technology would better tell whether bovine IgG occurs in non-bovine milk sample with denatured IgG protein or derived peptides binding to many bovine specific anti-peptide antibodies. Such developing procedure would enable analyst to screen a number of samples with just a few drops of diluted whey. A severe limitation of the methods based on whey protein detection is quantification of foreign milks as whey proteins irreversibly denature by heating aggregating with casein at isoelectric pH.

Quick tests based on casein detection

At our knowledge, no test kit enabling detection of bovine casein as rapidly as the IgG test does is currently available. However, the commercial ELISA developed by R-Biopharm (Bovine Casein Kit, cat. No. R5102) and Tepnel Biokits (Bovine Casein Kit, cat. No.902062W) [5] has recently judged unable to distinguish between cow’s milk and water buffalo milk only [4]. It is clear that no general reagent is available for milk speciation. The analytical problem is more easily solved if binary mixtures are to be distinguished.

Dot-blot with polyclonal anti-peptide antibodies against selected bovine casein components

To provide a simple, low-cost alternative to analytical procedures, a multi-well plate dot-blotting system was developed in our laboratory. The casein solution is transferred through vacuum blotting manifold on a nitrocellulose membrane sheet to create a dot blot. After exposing to the primary antibody specifically recognising a target casein component of binary milk mixtures, a secondary antibody/enzyme substrate complex is reacted with protein-antibody complex for quantification. The colorimetric signal is measured by a flat bed scanner capturing digital images and compared with those from mixtures at known content of the foreign milk simultaneously analysed on membrane wells.

Laboratory screening whey-based methods in samples of high interest

Rapid analytical systems that provide a reliable yes/no response are necessary and the most diffuse are the electrophoretic ones; the procedures based on gel isoelectric focusing, HPLC, capillary electrophoresis have been developed during the last years. Every positive result provided by the quick tests requires confirmation, usually by mass spectrometry in diverse versions.

Analysis by gel electrophoresis and immunoblotting with polyclonal antibodies against γ2-CN

The European Official Method of Analysis based on recognition of the bovine γ2-CN
has made highly specific and sensitive by using polyclonal antibodies specifically recognising γ2-CN from milk of any species. Immunoblotting serves to establish matches between suspect adulterated and abnormally degraded cheese casein and to distinguish between γ2-CN and similarly migrating bands. The relative amounts of this bovine marker and its ratio to homologous component of a non-bovine cheese are used to differentiate adulterated and genuine cheese and calculate the extent of the adulteration. The examples from our laboratory concern the detection of γ2-CN in water buffalo milk and derived Mozzarella cheese containing different amounts (0.2 to 5%) bovine milk. The focused profile after immunoblotting with polyclonal antibodies raised against peptide stretches of γ2-CN was clearer than that coloured with traditional Blue stain as it contained exclusively the γ2-CN and peptides containing the putative peptide stretch.

**Direct analysis by HPLC**

The method that uses HPLC and C18 column for separating β-Lactoglobulin (β-Lg) of different species is useful to detect bovine whey (β-LgA as marker) in water buffalo milk [6]. Similar methods have been developed for detecting binary mixtures by HPLC [7] or tertiary milk mixtures by CE [8].

**Novel methods based on on-line HPLC-ESI/MS detection of intact proteins and whole whey hydrolysates**

Rationalisation with ESI/MS of the efficiency in separating the homologous components from four species precluded the use of HPLC as global technique. Co-eluting proteins were identified according to the different molecular mass and, whenever possible, quantified by a LC-ESI/MS-SIM. Further improvements of the quantitative technique have been obtained by using as species marker a derived tryptic peptide instead of the parent protein. MALDI-TOF has shown also able to partly solve the problem of detecting binary mixtures of milks [9].

**Laboratory screening casein-based as confirmatory methods**

Mass spectrometric techniques are frequently used to identify proteins and peptides in proteomics. However, these techniques are too expensive for screening purposes and are less suitable for on-site monitoring. Instead, confirmatory methods, based on direct analysis or by LC-MS, could be used in laboratory to recognise casein from single species through multiple markers, either casein or whey fractions. An example of the application of MALDI-TOF in detecting simultaneously the four casein fractions from quaternary mixtures of milks is presented with the indication of the casein peptide markers.

**References**

Rapid testing for authenticity of (olive) oils

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Oils and fats constitute one of the three major classes of food constituents, the others being proteins and carbohydrates. Chemically oils are predominantly tri-fatty acid esters of glycerols and the relative proportion of each fatty acid together with minor compounds (e.g. sterols, alcohols, chlorophylls, hydrocarbons, waxes etc) are important not only in nutrition but also in authenticity.

Beyond the dictionary definition of the authenticity term, the criteria of edible oil authenticity are associated to terms such as adulteration, geographical origin, extraction system and variety (in the case of virgin olive oil) among others. However, the authenticity issues are evolving continually to situations that are basically governed by a global market trend. Thus, the analytical techniques have been developed or modified to give plausible solutions to the devious adulterations, the characterisation of premium oils or the chemical traceability at each moment.

Despite the inconvenience of the wide variety of vegetable products that can produce edible oils, it has been possible to have irrefutable analytical methods in adulteration (e.g., stigmastadienes) due to the large number of edible oil constituents. Furthermore, increasingly sophisticated methodologies are today capable of picking up minor differences associated with some authenticity issues, most the which are based on stable isotopic analysis, trace element analysis, pattern recognition, structural analysis and separation techniques.

On the basis of the expanding markets, the authentication issues have been focused on the analysis of vegetable oils, olive oil in particular, and fish oils. Some of the methods and techniques used in the authentication of these edible oils are described in the following tables. The information is clustered into three groups: fish oils, seed oils and olive oils. Tables 1-3 include classical chromatographic techniques, some of them being international standards, and sophisticated methodologies although most of these are circumscribed to the protection of olive oil and its categories (extra-, virgin, refined olive oils) against their adulteration.
Table 1. Overview of methods of fish oil authenticity testing.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte - Indicative information</th>
<th>Applicability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H NMR$^a$</td>
<td>Content of Total $\omega$-3 acids, DHA (C22:6n-3), EPA(C20:5n-3), cholesterol, and mono-, di- and tri-glycerides</td>
<td>Composition Process identification</td>
</tr>
<tr>
<td>$^2$H NMR</td>
<td>Deuterium distribution in fatty acids, fish oil</td>
<td>Origin</td>
</tr>
<tr>
<td>$^{13}$C NMR$^a$</td>
<td>Positional distribution of the $\omega$-3 fatty acids in the triglyceride moiety. A fingerprint that includes free fatty acids, fatty acids esterified to mono, di-, and tri-glycerides and phospholipids.</td>
<td>Species identification Process identification</td>
</tr>
<tr>
<td>GC</td>
<td>Fatty acid profile</td>
<td>Adulteration of fish oil with vegetable oil</td>
</tr>
<tr>
<td>SFC$^a$</td>
<td>Distribution of triglycerides</td>
<td>Species identification Process identification</td>
</tr>
</tbody>
</table>

Note: NMR, Nuclear Magnetic Resonance; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; GC, Gas Chromatography; SFC, Supercritical Fluid Chromatography; $^a$ Examination of intact product.

Table 2. Overview of methods of seed oils authenticity testing.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte - Indicative information</th>
<th>Applicability</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>Linolenic (C18:3) FA composition at TG 2-position</td>
<td>Presence of groundnut or sunflower oils in soya and rapeseed oils</td>
</tr>
<tr>
<td>GC</td>
<td>Brassicasterol</td>
<td>Presence of sunflower and groundnut oils in rapeseed oils</td>
</tr>
<tr>
<td>HPLC</td>
<td>$\gamma$-tocopherol</td>
<td>Adulteration of sunflower oil with soya oil</td>
</tr>
<tr>
<td>GC</td>
<td>C60/C58 ratio</td>
<td>Adulteration of safflower oil with sunflower oil</td>
</tr>
<tr>
<td>GC</td>
<td>C48 concentration $\times$ Palmitic (C18:3) enrichment factor</td>
<td>Detection of stearins or oleins in palm oil</td>
</tr>
<tr>
<td>GC</td>
<td>Palmitic (C16:0)</td>
<td>Presence of palm olein in cottonseed oil</td>
</tr>
<tr>
<td>GC</td>
<td>C33, C36, C38, C40</td>
<td>Detection of mixtures of palm kernel and coconut oil</td>
</tr>
<tr>
<td>GC</td>
<td>Oleic (C18:1) FA</td>
<td>Detection of palm kernel olein oil in palm kernel</td>
</tr>
<tr>
<td>IRMS</td>
<td>$^{13}$C/$^{12}$C ratios</td>
<td>Presence of maize oils with other commercial oils</td>
</tr>
<tr>
<td>GC</td>
<td>Linoleic (C18:4), Erucic (C22:1)</td>
<td>Presence of borage oil in evening primrose oil</td>
</tr>
<tr>
<td>GC</td>
<td>Linoleic (C18:2), Stearidonic (C18:4)</td>
<td>Presence of blackcurrant seed oils in evening primrose oil</td>
</tr>
<tr>
<td>GC</td>
<td>C18 (oleic, linoleic, linolenic) FA</td>
<td>Geographical origin (continent) of groundnut oil</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Tocopherols, tocotrienols</td>
<td>Characterisation of grapeseed, HOSO and soya oils</td>
</tr>
<tr>
<td>DSC</td>
<td>Information of the temperature curve</td>
<td>Characterisation of palm, palm kernel and coconut</td>
</tr>
</tbody>
</table>

Note: C, Carbon number triacylglyceride; DSC, Differential Scanning Calorimetry; FA, Fatty Acid; HOSO, High Oleic Sunflower Oil; IRMS, Isotope Ratio Mass Spectrometry.
Table 3. Overview of methods of olive oil authenticity testing.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte - Indicative information</th>
<th>Applicability</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Triacylglycerides</td>
<td>Olive oil categories: VOO, ROO and POO</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Selected wavenumbers</td>
<td>Olive oil categories: VOO and ROO</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Tocopherols, tocotrienols</td>
<td>Olive oil categories: VOO and ROO</td>
</tr>
<tr>
<td>GC</td>
<td>FA, Hydrocarbons, etc</td>
<td>European varietal olive oils</td>
</tr>
<tr>
<td>DHS-GC</td>
<td>Volatiles</td>
<td>European varietal VOO</td>
</tr>
<tr>
<td>HPLC</td>
<td>Chlorophylls</td>
<td>Spanish varietal VOO</td>
</tr>
<tr>
<td>GC</td>
<td>Diff. series chem. comp.</td>
<td>Non-natural products in VOO</td>
</tr>
<tr>
<td>13C-NMR</td>
<td>Selected δppm</td>
<td>Geographical origin of European olive oils</td>
</tr>
<tr>
<td>GC</td>
<td>Aliphatic alcohols</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>Terpenic alcohols</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>Stigmastadienes</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>Triacylglycerides</td>
<td>Presence of some refined seed oils (maize, cottonseed, sunflower, soybean, rapeseed) in olive oils</td>
</tr>
<tr>
<td>LC-GC</td>
<td>Sterols</td>
<td>Presence of refined seed oils (rapeseed, sunflower, soybean, palm and grapeseed) in olive oils</td>
</tr>
<tr>
<td>FT-IR</td>
<td>1st Derivative selected wavenumbers</td>
<td>Presence of seed oils (soybean, sunflower, rapeseed, coconut and walnut) in ROO</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Triacylglycerols</td>
<td>Presence of canola oil in ROO</td>
</tr>
<tr>
<td>NMR</td>
<td>Squalene, sterols</td>
<td>Presence of POO in ROO</td>
</tr>
<tr>
<td>LC-GC</td>
<td>Δ8, 14-Stigmastenol</td>
<td>Presence of desterolised sunflower oil in olive oils</td>
</tr>
<tr>
<td>GC</td>
<td>Ratio R1/R2</td>
<td>Presence of all the desterolised vegetable oils in olive oils</td>
</tr>
<tr>
<td>LC-GC</td>
<td>Palmitic in 2-position</td>
<td>Presence of esterified edible oils in olive oil</td>
</tr>
<tr>
<td>FT-Raman</td>
<td>Selected wavenumbers</td>
<td>Presence of HOSO or maize or POO in VOO</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Elements</td>
<td>Geographical origin</td>
</tr>
<tr>
<td>MOS sensors</td>
<td>Sensor response</td>
<td>VOO: varieties and categories</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>Triacyl- and diacyl-glycerols</td>
<td>Characterisation of several seed oils (hazelnut, pistachio, almond, palm, rapeseed, macadamia, soya, etc.)</td>
</tr>
<tr>
<td>GC</td>
<td>Sterols</td>
<td>Presence of hazelnut or sunflower oils in olive oil</td>
</tr>
<tr>
<td>SFE-GC, RPLC-GC, SPME-GCMS</td>
<td>Filbertone</td>
<td>Presence of crude hazelnut oil in VOO</td>
</tr>
<tr>
<td>13C-, 1H-, 2H-NMR, FT-MIR and FT-Raman</td>
<td>Spectrum peaks</td>
<td>Presence of some seed oils (e.g. hazelnut) in olive oil</td>
</tr>
<tr>
<td>δ2H-EA-Py-IRMS</td>
<td>Ion ratio</td>
<td>Presence of hazelnut oil in olive oil</td>
</tr>
<tr>
<td>δ2H-GC-Py-IRMS</td>
<td>Isotope ratios FAMEs</td>
<td>Presence of hazelnut oil in olive oil</td>
</tr>
<tr>
<td>HPLC and GC</td>
<td>Triacylglycerides</td>
<td>Presence of seed oils (e.g. hazelnut) in olive oil</td>
</tr>
</tbody>
</table>

Note: DHS, Dynamic Head-Space; FA, Fatty Acids; FT, Fourier Transform; HOSO, High Oleic Sunflower Oil; HPLC, High Performance Liquid Chromatography; HS, static headspace; LC, Liquid Chromatography; IR, Infrared Spectroscopy; MS, Mass Spectrometry; RP, Reverse Phase; VOO, Virgin Olive Oil; ROO, Refined Olive Oil; OO, Olive Oil; POO, Pomace Olive Oil.
Real-time PCR for meat and fish quantitation

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¹Central Science Laboratory, UK, ²Veterinary Laboratories Agency, UK and ³Campden and Chorleywood Food Research Association, UK

Quantitative assays to determine the species and content of meat in complex meat products are needed to protect the consumer and to enforce correct product labelling, especially with respect to recent QUID legislation. The most suitable analyte for this type of work appears to be DNA, which can be used in species-specific assays, and in some applications, has been shown to be capable of supporting quantitative assays. Currently, real-time PCR is the technology of choice for DNA based quantitative assays, and publications describing quantitative real-time assays appear in the literature, most notably in the field of GMO analysis. These types of assays are relatively accurate and form the basis of regulation for GMOs in food. It appears, therefore, that a similar approach might be applicable to the quantification of meat in complex food products. There are fundamental differences, however, between the aims for quantification of meat and GMOs; the percentage of a GMO variety in a raw material or ingredient from the same species has to be determined, whereas for meat the requirement would be the measurement of the total amount of meat from a species in a product. For the former, an endogenous gene present in both the GMO variety and the non-GMO of the same species is used to normalise the quantity of DNA added to the reaction. The percentage of the GMO can be established simply from a calibration curve of normalised values. Additionally there are certified reference materials, at a range of concentrations, which can be used for GMO analyses. In the example below, for RoundUp Ready soya, lectin is used as the species-specific endogenous gene and Roundup Ready is used as the GMO-specific gene. In meat products there may be differences in the source and processing of species tissues, for example liver or muscle tissue which may have been preprocessed before addition to the product, giving rise to non-uniform DNA degradation. A product may also contain plant-derived ingredients such as rusk and soya, which would be a source of non-meat DNA. The assay cannot therefore be normalised for the quantity of DNA added to the reaction using a species-specific gene but must be normalised with the use of a universal meat assay designed to a gene present in all meat species. Moreover there are no certified meat reference materials for meat available and therefore calibrants must be produced in-house. Additionally, processing of meat products can fragment DNA so that calibrants must be produced from similarly processed meats to be representative, or at least the fragmentation must be taken into account.

Recent reports in the literature have outlined both semi-quantitative [1] and quantitative [2] assays for meat quantitation. Both of these studies used universal and species specific primers in real-time PCR assays, together with raw meat calibrants, to demonstrate quantitation of beef in model samples. These reports illustrated the utility of the real-time PCR based approach for meat quantitation, describing proof of principle, but failed to demonstrate accurate quantitation in complex food products and in samples where the DNA had been degraded.
We have extended the work presented in these two reports and investigated the use of different calibrant types, including the use of processed meat calibrants, to increase the accuracy of real-time quantitative assays for meat. Calibrants studied included DNA extracts from raw, baked and canned meat admixtures, DNA admixtures and copy number standards consisting of plasmids containing a species-specific target sequences. These calibrants were used to determine the amount of pork and chicken in a raw meat sausage composed of pork and chicken at 90, 50 and 10% (w/w) chicken in pork using both nuclear GAPDH and mitochondrial 16S gene targets. DNA from the calibrants was extracted using the same method as the sausage sample. Due to the different origin of sample and calibrant it was important to establish that they amplified with similar efficiencies otherwise biased quantification results could be obtained.

Sample-derived factors that affect PCR amplification efficiency (E) include:

- PCR inhibitors;
- very low starting template copies;
- fragmented (non-detectable) DNA target and fragments causing non-specific priming events.

Using the chicken specific assays for the 2 gene targets, it was found that the sausage DNA amplified with a similar efficiency to the raw meat admixture and DNA admixture calibrants and the plasmid copy number.

Whilst it was found that the reproducibility of the assays was good in terms of Ct generated, the chicken and pork targets were not detected equally by the universal assays, used to normalise the amount of DNA applied to the assay. This is a fundamental requirement for accurate quantitation since the normalising assay must detect all species equally well to produce accurate quantitative results. In contrast, the plasmid calibrants offered a wider range of values however they gave conflicting copy number results depending on whether direct Ct or normalised delta Ct measurements were used. In all cases the 50% and 90% chicken sausage samples were difficult to distinguish. This is not unexpected, as there is 5 times the amount of template in the 50% sausage compared to the 10% but only 1.8 times the amount of template in the 90% sausage compared to the 50% sausage. A two-fold difference in target is approaching the detection limit of conventional quantitative PCR.

Examples of the most accurate results for each gene target are shown below. The GAPDH assays gave more accurate results with the normalised delta Ct measurement whilst the 16S assay performed better with direct Ct measurements. In summary, the quantitation of complex meat products using real-time PCR based assays has yet to be realised, however the use of plasmid based calibrants may represent the best alternative for controlling these assays.

There is also a need to quantify the amount of fish muscle in complex matrices, where traditional methods of analysis are unsuitable. Quantification of fish species in breaded products has routinely been carried out by nitrogen determination followed by the use of a conversion factor to relate nitrogen to fish content. White fish autolyse muscle tissue during periods of starvation, leading to seasonal variation in the protein content of fish muscle which has lead to some debate over the most appropriate nitrogen conversion factor to apply.

The quantitation of fish content in commercial products is particularly suited to DNA based

<table>
<thead>
<tr>
<th>sausage sample no</th>
<th>% chicken in pork</th>
<th>% calculated / raw meat calibrants</th>
<th>% calculated / raw meat DNA admix calibrants</th>
<th>16S Ct pork</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>16.69</td>
<td>44.41</td>
<td>34.21</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>9.76</td>
<td>36.59</td>
<td>38.52</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>53.64</td>
<td>86.25</td>
<td>75.81</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>52.94</td>
<td>85.39</td>
<td>66.14</td>
</tr>
<tr>
<td>19</td>
<td>90</td>
<td>54.95</td>
<td>87.65</td>
<td>74.44</td>
</tr>
<tr>
<td>20</td>
<td>90</td>
<td>57.64</td>
<td>90.78</td>
<td>70.45</td>
</tr>
</tbody>
</table>

GAPDH delta Ct (meatgen - pork) 16S Ct pork

GAPDH delta Ct (meatgen - pork) 16S Ct pork
methods. Unlike meat products, loss of target DNA associated with processing of meat products, is not applicable to the quantification of fish since fish flesh is used either raw or very lightly processed. Initially, we investigated the variability of nitrogen content / g of fish muscle and compared it with the variability of a single gene copy number / g fish muscle. The working hypothesis was that if the variation in copy number, as determined using real-time PCR, was equal to or smaller than, the variation in nitrogen content for the same tissue, then a real-time PCR method would offer a more accurate measure of fish content. To investigate this variability, 10 haddock were collected from 3 separate fishing grounds, 4 times, over the course of one year, giving 12 individual season-ground data sets (30 haddock were collected in each of April, July, October of 2001 and in January of 2002).

The average nitrogen content (X on figure) and Ct value (O on figure) for each fish, normalised against the average of the whole data set, was subjected to statistical analysis. The measurement of uncertainty associated with the analysis of the haddock DNA Ct and nitrogen showed similar uncertainties (15 and 14% respectively) and indicated that the variation in the DNA based results was similar to the variation in the nitrogen data. This indicated that quantification of fish flesh, based upon nitrogen or Ct values, would return data with a similar degree of accuracy. Furthermore, since these data also indicated that the relationship between gene copy number (Ct value) and haddock muscle weight was relatively constant, then a quantitative method, based on Ct values and haddock muscle weight was feasible. Having confirmed the robustness of the DNA based approach, we went on to design and optimise haddock specific primers and develop a quantitative assay for haddock in complex matrices. We investigated the construction of calibration curves using haddock muscle tissue added to white sauce for lasagne, breadcrumbs from turkey escalopes, cod muscle tissue or water at 90, 75, 50, 25 and 10 % (w/w) haddock. The calibration curves drawn for each matrix showed a good relationship between the % of haddock added and the Ct values returned and showed no marked matrix effects at 90, 75, 50 and 25% haddock however at the 10% level there were statistically significant matrix effects, indicating that quantification of haddock below 20% would be affected by matrix and would need a matrix matched standard curve.

The method was assessed for accuracy against model samples prepared by spiking tuna mayonnaise paste, or cod in crispy coating with known quantities of haddock tissue. These model samples were quantified against a calibration curve prepared from a second set of pastes and fish. Calculated percentages for the model samples compared well with the actual values and varied less than 7% from the true gravimetric values. We have therefore shown the utility of this method and established that model samples can be quantified to within 7% of the actual value [3].

<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Actual % haddock (w/w)</th>
<th>Calculated haddock% haddock (w/w) ± std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod in crispy coating</td>
<td>40</td>
<td>33 ± 8.8</td>
</tr>
<tr>
<td>Fish paste</td>
<td>25</td>
<td>29 ± 8.7</td>
</tr>
<tr>
<td>Fish paste</td>
<td>20</td>
<td>20 ± 6.5</td>
</tr>
</tbody>
</table>

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In conclusion, the quantification of mammalian meat in complex samples using DNA as an analyte remains to be achieved, however the quantification of fish flesh in complex samples has been achieved with good accuracy.

References


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DNA-IQ: development of molecular genetic methods for the identification and quantification of fish and seafood

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Addressing consumer concerns and sustainability of food, the European Commission, as part of the 5th framework program tendered for projects to identify and quantify commercially relevant fish species. Eurofins/GeneScan won and co-ordinated the project 'DNA-IQ: development of molecular genetic methods for the identification and quantification of fish and seafood'. As part of this project, new methods for the identification and quantification of seafood were developed for different analytical platforms: LightCycler, iCycler and ABI Prism. The developed systems were successfully tested and validated using processed and unprocessed products.

The presentation will give a short overview over the developments and inventions made in the course of this project.
Enforcement of food standards legislation using DNA-based techniques.

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Food Standards Agency, Enforcement Division, UK

Advances in DNA technology in forensics have opened up new possibilities of solving criminal investigations. Similarly applying DNA technology to determine the origin of foods or its ingredients has also assisted in checking or verifying labelling or description claims and enforcing food legislation.

The Food Standards Agency has a dedicated programme of research to check the authenticity of foods, and many of the approaches used are based on DNA technology. Methods are developed and validated, then used in national surveys to check a specific authenticity issue. Follow-up action will depend on results of the survey, and may involve prosecution of individual companies by local government authorities. The outcome of surveys may reveal a more widespread problem that requires central action, which may be a co-ordinated enforcement exercise, or development of a Code of Practice or guidance. DNA techniques are slowly being introduced as routine methods in enforcement laboratories (Public Analysts), and development of a portfolio of methods will assist the justification of the investment in these techniques.

Authenticity and food standards

There is a large amount of legislation that covers the description and composition of food that goes under the general umbrella of ‘food standards’. Most of the legislation is EC based, but there is still a few UK national rules covering labelling requirements of potatoes and meat products. The areas covered are summarised in the Table 1.

Table 1. Food standards legislation.

<table>
<thead>
<tr>
<th>Labelling or description</th>
<th>Legislation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the food – legal name</td>
<td>Food Labelling Directive, Vertical Directives, EC Marketing Directives, National rules on potatoes, meat products, and ice cream</td>
</tr>
<tr>
<td>List of ingredients</td>
<td>Food Labelling Directive – generic names</td>
</tr>
<tr>
<td>Mention of a process</td>
<td>Food Labelling Directive, Quick-frozen food Directive</td>
</tr>
<tr>
<td>Geographic origin</td>
<td>Food Labelling Directive, Protected Denomination of Origin (PDO), Protected Geographic Indication (PGI), EC Marketing Regulations, EC Beef Labelling Regulation</td>
</tr>
<tr>
<td>Quantitative Ingredient Declaration (QUID)</td>
<td>Food Labelling Directive, Vertical Directives</td>
</tr>
</tbody>
</table>

Incorrect use or declaration of any one of the above may be done for profit and is misleading the consumer.
Issues investigated by the agency using DNA techniques

In this presentation I would like to give a few of the examples where DNA techniques have been applied successfully. Table 2 summarises all the applications or projects using different DNA techniques.

Table 2. Completed DNA-based projects.

<table>
<thead>
<tr>
<th>Issue</th>
<th>DNA technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice varieties</td>
<td>Microsatellites or Simple-sequence length polymorphism (SSLP-PCR)</td>
</tr>
<tr>
<td>Potato varieties</td>
<td></td>
</tr>
<tr>
<td>Origin of tea</td>
<td></td>
</tr>
<tr>
<td>Olive varieties</td>
<td></td>
</tr>
<tr>
<td>Fruit species in jams and pulps.</td>
<td>Single Nucleotide Polymorphisms (SNPs)</td>
</tr>
<tr>
<td>Rice varieties</td>
<td></td>
</tr>
<tr>
<td>Olive varieties</td>
<td></td>
</tr>
<tr>
<td>Durum wheat pasta</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Quantitative meat and fish species</td>
<td></td>
</tr>
<tr>
<td>GM soya</td>
<td></td>
</tr>
<tr>
<td>Meat species</td>
<td></td>
</tr>
<tr>
<td>Differentiation of meat tissues, CNS,</td>
<td>Methylation events - PCR</td>
</tr>
<tr>
<td>offal</td>
<td></td>
</tr>
<tr>
<td>Fish species</td>
<td>Restriction fragment length polymorphism (RFLP-PCR) and single strand conformation polymorphism (SSCP-PCR)</td>
</tr>
<tr>
<td>Meat Species</td>
<td></td>
</tr>
</tbody>
</table>

Meat and fish speciation

The first use of DNA methodology goes back to 1998, when a survey of smoked salmon and salmon products [1] was carried out to check whether any substitution with trout, a cheaper fish, had occurred. Samples were screened using isoelectric focussing of the soluble proteins, but species confirmation used SSCP-PCR. The use of SSCP-PCR was developed from an EU Project (AIR 2-CT94–1126 (1994–97), which applied the technique to canned tuna species. The Agency also used this method for tuna species in a survey on canned tuna and canned tuna products reported in April 2000 [2].

Replacement of isoelectric focussing for fish species using an RFLP-PCR technique has been successful for identifying 10 white fish species. This method has been adapted to a simple format (the Agilent 2100 Bioanalyser CHIP capillary electrophoresis system), and shown to be robust in a collaborative trial [3].

Although an RFLP-PCR was used initially for a meat speciation survey [4], more sensitive species-specific methods have been developed in real-time PCR format.

Use of microsatellites

Microsatellites are base repeat sequences that occur in the portion of DNA between genes, and are often used to genotype closely related plant varieties. Agency funded research has developed methods to identify potato varieties using only five microsatellite markers and long grain rice varieties using 10 markers. The method for potatoes has been applied in two national surveys to check potato variety declaration at the point of sale. The surveys revealed a specific problem with a popular variety of potato in the UK, and its substitution
with a cheaper but similar variety [5]. In the case of Basmati rice varieties, the microsatellite method has been further developed to give a quantitative measure of the non-Basmati content. An extensive national survey revealed that substitution was occurring, and in a small number of cases the rice being sold had little Basmati content [6].

**Tissue differentiation**

One of the mechanisms of blocking gene expression is the methylation of certain parts of the gene initiator sequence. This mechanism can be used to show that certain tissue specific genes have been active and are in the non-methylated state, and hence the possibility that certain tissues such as brain or spinal cord are present in products containing muscle. This approach has been tested and is being adapted to a simpler format for wider use.

**Real-time PCR**

Real-time PCR is a sensitive technique for measuring the copy number of a specific target gene in a mixed matrix. Its main use has been the quantitative measurement of GM soya or maize in raw flour-form samples. Application to GM soya containing composite and processed foods has proved more difficult and matrix dependent, although the Agency carried out a limited survey on GM soya in baked products [7]. However, a real-time PCR assay has been developed to measure common wheat adulteration of durum wheat pasta, where processing is fairly consistent, and as a single ingredient food, the matrix effect is minimised, hence reproducible results can be obtained.

Quantitative PCR has proved the most difficult to achieve robust protocols and the Agency is examining alternative approaches based on mass spectrometry. However, these are still at the early stages but look promising for the future.

**References**

2. Food Surveillance Information Sheet No 1/00 April 2000 – Survey of misdescription of tuna products.
5. Food Surveillance Information Sheet No 45/03 October 2003 – Survey to investigate the varietal labelling of potatoes – Part 2.
Quality tracking & tracing: an overview

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Tracking and tracing, or as it is often called nowadays ‘traceability’, is an old concept, but it still means different things to different people. When applied to the food industry, it has traditionally been applied to techniques, which make it possible to find the origins of a product. A loose implementation of this concept has for sometime been present in food legislation, particularly applied to labelling. For example, a directive on lot marking of foodstuffs passed into European law in 1989 (89/396/EEC) in order to provide better information on ‘the identity’ of products. Through this means it was intended to be easier to find the manufacturer of the product, with some degree of precision regarding the nature and time of production.

Many, if not most, operators in the food industry still believe that this is the final goal of traceability. An official of a federation representing many of the major supermarkets in Europe recently said. “Why do we need more rules on traceability? Pick up any product in the supermarket and you can see exactly where and when it was manufactured”. Legislation has however moved on from this simple concept of traceability back to the source of supply of a marketable product. The European General Food Law (EC/178/2002) pursues the goal of ‘farm to fork’ traceability. Although the drafting of this law is flawed as an effective means to achieve full chain traceability, the principle of being able to find the current location of particular batch of products is clearly established.

This is a very different concept from finding the origins of a product. Some people distinguish these two concepts through their use of the words ‘track’ and ‘trace’, although it is a little difficult to determine the semantic basis for this distinction. Elaborating the terminology, traditional traceability has been concerned with ‘tracing back in time’ to the history of a product (otherwise called ‘upstream’ or ‘ascendant’ traceability) and the more recent use of traceability in the food industry extends to cover ‘tracking forward in space’ to the location of a product (otherwise called ‘downstream’ or ‘descendant’ traceability).

The reason that downstream traceability has become a legal requirement is twofold: the more obvious reason is that it provides the way to locate suspect food products once a problem has been detected, and therefore prevent any more of these products from being consumed; the less obvious reason is to avoid unnecessary disruption to the food industry and the general public by implicating products which are not actually contaminated, by executing precise and targeted withdrawals.

One would have though that food companies would have had exactly the same objectives. The larger the recall, the greater the cost and the greater the damage to brand image (not to mention potential claims from distributors and retail outlets). However food operators are very resistant to implementing precise downward traceability. As stated by the quality manager of one large food processor, which produces millions of products a day: "Why bother with tracking where the products have gone? All our products are consumed in a few days, and if we have a problem we can just recall all our products in distribution".

An explanation for this resistance is that downstream traceability is a lot harder than upstream traceability. In the latter case, you have a product in your hands when you ask...
questions about its origins. The basic information is already on the label, and this label can
tell you where to go and find more. This is not the case when you simply have a reference
number, and you want to know the current location of all products with this reference number
– which could be somewhere among a thousand locations. The only way to achieve this is by
systematic recording within a traceability database of all movements of all products,
combined with an effective means to establish the links between these records. Only very
few companies in the food industry have so far implemented this with a good level of
precision and an ability to extract the information rapidly.

However...both the concepts of traceability which I have described so far are conceived as a
kind of insurance policy to deal with emergencies. Companies are naturally reluctant to
invest in a system when their only justification is risk protection. The real value of a
traceability system is when it provides day-to-day operational benefits.

In fact, a well-implemented traceability system – perhaps originally conceived as a risk-
protection measure or to comply with legal obligations – can provide the foundations for
improvements in quality control and operational processes which would not be possible
without such detailed data gathering. Information gathered in a traceability system does not
have to be limited to product movements, nor to the association between ingredients and
finished products, it can be used to store all the parameters of processes or environment
which can affect the quality of the product. Furthermore, the detail gathered can be applied to
analyse automatically the effect of such parameters on final quality, thereby leading to
iterative cycles of improvement. For example, in the case of agricultural production, the
productivity and quality result can be tracked to the use of different treatments or cultivation
techniques. Similarly with livestock, records built up through traceability can demonstrate
which conditions provide the best body weight and meat quality, and quantitative analysis
can be done of financial return.

Nearly all inputs to food are organic, and to varying degrees food products delivered to the
final consumer are still changing. Traditional predictive techniques, based on controlled-
condition experiments or scientific theory, have been used to estimate the changing nature of
food products through the storage and distribution cycle. Traceability adds micro-level
empirical data to the knowledge base of exactly how products perform according to time and
environment. However it goes further than that, since traceability data can be used in real-
time to correct the conditions under which food is being handled.

Traceability systems are now beginning to emerge on the market, such as FoodReg
(www.foodreg.com), which treat traditional traceability functions as just one of the outputs
possible. Simple traceability functions can be integrated with the operation of a HACCP-
based control program, and can facilitate a general improvement of information flow in the
supply chain.

With quality data properly recorded, traceability can serve one of its traditional functions in
providing information about the history of a product, and go a step further. Issues of final
product quality which are not perceptible by the purchaser, or which may require a certified
level of proof, can be conveyed to the consumer. For these consumers, concepts of quality
might cover traditional values such as the denomination of origin, or more modern
requirements such as proof of organic production or nutritional characteristics, and might well
extend into issues such as environmental protection and farm-worker welfare.

Rapid methods can therefore interact with traceability systems in two ways. First, the ability
to detect and report on the characteristics of food ingredients and products, or of any other
factor which can affect these food products, can be fed into the traceability system to serve
later as historic data – or possibly to trigger immediate changes to environmental or process
parameters – leading to improvements in quality as well as food safety. Second, in the case
of a reported food problem, rapid methods can be integrated with traceability systems to create the fastest and most effective reaction: quickly diagnose the problem in the end product, move straight to the likely moment in the food supply chain where the problem was caused, rapidly analyse and confirm the hypothesis of the problem, and then within a matter of hours locate and place in quarantine all contaminated product.

Traceability and rapid methods is a partnership which will deliver improved food safety and a more defined quality of final product.
Quality tracking & tracing: technology

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Food production and distribution chains become more and more complex. As a consequence, the (potential) impact of food crises increases. In reaction to this threats governments and food chains define strict requirement on the tracking & tracing systems in the chains. These requirements force food and feed companies to do substantial investments. Because compliance to the tracking & tracing requirements is seen as a minimal ‘license to deliver’, traceable products do not guarantee a better price. To justify the traceability investments, a food company should be able to integrate tracking & tracing investments in the value-adding strategy of the organisation.

In this paper we discuss quality tracking & tracing as a way to use tracking & tracing technology to generate additional revenues. The organisation of this text is as follows. We will start with a short introduction of tracking & tracing. Then we will introduce quality tracking & tracing as an extension on the traditional view on tracking & tracing. We will provide an analysis of tracking & tracing technology that can accommodate the existing Tracking & Tracing concepts, and we will discuss the position of rapid methods in this playing field.

Tracking & tracing

Tracking & tracing, ‘following (in real time) or reconstructing (off-line) the logistic route of singular or compound products’, originates from logistics. In logistics, the main concern is efficient control of logistic processes. Consequently, many tracking & tracing actions that stem from logistics are justified by improved process planning and control. Due to various food incidents and under influence of consumer organisations and authorities, the focus of traceability in the food sector has shifted towards prevention and reactive control of food safety aspects.

The HACCP approach realises a preventive set of measures, while traceability ensures executable measures for reactive control, such as recall management, damage control and liability. Moe [1] distinguishes between chain traceability and internal traceability, dependant on whether or not the traceability covers more than one organisation. The General Food Law (GFL), effective in the European Union from January 1, 2005, includes the obligation for food companies to maintain traceability on a one-up-one-down basis: external traceability. Internal traceability is not compulsory, but can help companies to minimise the impact of a recall action. Furthermore the GFL prescribes the availability of procedures and systems to provide the proper authorities with specific traceability information [2].

Tracking & Tracing systems consist of four connected components. First, to enable proper traceability of products, the organisation of processes in the total production and distribution chain must be based on a clear definition of responsibilities and standards. Second, the process design has to enable traceability. Many processes in food producing chains have been designed with efficiency and cost reduction in mind. As a result, not all processes are in its current form suitable for tracking and tracing applications. Third, the information infrastructure in the chain has to allow exchange of the relevant information to couple product identities in successive processes. Finally, when all these factors are in order, traceability technology can enables identification, registration and processing of product flows.
Quality tracking & tracing

It was indicated before that traceability for food safety can be made more attractive for companies by embedding the traceability solutions in the business strategy. One of the main differences between food products and many other ‘industrial’ products is that food quality is a dynamic aspect that changes in the food distribution chain. This is both true for the consumption quality and for the food safety status. Traditional traceability systems are powerful in managing the logistic aspects of traceability (location of product at specific times, origin of product, destination of product), but they do not provide means for linking (changes in) product quality to the product history. By adding product quality parameters to a tracking & tracing system, decision making in the chain (keeping conditions, processing settings, logistic configuration) can be optimally fit to the product requirements. In order to do so, it is necessary to link quality information to the logistic information.

Tracking & tracing technology

Earlier we introduced a subdivision of tracking & tracing systems to cover technology, information, process and organisation. In this section we further detail tracking & tracing technology. Technology for tracking & tracing is described using two dimensions. The first dimension covers the purpose of the traceability system. This purpose can be:

- location registration;
- condition registration; or
- quality registration.

The second dimension is that of traceability function. We distinguish three functions of technology:

- identification;
- registration;
- processing (omitted in this text, as it does not include specific technology, but mere ICT).

These two dimensions span a matrix as shown in table 1. Each cell in the matrix can contain technology types, each with a variety of specific techniques. The table can best be understood as being built up from left to right. Tracking & tracing starts with the ability to register, throughout the entire process, identity, location and time/date of product flows. On top of that it can make sense to register chain conditions such as temperature, relative humidity, gas concentration, shocks etc. All these parameters operate on the product in the chain, and can influence the product quality. Often, chain partners apply quality standards for monitoring their process, e.g. maximum and minimum temperatures. By monitoring the process conditions, compliance to these standards can be established. This is important in making process quality accountable. Given the dynamic nature of product quality in food chains however, monitoring process quality does not guarantee a proper product quality. Modern techniques help to make internal quality measurable. By adding this technology to existing tracking and tracing infrastructure, one is able to document product flows in such a way that hazardous products can be withdrawn from the market, one is able to identify and optimise sub-optimal chain conditions, and one is able to configure production chains to fit the actual product quality instead of applying standard conditions.

Rapid methods for (quality) tracking & tracing

The development of rapid methods potentially has a large influence on tracking & tracing technology, especially in the areas of location and quality registration.

The main function of tags is to make products identifiable. Tags enable identification of products. Dependent on product type and tag technology however, tags can get lost, unreadable or falsified. By introducing biochemical tags, based on the ability to read them with rapid analysis methods, these disadvantages can be overcome. Providing cattle with earmarks is a common way of tagging, but earmarks can get lost. DNA samples of the
animals are inseparably attached to the animal, but time-consuming to read. By using immunological tagging technology, animals can be tagged and identified in a feasible way.

Table 1. Tracking & tracing technology.

<table>
<thead>
<tr>
<th>Function</th>
<th>Purpose</th>
<th>Location</th>
<th>Condition</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relevant information items</td>
<td>Identity, location, time</td>
<td>Conditions (temperature, relative humidity, gas concentrations ...)</td>
<td>Dynamic quality (ripeness, physiological state, ...)</td>
<td></td>
</tr>
<tr>
<td>Physical information carrier</td>
<td>• Alphanumeric tags&lt;br&gt;• Optical tags&lt;br&gt;• Electronic tags&lt;br&gt;• Biochemical tags</td>
<td>• TTI&lt;br&gt;• Data logger</td>
<td>• DNA/RNA expression&lt;br&gt;• Metabolites&lt;br&gt;• Physiological parameters</td>
<td></td>
</tr>
<tr>
<td>Registration</td>
<td>• Human data entry&lt;br&gt;• Optical readers&lt;br&gt;• Electronic, magnetic and RF readers&lt;br&gt;• Biochemical readers</td>
<td>• Registration interval&lt;br&gt;• Registered information&lt;br&gt;• Registration method&lt;br&gt;• Registration focus</td>
<td>• Microarrays&lt;br&gt;• Assays&lt;br&gt;• Physiological measurements</td>
<td></td>
</tr>
</tbody>
</table>

The main function of quality registration is to capture the dynamic quality development of agricultural products. This cannot be realised by simply measuring some physiological parameter, as these reflect the internal quality state, but not necessarily relate to dynamic aspects. By measuring the relevant biochemical processes, e.g. by measuring RNA expression, or the concentrations of specific metabolites, the internal product state can be assessed more reliably. This has been illustrated in various agricultural products, including iris, pine trees and tomatoes.

Some of the major problems in assessing rapid methods on routine base for tacking & tracing applications are costs, usability and automation. The commercial prize of current tests does not allow routine application. Current optical tags cost far less then € 0.01. Modern electronic tags cost around € 0.20, which is considered to high for product tagging. The current cost of rapid methods, in the order of € 2.00-€ 5.00 and higher, does not allow for routine testing. Moreover, routine application requires substantial labour costs. Further automation should make them at line available against reasonable costs. If so, the application potential is large for food quality and food safety purposes.

References
The identification of animal species in human food: an example of application of the DNA chip technology

What are 'DNA chips'? 
This generic term refers to an evolution in the reverse dot-blot hybridisation technique, which aims to analyse natural or amplified nucleic acids [1]. The words DNA 'micro-arrays' or 'biochips' are also used, although a biochip can also integrate other functions of molecular diagnostics such as the extraction and the amplification. DNA chips are flat surfaces, generally very small (less than 1 cm$^2$) that contain tens to millions of oligonucleotide DNA probes [2]. Therefore, in practice their manufacturing is a fantastic technological evolution combining the expertise of different fields. Biophysics and biochemistry is used to synthesise nucleic acids and control their adsorption on solid surfaces, to fragment and label amplicons, micro-manufacturing techniques to deliver small surface disposables with a high probe density, micro-optics to catch very low signals, bioinformatics technology to design these micro-arrays and to interpret the hybridisation data etc. The basic notion lying behind DNA chip is 'a lot of materialised genetic information'.

Multi-detection of animal species in food with FoodExpert-ID®

Authenticity testing of the animal species present in food is important for economic, safety, legal, religious and health reasons. It is a major consideration during the purchasing of raw materials, process management and labelling of finished products. Furthermore, this knowledge can be used as primary information in the traceability of ingredients and products from 'farm to fork'.

Existing techniques based on the use of species-specific protein characterisation (electrophoresis, immuno-precipitation or ELISA) do not work well on processed food into which the epitope is destroyed (heat, salt, etc.). Furthermore specificity is far from being optimum since cross-reactions have been observed with neighbouring species. DNA is the ideal biomarker for food species composition analysis to validate authenticity, safety and value for money. PCR DNA tests detecting one species do exist but can only answer closed questions like 'is there pork in this sample?' The development of solid phase micro-arrays, which can support up to 200,000 probes, has increased the resolutive power of analysis faced with multidetection. One key advantage is its capability to answer both closed and open question such as: is there beef in this sample? is there only beef in this sample and is not, what else? what is the animal composition of this sausage?: is this processed white fish made of pure cod?

We developed FoodExpert-ID®, a rapid automated system for the screening and identification of animal species, pure or in mixtures, in raw and processed food. It is based on the use of a high density Genechip micro-array having 80,000 probes of 17 base pairs long and reconstituting the sequence polymorphism of the cytochrome b gene that differentiates vertebrate species [3]. It allows the specific detection of species alone or in mixtures (12 mammals, 5 birds, and 16 fish) in a single test. In addition, a combination of single nucleotide
polymorphisms (SNPs) are used to identify the 3 classes of vertebrates potentially present.

Figure 1 describes the hybridisation of cytB amplicons on this micro-array, whereas Figure 2 shows an example of result report by the dedicated software.

Based on the analysis of exclusivity and inclusivity, 33 species of commercial interest (Table 1) have been validated for detection and identification in mixed and pure products. We will describe and discuss the results obtained with reference and commercial food products, in terms of sensitivity, capacity for multidetection of several species in one sample, as well as range of food matrices applicability.

Figure 1. Hybridisation of cytB amplicons on the FoodExpert-ID DNA chip.

![Figure 1](image1.png)

Figure 2. Specimen of result report generated by the dedicated software.

![Figure 2](image2.png)
Table 1. Specificity of detection of the FoodExpert-ID DNA chip.

<table>
<thead>
<tr>
<th>Inclusivity</th>
<th>Exclusivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct identification / samples tested</td>
<td>Cross reactions / species tested</td>
</tr>
<tr>
<td>Beef (Bos taurus)</td>
<td>3/3</td>
</tr>
<tr>
<td>Mule deer (Odocoileus hemionus)</td>
<td>7/7</td>
</tr>
<tr>
<td>Virginian deer (Odocoileus virginianus)</td>
<td>NR</td>
</tr>
<tr>
<td>Reindeer (Rangifer tarandus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Goat (Capra hircus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Sheep (Ovis aries)</td>
<td>3/3</td>
</tr>
<tr>
<td>Pig / Boar (Sus scrofa)</td>
<td>3/3</td>
</tr>
<tr>
<td>Rabbit (Oryctolagus cuniculus)</td>
<td>3/3</td>
</tr>
<tr>
<td>European hare (Lepus europaeus)</td>
<td>3/3</td>
</tr>
<tr>
<td>Cat (Felis catus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>2/2</td>
</tr>
<tr>
<td>Rat (Rattus norvegicus)</td>
<td>5/5</td>
</tr>
<tr>
<td>Mouse (Mus musculus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Ostrich (Struthio camelus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Goose (Anser anser)</td>
<td>2/2</td>
</tr>
<tr>
<td>Turkey (Meleagris gallopavo)</td>
<td>3/3</td>
</tr>
<tr>
<td>Guinea fowl (Numida meleagris)</td>
<td>2/2</td>
</tr>
<tr>
<td>Chicken (Gallus gallus)</td>
<td>3/3</td>
</tr>
<tr>
<td>American eel (Anguilla rostrata)</td>
<td>NR</td>
</tr>
<tr>
<td>European eel (Anguilla anguilla)</td>
<td>2/2</td>
</tr>
<tr>
<td>Japanese eel (Anguilla japonica)</td>
<td>1/1</td>
</tr>
<tr>
<td>Mozambican eel (Anguilla mossambica)</td>
<td>1/1</td>
</tr>
<tr>
<td>Atlantic bonito (Sarda sarda)</td>
<td>1/1</td>
</tr>
<tr>
<td>Pacific bonito (Sarda chiliensis)</td>
<td>NR</td>
</tr>
<tr>
<td>Skipjack tuna (Euthynnus pelamis)</td>
<td>0/5</td>
</tr>
<tr>
<td>Mackerel (Scomber scombrus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Tuna (Thunnus sp.)</td>
<td>6/6</td>
</tr>
<tr>
<td>Spotted tuna (Euthynnus alletteratus)</td>
<td>1/1</td>
</tr>
<tr>
<td>Hake (Merluccius merluccius)</td>
<td>2/2</td>
</tr>
<tr>
<td>Atlantic cod (Gadus morhua)</td>
<td>2/2</td>
</tr>
<tr>
<td>Greenland cod (Gadus ogac)</td>
<td>1/1</td>
</tr>
<tr>
<td>Arctic char (Salvelinus alpinus)</td>
<td>2/2</td>
</tr>
<tr>
<td>Brook trout (Salvelinus fontinalis)</td>
<td>2/2</td>
</tr>
<tr>
<td>Atlantic salmon (Salmo salar)</td>
<td>2/2</td>
</tr>
<tr>
<td>Rainbow trout (Oncorhynchus mykiss)</td>
<td>3/3</td>
</tr>
<tr>
<td>Sea trout (Salmo trutta)</td>
<td>2/2</td>
</tr>
<tr>
<td>Species of the mammals class</td>
<td>41/41</td>
</tr>
<tr>
<td>Species of the birds class</td>
<td>15/15</td>
</tr>
<tr>
<td>Species of the fish class</td>
<td>44/49</td>
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NR: test not realised

References


Industrial vision on tracking & tracing and rapid methods

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New control methods need to be assessed not only on the ground of their sole technology and scientific merits, but primarily by the value added they provide to operators and consumers in the food chain and. This leads us to consider:

- current issues in food chain industries;
- relevance of tracking, tracing and rapid methods;
- assessment of current techniques and tools;
- requirements towards new developments.

Industry issues

In the EU, food chain industries are characterised by low growth prospects in terms of population and food budget per capita. Hence, there is a challenge about achieving growth in terms of turnover, profit and added value. Besides, there are constraints, especially on the regulatory side: food safety, which is heavily regulated, includes stringent requirements on traceability in the food chain and responsibility. Innovation is often confined into product peripherals, like packaging or combination with services. Functional food allegations are severely limited, which also limits product innovation. As far as industry structures and strategy are concerned, we have a combination of:

- consolidation into a small number of large multinational companies;
- national and regional segmentation of many markets still running high, in spite of internal market progress;
- growing role of the retail and their own brands;
- globalisation of supply chains;
- increased value chain integration;
- shift from push towards pull supply chains;
- alliances in terms of strategic capacities.

Typical relevant success indicators in food chain industries are:

- risk control;
- performance of supply chain: cycle time, cost, throughput;
- value creation for consumers and value capture.

Moreover, consumer requirements are difficult to interpret, displaying tendencies towards:

- individualism;
- demand for service as an integral component of the product;
- security and safety consciousness;
- lack of awareness or interest for product information.

Translation into tracking & tracing and rapid methods

Emerging profile of supply chain implies specific requirements and constraints on tracking, tracing and rapid methods:

- regulatory compliance: rests on tracking and tracing, including authentification;
- minimising cost of recall;
- maximising cost-efficiency of supply chain: stock, selling dates, minimising frauds, authentification;
minimising risk: safety, non-compliance (e.g., GMO);
trading off traceability performance versus the cost operations, often related to lot sizing;
value added information to the consumer.

Food chain globalisation results in new operational conditions, including:
real-time qualitative testing (e.g., maturation, mycotoxin);
bridging gaps in information chain, especially in upstream segments of the food chain;
co-operative management of food chain;
country of origin labelling;
seamless management of supply chain without storage;
control on far away production sites;
continuous monitoring;
integration of HACCP and traceability.

Matching requirements and current offerings

Current approaches aim at integrating: IT solutions, organisation, sensors and testing, management or control and organisation. They impose an unequal weight to partners in the supply chain, the upstream operators typically being more impacted than downstream and retail. On the other hand, tracking & tracing requirements tend to be generated by these very downstream operators. This might create pressure since high traceability standards typically result in smaller lot sizes and therefore in higher operations cost.
There is a need for developments such as:
portable testing at farm level;
low cost real-time tracing (RF);
solutions for bulk;
interoperability;
integration of tracking & tracing and decision support;
applicability in small-scale operations.

Openings for the future

In the future, there will be more need for:
integration;
value added to consumers;
co-operative management of issues such as innovation and food safety across the supply chain;
continuous adaptation to forthcoming regulatory developments in food safety.
From an industry viewpoint, this opens many avenues to monitoring, R&D and organisational development.
Scientific vision on tracking & tracing and rapid methods in food supply chain networks

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Since the 1980s, literature on Logistics and Information & Communication Technology (ICT) has stressed the need for collaboration among successive actors in the supply chain, from primary producers to final consumers, to better satisfy consumer demand at lower costs (Lambert et al. 1998; Van der Vorst, 2000). This new way of managing the business within each link and the relationships with other members of the supply chain has been named Supply Chain Management (SCM). A driving force behind SCM is the recognition that sub-optimisation occurs if each organisation in a supply chain attempts to optimise its own results rather than to co-ordinate its goals and activities with other organisations to optimise the results of the chain (Cooper et al., 1997). This holds true especially in food supply chains because of shelf-life constraints of food products and increased consumer concern for safe and environment/animal-friendly production methods.

Recent events have increased the interest in SCM as a means of improving the strength of the chain. Examples are the BSE crisis in the United Kingdom, and Classical Swine fever and Avian Influenza in, amongst others, the Netherlands, which made producers aware of the necessity of chain control and intensified chain co-operation. Furthermore, the increased interest in SCM has been spurred by intensified competition (due to open EU-markets), demographic and market developments (such as product proliferation and shorter product life cycles), combined with developments in ICT that enable more efficient execution of processes and more frequent exchange of huge amounts of information for co-ordination purposes. Consumers in Western-European markets have become more demanding and place new demands on attributes of food such as quality (guarantees), integrity, safety, diversity and associated information (services).

These developments put dynamic requirements on the performance of the food system initiating a re-orientation of companies in the Dutch agriculture and food industry regarding their roles, activities and strategies. Demand and supply are no longer restricted to nations or regions but have become international processes. We see an increasing concentration in agribusiness sectors, an enormous increase in cross-border flows of livestock and food products, and the creation of international forms of co-operation. The food industry is becoming an interconnected system with a large variety of complex relationships, reflected in the market place by the formation of (virtual) Food Supply Chain Networks (FSCN) via alliances, horizontal and vertical co-operation, forward and backward integration in the supply chain and continuous innovation (Beulens et al., 2004). The latter encompass the development and implementation of enhanced quality, logistics, and information systems. In order to satisfy the increasing demands of consumers, government, business partners, and NGOs, and to obtain the 'license to produce and deliver', companies continuously have to work on innovations in products, processes and forms of Cupertino in the FSCN.

When we evaluate these innovations and look at their essence, we find the following generic characteristics (Van der Vorst et al. 2005):
• a focus on cost effectiveness with more and more attention to the combination of Profit, People and Planet;
• an increase in demand for guarantees relating to food quality and safety;
• an increase in (international) co-operation in supply chains whilst maintaining a high flexibility in partner selection;
• a decoupling of processes via modularisation and the creation of dynamic relationships between processes, which increases the complexity of traceability;
• a speeding up of processes via rapid fulfilment techniques and parallel processing;
• possibilities to have a more detailed view on lot sizes with techniques to identify individual products (via Radio Frequency Identification techniques and Electronic Product Code standard);
• an increased use of the potential of new rapid methods for product quality identification, and information capturing and processing capabilities;
• a consolidation of product and information flows within organisations, supply chains and FSCNs; and
• an introduction of new packaging and processing technologies that facilitate new logistics concepts with different transitions in form, place and time.

In short, we see a continuous change in the network structure, the processes, the management structures, and the resources used.

One of the main instruments to guarantee food safety and reduce the size of a product recall is traceability. Traceability is defined as ‘the ability to document and trace forward and backward a product (batch) and its history through the whole, or part, of a production chain from harvest through transport, storage, processing, distribution and sales’. With regard to traceability, the General Food Law states that companies must be able to identify the suppliers of its raw materials and the customer of its end-products on a transaction basis, to be implemented as of 1 January 2005. Traceability allows to closely monitor properties of objects as they are made and move through FSCN thus allowing early warning for quality problems and hazards, to avoid these problems and efficient recall and action when needed.

Traceability is of importance on chain level, as well as on company level. On company level a system should provide information on the location of the product and on the history of the product (product and process information). On chain level, besides information on the location of products, also information on the origin of the product is of importance. In this regard it is important to identify the current unique characteristics of lots (components) and the historical relationship between lots. When chain information systems are in place to enable this information exchange, transparency is created, which makes it possible to offer specific information to buyers and consumers. This again can play a major part in (re)gaining the trust of the consumer. Moreover, by sharing information between partners information flows can be better managed with as a result lower costs and more flexibility throughout the chain. The development of such chain information systems is however not an easy task.

When we translate these developments to the subject of this conference, we can identify a number of challenges for traceability and rapid methods:
• How to guarantee composition, origin, safety, integrity and other properties of products at all stages in the FSCN to show due diligence in the control of food quality?
• How to minimise the risk on incidents (and its effects)?
• How to trace origin and cause of problems when they occur as fast and accurate as possible and how to perform an effective recall?
• How to inform stakeholders of FSCN about properties of products, processes and production means (to create transparency)?

These challenges can only be met by (joint or co-ordinated) development of business processes and products in FSCN, having necessary properties and associated quality and transparency systems.

Companies in FSCN are trying to organise traceability but they are held up by a number of important questions. For example, what’s the maximum recall batch size the company of
supply chain can handle? This is determined by a number of factors:
- the chance of an incident occurrence and the severity of the incident (this depends on the risk analysis);
- the volume of an infected lot (which is determined by the lot segregation); and
- the reaction time (how fast can one identify the problem, isolate the infected lots and recall those lots?).

This means companies have to identify the optimal batch size in each stage of the production and distribution process. Based on the risk analysis they have to define the sampling regime. What is the smallest traceable unit? And therefore, it has a direct impact on the performance requirements of rapid identification methods. Because of the internationalisation, increase of clock speed of the FSCN and decrease of lot sizes, there is a need for:
- fast, cheap, robust, sensitive, real-time, accurate and validated testing methods for food safety/quality;
- unambiguous relationships between samples and logistic lot sizes;
- identification methods at different levels (e.g. flock of birds versus an individual cow);
- international standards/norms;
- fast and reliable communication with principal (recall!).

We conclude that developments in FSCN put stringent requirements on traceability and rapid methods to assist in fast and accurate food quality determination. Companies should perform risk analyses with specific focus on lot sizes and maximum (allowed) recall volumes. This determines the inspection/sampling regime and its requirements, hence the usefulness of rapid methods. Because of the globalisation we have to develop international standards for ICT systems and rapid identification methods. Future research should focus on the development of early warning systems (rapid methods + processes + ICT) and pro-active planning concepts.
SPOTLIGHT PRESENTATIONS

Rapid and reliable determination of food safety and quality

K. Jacobson and John Butler

Biacore AB, Sweden

Market demands
- Analysing food products accurately and meeting the demands of quality and legislative controls are crucial.
- Farm animals and fortified food products need to be rapidly and cost-efficient tested using sensitive and reliable detection methods.

Increase productivity and reduce costs
Biacore®Q and Qflex® Kits increase speed, sensitivity and reliability of food analysis by offering:
- Minimal sample preparation;
- Unattended multi-sample analysis;
- Same day results;
- Easy-to-use assay kits;
- Minimal re-testing;
- One platform for many different assays.

Minimal sample preparation
- Simple pre-treatment, no labelling or tagging required.
- Quickly analyse coloured or opaque samples from a variety of foodstuffs such as infant formula, meat and sport drinks.

Unattended multi-sample analysis
- Full automation from sample injection through to data analysis and report generation.
- Wizard-driven software.

Same day results
Samples analysed within minutes, significantly reducing the time compared to traditional methods.

Easy-to-use assay kits
- For sensitive and reliable detection of a wide range of vitamins and veterinary drug residues.
- Qflex Kits include critical reagents and components for use in Biacore Q.
- Standard and easy to use protocols included.

Minimal re-testing
Robust technology generating sensitive, reliable and consistent results.
One platform – a variety of assays
Switch easily from one analysis to another with ready-to-use kits using the same instrument:
- Qflex Kits for veterinary residues: clenbuterol; ractopamin; β-agonists; sulfadiazine; sulfamethazine; streptomycin; chloramphenicol; sulfonamides.
- Qflex Kits for vitamins: pantothenic acid; folic acid; biotin; vitamin B$_{12}$; vitamin B$_{2}$.

Some of our customers
- Food processing companies.
- Agrifood institutes.
- Dairies.
- Contract service laboratories.

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Many food-testing laboratories worldwide already benefit from higher productivity and subsequent cost reductions using Biacore Q and Qflex Kits.

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Rapid methods for the detection of food adulteration: the R-Biopharm solution

Ronald Niemeijer
R-Biopharm AG, Germany

Food products can be adulterated either accidentally during production, storage and transport or purposely by fraudulent producers. Any food laboratory might face the question whether the products to be monitored are adulterated or not. Examples of these questions are:

- adulteration of goats milk with cow’s milk;
- adulteration of semolina with non-durum wheat;
- contamination of poultry products with proteins from different species;
- adulteration of orange juice by adding excessive amounts of water.

Several sophisticated but quite laborious, time-consuming and expensive methods have been developed for these issues. To answer this question in a rapid, user-friendly and cost-effective way, R-Biopharm offers several analytical methods based on ELISA or other immunochemical methods, PCR and enzymatic methods.

Adulteration of goat’s or sheep’s milk and cheese with cow’s milk can be detected within 2 hours even at a level of 0.1% with the RIDASCREEN® CIS test and within 10 minutes with the RIDA® QUICK CIS, adulteration of both pasta and semolina can be detected within 40 minutes with the R-Biopharm Rhone Pastascan and Durotest S respectively. Both test methods are based on immunochemical methods. PCR based methods like the SureFood® Animal ID kits are an excellent and very sensitive tool for the detection and identification of animal proteins in various products, e.g. brine injected chicken fillets. Finally enzymatic methods like the Roche enzymatic tests for the detection of sugars and organic acids allow the detection of adulteration of fruit juices like orange juice.

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CarboDeep™ – NutriCognia’s technology platform

Ofer Markman
NutriCognia, Israel

CarboDeep™ is an innovative proprietary technology for rapid analyses of complex-sugars namely glycoproteins and glycolipids, in complex solutions.

The analysis is carried out directly on unpurified samples, such as dairy ingredients, dairy products and dairy fermentation products, obviating the need for time-consuming purification and degradation steps.

The CarboDeep™ technology allows a parallel analysis of many samples to be performed within 3 hours.

The technology is suitable for on-line / at-site monitoring of food (and dairy) processing and manufacturing, process development, manufacturing and QC. The technology provides also efficient tool for tracking the quality of incoming ingredients, aid in product's development and product analysis / evaluation. NutriCognia's technology dramatically decreases the product development duration of off-the-shelf products.

The method is based on lectins arrayed in groups with overlapping recognition specificities. A sugar or soluble food sample is applied to the lectin array, and detected by one or more labelled probes. The probes can be lectins and/or other chemical entities that recognise any of the components. The result is a characteristic fingerprint of the sugar's profile of the mixture. This fingerprint is highly sensitive to minor changes in the sugar composition.

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Microscreen – total solutions on microbiological diagnostics using FISH and qPCR

Ron Wolbert
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We offer excellent service, knowledge and products for an efficient safety assurance in your laboratory through our subsidiaries and selected distributors around the world. In broader perspective Ribo helps you to decrease the risk of food-borne illness.

Increasingly powerful and vociferous consumer groups are making more and more demands regarding quality and safety. Until recently, however, producers and importers have had rather cumbersome tools at their disposal to guarantee this safety and quality.

Our FISH and SCAN solutions, provides you rapid, reliable solutions for the determination and detection of pathogenic organism in a wide variety of food-related products, tap-water, bear, blood or faecal-flora. Assays are faster and more reliable than traditional methods.

As shown in our presentation we already established kits for different microorganisms/pathogens or we are able to design on customer request dedicated analysis test of their QA.

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A multi-antibiotic enzyme-linked immunosorbent assay for screening five banned antimicrobial growth promoters in animal feedingstuffs

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¹Euro-Diagnostica BV, the Netherlands, ²Queen's University of Belfast, Department Veterinary Science, UK and ³Ghent University, Department of Bio-Analysis, Belgium

Antibacterial agents used as feed additives to promote growth in animal husbandry contribute to the ever rising burden of resistant bacteria. Since 1999, the European Union (EU) has decided to ban the use of five antibacterial growth promoters (AGPs) in animal feedingstuffs, i.e. zinc bacitracin, spiramycin, tylosin, virginiamycin and olaquindox.

In order to effectively monitor compliance with the ban, an EU funded project QLK5-CT-2000-00900 entitled Feedstuff-RADIUS (co-ordinator Dr. C.T. Elliott) started in 2001. During the course of this project a control strategy to allow the detection of the banned AGPs was developed and consisted of two distinct analytical approaches: a multi-antibiotic screening procedure backed up by a multi-antibiotic confirmatory method.

For the screening method a competitive enzyme-linked immunosorbent assay (cELISA) kit format was used. The assay consists of a single microtitre plate test. The sample preparation involves a single extraction procedure followed by a rapid SPE clean up. The target detectable concentrations of 3 mg.kg⁻¹ (ppm) for olaquindox and 1 ppm for the other compounds were set for the study that equates to 20% of the previously authorised minimum feed inclusion rates. The detection capability of the ELISA was shown to be 1 ppm for all antibiotics included in the project.

The performance of the developed ELISA was assessed in a collaborative study. Six incurred materials of three different feed types were analysed. The analysis of 231 reported results revealed an overall false non-compliant rate of 1.9% and a false compliant rate of 0% at concentrations of antibiotic in the region of 20% of their previous minimum inclusion rates. It can be concluded that the developed ELISA is a very reliable tool to monitor the presence of the five AGPs in animal feedingstuffs.
AK-Phage™ assay: a fully automatic method for the detection of very low levels of pathogens in food within a working day

Malcolm Walpole
Alaska Food Diagnostics, UK

Alaska Food Diagnostics plc, a joint-venture company with the UK Ministry of Defence, has developed a series of ultra-rapid assays for the detection of pathogenic micro-organisms in a broad range of foods and beverages. The technology used for these assays, is based on the AK-Phage™ technology used by the UK armed forces during the first Gulf war in the early 1990’s, to detect biowarfare agents in the air and environment in battleground situations.

This technology, which is covered by five patent families, uses two selective pressures to isolate the target markers: antibody coated paramagnetic microspheres, and appropriate lytic bacteriophage. The microspheres effectively capture the target from the foodstuffs, and then they are washed and exposed to bacteriophage specific to the species, genus, or even serotype of the target. The bacteriophage infects the cell and turns it over to the production of more copies of itself. Once critical mass has been achieved, the phage produces a lysin, breaks open the cell, and the contents are released into the surrounding fluid. This fluid, which contains the enzyme adenylate kinase (AK), is then mixed with ADP solution. The AK catalyses the production of ATP from the ADP. The resulting mixture is then mixed with firefly luciferase, and produces light. Light equals the presence of the target pathogen.

The company is producing three distinct products from the base technologies:

- manual assays in a 10-test kit format;
- high throughput instrumented assays in a 32-test microtitre tray format;
- a fully automated, random access system with its own dedicated consumable.

The remarkable sensitivity and specificity conferred on the product by the enabling technologies, allows the detection of pathogenic Salmonellae and E. coli O157 within 8-9 hours, and Listeria spp. and L. monocytogenes within 12 hours. This has great economic implications for food producers who are tying up warehousing space, often chilled, with products waiting for test results. Short shelf-life products such as ready-to-eat (RTE) cakes, sandwiches, and sliced cooked meats may be released up to 5 days earlier than is currently possible. In addition, public health bodies trying to trace the source of pathogenic bacterial food poisoning, can screen candidate foods within their working day, making the tracking down of the offending food much more rapid than is currently possible. This will almost certainly lead to a reduction in the cases occurring, because the problem foods may be removed from sale up to four days sooner than the time taken now.
POSTERS

P1  **SNPWave™: flexible multiplex SNP genotyping with automated scoring software**  
M. van Haaren, J. Broekhof, H. van der Poel, R. Hogers, J. van Aart, R. Koomen, J. Buntjer, H. Verstegen and M. van Eijk  
Keygene, the Netherlands

P2  **First steps towards the development of immunosorbents by using plasma treated beads**  
A. Rodriguez, M. Manso, J. Barrero and F. Rossi  
EC-DG JRC Institute for Health and Consumer Protection, Italy

P3  **FLORIDA: measuring parts per trillion with a rapid immunoassay**  
J. Polackova, S. Hagenmaier, J. Bonenberger  
Cibitest, Germany

P4  **Online non-contact NIR transflection system for monitoring fat content in consumer-packed salmon fillets**  
B. Narum, V. Segtnan and J.P. Wold  
Norwegian Food Research Institute (MATFORSK), Norway

P5  **Simultaneous detection and identification of micro-organisms by electrical biochips**  
A. Breitenstein, H. Zinecker, S. Bau and H. Maucher  
Scanbec, Germany

P6  **The application of flow cytometry as a fast microbial analysis technique in the food industry**  
T. Ruyssen, T. Vanheuverzwijn, G. Vlaemynck and P. Van Assche  
Hogeschool Gent, Biotechnological Sciences, Belgium

P7  **A rapid assay to monitor Clostridium difficile Toxin A specific IgA antibody production in bovine milk as an active ingredient for functional food**  
A. van Amerongen¹, C.M.H. Hensgens², N. de Groot², M. van der Eijk², L.B.J.M. Berendsen¹ and J.H. Wiggers¹  
¹Agrotechnology & Food Innovations (A&F), the Netherlands and ²MucoVax, the Netherlands

P8  **Application of a multiplex PCR for detection of Clostridium botulinum in foodstuffs**  
G.M. Wyatt¹, J. Plowman¹, C. F. Aldus¹, M. W. Peck¹ and W. Penaloza Izurieta²  
¹Institute of Food Research, Food Safety and Computational Microbiology Group, UK and ²Nestlé Research Center, Quality and Safety Department, Switzerland

P9  **PCR and duplex PCR in detection of Campylobacter spp. in food**  
Z. Šabatková, J. Pazlarová and K. Demnerová  
Institute of Chemical Technology, Department of Biochemistry and Microbiology, Czech Republic

P10 **Detection of mycobacteria in the pork production chain**  
T. Nieminen¹, J. Pakarinen², T. Tirkkonen³, J. Ahilgren³, M. Salkinoja-Salonen², P. Neubauer¹ and T. Ali-Vehmas³  
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Multiplex detection in the food production chain
J.H.W. Bergervoet¹, J. Peters¹, J.M. van der Wolf¹, W. Haasnoot², M.E. Bienenmann², J.G. du Pre³, R. Wessels³, E. Meulenberg⁴
¹Plant Research International, the Netherlands, ²RIKILT-Institute of Food Safety, the Netherlands, ³Bakker Centrale Inkoop, the Netherlands and ⁴ELTI Support, the Netherlands

Novel developments in rapid mycotoxin detection
S. De Saeger¹, L. Sibanda², C. Paepens¹, M. Lobeau¹, B. Delmulle¹ and C. Van Peteghem¹
¹Ghent University, Laboratory of Food Analysis, Belgium and ²Toxi-Test, Belgium

Recent developments in application of solid phase clean-up procedures for rapid analysis of difficult matrices
L. Sibanda¹, S. De Saeger², C. Paepens² and C. Van Peteghem²
¹Toxi-Test, Belgium and ²Ghent University, Laboratory of Food Analysis, Belgium

Development of a new field test: a clean-up tandem assay column for the detection of ochratoxin A in roasted coffee
M. Lobeau¹, S. De Saeger¹, L. Sibanda², I. Barna-Vetró³ and C. Van Peteghem¹
¹Ghent University, Laboratory of Food Analysis, Belgium, ²Toxi-Test, Belgium and ³Agricultural Biotechnology Center, Institute for Animal Sciences, Hungary

Development of an immunoassay based lateral flow dipstick for the rapid detection of aflatoxin B1 in feed
B. Delmulle¹, S. De Saeger¹, L. Sibanda², I. Barna-Vetro³ and C. Van Peteghem¹
¹Laboratory of Food Analysis, Ghent University, Belgium, ²Toxi-Test, Belgium and ³Agricultural Biotechnology Center, Institute for Animal Sciences, Hungary

Application of the flow-through enzyme immunoassay in a market orientated supply study for the evaluation of fumonisins in cornflakes
C. Paepens¹, S. De Saeger¹, L. Sibanda², I. Barna-Vetró³, M. Anselme⁴, Y. Larondelle⁴ and C. Van Peteghem¹
¹Ghent University, Laboratory of Food Analysis, Belgium, ²Toxi-Test, Belgium, ³Agricultural Biotechnology Center, Institute for Animal Sciences, Hungary and ⁴Catholic University of Louvain, Nutritional Biochemistry Unit, Belgium

In vitro detection of fumonisin exposure by the measurement of sphinganine 1-phosphate: a potential application to the screening of fumonisin-contaminated foods
H.-W. Shin¹, D.-H. Kim¹, S.-Y. Ko¹, J.-E. Kweon¹, Y.-M. Lee¹, H.-S. Yoo¹, S. Oh²
¹Chungbuk National University, College of Pharmacy, Korea and ²Ewha University, Department of Neuroscience, Korea

Elevated sphingoid bases 1-phosphate in mouse serum: in vivo estimation of fumonisin exposure as a corn-based food contaminant
D.-H. Kim¹, H.-W. Shin¹, S.-Y. Ko¹, J.-E. Kweon¹, Y.-M. Lee¹, H.-S. Yoo¹, S. Oh²
¹Chungbuk National University, College of Pharmacy, Korea and ²Ewha University, Department of Neuroscience, Korea

Determination of residues in spiked samples and incurred tissue using the Premi®Test
E.M.L. Geijp¹, A. de Rijk¹, F. de Goeijen² and J. Stark¹
¹DSM Food Specialties, R&D, the Netherlands and ²DSM Premitest, the Netherlands
P20  Determination of antibiotic residues in fish using the Premi™Test
E.M.L. Geijp¹, A. de Rijk¹, F. de Goeijen² and J. Stark¹
¹DSM Food Specialties R&D, the Netherlands and ²DSM Premitest, the Netherlands

P21  Lateral flow device for the detection of nicarbazin in avian meals
K. Campbell and C.T. Elliott
Queen’s University of Belfast, UK

P22  Optical biosensor detection of tetracycline residues in foodstuffs
T. McGrath
Xenosense, UK

P23  Spreeta-based biosensor immunoassays to detect adulterants in milk products
W. Haasnoot¹, G.R. Marchesini¹ and K. Koopal²
¹RIKILT-Institute of Food Safety, the Netherlands and ²Analytic Devices, the Netherlands

P24  Species specific detection of chicken in meat models and commercial products by
PCR-RFLP analysis
A. Jánosi, G. Ujhelyi and É. Gelencsér
Central Food Research Institute, Department of Biology, Hungary

P25  Detection of chicken meat in meat products by real-time PCR method
E. Rencova and P. Krcmar
Veterinary Research Institute, Czech Republic

P26  Qualitative and semi-quantitative GMO detection from food samples derived from the
Hungarian market
G. Ujhelyi, A. Jánosi and É. Gelencsér
Central Food Research Institute, Department of Biology, Hungary

P27  Characterisation of the geographical origin of coffee by pattern recognition techniques
R.M. Alonso-Salces, F. Serra, F. Reniero and C. Guillou
EC-DG JRC Institute for Health and Consumer Protection, Italy
SNPWave™: flexible multiplex SNP genotyping with automated scoring software

Mark van Haaren, J. Broekhof, H. van der Poel, R. Hogers, J. van Aart, R. Koomen, J. Buntjer, H. Verstegen and M. van Eijk
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The SNPWave technology is suitable for genetic screening and diagnostic applications in plants, animals, human and micro-organisms.

SNPWave is based on highly multiplexed (10-100) allele-discrimination, followed by selective amplification of 10-30 SNP loci in a single polymerase chain reaction (PCR) using the amplified fragment length polymorphism (AFLP) technology. The use of AFLP allows the amplification of various subsets of SNPs, including 10 subsets for whole genome screening which each contain 1 SNP of every chromosome arm, or 10 subsets for fine mapping which each contain 10 SNPs derived from a single chromosome arm.

To streamline these powerful diagnostic screening technologies, automated scoring is essential. Therefore, we developed user-friendly software application that combines automated scoring with the ability to flag suspect data for manual quality control. Currently, this software supports the MegaBACE capillary electrophoresis platform (GE, Amersham Biosciences) as well as polyacrylamide slab-gels. Additional support of other platforms is in progress.

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First steps towards the development of immunosorbents by using plasma treated beads

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Rapid detection methods are gaining popularity and importance in the field of food control because they are fast, cost-effective and time-saving compared to traditional methods. Nowadays they are widely used as screening tools as excellent complement to confirmatory methods. Several rapid detection kits like ELISA, PCR, biosensors, and solid phase extraction (SPE) based on immunoaffinity columns (IAC) have been developed and are commercially available for a wide variety of food and environmental contaminants.

The use of SPE and in particular IAC tremendously improves the efficacy and simplicity of the cleanup step when dealing with complex matrices. One disadvantage that they could present is the variability on the analyte recovery when using different manufacturers and even within different batches of the same manufacturer.

Provision of active functional groups by plasma treatment, instead of wet chemistry, could be a mean to control the activity of the surface and therefore its reactivity. In this study a first step towards the development of immunosorbet by using plasma treated materials is presented. Several plasma modified polymer beads were characterised and their interaction with BSA-FITC fluorescence conjugate/protein with ($\lambda$ exc 490nm and $\lambda$ em 518nm) was investigated. The protein retention was quantified by monitoring the fluorescence intensity on the solid material. The results obtained indicate high protein retention on all plasma-treated while only minor retention has been observed on the raw materials.

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In contrast to conventional rapid immunoassays, which are labelled with gold or latex beads, FLORIDA (Fluorescence Labelled Optical-Read Immuno Dipstick Assay) uses fluorescent dyes. The fluorescent signals are detected visually with a sensitivity of only parts per trillion (ppt). Due to its bright signals, FLORIDA facilitates a rapid immunoanalysis of strongly coloured specimens, even under bad light conditions.

FLORIDA is based on a special method of labelling biomolecules, which has been applied for a patent by Cibitest. The extreme sensitivity of FLORIDA can be achieved by conjugation of certain fluorophores in high density to carrier molecules and conjugating subsequently the antibodies to this fluorescent complex. The enhancing effect depends on the very high ratio of fluorophores conjugated to a single antibody. Compared with the direct conjugation of fluorescent dyes to antibodies, e.g. via NHS-esters of the dyes, an increase in the sensitivity of three orders of magnitude can be achieved. Examplarily, this sensitivity was determined with an affinity-purified antiserum raised against β-lactam antibiotics. A cut-off value of 10 pg/ml (10 ppt) was determined using these antibodies in a FLORIDA test. But this finding differs approximately three orders of magnitude from the average sensitivity of conventional rapid immunoassays. Comparable sensitivities can routinely be achieved only by radio-immunoassays in a laboratory environment.

In contrast to laboratory methods, FLORIDA uses a strongly simplified instrumentation. The test signal is generated by a competitive immunoassay on a lateral flow teststrip. Evaluating the test strip is only possible by the excitation of the fluorophores. After inserting the lateral flow teststrip into a handheld lamp, the test signals generated at the capture and the control line can be detected visually on site of carrying out the test. Moreover, the fluorescent signals have great advantage if the teststrip has to be evaluated under bad light conditions or even in complete darkness.
A new and highly specific declaration system for foods has been introduced in some countries, and is about to be introduced in others. This declaration system is an important information source for the consumers and meant to be a preventive aid against the increasing amount for patients suffering from inter alia overweight, diabetes and cardiovascular disease. In Norway, a declaration suggestion has been introduced, that is based on giving the consumers inter alia information about e.g. the product’s amount of fat, carbohydrates, proteins, cholesterol and salt, and even the type of fat.

To be able to declare, e.g. the total fat content in a whole piece of food, one needs to analyse the food sample non-destructively. We have performed an on-line feasibility study on salmon fillets with promising results. For this work we used an on-line scanner based on NIR spectroscopy. The scanner is based on a spectral line camera, which measures transflectance NIR spectra from the entire fish. The scanner is generally very well suited for NIR measurements on heterogeneous products. Fish is automatically segmented from a conveyer belt were the speed can be as fast as 1 fish per second.

In this study each of 20 salmon fillets were segmented into 5 pieces representative of consumer packages. With good results we predicted the fat content in each of the 5 pieces in every fillet using PLS regression.
Simultaneous detection and identification of micro-organisms by electrical biochips

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Nucleic acid micro-arrays represent the latest advance in molecular technology, providing unparalleled opportunities for multiplexed detection of nucleic acids. Originally designed for large-scale expression analyses, clinical diagnostics, and genetic analyses, microarrays likewise offer tremendous potential for microbial community characterisation, process monitoring, and as well specific pathogen detection in phylogenetically diverse microbial consortia. On a practical level, those special detection objectives include the following requirements: (i) detection of many different micro-organisms simultaneously, (ii) conduciveness to automation, (iii) monitoring of RNA as a qualitative indicator of microbial activity, and (iv) quantification of RNA levels and/or the extent of microbial activity.

Biochip arrays with electrical signal read-out represent a novel and powerful technology platform meeting all those objectives. Electrical biochip arrays are an innovative tool for the parallel detection and identification of micro-organisms providing reliable and easy analyses for microbial diagnostics applicable in food and environmental diagnostics, medicine and bioprocess control. A 16-position biochip array with electrical signal read-out was used for a simultaneous detection and identification of micro-organisms by means of a sandwich hybridisation method using the 16S ribosomal RNA as target molecule.

The detection principle is based on an enzyme-linked sandwich hybridisation assay with capture probes immobilised on the biochip surface and Biotin labelled detection probes, both specifically hybridising to the target molecule of interest. The detection is performed by an enzymatic reaction of the streptavidin-alkaline-phosphatase coupled to the detection probe and releasing molecules, which are redox recycled at the electrodes on the chip and yielding current responses, which are measured position specific by a sensitive multi-channel potentiostat performing quantitative multi-channel readout.

The used biochip arrays made in silicon chip technology are based on interdigitated gold ultramicroelectrode arrays having 8 or 16 measuring positions. Using that technology up to 16 different targets can be identified simultaneously. The electrical biochip array system is characterised by the following advantages:

- high specificity (accurate taxonomic identification of the micro-organism of interest);
- high sensitivity (< 8x10⁸ target molecules; < 90,000 cells);
- opportunity for a quantitative detection by use of in vitro standards;
- option for automation of the method.

In contrast to conventional microbial detection and identification methods performed especially in food diagnostics, the use of the electrical biochip technology is of great advantage since it is highly sensitive and selective as well as considerably less time-consuming (analysis time is reduced from several days to a few hours). Since the test targets are RNA molecules, only living cells as well non-culturable organisms will be detected. Furthermore, the biochip array system with electrical signal read-out provides an accurate taxonomic identification of the micro-organisms of interest.
The application of flow cytometry as a fast microbial analysis technique in the food industry

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Microbial contaminations in food cause important economical losses and pose a threat to public health. Suggested critical limits within the production process and control of relevant parameters allow process control. Today it is almost impossible to use microbial parameters as critical control points, since the current methods are too slow to quickly provide the necessary feedback within an HACCP-system. Most times a fast determination of the microbial quality of the raw materials and the final products is impossible for the same reasons. That is why at the moment a lot of efforts are made in developing methods that allow a fast guarantee of the microbial quality and safety of food products. In this case flow cytometry offers an alternative towards standard methods. It is a fast and sensitive method, which can also provide useful quantitative and qualitative information concerning the microbial population. The wide range of available fluorescence probes allows the analysis of many different characteristics of micro-organisms. Therefore the use of flow cytometry can provide new applications within the quality- and process control of food products and in the control of fermentation processes.

The purpose of this study is to evaluate a compact flowcytometer (green solid state laser, 532 nm, 100 mW, 4 parameters) and at the same time to investigate its application possibilities as a microbiological quality-, process- and hygiene control device in the food industry. Within this part of the research project three domains can be distinguished:

- Evaluation of the performances of a flowcytometer and the matching fluorochromes (PI, Syto81, JOJO-1, DiSBAC\(_2\)(3), C12-resazurine).
- The evaluation and development of fast and reliable methods for the quantitative and qualitative determination of micro-organisms (total, specific groups) in food related matrices and comparing the results of different fluorochromes in the determination of the physiological state and its relevance in the quality control of food products.
- Tracing cellular damage caused by processing factors through flow cytometry.

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A rapid assay to monitor *Clostridium difficile* Toxin A specific IgA antibody production in bovine milk as an active ingredient for functional food

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MucoVax generates antibodies in the milk of normal cows with unprecedented high titres, directed against disease causing pathogens of the human gastro-intestinal tract. By stimulating the natural immune system of cows using techniques adopted from and developed in the field of mucosal technology MucoVax is able to produce biological active milk. This active milk (MucoMilk®) is supplied as a whey powder and can be used as nutritional therapy (functional food ingredient) for risk reduction or prevention of gastro-intestinal tract infections (bacteria and their toxins as well as viruses and fungi) both in humans and animals. MucoVax supports the safety and efficacy of its ingredients through clinical case reports.

Agrotechnology & Food Innovations has been developing lateral flow immunoassays (LFIAs) for over 15 years. The LFIAs are applied to detect ligands such as antibiotics, toxins, allergens and micro-organisms in raw materials and in feed and food products. In addition amplified DNA/RNA material can be detected in a modified assays, the nucleic acid LFIAs (NALFIAs). The assays are simple and straightforward, easy to interpret and results are visible in 10 to 15 minutes. Neither expensive equipment nor special skills are necessary and, therefore, the assays are especially suited for low-facility laboratory and field settings. Colloidal carbon particles, the immunolabels applied, show an excellent signal-to-noise ratio (black on a white membrane), making it for example an ideal label for the blood matrix.

The first whey ingredient product of MucoVax, designed against *Clostridium difficile* associated diarrhoea, is produced by immunising cows with *C. difficile* toxoid, containing Toxin A. The characteristics of the specific polyclonal immunoglobulins in the milk, particular of the IgA class, control the formulation possibilities for a consumer end-product. Therefore, the analysis of the Toxin A specific immune response is very important to maintain a high quality of the end product.

To enable cow-side analysis rapid LFIAs were developed to monitor Toxin A specific IgA antibodies, in bovine milk. In the LFIAs Toxin A coated colloidal carbon particles are used as detection conjugates, whereas anti-bovine IgA monoclonal antibodies, are used as capture ligands immobilised onto the nitrocellulose membrane. Diluted milk samples (10 times in running buffer) are applied to the membrane and upon absorption of the total volume (50 µL) another 50 µL of running buffer containing the colloidal carbon particle Toxin A conjugate is added. The signal is visible as a grey/black line and the correct test performance is indicated by a control line upstream from the specific line. The test can be quantified in seconds by a simple apparatus measuring the reflectance of the specific line.

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Application of a multiplex PCR for detection of *Clostridium botulinum* in foodstuffs

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The presence of spores of *Clostridium botulinum* in raw materials to be used for minimally heat-treated refrigerated food products presents a potential safety hazard, as the organism can produce a highly potent neurotoxin during growth. In most cases the spores originate either from contact between the raw material and soil or, particularly in the case of seafood, are present in contaminated water. Although the number of spores in raw materials is generally low (<10⁵/kg), the heat resistant nature of spores means that they can survive short heat treatments such as pasteurisation. It is necessary to monitor shelf life of minimally heat-treated products strictly to ensure safety as non-proteolytic *C. botulinum* can grow at refrigeration temperatures.

Quantification of the spore load in raw materials is necessary to enable meaningful risk assessment when, for example, proposing a new food product. However, this presents technical difficulties in determining the presence of low numbers of spores, and it is necessary to use anaerobic enrichment culture techniques, combined with a most probable number statistical assessment.

A further complication is presented by the range of toxin types that occur and, clearly, improved detection methodology is needed. In this poster we show initial work on the use of a multiplex PCR [1] for simultaneous detection of four *C. botulinum* toxin types in foods. The PCR amplifies the genes for *C. botulinum* neurotoxin types A, B, E and F and was used on extracts of culture fluid from enrichment cultures of various foodstuffs. A new internal control was devised for the assay, in accordance with the recent draft ISO standard on the use of PCR in food diagnostics. Certain foods were found to be inhibitory to the PCR and it was thus necessary to optimise the ratio of food:culture medium in the enrichment cultures to minimise this effect. Data on the minimum detectable level of *C. botulinum* spores in various foods using this method is presented. It was found that the PCR method would not detect the rare A2 toxin variant due to sequence differences at one primer site.

References

PCR and duplex PCR in detection of *Campylobacter* spp. in food

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The members of genus *Campylobacter* are gram-negative, micro-aerophilic, thermotolerant organisms. Thermophilic *Campylobacter* spp., particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognised as one of the etiologic agents of acute diarrheal disease in humans worldwide. They spread from their primary natural sources into the food chain and induce the strong diarrhoeic illness in human. *Campylobacter jejuni* and *Campylobacter coli* are the commonest species occurring in food.

We started our project by preparation the rapid detection method for the identification of three campylobacters, the common agents of acute gastrointestinal illnesses in humans. We developed another detection method because the requirement was to recognise *C. jejuni* and *C. coli* in the sample. In both cases polymerase chain reaction (PCR) was used. A PCR based on specific amplification of the variable sequence of 16S rRNA gene, that is specific for *C. jejuni*, *C. coli* and *C. lari* was performed using Tth DNA polymerase and the PCR products were visualised after agarose gel electrophoresis. Duplex PCR for separation *C. jejuni* and *C. coli* was performed using Tth DNA polymerase too. The specific primers {1,2} were previously checked for individual strains. Real food samples were tested according these two protocols and compared by standard normative methods.

**References**


Detection of mycobacteria in the pork production chain

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Many species belonging to environmental mycobacteria are capable of causing illness in humans and animals. Mycobacteriosis has been diagnosed in pigs in several European countries and the infections cause major economical losses. There is evidence indicating that pigs may be an important vehicle for environmental mycobacteria infection in humans or that pigs and humans share common sources of infection. The infection sources of environmental mycobacteria in humans and animals are poorly known. The detection and quantification of mycobacteria in the environment has been hampered by the lack of suitable methods. Mycobacteria grow slowly and the cultivation may take up to several months. Therefore rapid methods that don’t require cultivation are highly beneficial.

We investigated a 16S rRNA fluorescent sandwich hybridisation and real-time PCR as rapid tools for the detection and quantification mycobacteria in piggery environment and pig organs. Cultivation was used as a reference method. The methods were applied for the identification of infection sources in order to decrease the number of pig infections early in the pork production chain.

Mycobacteria were detected in piggery environment using real-time PCR and cultivation. The PCR method was successfully applied for quantification of mycobacteria in complex environmental samples. High numbers of mycobacteria were detected in feed, water and straw, peat and wood chips used as beddings. Mycobacteria were measured from the same stock of feed and bedding material both before and after the material was taken into piggeries. Higher numbers were detected in used material. The results show that there are many external sources of mycobacteria in piggeries. Mycobacteria multiply in piggeries and contaminate feed and water.

Mycobacterial 16S rRNA was detected in peat with fluorescence sandwich hybridisation assay without an amplification step. The number of mycobacterial 16S rRNA molecules measured in the same peat after heat treatment was below detection limit indicating that the heat treatment was an efficient way to decrease the number of viable mycobacteria. A hybridisation signal above the detection limit was measured from straw when 10⁸ cells were added per gram of sample indicating that the method was applicable for other piggery samples than peat but the sensitivity needs to be improved.
Multiplex detection in the food production chain


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At Plant Research International multiplex test are developed for fast and cost effective detection of pathogens or contaminants. Multiplex detection is the simultaneous assessment of a large number of parameters in one single sample. This can be achieved by using ‘microspheres’, polystyrene beads (ø 5.6 µM) on which covalently antibodies or other substances can be coated. There are 100 different sets of these microspheres, and 100 tests can be conducted at the same time in one single sample. So in a 96-well microtiterplate theoretically 9600 tests can be done.

In general the test procedure is simple, sample extracts are transferred to a microtiterplate and the microspheres are added to the sample. After 20 minutes the secondary antibodies are added without washing. The samples are analysed after another 20 minutes of incubation.

To summarise, the Luminex technology offers a no-wash procedure for fast, cost effective HTP screening. The sensitivity of the method is at least comparable to standard ELISA techniques but has a reduced use of consumables (microtiterplates), buffers and antibodies. At the moment several tests are developed for the detection of:

- pathogenic bacteria and viruses;
- contamination of milk powder by plant proteins;
- sulphonamides in the food chain;
- pesticide residues in vegetables.
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Novel developments in rapid mycotoxin detection

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In this poster recent developments in rapid antibody-based mycotoxin screening techniques will be highlighted. Such tests are designed to be used outside a laboratory environment, at the place of sampling. Results are expected immediately, so that commodities can be further processed without delay. Because they are used for mycotoxin analysis, very low levels (ppb and ppt range) should be detected. A further requirement is that the obtained results are accurate with a false compliant rate of < 5% at the level of interest [1]. All these requirements make the development of such tests rather difficult.

At first, plastic microtiter plates were used as solid phase materials for immobilising antibodies. However, to increase speed and user-friendliness, plastics were replaced by microporous membranes. Different signal generation systems to enable visual evaluation of results have been used. Enzyme labels, however, do not seem to be completely satisfactory in terms of stability and repeatability of the generated signal. Therefore microparticle labels such as colloidal gold particles are used more and more.

Sample pretreatment for screening techniques should be rapid and simple, consisting of only a very limited number of steps. Preferably a simple solvent extraction is used, followed by a filtration and dilution step. However, for strongly coloured or complex food matrices, this approach does not seem to work. An additional clean-up step is required which makes the test too complicated for field use. This problem can be solved by combining clean-up and detection in one single test device [2]. Therefore, a combination of silica derived solid phase material and immunoaffinity-based gel is used.

This evolution in rapid antibody-based mycotoxin detection tests will be outlined. Advantages, drawbacks and difficulties in developing rapid mycotoxin detection techniques will be discussed by using examples of flow-through, lateral flow and gel-based rapid field tests, which were developed, in our laboratory.

References
Recent developments in application of solid phase clean-up procedures for rapid analysis of difficult matrices

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An extraction and clean-up method for ochratoxin A (OA) in roasted coffee and aflatoxin B\textsubscript{1} (AFB\textsubscript{1}) in spices has been developed and the HPLC method optimised. The Amino SPE column was applied as a clean-up step for difficult matrices like roasted coffee, spices and cocoa. An interfering compound with a similar retention time as OA was adsorbed by the Amino SPE material at ≤5% NaHCO\textsubscript{3}. The Amino SPE solid-phase clean-up was developed to chromatographically elute OA and AFB\textsubscript{1} but retain cross-reacting compounds. A high partition co-efficient for OA and AFB\textsubscript{1} in the mobile phase was achieved by using methanol/5% aqueous NaHCO\textsubscript{3} as the sample extraction and clean-up solvent. Residual OA or AFB\textsubscript{1} on the column was recovered by washing the column with the extraction solution followed by methanol. Fractions were mixed together for further clean-up with Ochratext\textsuperscript{\textregistered} and AflaStar\textsuperscript{\textregistered} immunoaffinity columns (IACs). Analysis by HPLC resulted in a well resolved OA peak and reduction in matrix interferences. Recoveries ranged from 72 to 84\% and 75 to 91\% for OA and AFB\textsubscript{1}, respectively.

Membrane-based flow-through enzyme immunoassays (Patent No. EP 089 36 90) for the detection of OA in roasted coffee was developed. First, an extraction and solid phase clean-up method was developed. Without the use of the Amino SPE clean-up cross-reacting compounds caused the assay to result in 100\% false positive responses for both the flow-through and the HPLC methods. Difficult matrices are coloured and this causes intense background on the flow-through membrane system. The Amino SPE absorbed the coloured matrices allowing for high resolution of the results. After clean-up with Amino SPE no false positives were observed. The flow-through test results were visually evaluated. The sensitivity achieved for the flow-through was 4 ppb in spiked roasted coffee. The assay used to screen roasted coffee samples for OA and the results were confirmed with HPLC.

The Amino SPE column is now a constituent of our general product for mycotoxin screening in difficult matrices. It offers, in combination with an elutropic solution, a rapid clean-up system that is compatible with our fast tests.
Development of a new field test: a clean-up tandem assay column for the detection of ochratoxin A in roasted coffee

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Because the mycotoxin ochratoxin A (OTA) can occur in roasted coffee, regulations and maximum limits are necessary to protect consumer’s health. The clean-up tandem assay column for the detection of OTA in roasted coffee uses simple equipment and limited operational steps, allowing a rapid method of screening samples for compliance or non-compliance.

Clean-up and detection were performed on one single column device. The column described comprised two superposed layers: an aminopropyl derived silica layer capable of adsorbing the interfering fraction and the brown colour of the roasted coffee sample and a gel detection layer containing antibodies able to capture the analyte OTA. No expensive instruments were needed as results were visually evaluated after the application of an OTA-horse radish peroxidase-conjugate and the enzyme substrate tetramethylbenzidine. The intensity of the developed colour in the gel detection layer decreased with increasing concentrations of OTA. The cut-off level i.e. the smallest OTA concentration resulting in no colour development, was 6 µg kg⁻¹ OTA in roasted coffee. The result of the analysis can be binary only: presence/absence or yes/no response indicating whether OTA is present or not above the cut-off level. Assay validation was performed using samples fortified with OTA. The method gave a low percentage of false compliant results and no false non-compliant results. Assay performance was evaluated by screening naturally contaminated samples.

The described method offers a rapid, simple and cost-effective screening tool for OTA in roasted coffee, contributing to more effective quality control procedures and to consumers’ health protection.

References
Development of an immunoassay based lateral flow dipstick for the rapid detection of aflatoxin B1 in feed

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The development of a new immunoassay based lateral flow dipstick for the rapid detection of aflatoxin B1 (AFB1) in feed is presented. The test consists of three main components: conjugate pad, membrane and absorbent pad. The membrane is coated with two capture reagents, i.e. AFB1-bovine serum albumin conjugate (test line) and rabbit anti-mouse antibodies (control line). The detector reagent consists of colloidal gold particles coated with affinity purified monoclonal anti-AFB1 antibodies which saturate the conjugate pad.

A comparison of several AFB1 extraction methods for the feed matrix is presented. A mixture of methanol:water (80:20; v/v) gives the best recoveries. After sample extraction and dilution, the dipstick is put in the crude sample solution at the conjugate pad side and allowed to develop for ten minutes. Analyte present in the sample competes with the AFB1 immobilised on the membrane for binding to the limited amount of antibodies in the detector reagent. Thus, the test line of a blank sample shows the most intense pink colour because of the inverse relationship between toxin concentration and colour development. The smallest toxin concentration that results in no colour development is considered the visual detection limit. The control line is a check for the good performance of the test to ensure that the colloidal gold conjugate antibodies migrate throughout the system. If no control line is present the test is considered invalid. The visual detection limit for AFB1 in feed is at 5 µg/kg. The assay is accurate and reliable giving no false compliant (false negative) results and only a low percentage (10%) of false non-compliant (false positive) results. The lateral flow dipstick is also evaluated with certified reference materials (IRMM, Institute for Reference Materials and Measurements, Geel, Belgium). All tests with compound feed containing 9.3 µg/kg ± 0.5 AFB1 (n=7) show one pink line while blank compound feed (n=5) shows two pink lines.

The major advantages of this one step striptest are that results can be obtained within 10 min and that all reagents are immobilised on the lateral flow dipstick. It can be concluded that the described assay format offers potentials as a reliable on-site screening tool.
Application of the flow-through enzyme immunoassay in a market orientated supply study for the evaluation of fumonisins in cornflakes

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Fumonisins are toxic metabolites of naturally occurring moulds, principally *Fusarium verticilloides* and *F. proliferatum*, present ubiquitously in our environment and presenting a significant health risk to people and live stock. Fumonisin B₁, B₂ and B₃ are the major mycotoxins found in maize. When contaminated raw material is processed to cornflakes, fumonisins are carried over into the finished product.

Cornflakes brands (7 conventional and 2 organic) were purchased from retail stores in the Flemish and Walloon part of Belgium during the years 2002-2003-2004. A total of 90 samples were screened using the flow-through enzyme immunoassay technique described by Paepens et al. [1]. The samples were subjected to the previously described sample preparation procedure and the flow-through assay was adapted for the cornflakes matrix by lowering the visual detection limit to 275 µg FB₁ equivalents/kg. For samples contaminated with fumonisin B₁, B₂ and B₃ equal to or above the abovementioned visual cut-off, no colour (average ΔÊ*ab = 6.35 ± 0.15, n = 12, Minolta CR 321 colorimeter) appeared on the membrane and they were indicated as 'non-compliant'. When a blue coloured spot appeared, even substantially lighter coloured than the blank control sample (average ΔÊ ab = 10.45 ± 1.23, n = 12), the sample was considered to be 'compliant'. It should be emphasised that these quotations are only related to the visual detection limit and do not indicate containing a violative concentration, since the European Regulation (EC) No 466/2001 does not include the fumonisins. An intralaboratory validation study was performed according to the AOAC test kit procedures for qualitative tests. In order to determine the accurateness of the assay, all cornflakes samples were re-analysed with a validated quantitative LC-MS/MS method. Comparison of visual test results with LC-MS/MS values showed a good assay precision with no false compliant results and a rate of false non-compliant results of 18%.

This study demonstrates the feasibility of a rapid extraction technique combined with a rapid enzyme linked flow-through assay in order to meet the upcoming consumer protection regulations and quality control measurements in the food industry.

References


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In vitro detection of fumonisin exposure by the measurement of sphinganine 1-phosphate: a potential application to the screening of fumonisin-contaminated foods

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Fumonisins are a family of mycotoxins produced by \textit{Fusarium verticillioides}. Fumonisins are known to cause toxicities in animals and possibly humans associated with the consumption of mouldy corn contaminated with fumonisins. In this study we demonstrated that fumonisins accumulated not only sphinganine but also sphinganine 1-phosphate, which was typically observed in fumonisin-sensitive pig kidney epithelial cells (LLC-PK1 cells). Moreover, sphinganine 1-phosphate was shown to be a new indicator for fumonisin exposure as well as toxicity using fumonisin-sensitive (LLC-PK1 cells) and –resistant cells (Chang liver cells and Chinese Hamster Ovary cells). For further study to elucidate the toxic mediator of fumonisins, we used mouse F9 embryonal carcinoma cells, which exhibits SPL -/- stable transformant (SPL lyase KO) and murine sphingosine 1-phosphate phosphohydrolase (mSPP1) stably overexpressed transformant (SPL lyase KO + mSPP1). Surprisingly, overexpression of mSPP1 in SPL -/- stable transformant showed strong resistance to fumonisin B1. These results suggested that the elevation of sphingoid bases and their 1-phosphate in culture cells would be very useful for the potential and specific detection of fumonisin contamination in corn and corn products. In additional conclusion, sphinganine 1-phosphate may be the key differentiator between simple contamination and toxicity of fumonisins in corn-based foods as well as feeds.
Elevated sphingoid bases 1-phosphate in mouse serum: \textit{in vivo} estimation of fumonisin exposure as a corn-based food contaminant

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Fumonisins are a group of structurally related compounds produced by \textit{Fusarium verticilloides}, which is common fungal contaminants in corn and corn products. Fumonisins are known to cause several animal diseases such as equine leukoencephalomalacia, porcine pulmonary oedema, and liver cancer, hepatotoxicity and nephrotoxicity in rat and possibly human oesophageal cancer associated with the consumption of mouldy corn contaminated with fumonisins. Fumonisins are inhibitors of ceramide synthase in sphingolipid metabolism. The objective of this study was to develop the sensitive biochemical marker for the prediction of fumonisin contamination in corn-based foods as well as toxicity. When mice were treated with FB1 (10 mg/kg, i.p/day) for 5 days, the serum levels of sphingoid bases and their 1-phosphate were remarkably elevated. The accumulation of sphingosine 1-phosphate (So-1-P) and sphinganine 1-phosphate (Sa-1-P) in serum by FB1 treatment was much more apparent than the elevated levels of sphingosine (So) and sphinganine (Sa). Sa-1-P/So-1-P ratio in serum were much more elevated than Sa/So ratio following fumonisin B1 treatment, indicating that phosphorylation of sphingoid bases may be a sensitive biomarker for fumonisin exposure. In addition, the tissue levels of Sa and Sa-1-P were also remarkably elevated in kidney, liver, heart, lung and brain. FB1-induced toxicity was confirmed microscopically in both liver and kidney. Liver lesions consisted of centrilobular hypertrophy and cytoplasmic vacuolisation. In addition, hepatic binucleated cells were increased and acidophilic body was observed in FB1-treated mouse. Kidney lesions were consistent with tubular nephrosis, and tubules were dilated and contained cellular debris in FB1-exposed mouse. The interstitial fibrosis and tubular atrophy were observed in the kidney cortex of FB1-treated mice. These results suggested that the increased ratio of Sa-1-P/So-1-P in serum would be a specific biomarker for predicting fumonisin exposure, and elevated tissue levels of Sa-1-P as well as Sa may be related to fumonisin toxicity in animals.
Determination of residues in spiked samples and incurred tissue using the Premi®Test

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There is a continual need for improved antimicrobial screening procedures for use in veterinary drug residue surveillance programs because of the large amount of such testing required and the limited capabilities of existing methodology.

Premi®Test is a new broad-spectrum screening test for the detection of antibiotic residues in meat, meat products, eggs and urine. The test is based on inhibition of the growth of *Bacillus stearothermophilus*, a thermophilic bacterium very sensitive to many antibiotics. Premi®Test can detect the most relevant compounds at or below the EU and U.S. Maximum Residue Level (MRL).

This poster summarises the results of validation studies executed at the Central Science Laboratory (UK), the Workgroup 'Pharmakologisch Wirksame Stoffe' (Germany), TNO Quality of Life (the Netherlands) and École Nationale Vétérinaire de Toulouse/ AFSSA Fougères (France).
Determination of antibiotic residues in fish using the Premi®Test

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Aquaculture is a fast growing industry. As in many other intensive farming systems, the use of antimicrobial compounds to prevent or treat fish diseases is necessary. Residues of medication can therefore be expected in edible fish tissue.

Premi®Test, an antibiotic residue screening test, can detect the most important antimicrobial substances in fish. At the Central Science Laboratory (UK) the sensitivity was examined for salmon and white fish using spiked samples. At Utrecht University (the Netherlands) the suitability of Premi®Test with spiked muscle tissue from eel, salmon and trout was examined. Also incurred tissue of rainbow trout was examined.
Lateral flow device for the detection of nicarbazin in avian meals

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A rapid analytical tool for the detection of contaminating concentrations of the coccidiostat nicarbazin in avian feeds using lateral flow technology has been developed. This tool, based on lateral flow technology, similar to those used in-home pregnancy tests consisted of a small plastic device containing a sample pad and a viewing window. Solvent partitioning was performed to extract nicarbazin from the avian feeds. A few drops of this extract was added to the sample pad, and after 5 minutes the result was visually determined by looking for the presence of blue lines in the windows. The first line, a control, indicated that the test has been performed correctly. A second blue line, the test line, indicated that the level of nicarbazin was below the detection limit of the method (<1 ppm). The absence of this second (test) line denoted a level of nicarbazin above the detection limit. Previous studies have shown that concentrations of nicarbazin around 2 ppm in feed will result in violative concentrations of the drug in avian liver samples.

The test is based on a competitive assay; therefore, unlike the conventional lateral flow device format, a negative result is indicated by the presence of two lines and a positive detection by a single line. This device will enable people with no scientific training to determine if significant cross contamination with nicarbazin has occurred. Ultimately, this procedure could provide a fast, cheap, disposable, and user-friendly method of preventing violative concentrations of the drug in avian samples due to contaminated feeds.
Optical biosensor detection of tetracycline residues in foodstuffs

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Tetracyclines are broad-spectrum antibiotics with activity against gram-positive and gram-negative bacteria and so have numerous applications in veterinary medicine. They inhibit protein synthesis by binding reversibly to receptors of the 30 S ribosomal subunit of susceptible microorganisms. Annex 1 of Council Regulation (EEC) No 2377/90 defines the following maximum residue limits (MRL) for all food producing species: Tetracycline (Tc), Chlortetracycline (CTc) Oxytetracycline (OTc) and Doxycycline (Dc) 100 μg/kg in muscle and milk, 200 μg/kg in eggs, 300 μg/kg in liver and 600 μg/kg in kidney for the sum of parent drug and its 4-epimer. No permitted levels have been set in this legislation for anti-infectious agents in honey therefore the use of antibiotics, such as tetracyclines, is not permissible in European apiculture although tetracyclines have been permitted in countries such as Canada and the USA. Some bacteria have acquired resistance to the antibiotic due to, among other things, overuse and insufficient adherence to legislative control. Susceptible cells concentrate the antibiotic while resistant strains appear to carry an R-factor that inhibits uptake of the drug. Laboratory testing is performed on samples to help enforce the legislation and minimise the risk of tetracyclines entering the human food chain. Screening tests are used to filter out the majority negatives while suspect samples can be forwarded for confirmatory analysis.

Biacore® Q is a platform that can be used for developing a screening test. The Biacore Q can provide a rapid, reliable solution for determination of veterinary drug residues, other adulterants and vitamins in a wide variety of food-related products. Assays can be faster and more reliable than traditional screening tests. No labelling or tagging is required and so sample preparation can be kept to a minimum.

Preliminary results will be presented for the development of a tetracyclines assay in foodstuffs such as honey and kidney.
Spreeta-based biosensor immunoassays to detect adulterants in milk products

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Optical biosensors of Biacore (Uppsala, Sweden) proved to be robust analytical tools for the automated and rapid (5 min/sample) immunochemical detection of different adulterants and contaminants in milk and milk powder. However, the high price of these biosensor systems is a disadvantage for the wide application in food control laboratories. Therefore, a low-cost alternative optical biosensor (Spreeta™ biosensor of Texas Instruments, USA) was built into an affordable prototype liquid handling system.

In this prototype biosensor system, inhibition immunoassays, with anti bovine κ-casein monoclonal antibodies and a bovine κ-casein-coated CM5 chip of Biacore, were evaluated for the measurement of cows' milk in ewes' and goats' milk and for the detection of bovine rennet whey powder in milk powder. For these potential adulterants, comparable and useful sensitivities were obtained in both the Spreeta-based prototype biosensor system and the Biacore 3000. The limit of detection for cows' milk in ewes' and goats' milk was 0.17% and bovine rennet whey powder could be detected in milk powder above 1% (comparable with HPLC). For the applications shown, the prototype biosensor system proved to be a promising affordable alternative for the Biacore biosensor and it is a worthwhile option to be evaluated for other applications.

Figure 1. Scheme of the Spreeta-based prototype biosensor system.
Species specific detection of chicken in meat models and commercial products by PCR-RFLP analysis

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To meet requirements in the field of food analysis, application of specific, reliable, quick and sensitive methodologies are of increasing importance. One of the high sensitivity methods is the PCR, the use of which for detection of food components has been successful even in the investigation of heat-treated foodstuff. Micro-organisms, genetically modified organisms, raw and processed foods can be detected by PCR.

The Polymerase Chain Reaction - Restriction Fragment Length Polimorfism (PCR-RFLP) was applied for species identification of chicken species from raw meat (beef, pork, chicken, turkey), heat-treated complex model matrix (meat, water, fat, soy protein isolate) and red meat products (20 sausages). The selected sequence for amplification was a 359 base pair fragment of the mitochondrial cythochrome b gene as a part of the template DNA. The DNA was extracted from raw meats, models and commercial products using Wizard DNA binding resin and subjected to polymerase chain reaction.

Each batch reaction mixture contained Taq DNA polymerase, buffer, nucleotide triphosphate mix, DNA template (except the negative control) and a pair of primers in a final volume of 50 µL. 8µL Of the PCR product was digested by addition of 10 U of RsaI restriction endo-nuclease. Samples were digested for 2 h at 37°C and fractionated on a 10% polyacrylamide gel electrophoresis. The gels were performed in TRIS-Borate-EDTA puffer for 1h at 200 V. The bands were visualised by staining with ethidium-bromide and photographed under UV transillumination.

The 359 bp fragment was amplified as a product of PCR from each species, but only the chicken and turkey PCR product had a restriction site. The detection limit was 0,5% from models and three positive samples (sausages) were found among the commercial products where labelling was not found on the package. The results have showed that the PCR-RFLP is a useful method for the selective identification of chicken in raw meat, heat-treated models and in processed meat products.

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Detection of chicken meat in meat products by real-time PCR method

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To protect consumers' health and economic interests and to ensure adherence to liabilities in domestic and international trade and to customs tariffs, food products must be controlled not only for safety, but also for starting materials and for correspondence of the product to the declared composition. Species adulteration in meat products has become a wide problem in retail markets. Single and even multispecies adulteration has been reported in commercial meat products. Poultry proteins, which are cheaper than that of mammalian species, are often used for adulteration of meat products.

For the determination of chicken meat in meat products number of methods have been reported: agar gel immunodiffusion, indirect ELISA technique, PCR-restriction fragment length polymorphism technique (RFLP) and intra-short interspersed element PCR.

The real-time PCR method for the detection of chicken meat in retail meat products has been developed with the detection limit of 0.5% chicken meat in meat mixture, respectively. The primer pairs and probe for RT-PCR were designed for the region of chicken mitochondrial ATPase subunits 8 and 6 and the size of the amplification product was 113 bp. The specificity of the designed primers and probe were tested using samples of DNA of eleven animal species that may be present in meat samples. The designed primers and probe yielded only chicken specific amplification product. Twenty two meat product samples from the firm Krahulci of the Czech Republic have been tested by real-time PCR method. In four cases chicken meat was detected in discrepancy with the declared composition.

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Qualitative and semi-quantitative GMO detection from food samples derived from the Hungarian market

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The European Union (EU) has had regulation for the labelling of novel foods since 1997. Current EU regulations stipulate that products containing an ingredient of which >0.9% originates from a GM product must be labelled. The specific labelling requirements must ensure that the consumer is informed of any change in the characteristics or food properties such as composition, nutritional value or nutritional effects that render the novel food no longer equivalent to an existing food. For this reasons our goals were to perform risk assessment whether the Hungarian consumers are exposed to consume genetically modified foods and to what extent.

97 Food samples from the Hungarian market were bought and examined. The examinations were carried out with the help of Polymerase Chain Reaction (PCR) screening method and the quantification were perform by means of lab-on-a-chip technology. DNA from the commercial samples was extracted by WIZARD and CTAB method. Purity and concentration of the isolated DNA solution were controlled by spectrophotometer (R=A\text{260}/280). The suitability of the isolated DNA for the PCR reaction was assured by specific lectin-gene amplification. The presence of soy in the food products has been controlled with the amplification of this gene. Two specific PCR reactions (35S promoter, NOS terminator) were used for the GMO screening of the food samples. For gel analysis 10 µl of PCR products were analysed on 10% polyacrylamide gel. Following electrophoresis, the gels were stained with SYBR Green staining and the PCR products were analysed using a gel documentation system (Kodak EDAS 290). In the course of quantitative measurements the PCR products were analysed using Bioanalyser 2100 system and LabChip DNA 500 kit. 1 µL Of PCR products were analysed on the chip.

Evaluating the results, DNA was isolated successfully from 77 samples out of 97 food products. Lectin-gene was detected in case of 62 samples and in 7 cases we did not find any labelling concerning the soy content on the packages. 32 GM positive samples were detected out of 77 samples. In this 32 cases there was not found any labelling on the packages that refer to the GMO content.

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Characterisation of the geographical origin of coffee by pattern recognition techniques

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Due to its broad diffusion and high price, it is not unusual that coffee is subject to adulteration. In the strict sense of consumer safety, a coffee blended with cheaper products ( chicory, cereals and leguminous plants) represents a fraud affecting the basic quality of the product when the ingredients listed on the label offer an untrue description of the content on the package. More often frauds are related to the intrinsic quality, concerning the sensorial properties associated with the geographic origin of the coffee.

Several attempts have been made to develop analytical tools that are able to confirm appellation systems already in use. These approaches deal with the analysis of coffee tocopherols and triglycerides by HPLC [1], the determination of more than 50 elements in coffee by semi-quantitative scan ICP-MS [2], the analysis of δ^{13}C and δ^{15}N coupled with the site-specific (D/H) isotope ratio by ²H-NMR on caffeine extracted from green coffee [3], or the characterisation of green coffee by δ^{13}C, δ^{15}N and δ^{11}B analysis [4]. Total polyphenols together with other chemical descriptors (metals, amino acids, furfurals, caffeine) has been used to perform varietal classification of coffee or discriminate among different kinds of roasted coffees [5].

In this study, a huge sample set covering all the regions producing coffee in America, Africa and Asia are studied. The polyphenolic profiles analysed by HPLC-DAD-MS and the isotopic content by IRMS of coffee beans, are evaluated by pattern recognition techniques in order to classify coffees according to their geographical origin.

References