INCO COPERNICUS News Issue 2

Quality Control in Dairy Industry December 1999

Editorial

Welcome to the second issue of INCO COPERNICUS News, the newsletter of the EU-funded project PL 979012 "Rapid, specific detection of *Listeria monocytogenes* by antibody-based techniques and on-line sensor technology; development of improved control of food safety for industry and the consumer".

Those of you who saw the first issue will already know the aims and objectives of our project, and will have read an overview of the organism, *Listeria*, at the centre of this research. That newsletter also contained an article on the innovative way we are approaching development of a *Listeria* detection method, i.e. use of antibodies produced to synthetic peptides representing important *Listeria* virulence determinants.

In this issue we have an article on the most used cultivation methods for *Listeria* isolation and on existing rapid detection methods for *Listeria*; we hope that our project will improve on these methods, especially relating to specific detection of the species *L. monocytogenes*. There is also an article on biosensors, one of the detection techniques in which we plan to employ our antibodies. Although these devices are currently expensive to buy, and generally limited to large organisations, much cheaper versions are under development — these are very likely to become more widely used in the future. We will, of course, also be producing our detection test in a microtitre plate ELISA format, suitable for use in smaller-scale laboratories.

We also report here on the second project planning meeting, excellently hosted by the Dairy Research Institute, Zilina, Slovakia (partner P6 in our project) and about strategies of controlling *Listeria* in USA as well as about the outbreaks of listeriosis in North America and Europe. In USA more frequent testing for *Listeria* occurence results in increasing the number of *Listeria*-positive food samples identified. The last food-born listeriosis outbreaks also in Europe very clearly prove the need for sensitive, fast and simple method for serial assaying *L. monocytogenes* in a large number of samples to help monitoring and controlling this pathogen in food processing and supply system.

We hope that you find this newsletter interesting, and we would be pleased to discuss further any aspect of our project. Please use the contact information given at the end of the newsletter.

Gary Wyatt, Katarína Horáková

Isolation of *Listeria* from foods

General acceptance that food is the principal vehicle of transmission of *Listeria* followed a series of outbreaks in North America and Europe in the 1980s. *L. monocytogenes* is videspread in the envirement and consequently there is considerable opportunity for foods to became contaminated and for *Listeria* to be disseminated by cross contamination during processing and storage. The organism tolerates widely ranging salt concentrations, pH and temperature It is capable of growing well at ordinary refrigerator temperatures, the low temperatures giving it competitive advantage over the accompanying mesophilic flora. The recognition that *Listeria* infection can have very serious consequences and the knowledge that refrigeration can actually encourage the growth of *L. monocytogenes* has made it necessary for food manufactures to make great efforts to exclude the organism from their products.

Much work in recent years has been devoted to formulating improved culture media and great strides have been made in increasing the ability to detect very small numbers of *Listeria.* National standard methods and procedures tend to be broadly similar. The intention of this contribution is to describe the most used culture media and procedure methods for the detection of *Listeria monocytogenes* in different food products.

Enrichment media

Enrichment of bacterial cells is a common protocol for all cultural methods and for both antibody- or DNA-rapid pathogen detection models. Much of the most recent media development has been directed towards optimising performance of existing media by varying the concentration of the selective agents they contain.

Selective Enrichment Media currently used in the major isolation procedures are divided into three groups:

• **LEB** (Listeria enrichment broth [1], Listeria enrichment broth modified [2] and Buffered Listeria enrichment broth [3]). The formulation of the broths [1]—[3] is a modified Tryptic Soy Broth with additional of yeast extract, which are rich sources of the biochemical components and trace nutrient required for bacterial metabolism. Enrichment properties of buffered LEB can be improved by the addition of potassium di-hydrogen orthophosphate and disodium hydrogen ortophosphate. The inhibition of unnecessary accompanying flora (selectivity) is achieved by addition of nalidixic acid, cyclohexamine and acriflavine hydrochloride.

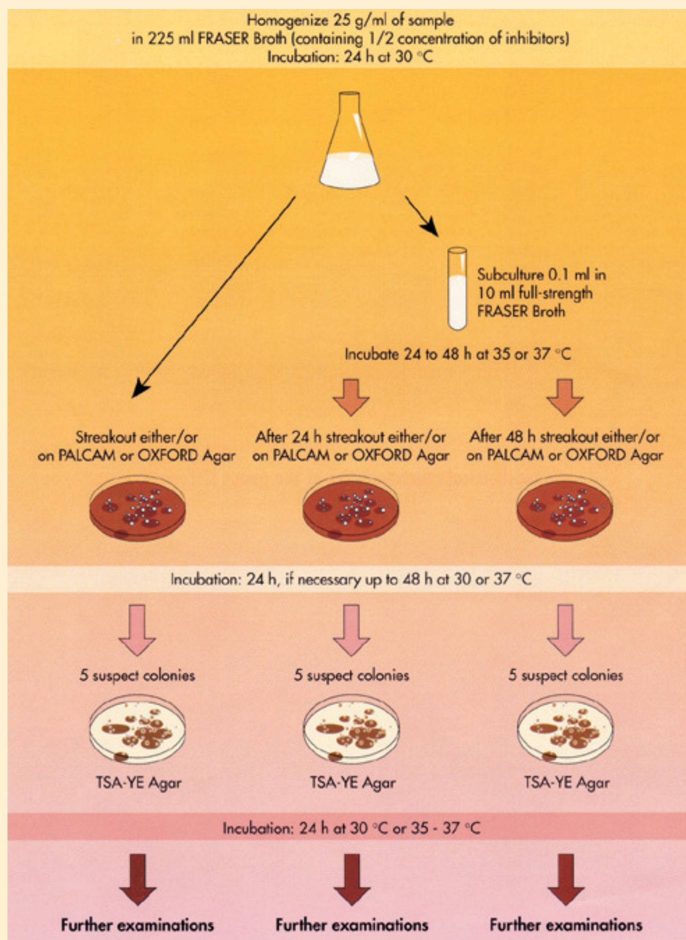

Fig. 1: *Diagram of procedure according to International Standard Organisation/ Commitee Draft (ISO/CD) 11290 and methode de routine Association Francaise de Normalisation (AFNOR) V08–055 Detection of L. monocytogenes.*

Table 1: *Double indicator system — Aesculin and ferrous system, mannitol and phenol red.*

OXFORD Agar		
	growth	Aesculin reaction
Listeria	+	+
G^- spp	–	–
unwanted G^+ spp	–	–
some *enterococci*	poorly	weak (after 40 h)
staphylococci	+	–

Fig 2a: Colony appearance of Listeria species on Oxford Agar.

PALCAM Agar			
	Fermentation of manitol	pH indicator	Aesculin reaction
Listeria	–	red	+
enterococci	+	yellow	+
staphylococci	+	yellow	–

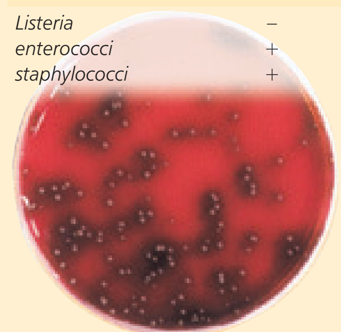

Fig 2b: Colony appearance of Listeria species on Palcam Agar.

- **UVM** (UVM primary and secondary Listeria enrichment broths). Subsequent modification of UVM formulations reduced the nalidixic acid concentration in both the primary and secondary selective enrichment media and increased the concentration of acriflavine hydrochloride in the secondary selective enrichment medium. The combination of various peptones, extracts, salts and buffer substances enables a very good growth of *Listeria*. The two-stage enrichment method (Fig 1) has demonstrated its value especially in sample materials (meat and meat products) that are characterized by a high level of accompanying flora.
- **FRASER** medium is modification of UVM secondary enrichment broth. Optimum growth conditions are created for *Listeria* due to the high nutrient content and the large buffer capacity. The growth of accompanying bacteria is largely inhibited by lithium chloride, nalidixic acid and acriflavine hydrochloride. The detection of the β-D-glucosidase activity of *Listeria* is possible by the addition of esculin and ammonium iron (III) citrate. The glucoside esculin is cleaved by β-D-glucosidase into esculetin and glucose. The esculetin then forms an olive-green to black complex with the iron (III) ions. Therefore, during the growth of *Listeria* in Fraser broth, usually a blackening of the broth is observed.

Isolation

The recognition that foods can be a vehicle for infection by *L. monocytogenes* has brought with it the necessity for isolation procedures more appropriate to food microbiology.

Almost all of the methods developed for isolation of *L. monocytogenes* use one or both distinct characteristics of the bacterium. The **first** characteristic, the ability to grow at refrigerator temperatures, has been exploited in cold enrichment broth culture, but is not applied to plating media. The **second**, resistance to many antibiotics, is exploited in recent plating medium formulations. Antibiotics, including nalidixic acid, moxalactam and cefotetan are used in conjunction with other selective agents. Blood was added to detect haemolytic strains of *Listeria*. Glycine and lithium chloride were also included to enhance selectivity. The introduction of formulae containing aesculin and ferric citrate was a further significant advance. It is now possible to detect colonies of *Listeria* spp. by the colour of hydrolysed aesculin. A further refinement, designed to differentiate enterococci, which also hydrolyse aesculin, from *Listeria* spp. was the incorporation of mannitol in Palcam Agar so that the enterococci could be differentiated by mannitol fermentation.

OXFORD and PALCAM agars have emerged as highly effective plating media which are widely specified in official methodology. Double indicator system of both media are given in Fig 2 and Table 1.

In general the cultivation methods can be characterised by the next following operations (Fig 2)

- selective growth in enrichment broth for 24—48 h at 30 and 37 °C which enable resuscitation of stress damaged *Listeria* cells as well as inhibition of accompanying flora to occur
- streakout on selective-diagnostic agars
- to prove the characteristic colonies on nonselective plating media
- identification of the *Listeria* spp. by appropriate rapid methods

Conclusion

It has been recognized that production of the genus-specific antigens by *Listeria spp.* can vary with growth conditions, including temperature, pH and the salt or preservatives present. While enrichment of samples in *Listeria*-selective media are widely used in conjunction with antibody-based *Listeria* diagnostic assays, the detectability of *Listeria spp.* in an ELISA format will depend upon the chois of broth medium used for the isolation and growth of cells from suspect food samples prior to testing. Therefore until now only limited success has been achieved in efforts to develop species-specific polyclonal antibody or monoclonal antibody probes capable of detecting most serotypes of *L. monocytogenes* and most nonpathogenic *Listeria species*.

K. Horáková, M. Greifová

Rapid methods for detection of *Listeria* in foods

This brief review will cover the rapid methods for detection of *Listeria* in foods that are available commercially, at least in western Europe.

It will be divided between those that are intended to detect the genus *Listeria*, and those that are aimed at *L. monocytogenes* specifically. It will be further divided between nucleic acid-based methods and antibody-based methods, with the emphasis on the latter. Consideration will be given primarily to those methods that have received validation in a recognised scheme, such as that operated by the Association of Analytical Chemists (AOAC); validation schemes will be the subject of a future article in this newsletter. Kits available at the time of writing are shown in the table.

General comments

To begin with, a few comments on the use of kits & antibodies are necessary. A commonly accepted standard for pathogens is that the method must be able to detect 1 cell in 25g food. In theory, it would be possible to build sufficient amplification into a kit such that it could detect a single cell. However, finding that cell in the food sample would be difficult, so the usual approach is to culture the food, thus allowing any cells present to multiply to a level at which they are more easily detected. This applies to almost all of the kits discussed here.

Next, the user-friendliness of the kit must be considered. Most kits will contain all the reagents and other components necessary to carry out, for example, 100 tests. Nucleic acid-based methods are potentially extremely sensitive, but often require a lot of specialised training and, currently, much manipulation is required for tests based on this technology. Antibody-based methods are relatively simple to use and need less operator training. In fact, some formats, such as the self-contained dipstick devices, can be used with little or no training. At the moment, therefore, these methods have many advantages over nucleic acid-based kits.

Commercially available kits

Now we will look in detail at some of the commercially-available kits.

First, methods for the species *L. monocytogenes*. Here, three commercial kits are available, two based on nucleic acid technology and one on antibodies. The *Gene-Trak* assay (DiffChamb AB) uses an rRNA probe, and the *BAX* assay (Qualicon) is a PCR-based method; further details will not be discussed here. The antibody-based *VIDAS L. monocytogenes* kit is part of the *VITEK* series of semi-automated immunoassays (bioMerieux); these use a disposable solid-phase device coated with antibodies. The food sample to be tested is cultured in an enrichment broth and a sample of the broth is then applied to a reagent strip. The analysis is carried out automatically by the *VITEK* instrument, using fluorescent end-point detection.

Second, and much more numerous, are kits aimed at detection of the genus *Listeria*. Two of these are nucleic acid-based, a *Gene-Trak* kit, and the *Probelia PCR System* (BioControl). As before, the details will not be discussed here. The remaining kits are antibody-based, and a selection of those that have completed a validation scheme will be mentioned.

A *VIDAS* kit for the genus is also available for the *VITEK* instrument, operating in exactly the same way as the *L. monocytogenes*-specific test (see above). This has received AOAC approval as a Performance Tested Method (PTM; licence number 981202). Another automated immunodiagnostic system, the *EIAFoss* (Foss Electric) has a *Listeria* detection module available; this is AOAC certified as PTM 980801 for meat and milk. Other than these automated systems, the test kits are divided into Enzyme-Linked Immunosorbent Assays (ELISAs), with visual or instrumental assessment of the end-point, and other formats such as magnetic beads assay and dipsticks. The *Tecra Visual Immunoassay* (Tecra) is formatted as a microtitre plate ELISA (divided into single strips), based on polyclonal antibodies, with the end-point being measured by eye. This kit has AOAC approval as an Official Method (OM; number 995.02) for detection of *Listeria* species in foods. The *Assurance EIA* (BioControl) is similar, but the end-point is measured in a spectrophotometer; this is AOAC certified as OM 996.14. *Listeria-Tek* (Organon-Teknika) is a microtitre plate ELISA based on monoclonal antibodies, with the end-point instrumentally determined. This has AOAC approval (OM 994.03) for use in dairy products, seafoods and meats. Other similar ELISA-based kits are available (see table).

As mentioned above, formats other than these ELISA types are available commercially. For example, the *Unique* test (Tecra) uses a plastic dipstick with immobilised antibodies to capture antigens from an enrichment culture of the food sample; the end-point is recognised as a coloured line on the dipstick surface. Lateral-flow test devices are also available; for example, the *Listeria Rapid Test* (Oxoid) and the *Visual Immunoprecipitate Assay* (BioControl). Theses are immunochromatographic systems using antibodies immobilised on a membrane; after application of an aliquot of enrichment broth, a positive result is indicated by the appearance of a line in a window of the plastic cassette. Both kits have AOAC approval — Oxoid as PTM 960701 and the BioControl test as OM 997.03.

Finally, in a completely different approach, antibodies immobilised on magnetic beads are used to capture *Listeria* cells from a prepared food sample in the *ListerTest* (Vicam). The beads, with the captured cells, are then plated onto an appropriate agar medium; after incubation, the plate is examined for characteristic colonies of *Listeria*. These latter tests are a good illustration of the flexibility of formatting possible using antibodies in rapid microbiological tests.

G. M. Wyatt

Commercial test kits for *Listeria* in foods

Test	Analytical Technique	Approx. Total Test Time[1]	Supplier
Assurance Listeria EIA[2]	Enzyme immunoassay	50 hours	BioControl Systems, Inc, web: www.rapidmethods.com
BAX for L. monocytogenes	Polymerase chain reaction	45 hours	Qualicon, Inc., web: www.qualicon.com
EIA-Foss[2] for Listeria	ELISA		Foss North America, Inc., web: www.fossnorthamerica.com
GENE-TRAK Listeria monocytogenes Assay	Nucleic acid hybridisation	48 hours	GENE-TRAK Systems, web: www.diffchamb.com
GENE-TRAK Listeria Species Assay[2]	Nucleic acid hybridisation	48 hours	GENE-TRAK Systems, web: www.diffchamb.com
Listeria Rapid Test[2]	Lateral flow cassette	42 hours	Oxoid, Inc., web: www.oxoid.co.uk
Lister-Tek[2]	ELISA	48 hours	Organon Teknika Corp., web: www.organonteknika.com
ListerTest[2]	Immunomagnetic separation	24 hours	Vicam, web: www.vicam.com
Probelia PCR System	Polymerase chain reaction	30 hours	BioControl Systems, Inc., web: www.rapidmethods.com
Reveal for Listeria[2]	Sandwich ELISA	48 hours	Neogen Corporation, web: www.neogen.com
TECRA Listeria Visual Immuno Assay[2]	ELISA	42—50 hours	Tecra International Pty, Ltd., web: www.tecra.net
TECRA Unique for Listeria	Immunoenrichment/Solid-phase dipstick	32 hours	Tecra International Pty, Ltd., web: www.tecra.net
Vidas (LIS)[2] for Listeria	Enzyme linked fluorescent assay	48 hours	bioMérieux Vitek, web: www.biomerieux.com
Vidas (LMO) for Listeria monocytogenes	Enzyme linked fluorescent assay	48 hours	bioMérieux Vitek, web: www.biomerieux.com
Visual immunoprecipitate (VIP) for Listeria[2]	Lateral flow cassette	48 hours	BioControl Systems, Inc., web: www.rapidmethods.com

[1] Includes enrichment [2] AOAC Approved (Web: www.aoac.org)

Characterisation and Application of Immunoassay Reagents for Rapid Detection of *Listeria monocytogenes* using Biosensor Technology

Considerable development of transducers and biointerfacial chemistry over the past few years has given rise to a new generation of portable biosensors for rapid detection of analytes. Despite these developments, the ultimate performance of such biosensing technologies is dictated by the quality of the affinity-based recognition ligand. Hence, the generation of antibodies with high specificity for *L. monocytogenes* is a prerequisite for the successful development of biosensor-based or microtitre plate-based immunoassays. Polyclonal antibodies have been raised against multi-antigenic peptides (MAPs) whose amino acid sequence was selected from a pathogenicity protein (i.e. Internalin) unique to *L. monocytogenes*.

The widely employed BIACORE 1000™ biosensing system will be used to evaluate the binding characteristics of these antibodies to the MAPs, purified internalin (whole molecule) and whole *Listeria* cells. Antibodies possessing suitable binding characteristics will be employed in direct and indirect immunoassay formats. A pre-enrichment broth will be required to amplify the *Listeria* load of samples to detectable levels. However, direct analysis of the crude broth samples using ELISA or the fibre optic-based biosensor under development will eliminate time-consuming sample preparation.

'Real-time' Biomolecular Interaction Analysis

'Real-time' biospecific interaction analysis (BIA) using BIACORE technology has become a well-established technique over the past decade. The BIACORE 1000/2000/3000™ systems are generic refractive index sensors that employ angle-dependent surface plasmon resonance (SPR) detection and a monochromatic light source.

Mass changes caused by binding of the analyte (e.g. *Listeria* cell) to the immobilised ligand (e.g. anti-MAPs antibody) result in a change in refractive index that is monitored through the SPR phenomenon in 'real-time'. Briefly, surface plasmons are longitudinal oscillations of free electrons at the surface of a thin metal film. Resonance occurs when the wave vector of the incident light matches that of the surface plasmon thus causing the adsorption of light. However, the resonance condition is critically dependent on the refractive index of the biointerfacial layer. Hence, the progress of a biomolecular interaction is made possible by continuously monitoring the position of the reflectance minimum. Analysis of the 'real-time' interaction curve (i.e. plot of the biosensor response (RU) against time (sec)) reveals the rate at which the analyte-ligand complex both forms and dissociates, thus providing a stringent measure for selecting reagents.

Visual ranking of the rate of association and dissociation from these 'real-time' interaction curves provides a rapid means of comparing the binding performance of a panel of antibodies. More comprehensive characterisation requires calculation of the observed rate constants, while estimation of kinetic constants demands careful experimental design and data analysis.

Novel Sensor Technology

Similar to the BIACORE systems, the novel fibre optic-based biosensor is also a generic refractive index sensor that employs SPR detection. However, it has been configured to accommodate wavelength-dependent SPR employing a polychromatic light source (Fig). This sensor configuration allows 'dip-stick' sampling thus facilitating rapid analysis of crude samples. Disposable optical fibre sensing elements, precoated with the chosen affinity-ligand, can be manufactured at low cost and stored for several months at 4 °C until required. Immunoassay development using 'real-time' BIA technology obeys many of the principles applicable to enzyme-linked immunosorbent assay (ELISA) but does not require labelling procedures. Moreover, the biointerface can be regenerated by removing the affinity-captured analyte using pH shock (i.e. 10 mM NaOH) thus allowing the ligand-coated sensing element to be used repeatedly. There are many factors to consider during assay design including the fundamental sensitivity of the transducer, interference from non-specific binding of components contained in crude sample matrices and surface regeneration. A versatile array of assay formats are possible, the simplest of which is the direct detection of free *Listeria* by exposing samples to a surface pre-coated with anti-*listeria* antibody. Indirect detection can either be competitive - in which a competition between free antibody and immobilised antibody for binding to free *Listeria* is established - or inhibitive - where the presence of free *Listeria* cells inhibits binding of free antibody to immobilised MAPs. In conclusion, 'real-time' BIA will be applied to the selection of suitable antibodies for the development of immunoassays that specifically detect *L. monocytogenes*. Furthermore, a novel fibre optic-based biosensor will be employed for the rapid detection of this pathogen. Clearly, direct feedback from rapid *L. monocytogenes* testing would enable food producers to respond more quickly to unwanted contamination thereby increasing food quality and lowering production costs.

J.Quinn

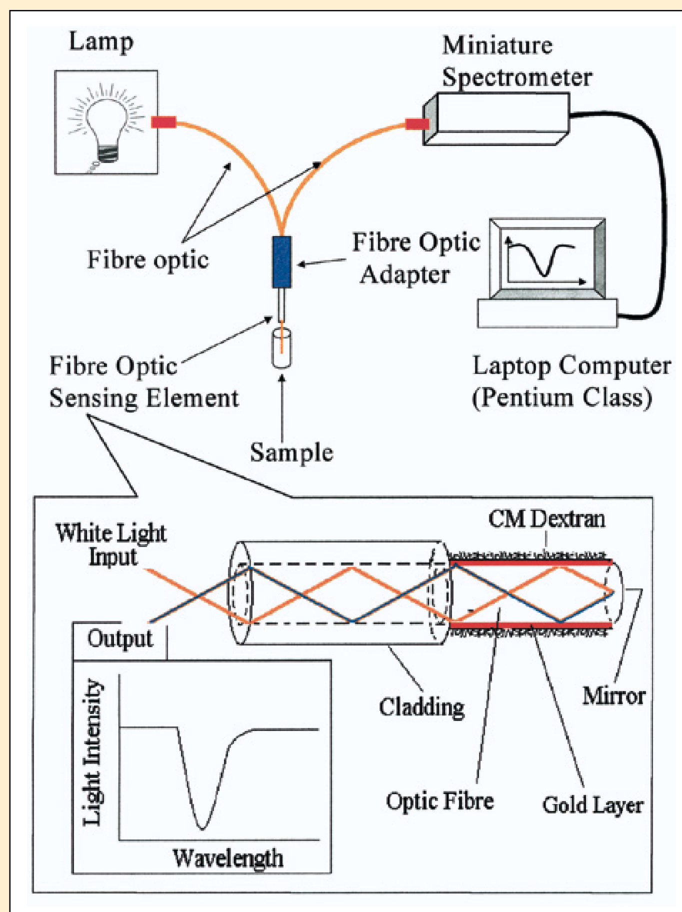

Figure: Schematic illustration of the optical configuration for the fibre optic-based biosensor with an optical fibre SPR sensing element. The carboxymethylated (CM) dextran layer forms an excellent biointerfacial layer as it allows covalent attachment of ligands, inhibits non-specific binding and facilitates a high analyte binding capacity.

Listeria in USA — Occurence, Control, Analysis

In the period from early August 1998 through springs of 1999, USA experienced an outbreak of listeriosis caused by a rare strain of Listeria monocytogenes (Pattern E) of serotype 4b. Hot dogs and possibly deli meats produced by Bil Mar Foods, a subsidiary of Sara Lee Corp. were identified as the source. Approximately 100 people were affected with 21 death cases. The probable source of contamination was dust generated during a summer maintenance project at the company's plant in Zeeland, Michigan. This huge outbreak had shown that listeriosis still represents a serious risk for consumers in USA in situation that a growing number of Americans have weakened immune systems and are more vulnerable to food poisoning and the shelf life of refrigerated processed foods has been extended to several months instead of weeks.

Strategies for controlling *Listeria*

Federal agencies pay attention to this pathogen for several years. Since 1987, there is the "zero tolerance" for *Listeria monocytogenbes* in ready-to-eat, cooked foods by Food Safety and Inspection Service (FSIS) of USDA (US Department of Agriculture) and Food and Drug Administration (FDA). FSIS is responsible for inspecting plants that slaughter and/or process meat, poultry, and egg products. The FDA oversees the safety of most other foods, including dairy products.

Both agencies have testing programs for *L. monocytogenes*. The goal of these programs is to help government and industry identify the causes of contamination in processing plants and to make permanent changes that will reduce *L. monocytogenes* contamination, prevent problems and ensure a safe food supply. Both agencies can hold or detain products at the food processing plant, request a voluntary recall of the product or seize products through court order if necessary. However, situation regarding last outbreak forced FSIS to re-examine its approach to combat listeria, as well as the approach taken by industry. Possible changes include:

- FSIS had published a notice in the Federal Register advising plants to reassess their HACCP preventive control plans to ensure they are adequately addressing the pathogen.

The prevention-oriented HACCP system requires plants to identify critical control points along their production lines and ensure that practices at those points minimize or prevent the likelihood of bacterial contamination or growth.

- The agency is providing guidance to industry recommending environmental and end-product testing.

Because a number of trade associations have developed guidelines that cover areas such as sanitation, the handling of raw materials, and employee hygiene, FSIS is complementing those materials by focusing on testing by plants to verify that their HACCP plans and sanitation procedures are producing pathogen-free product. Recommendations for plants are following:

to conduct environmental testing for general *Listeria* species;
to test product contact surfaces for general *Listeria* species;
to test end-products for the pathogen *L. monocytogenes*.

- FSIS is carrying out extensive educational efforts targeted to "at risk" consumers. FSIS also has the following four longer-term initiatives:

a) the agency is drafting a protocol to study the post-production growth of *L. monocytogenes* in a wide variety of ready-to-eat products and asks USDA's Agricultural Research Service to conduct the study during Fiscal Year 2000.
b) FSIS is developing an in-depth verification protocol that can be used to evaluate plants' HACCP plans for ready-to-eat products, particularly regarding *Listeria monocytogenes*.
c) a risk assessment of *L. monocytogenes* in conjunction with the Food and Drug Administration will focus on all foods, but in particular refrigerated, ready-to-eat foods.
d) FSIS is developing food safety standards for ready-to-eat products that will address the need to control all pathogens, including *L. monocytogenes*.

The above mentioned strategies put strong accent on microbial testing which is however opposed by meat companies, saying the focus should be on ways to improve the manufacturing process enabling to control pathogens. Technology including treatment of packaged foods with combination of high temperatures for pasteurization and of irradiation seems to be promising.

Testing methods for *L. monocytogenes:*

FSIS and FDA had developed two similar culture procedures for detection of *Listeria* that rely on the presence of antibiotics in the medium, which allow multiplication of *L. monocytogenes* but inhibit multiplication of competing organisms. In 1986, independent laboratories verified the accuracy of the new FSIS method for meat and poultry. This method was revised in 1998.

The FDA method (applicable to dairy products, seafood and vegetables) has also been reviewed and accepted. FDA invented DNA probe identification method as well.

Potential sources of *Listeria*

L. monocytogenes has been associated with such foods as raw milk, supposedly pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish. However, thermally processed meat products as hot dogs and luncheons belong to sources of pathogen as well due to the possibility of recontamination as is shown in the last outbreak.

Occurrence and growth on soft cheeses is enabled due to neutral pH. That is why French (both soft and usually from non-pasteurized milk) as well as Mexican-type cheeses are not recommended for at-risk consumers. Hard cheeses and fermented milk products do not represent serious risk since they have higher acid level which kills the bacteria.

Conclusion

Last outbreak of *Listeria* had changed the view on its treatment in US food industry. More frequent testing for its occurrence results in increasing the number of *Listeria*-positive food samples identified. From among 47 recalls for food products published in first ten months of this year, 24 were associated with *Listeria* contamination. This situation proves the need for sensitive, fast and simple method for serial assaying *Listeria* in a large number of samples to help monitoring and controlling this pathogen in food production.

V. Mastihuba

Minutes of meeting for INCO COPERNICUS project PL 979012 “Rapid, specific detection of *Listeria monocytogenes* by antibody-based techniques and on-line sensor technology”

Present

ICT, Prague	Pavel Rauch (PR), Ladislav Fukal, Ludmila Karasova, Igor Hochel
IFR, Norwich	Mike Morgan, Gary Wyatt (GW), Gary Brett (GB)
STU, Bratislava	Katarína Horáková (KH), Mária Greifová, Andrea Šovčíková, Ján Tkáč
DCU, Dublin	Richard O’Kennedy (R’OK), John Quinn (JQ)
Milcom, Prague	Petr Roubal, Jan Drbohlav
DRI, Žilina	Karol Herian, Martin Tomáška (MT), Anna Slottová, Andrea Závodská
Lipotovská mliekáreň	Ján Kresák (JK), Dašena Sliacka, Mária Mikytová

The second project meeting was held at the Dairy Research Institute in Žilina on the 22nd and 23rd April 1999.

The participants were welcomed to Žilina by Karol Herian, head of the dairy Research Institute. Pavel Rauch opened the meeting, thanked the organisers and outlined the project.

Scientific Presentations

1. Institute of Food Research, Norwich, UK

The role of IFR is the identification and production of proteins and peptides for immunisation.

GW outlined the factors affecting peptide selection and the method used to produce purified proteins from an over-expressing *Listeria* strain.

GB reported the production of synthetic MAPs, all peptides had been successfully synthesised and distributed to partner 1 for immunisation. GW described some preliminary characterisation of PR’s anti-sera using immunoblotting. Some anti-sera recognised Internalin B protein in a crude protein extract. It was suggested that all partners should work with the same *Listeria* samples and cultures of *L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri and L. seeligeri* were distributed to the relevant partners.

2. Institute of Chemical Technology, Prague, Czech Republic

PR outlined the immunisation protocol for generation of polyclonal antisera. The 3 month procedure used aluminium hydroxide as an adjuvant. Antisera were checked by direct ELISA on MAP coated plates. IgG was protein A affinity purified before lyophilisation. So far antisera have been produced against 8 MAPs, antibody production to the remaining 2 MAPs, Inl B and crude protein extract has begun and the results will be presented at the next meeting. Antisera characterisation included testing different microtitration plates, coating protocols and competing protein. High background in the assay appeared to be a problem when analysing whole cells but the results with proteins and MAPs were encouraging. Two antisera (anti-MAP4 and anti-MAP9) were able to recognise the MAP, protein and whole *Listeria* cells. The possibility of using other assay formats was discussed.

3. Slovak Technical University, Bratislava, Slovakia

KH gave a detailed outline of the methods for the cultivation of *Listeria*. The four-step procedure for the identification of *Listeria* involved (i) growth on selective LEB media, (ii) streaking out on selective plates. The selective media contain different concentrations of essential nutrients, antibiotics and selective agents such as LiCl, 2-phenyl ethanol, aesculin and ferric citrate. The next step (iii) is to prove the presence of *Listeria* on non-selective media and finally (iv) identification by microscopy, carbohydrate fermentation, heamolysis and CAMP test. KH stressed that new methods of identification are very important.

4. Dairy Research Institute, Žilina, Slovakia

MT outlined the steps being taken to develop the methodology based on ISO standards 10560 and 11290 part 2 using *L. monocytogenes* reference standards from the Czech Republic and The Netherlands. The 3 step method involved (i) an enrichment step, either Fraser or Half Fraser method, (ii) plating out and identification on Oxford or Palcam agar and (iii) species confirmation based on TSYEA plates, microscopy, Gram stain, catalase reaction and motility, CAMP test and heamolysis. DRI are continuing to monitor *L. monocytogenes* in Slovak dairy products especially cheeses made from unpasteurised goats’s and ewe’s milk. There was some discussion on the inhibitory effects of LEB on the expression of cell-surface proteins. When antibodies become available, methods of improving the procedure can be explored.

Pavel Rauch during his lecture. Pictured by M. Tomáška.

5. Dublin City University, Dublin, Republic of Ireland

JQ gave an overview on the projected use of Biacore 1000 in the real time Biomolecular Interaction Analysis of *Listeria* cells both in the development of a quantitative assay and a fibre optic based dipstick test. The fundamentals of the SPR based technique were described along with the importance of immobilisation and kinetic factors. Analysis of red blood cells was described as a model for cell detection. Requirements of the technique include affinity purified antibody, the presence of a functional group or affinity handle on the peptide to allow specific immobilisation and some sample pre-enrichment. The sample pre-enrichment could be simply culturing in LEB and concentration by centrifugation. Sensitivity depends on the fractional occupancy of the sensor surface, therefore in theory the smaller the surface the better the detection of a given number of cells.

Other matters

Finantial situation

GW announced that all partners should receive payments shortly.

Liptovská mliekáreň, Liptovský Mikuláš

JK gave a summary of the activities of his company (the biggest producer of Slovak cheeses) and his position within the meeting. He reported that *Listeria* contamination is a significant problem and that his company would be keen to test the products of the project.

Participants of the meeting visiting Vrátna Valley near Žilina, where the afternoon programme of the meeting has taken place. Pictures by M. Tomáška.

Future work

Partners outlined their workplan for the next six months. It was agreed that a standard pasteurisation treatment of 7 mins/72 °C would be used if it is necessary to kill cells for safety reasons. Partner 1 will continue to refine ELISA conditions. By the end of 5/99 the new antisera should be ready for characterisation. Partner 2 will prepare more MAPs and proteins as well as derivatised peptides as requested by other partners. The possibilities of detection of specific proteins rather than whole cells will be investigated. GW will find out about the history of the IFR isolated *Listeria* samples. The possibility of raising monoclonal antibodies outside the project was discussed. Partner 3 had actually started work earlier than proposed but there had been problems equipping a suitable laboratory, these are expected to be resolved shortly. Protein fractions will be prepared according to suggestions from partner 2 and sent to PR and RO'K. Partner 4 will begin to characterise antisera. Partner 5 will prepare and compare samples and detection of *Listeria;* they will also investigate artificial contamination of cheeses, and provide milk samples with known microbiological parameters. Partner 6 will continue monitoring of milk and cheese samples.

Newsletter

KH reported that the first issue of the newsletter had been distributed. The cost was about 2.5 Euro/copy. There was general agreement that the quality of the newsletter was of a high standard and well received by Brussels. There was some discussion about the format and content of future issues. It was agreed that future issues would also be in colour and there would be two issues per year. Proposed articles were "Existing rapid detection methods of *Listeria*" (GW), "Biosensors" (RO'K), "Editorial" (GW). These articles are to be in Bratislava by 15/6. Other articles proposed were, "Listeria in the Czech and Slovak dairy industry especially in relation to raw milk"(P5 and P6), "Review of the situation in UK" (GW), "Review of the situation in the Republic of Ireland" (RO'K).
KH also requested news items, adverts and photographs.

Report

PR asked for contributions for the first report to be in Prague by 4/11. Copies of the 2nd newsletter should be in Prague by mid-December.

Next meeting

The next meeting will be in Norwich to coincide with the conference Agri-Food Antibodies '99. The Copernicus project meeting will be on Sunday 12th and Monday 13th September.

Future meetings will be
DUBLIN – May 2000 • BRATISLAVA – September 2000

Listeria outbreaks in North America

Five reported outbreaks of listeriosis in North America in the past two decades are either known or suspected to have been caused by *L. monocytogenes* in food.

- An outbreak in **1981** in Nova Scotia, Canada resulted in 41 cases of listeriosis including 18 deaths; 83 percent of the cases were perinatal (occurring near the time of birth). The outbreak was traced to *L. monocytogenes* on coleslaw that had been made from cabbage grown in field fertilized with manure from *Listeria*-infected sheep.
- An outbreak in **1983** in Boston resulted in 49 cases of listeriosis including 14 deaths; 14 percent of the cases were perinatal, the remainder in immunocompromised adults. Although pasteurized milk from *Listeria*-infected dairy cows was linked to the outbreak, *L. monocytogenes* was not found in the suspected brand of milk.
- An outbreak in **1985** in Los Angeles resulted in 142 cases of listeriosis including 46 deaths; 85 percent of the cases were perinatal. The outbreak was traced to *L. monocytogenes* on soft, Mexican-style cheese, manufactured with contaminated milk.
- An outbreak in Philadelphia in **1987** resulted in at least 32 cases of listeriosis, including 11 deaths. The cause was never identified.
- An outbreak in 22 states in August **1998**—February 1999 resulted in approximately 100 cases of listeriosis including 21 deaths (15 adults and 6 miscarriages/stillbirths). The outbreak was traced to a rare strain of *Listeria monocytogenes*, serotype 4b on hot dogs and possibly deli meats.

V. Mastihuba

Listeria outbreaks in Europe

A number of listeriosis outbreaks linked to the consumption of food products has occurred in the last two decades. A significant feature of many of these outbreaks is the high level of mortality associated with them.

The outbreaks very clearly demonstrate that control of *L. monocytogenes* in food processing and supply systems is absolutely essential. Outbreaks are usually investigated from a clinical perspective and complete information about the nature of the food involved and the production processes used is often not recorded. Data in the table represent a combination of the known information together with the most likely reason why these incidents occurred.

Foodborne outbreaks of listeriosis

Product type:	Surface-ripened soft cheese	Cooked prepacted pâté	Pork tongue in aspic	Row-milk soft cheese
Year:	1983—87	1987—89	1992	1995
Country:	Switzerland	UK	France	France
Levels:	10^4—10^6/gram	<10^2—10^6/gram	Not known	Not know
Outbreak serotype:	4b	4b	4b	4b
Cases (deaths):	122 (34)	>350 (>90)	279 (63)	20 (4)

- Between 1983 and 1987 a total of 122 cases of listeriosis, resulting in 34 deaths, was recorded in the western part of Switzerland (canton de Vaud). On detailed investigation these cases were epidemiologically linked with the consumption of Vacherin Mont d'Or cheese. The outbreake strain of *L. monocytogenes* was isolated from a surface-bacterial-ripened soft cheese, from a piece of cheese obtained from a patient suffering listeriosis and from areas in the cheese production environment, (with the wooden shelves and brine brushes used in the ripening rooms being particularly heavily contaminated).

- Between 1987 and 1989 the UK experienced a large increase in incidents of listeriosis rising from approximately 100 cases per year to a peak of nearly 300 cases in 1989. It was concluded that the increase was almost entirely due to the consumption of a contaminated pâté imported from a Belgian manufacturer. Following a general warning issued by the Department of Health in 1989 to avoid eating pâté and soft cheese together with the removal of sale of pâté from the implicated manufacturer, the level of reported listeriosis dramatically reduced. Since remained stable at approximately 100 cases per year in England and Wales.

- A listeriosis outbreak which resulted in an extremely high number of deaths occurred in France between March and December 1992. During the outbreak investigation over 12 000 isolates of *L. monocytogenes* were obtained from product and environmental samples taken from manufacturers, retailers and consumer homes. 203 samples were found to be contaminated with the epidemic strain, mainly in ham, pâté, products in jelly and several cheeses.

K.Horáková

Notice board

The 6th International Conference and Industrial Exhibition

SYROTECH 2000

3rd—5th May 2000
City Council Hall, Žilina
Slovak Republic

Conference is oriented on cheese area, for example:

- progress in science and technology
- quality assurance
- analytical methods — development and validation
- legislature and standard making
- marketing strategy

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...AND MORE PROJECTS

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FIELDS OF RESEARCH

Biotransformations and chemical modifications of natural compounds (alkaloids, flavonoids) and carbohydrates, preparation of substrates and enzymes and screening of microorganisms for biotransformations.

MAIN TOPICS

Enzymatic synthesis of bioactive carbohydrates mostly by glycosidases (e.g., derivatives of chitooligomers, fucose derivatives) for the use in immunology and receptor research. Preparation of new enzymes (glycosidases) for carbohydrate biotransformation. Chemical synthesis of carbohydrate-based targeted drugs and prodrugs. Use of nitrilases in biotransformation. Preparation of yeast glucan and derivatives (also large scale).

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