INCO COPERNICUS News

Issue 4

Quality Control in Dairy Industry

January 2002

Editorial

It is with some excitement that we move into the final stages 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 three issues of INCO COPERNICUS News will already know that overall objective of the project is to develop antibody-based methods for specific detection of *Listeria monocytogenes* in foods, with the aim of introducing appropriate quality control into food production processes of the CCE partners.

Third year of the project the progress has been so rapid that two workshops were held in Bratislava focused on optimisation of the pre-cultivation method and training of final version of both developed ELISA and dipstick assays with partners P1, P3, P5, and P6 taking part in the training. During the workshops high level of collaboration including common laboratory work, exchange of results and critical discussion of results was achieved. A feature of the project has been the synergistic interaction of the partners; it is a pleasure to acknowledge this, and predict that a number of collaborative publications will result.

Minutes of two interim project meetings held in Bratislava and Zvíkov as well as the final project meeting held in Prague are reported on pages 4—7. We recommend you not to miss the two main articles, report from conference on Agri-Food antibodies, and the summary of results and achievements announcing besides others how our project objectives have been met successfully. See us also on Web page: listeriainco.tripod.com In the frame of INCO COPERNICUS contract ERBIC15–CT98–0902 (PL 979012) this is our last issue. We thank to all of those who enabled us to realise this international project. If anyone requires any further information, please contact any of the project team or myself. We would like to thank also all contributors as well as readers for paying attention to our newsletter.

Katarína Horáková, editor

Listeria monocytogenes — significance, ecology and occurrence

Listeria momocytogenes is a Gram positive bacterium, motile by means of flagella. Some studies suggest that 1—10 % of humans may be intestinal carriers of *L. monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. It can be isolated from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. Most of *L. monocytogenes* strains are pathogenic to some degree. Listeriosis is the name of the general group of disorders caused by *L. monocytogenes*. Listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site (e.g. placenta, fetus).

Ecology and Food Vehicles

Listeria species have been isolated from a large variety of sources including soil, rotting vegetation, sewage water, rivers and salt estuaries. It has been suggested that the bacteria exists in a saprophytic environment involving plants and soil, which serves as a reservoir for later infection transmitted to animals and humans via faecal material, foods, insects etc. (Figure 1). Improperly fermented silage, for example, is an important niche for *L. monocytogenes* and has been cited as a frequent cause of encephalitis in domestic farm animals.

L. monocytogenes can be found as part of the normal flora of many animal species and in humans. In two Danish studies, about 1 % (3/348) of apparently healthy individuals were found to excrete *L. monocytogenes* (Figure 1). Possible routes for transmission of *L. monocytogenes* in faeces, whereas the corresponding figure among patients with listeriosis was 21.6% (16/74). In household contacts of patients with listeriosis the faecal carrier rate was reported to be 18 %. About 10 % of healthy cattle tested in the Netherlands were positive for *L. monocytogenes* in their faeces.

Because *Listeria* can survive and grow under many adverse conditions including low pH, refrigeration temperatures and high salt concentrations, they can easily contaminate food. This is of great concern to the food industry, since a low inoculum can translate into a substantial dose of *Listeria* for the consumer, depending on the shelf life and handling of a particular product.

Milk and dairy products

As mentioned above, healthy cows can serve as reservoirs for *L. monocytogenes* and secrete the organism in milk. Contamination of milk may also occur through accidental contact with faeces and silage. It was investigated the incidence of *L. monocytogenes* in bulk tank milk from 160 producers in Scotland over a year. Over this time-period twentyfive producers tested positive (15 %), but concentrations in the bulk tanks were low, usually less than one colony forming unit (CFU) per ml; the highest concentration being 35 CFU per ml. Nevertheless, raw milk must be considered by the dairy producers as a potential source of contamination coming into the plant. In the period 1986—87, *L. monocytogenes* was estimated by the FDA to be present in approximately 0.6% of the pasteurized milk available in the United States. It has been suggested that the current method for pasteurising milk, may not be effective against *L. monocytogenes*. However, more current research shows that unless the pasteurization process is defective, the level of heat-treatment the milk receives is sufficient to eliminate the organism to levels that do not pose an appreciable risk. Efforts in preventing contamination should therefore be concentrated at ensuring proper pasteurization and avoiding post-pasteurization contamination.

Situation in Czech Republic:
Listeria monocytogenes determination in Czech Republic:

Listeria monocytogenes — limits according to the national laws	
foods ready for use	neg./25g
meat products ($a_w < 0,92$)	< 100/g
cheeses made from raw milk	neg./25g
soft ripened cheeses	neg./25g

Reference Laboratory for *Listeria monocytogenes*:
State Veterinary Institute, Jihlava

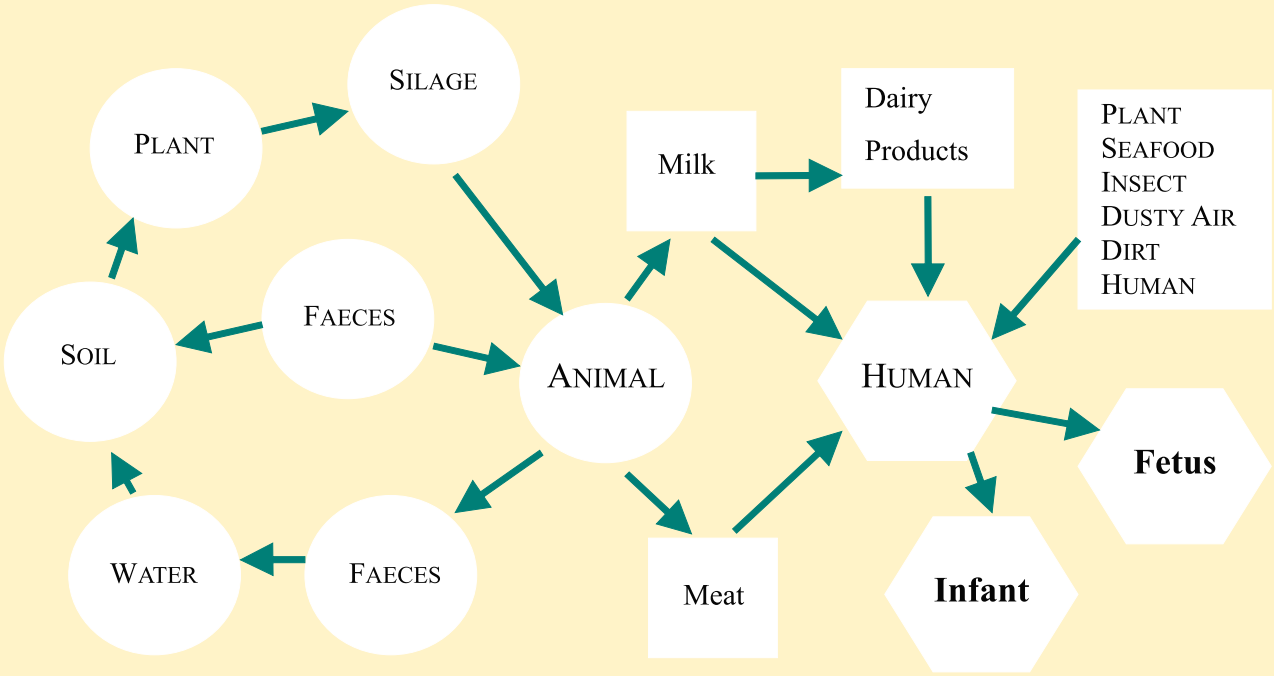


Figure 1: Possible routes for transmission of *L. monocytogens* to humans (adapted from Ryser and Marth, In: Listeria, listeriosis and food safety, M. Dekker, 1991)

Results of State Veterinary Service: Food and commodities of animal origin — microbiological detection of *Listeria monocytogenes* — years 1998—1999

Number of samples with positive detection of *L. monocytogenes*					
Kind of sample:	1998	1999	Kind of sample:	1998	1999
Fresh meat	0	2	poultry meat separated	0	1
Entrails	0	0	semi–finished poultry products	0	1
Semi-finished meat products	6	45	poultry products	0	1
Meat products — ripened	4	0	corned poultry meat	0	0
Meat products — not-ripened	8	0	eggs (yolk+white)	0	0
Other meat products	6	28	eggs (shell)	0	0
Corned meat	0	0	egg products pasteurised	0	0
Semi-corned meat	0	0	egg products not–pasteurised	0	0
Lard, tallow	0	0	mayonnaise	0	0
Raw milk	0	24	fresh water fish	0	0
Liquid milk	0	0	sea fish	0	0
UHT milk	0	0	fresh water fish products	0	0
Cultured milk products	0	0	sea fish products	0	0
Other liquid milk products	0	0	corned fish products	0	0
Dried milk powder	0	0	deep frozen products	0	0
Curd	1	0	delicatessen with mayonnaise	0	0
Butter	0	0	delicatessen without mayonnaise	1	0
Cheese	38	152	venison	0	0
Ice-cream	0	22	auxiliary products	0	0
Other solid milk products	0	2	honey	0	0
Poultry	0	0	other food of animal origin	0	1
Poultry — edible entrails	1	0	other food of vegetable origin	1	1
Total number of positive samples:		1998	66		
		1999	280		

Another state organisation involved into food safety control is **Czech Agricultural and Food Inspection** (CAFI). Assessment of microbiologic requirements for food in conformity with Decree No. 294/1997 Coll., issued by the Ministry of Health, is carried out on the basis of microbiologic analyses performed in laboratories or directly in the place of inspection, i.e. in the production premises or stores.

It is determined in the place of inspection whether there are evident visible changes in food caused by microbial activities, or even undesirable growth of microorganisms (such as the growth of moulds visible with the naked eye, or rottenness).

Microbiologic laboratories in the CAFI inspectorates (i.e. in Prague, Tábor, Ústí nad Labem, Hradec Králové, Brno and Olomouc) perform determinations of bacterial causative agents of food diseases (such as *Staphylococcus aureus*, ***Listeria monocytogenes***, *Salmonella spp.* etc.) and causative agents of food perishing (e.g. moulds, fungi, etc.). The criterion for evaluation of results obtained are "the maximum limits for the number of microorganisms" and "the tolerated values set for the individual types, groups or subgroups of foodstuffs" that are specified in the Ministry of Health Decree No. 294/1997 Coll.

Notifications of listeriosis disease infections in the Czech Republic in the years 1993 – 2001:

	1993	1994	1995	1996	1997	1998	1999	2000	I–X 2001
cases	9	8	11	10	10	10	13	23	13

Source: EPIDAT, 2001

Notifications of listeriosis disease in the Czech Republic – morbidity per 100 000 population in the years 1993 – 1999:

	1993	1994	1995	1996	1997	1998	1999
cases	0,1	0,1	0,1	0,1	0,1	0,1	0,1

Source: EPIDAT, March 2000, National reference centre for analysis of epidemiological data, NIPH Prague

In checking the microbiologic requirements, CAFI focused especially on products included in the list of food groups with epidemiological hazards that has been created as part of an agreement concluded between the Ministries of Health and Agriculture, such as cold meals, confectioneries, ice creams, etc.

The microbiologic analyses were carried out in the total of 8,755 food samples. Of which 1,978 samples were found non-complying, this represents 22.5 % of the total number of samples. The highest numbers of non-complying samples were detected in cold meals (489), confectioneries (449), fresh vegetables (321), fresh fruit (182), milk products (111), meat and meat products (102). In fresh vegetables and fruit, some undesirable microbiologic changes were detected in the places of inspection, without performing laboratory analyses. They were, for example, mouldy or rotten fruits.

National Institute of Public Health (Centre of epidemiology and microbiology) in Prague is charged by state authorities with surveillance of infections in the Czech Republic and among others:monitor the incidence of infections and monitor of morbidity and mortality of infectious diseases

Petr Roubal

Detection of *Listeria monocytogenes* in Cultured Cells by Fluorescent Microscopy

Viral, bacterial or protozoan intracellular infection usually involves subversion of mammalian signalling pathways. Often, microbial pathogens are swallowed up only by phagocytes (macrophages and neutrophils). However, some pathogens can induce their own internalisation into non-phagocytic mammalian cells by stimulating host cell tyrosine phosphorylation, phosphoinositide (PI) 3-kinase activity and rearrangement of actin cytoskeleton.

After invading a mammalian cell *Listeria monocytogenes* (*Lm*) spreads directly from cell to cell through protrusions consisting of host cell actin molecules. Initiation of protrusion or tail formation is enhanced by a bacterial surface protein called ActA. ActA is capable to induce localised actin assembly at the rear end of the bacterial body what generates a force for moving the bacteria forward through the cytoplasm of the host cell. Actin assembly involves continuous polymerisation and release of actin filaments at the bacterial surface while actin microfilaments remain stationary in the cytoplasm as the *Lm* moves forward. The dynamic behaviour of actin microfilaments in *Lm* tails is similar to the behaviour of microfilaments in the lamellipodia of motile keratinocytes and fibroblasts: short actin microfilaments are formed preferentially at the front of the actin-rich structure. Microfilaments are released after nucleation and cross-linked into loosely ordered meshwork; once in the network, their turnover rate is high. Turnover releases globular G–actin which can then difuse to the front of the array and polymerise again. The role of the *Lm* tail like the lamellipodial network is to generate protrusive force at the front of the array. The length of the tail is linearly proportional to the rate of the movement as well as the rate of the microfilament generation is related to the rate of the movement. Actin polymerisation is the constituent part of bacterial infection. Therefore, this process or the result of this process, i.e. actin tails can serve as good objects for detection of *Lm* infection. Moreover, *Lm* soon after entering the cytoplasm and usually before the beginning of actin tails formation is surrounded by host-cell cytoplasmic actin filaments. This phenomenon can be also used for detection of *Lm* infection without having specific antibody to *Lm*.

Fluorescent microscopy is a very sensitive method which has been used for detection and localisation of different cellular structures or proteins for many years. An extraordinary feature of fluorescent microscopy which distinguishes it from most of other methods, e.g. flow cytometry, electrophoresis, etc., is that by fluorescent microscopy we can observe selected proteins and structures not only in the whole population but also in individual cells. Usually polyclonal or monoclonal antibodies are used for detection of different cellular proteins and structures. If specific antibodies to *Lm* proteins are used (primary antibody) they can be linked directly to fluorescent dye. The procedure has only one step and calls the direct immunofluorescence (Fig. 1). However, a stronger signal is achieved by using unlabelled primary antibody and then detecting it with secondary antibody conjugated with fluorescent dye. This two steps-procedure calls indirect immunofluorescence. For detection of *Lm* surrounded by actin microfilaments or for detection of actin tails **phalloidin**, an alkaloid isolated from *Amanita phalloides,* which is capable to bind specifically to filamentous actin (F-actin), can be used after conjugation it with fluorescent dye (Fig. 2). Several frequently used fluorescently labelled phalloidins are commercially produced, e.g. fluorescein isothiocyanate (FITC)-phalloidin, coumarin phenyl isothio-cyanate (CPITC)-phalloidin, N-7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-phallacidin, rodamine-phalloidin.

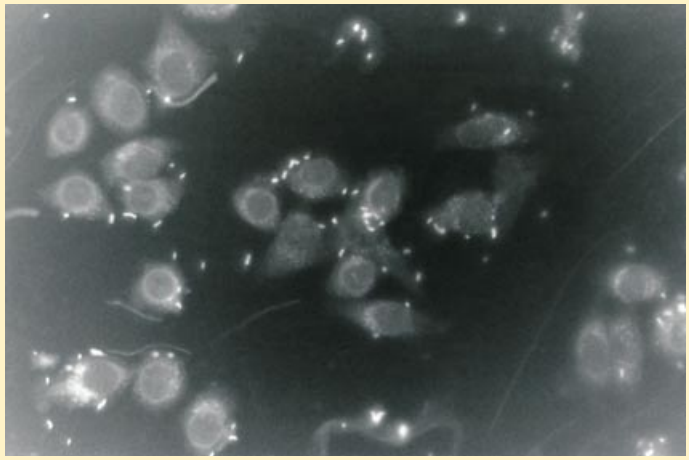


Figure 1. Indirect immunofluorescence of HepG2 cells infected with *Listeria monocytogenes*. Polyclonal antibody to whole *Listeria monocytogenes* developed by Partner 1 was used as the primary antibody and the goat IgG to rabbit antibody labelled with FITC as the secondary antibody. Stained bacteria are observed. Magnification × 400, orig.

The signal which gives phalloidin labelled with fluorescent dye is even higher than the signal which would be obtained by indirect immunofluorescence using specific antibody raised to actin. The procedure used for detection of actin tails localised in cells infected by *Lm* is identical with the procedure for detection of other cellular structures formed by filamentous actin (a contractile ring in dividing cells, an adhesion belt in epithelial cells, stress fibres and lamellipodia in fibroblasts, etc.).

The same protocol is used for detection of specific proteins of *Lm* and is as follows:

Cultured mammalian cells which were successfully infected with *Lm* are fixed with formaldehyde. This step necessary for visualisation of actin structures serves in this case also for killing pathogenic bacteria. So this is very important step in an experiment because from this moment the sample can be considered as non-infective. Then cells have to be gently permeabilised by TritonX-100. As actin cytoskeleton is TritonX-100- resistant most of non-cytoskeletal proteins and structures are released from cells during this process. The cells are then stained with unlabelled primary antibody specific to *Lm* proteins. Then secondary antibody labelled with fluorescent dye is added to the sample. If primary antibody are polyclonal rabbit antibody usually the goat antibody against rabbit IgG is used as the second antibody. During incubation of cells with fluorescently labelled antibody the sample has to be kept at dark to prevent spontaneous reduction of the sample fluorescence (photobleaching). Samples are observed with epifluorescence on a fluorescent microscope using camera coupled to an image-analysis system. For documentation samples are photographed using a very sensitive 400 ASA film.

Within 1 h of cell exposure to *Lm* almost a half population of bacteria are surrounded by F-actin. After 4 h all bacteria colocalise with F-actin. Moreover, some of *Lm* have already projections stained by fluorescently labelled phalloidin extending from one end of bacteria. These tails colocalise also with other actin-binding proteins, e.g. a-actinin and tropomyosin. Flourescence intensity profiles of actin tails show that there is a pronounced gradient of actin filament density through the tail: the filament density is the highest when the closest to the bacterium and decreases exponentially towards the distal end of the tail. The length of tails varies depending on the rate of movement. The largest tails can reach as much as 30-40 mm. *Lm* which distinguish with such a long tail belong to the most rapidly moving bacteria (formation of F-actin tails is proportional to bacterial movement). Intracellular bacterial movement depends on a cell type and can vary within a cell from time point to time point. The speed of bacteria migration can be as high as 1.46 mm/sec.

Detection of *Lm* by fluorescent microscopy is a very sensitive and useful method in determination of *Lm* infection or characterisation of antibody specific to *Lm*. Moreover, *Listeria monocytogenes* in combination with cultured cells and fluorescent microscopy provide an excellent model system for studying mechanism of signal transduction as well as universal propulsive mechanism based on actin polymerisation.

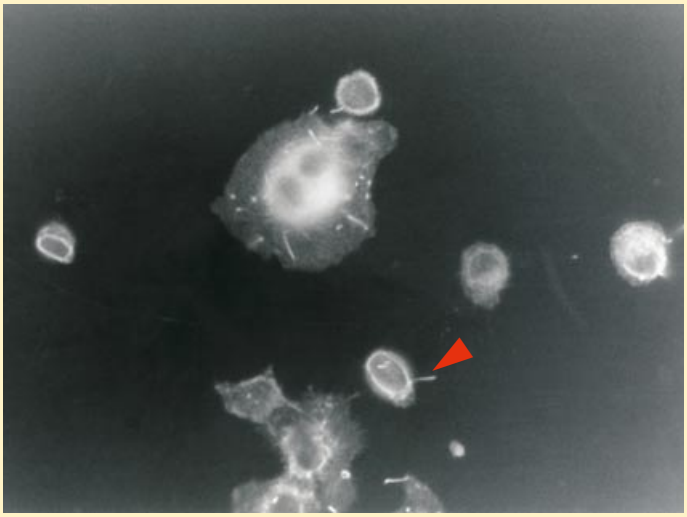


Figure 2. Direct immunofluorescence of MK2 cells infected with *Listeria monocytogenes*. Phalloidin conjugated with FITC was used to visualise actin tails (pointed by arrows) formed by bacteria. Magnification × 400, orig.

Cultured cells in combination with classical microbiological methods can be used for more detail characterisation of individual *Lm* strains, namely for determination of their invasiveness.

Miroslava Urbančíková

Minutes of meetings of COPERNICUS project PL979012
Rapid, specific, detection of *Listeria monocytogenes* by antibody-based techniques and on-line sensor technology
(shortened)

Bratislava, Slovak Republic, 23rd—24th November, 2000

Chairman
Pavel Rauch (ICT, Prague, P1)

Present
Lída Karasová (ICT, Prague, P1); Gary Wyatt (IFR, Norwich, P2); Mike Morgan (University of Leeds, P2); Katarína Horáková, Andrea Šovčíková, Zuzana Laurincová, Mária Greifová, Vladimír Mastihuba (STU, Bratislava, P3); Stephen Hearty (DCU, Dublin, P4); Petr Roubal (Milcom, Prague, P5); Martin Tomáška (DRI, Žilina, P6); Miroslava Urbančíková (IPCM, Bratislava, subP3)

Scientific presentations

ICT, Prague (partner 1)
LK described the production of three new batches of polyclonal antibodies – i) to MAPs 1, 5, 7 and 9; ii) to purified internalin B protein, and iii) to pasteurised cells of *L. monocytogenes* and their characteristics.
The new anti-MAP 1 antibody binds to whole cells of *L. ivanovii* 88/40 (absorbance 1.68 at 1/1000 dilution of antibody), and to *L. monocytogenes* 86/10 (absorbance 1.06), but not to *L. monocytogenes* 88/13; a similar pattern of binding was seen for anti-MAPs 7 and 9. The new anti-internalin B serum binds strongly to *L. ivanovii* and *L. monocytogenes*, but not to control *Lactobacillus* cells. The latest (boosted) sera from partner 2 were also tested against cells; R605 and R606 (anti-MAPs) did not bind, whereas R607 and R608 (anti-whole proteins) gave excellent recognition.
Using these antibodies, ELISAs (in competitive and sandwich formats) for *Listeria* were produced; where required, antibodies were labelled with enzyme. These assays were used during a period of work in the laboratory of partner 3 (see below).

IFR, Norwich (partner 2)
GW gave some background information on the commercial 'PathAlert' *Listeria* ELISA that had been tested by partner 3. He then summarised the current situation with respect to antibody production, using a chart outlining the decision points to be considered along the route from antibody recognition of MAPs to recognition of whole cells.
Characterisation of available antisera (except for the new partner 1 antibodies) by dot-blotting was now complete; with the exception of MAP 5, all peptides and proteins were recognised by at least one serum; by this

Participants of the Bratislava meeting in front of the Slovak University of Technology, Slovak Republic

method, very good binding to whole proteins was seen with sera R607/R608, and a much smaller amount of binding with R605/R606.
With boosting, a much improved titre of anti-internalin serum R608 against *L. monocytogenes* cells was seen; however, this serum still recognised *L. innocua*.

STU, Bratislava (partner 3)
KH reported on a new microplate method for characterisation of the growth patterns of *Listeria*, its use for measure the lag phase and growth rate in six culture media, and the effect of inoculum level on these parameters. The method correlated well with the standard method when the inoculum level was 10^6 cells/ml, but not when the level was 10^3/ml — this was attributed to the different rates of aeration and mixing.
Associated work included the production of cultures and cell pellets for other partners, and preparation of *Listeria* for examination of actin-tail formation by an external collaborator.

ICT, Prague — work carried out at STU
LH described a comparison of growth rates of *L. innocua* and *L. monocytogenes*. She had also purified internalin B protein from the over-expressing recombinant strain of *L. monocytogenes*, and used ELISAs to quantify the protein. The effect of growth in different culture media on detection of *Listeria* cells by ELISA (using the internalin B antiserum) was investigated; differences in detection were seen, but *L. innocua* was always also detected. Cells of an *Enterococcus* species also cross-reacted, but this organism is known to have proteins with homology to internalin B. Immunoblotting was used to investigate the specificity of the internalin B serum, and a band of 65 kDa was identified in both *L. monocytogenes* and *L. innocua* — this unexpected find needs further investigation.

IPCM, Bratislava (external assistance to STU)
MU described a method for investigation of actin-tail formation by *Listeria* in mammalian cells, using fluorescence microscopy. She had investigated the project *Listeria* collection; and all 13 *L. monocytogenes* strains were positive for actin-tail formation and, of the other species, only *L. ivanovii* was positive, as expected only in monkey cells.

DCU, Dublin (partner 4)
SH described his cross-reactivity studies with antisera R607/R608. He found that these cross-reacted with *L. innocua* (titre 1/5000 — 1/10000), and also with the other *Listeria* species. There was (possibly) some binding of R606 serum (anti-mixed *inl* MAPs) to *L. monocytogenes*, but the maximum titre was only 1/1000.
He then described the genetic control of internalin production and outlined a fibre-optic SPR biosensor which uses a 20 μl sample volume. Conditions for immobilisation of protein A and antibodies on the sensor had been investigated; a signal was achieved with binding of 11 μg/ml IgG to the protein A.

K. Horáková starting the program of the meeting.

Milcom, Prague (partner 5)
PRo described results from sampling of foods in the Czech Republic by the State Veterinary Service. In total, 66 samples from approximately 121,000 tested were positive for *L. monocytogenes*; of these, the highest incidence was from cheeses (38/4284 samples were positive).

DRI, Žilina (partner 6)
MT showed results from verification of the project *Listeria* collection. Three testing protocols were used — the 1996 ISO standard, the API *Listeria* miniaturised biochemical array, and Rapid-L-Mono, a culture plate method.

Zvíkov, Czech Republic, 10th—11th May, 2001

Chairman
Pavel Rauch (ICT, Prague)

Present
Lída Karasová, Barbora Mickova (ICT, Prague, P1); Gary Wyatt (IFR, Norwich, P2); Katarína Horáková, Mária Greifová, Vladimír Mastihuba (STU, Bratislava, P3); Paul Leonard (DCU, Dublin, P4); Petr Roubal (Milcom, Prague, P5); Martin Tomáška (DRI, Žilina, P6)

Scientific presentations

ICT, Prague (partner 1)
LK had confirmed the identity of her *Listeria* strains whilst working in Bratislava and Žilina. She isolated Internalin B (IB) from the verified cultures, and carried out Western blotting using anti-IB antibodies. This showed that *L. monocytogenes* (*Lm*) has a very strong band at the expected 65 kDa, but *Lin* (and other *Listeria* species) showed a different band, approx. 5 kDa heavier. It is not clear if blotting of this band is due to cross-reactivity of the IB antibody with the unknown protein, or if the antibody (which is polyclonal) also shows specific binding to the second protein.
LK had done competitive ELISAs with the anti-IB polyclonal, using plates coated with *Lm* cells. However, the assays recognised all *Listeria* species, with similar sensitivity (approx. 10^7 cells/ml). Sandwich ELISAs, using anti-whole cell antibody as both capture and detector, detected all *Listeria* species. However, when anti-IB antibody was used in the sandwich, the assay detected *Lm* (88/49) but not *Lin* (88/25); this confirmed the result found earlier by partner 2 (reported below).
When working in Bratislava, **LK** had studied the efficiency of pasteurisation of *Lm*. She found that i) in some experiments, *Lm* survived 76 °C/7 minutes in milk, and ii) survival was greater in milk > PBS > LEB medium. Pasteurised cells could be used successfully in ELISAs.

STU, Bratislava (partner 3)
KH had continued with the pasteurisation studies after **LK** had left. She found that, with heat treatments in milk, survival depends on inoculum size.
She used *Lm* heated in skimmed milk for 76 °C/7 minutes in a sandwich ELISA; the sensitivity was 10^5—10^6 cells/ml, and the milk background was good.

P. Roubal was the host of meeting in Zvíkov.

IFR, Norwich (partner 2)
GW described characterisation of the 'new' Prague antibodies (distributed at the Bratislava meeting). ELISAs showed high titres for the anti-IB and anti-*Lm* antibodies, against immobilised *Lm* cells. These titres were much higher than for the first batch of antibodies; however, binding to *Lin* cells was also seen. The new antibodies, and antibodies from further bleeds of IFR rabbits, were then labelled with HRP and used in sandwich ELISAs. After some adjustments to antibody and label concentrations, one combination of antibodies (Prague new anti-IB, as both capture and detector antibody) gave an assay that distinguished between *Lm* and *Lin*. Further work showed that this assay detected all *Lm* strains tested (to a variable extent) and did not detect 5 other species of *Listeria*; this was the first assay in the project to be specific to *Lm*. All cells were live and unpasteurised.
A brief investigation using growth of *Lm* in tissue culture media showed a slight improvement to the signal, but there were still differences between strains.

DCU, Dublin (partner 4)
PL reported that IFR serum number R607 (anti-whole cell protein) cross-reacts with *E. coli* and *B. subtilis*; serum R608 (anti-IB) showed very little cross-reactivity. A competitive ELISA using immobilised IB protein and anti-IB antibody could detect 150 μg/ml IB extract. He then described the new prism-based SPR biosensor which has user-friendly software; this can detect IB extract at 1—3 μg/ml. He had cloned p60 and IB genes from genomic DNA into *E. coli* using the *QIAexpress* system, which allows rapid purification of His-tagged proteins. Gels of products show a 65 kDa band, but this has not yet been blotted.
A naive library has been screened for scFv antibodies against IB, but nothing was found. He is currently making a new library from mice immunised with IB.

Milcom, Prague (partner 5)
PRo described data for Czech raw milk quality 1997—2000. The total bacterial count has continued to fall, but antibiotic residues are still high compared to EU countries. The State Veterinary Service had found *Lm* in 66 of ~120, 000 samples in 1998, and 280 in ~110,000 in 1999 — it is not clear whether this is a real increase, or better detection technology.

The meeting organized by Czech partners was held in a nice hotel near Zvíkov castle.

DRI, Žilina (partner 6)
MT presented the final results of characterisation of the *Listeria* collection using *API Listeria* and other methods and determined the identity of strains 89/5, 88/40, and 88/10.

KH & **VM** asked about availability of a cheap version of a biosensor that could be used in Bratislava. **PL** said he would discuss this with R. O'Kennedy in Dublin. One other possibility would be for **VM** to visit Dublin to gain experience of the biosensor there.

Prague, Czech Republic, 1st—2nd October, 2001

Chairman
Pavel Rauch (ICT, Prague)

Present
Lída Karamonová (ICT, Prague, P1); Gary Wyatt (IFR, Norwich, P2); Katarína Horáková, Mária Greifová (STU, Bratislava, P3); Richard O'Kennedy (DCU, Dublin, P4); Petr Roubal (Milcom, Prague, P5); Martin Tomáška (DRI, Žilina, P6); Miroslava Urbančíková (IPCM, Bratislava, subP3)

Scientific presentations

ICT, Prague (partner 1)
LK described her work on labelling the *L. monocytogenes* (*Lm*)-specific anti-internalin B (InlB) antibody during her stay in Norwich in August 2001, and use of the antibody in assays when working at STU, Bratislava. The main findings were i) using a sandwich format and *Lm* grown in TSB, she confirmed the specificity for *Lm* ii) for unknown reasons, there was assay signal variation between the 3 labs iii) as previously, different *Lm* strains were recognised to different levels; however, growth in different culture media had a much larger effect, with Fraser and UVM media giving the best signal iv) unlike the situation reported previously with the anti-whole cell antibody, with the anti-InlB antibody pasteurising cells at 76 °C for 7 minutes destroyed binding v) the assay specificity was confirmed by testing all *Listeria* species grown in Fraser broth; also, no binding was seen to *Lactobacillus*, *Enterobacter*, *Lactococcus* and *Bacillus* species. It was observed that there was much less strain-to-strain variation for *Lm* when grown in Fraser broth.
LK then described her work on transferring the assay from microplate format to dipstick format.

IFR, Norwich (partner 2)
GW summarised the objectives and results from the project. His time allocation on the project since the last project meeting had been spent mainly on preparation of manuscripts and conference talks/posters, hosting LK's visit to Norwich and development of follow-up project proposals.

IPCM, Bratislava (sub-contractor to partner 3)
MU had studied the immunoreactivity of four new antibodies to *Lm* by indirect immunofluorescence. The experimental system consisted of two mammalian cell lines (MK2 and HepG2) and 13 strains of *Lm*. The anti-*Lm* whole cell antibody recognised all the *Lm* strains, anti-InlB and MAP 5 antibodies recognised only the over-expressing recombinant strain, and anti-MAP 7 recognised no strain of *Lm*.
The invasion of MK2 cells by 13 strains of *Lm* was determined by plating infected MK2 cells on Oxford agar and counting colonies after overnight culture. The range of colony forming units (CFU) was from 0.45×10^6 CFU/ml to 3.75×10^6 CFU/ml (average 1.56×10^6 CFU/ml) for most of *Lm* strains. Three strains expressed very different values representing 13.49×10^6 CFU/ml, 39.29×10^6 CFU/ml and 1.19×10^5 CFU/ml.

STU, Bratislava (partner 3)
KH described the composition of the culture media used in the work reported by LK; confirmation of *Listeria* heat resistance studies was found in literature.

DCU, Dublin (partner 4)
RO'K described an inhibition assay format used in biosensor development. He described the response profiles and the re-generation characteristics of this sensor.
RO'K also illustrated the 3 types of sensor system under investigation i) Biacore flat-chip system which has very high cost, and a flow which can be impeded by cells ii) a probe-based biosensor using a gold layer and

The final project meeting was hosted by Institute of Chemical Technology, Prague, Czech Republic.

dextran coating; this type is not yet as sensitive as the Biacore system iii) a miniature prism-based biosensor with a protein A coating. Custom-designed software has been developed to control the last 2 systems.

RO'K then discussed monoclonal antibody production being carried out in conjunction with the University of Giessen, Germany — cell lines reacting with a 60—66 kDa protein thought to be InlB are undergoing further cloning.

Production of recombinant InlB and p60 proteins is also under development; cloning of sequences into *E. coli* has been successful, and optimisation of expression and purification is underway.

An scFv antibody phage-display library is under construction; both naive and immunised libraries are being screened.

Milcom, Prague (partner 5)

PRo described the training in the use of the new assay received in Bratislava by the industrial partners. UHT milk was inoculated with *Lm*, and cultured in Fraser broth at 37 °C for 18, 24 and 42 h; growth of *Lm*

P. Rauch opened the meeting.

was monitored by turbidity, plate count and ELISA. Growth was slow and needed 24h to reach 3×10^5/ml from the lowest inoculum. The ELISA was just positive (absorbance 0.3) at 24h from an inoculum of 10^3/ml; from inocula of 10^3 and 10^4/ml absorbances were 0.6; at 42h much higher absorbances were seen from all samples. Future work will include pre-incubation of milk samples with Fraser broth before transfer to fresh broth; optimisation of time for ELISA and dipstick, and testing of more foods.

DRI, Žilina (partner 6)

MT described *Lm* reference materials that are available from RIVM, The Netherlands. There are two types i) a cheaper reference material (5 cfu/ml) with a lower statistical certainty and variability from batch-to-batch ii) a more expensive official certified EU material (7.2 cfu/ml, with a range of only 6.8—7.6). Twenty capsules of the first type had been ordered and will be used in further trials of the ELISA in Bratislava.

Technology Implementation Plan (TIP)

This has to be submitted with the final report. Our results will be in exploitation category A. After much discussion, a list of results for inclusion in the TIP was agreed. These are —

1. An antibody specific for *Lm*
2. An antibody generic for *Listeria*
3. Monoclonal antibodies to internalin B
4. Antibodies to *Listeria* virulence proteins
5. An ELISA that is specific to *Lm*
6. A dipstick that is specific to *Lm*
7. A chip-based biosensor for *Listeria*
8. A probe-based biosensor for *Listeria*
9. A novel biosensor-based immunoassay format for *Listeria*
10. A method for quantifying invasiveness of *Lm* using *in vitro* mammalian cell system.
11. A microplate method for determining growth characteristics of *Lm*
12. A novel approach to generation of peptides for production of antibodies to proteins
13. A method for determining the effect of culture media on expression of p60 protein
14. A partially-validated method for detection of *Lm* in dairy products

Other matters

i) Task 18 of the project is –

T18	*months 34—36*	*P1*	*Application for funding from Accompanying Measures to hold workshop for reporting/discussion of the complete project with other potential industrial users*

GW will co-ordinate this application with the other partners. It was suggested that the workshop be held in Spring 2002 in or near Žilina, and will be arranged by partners 3 and 6.

ii) Paul Leonard (Dublin) will visit Bratislava for collaborative work using a portable biosensor.

iii) The final experiments in Bratislava on detection of *Lm* by the new assays will be with milk and cheese, using both direct inoculation and the reference capsules.

Photos by M. Tomáška.

Report from the conference by Gary Wyatt

This meeting was the sixth in the international series of *Society of Food and Agricultural Immunology* meetings; the previous meetings in the series were at Windermere, UK (1991), Madrid, Spain (1993), Canberra, Australia (1995), Durham, USA (1997) and Norwich, UK (1999). The meeting was organised jointly by the *Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague* and the *Czech Society for Biochemistry and Molecular Biology*.

About 120 scientists participated in the conference, mainly from Europe; also, despite the recent tragic events, several delegates from the USA attended, and there was even a contingent from as far away as Brazil. The conference was opened by **Prof. Pavel Rauch** of the Institute of Chemical Technology (ICT), Prague, who asked participants to observe a short silence in support of American colleagues.

As in previous meetings of this series, a wide variety of antibody-related science topics was presented in the talks. At the fundamental level, strategies for production of recombinant antibodies and antibody-like peptides from libraries (aimed particularly at hapten recognition) again featured strongly. Instrumental methods of analysis using antibodies obviously continue to make good progress and, in contrast to these highly sophisticated methods, simple dipstick-based techniques also show considerable advances.

At the food level, methods for detection of antibiotic and pesticide residues were presented, and it was interesting to note that radio-immunoassays have not been entirely replaced in food analysis, at least in measurement of plant secondary metabolites. Detection of genetically-modified proteins in food for regulatory purposes by antibody-based methods seems to be developing rapidly, and this is also the situation with diagnostic tests for allergenic proteins. The final area of work featuring at the conference, and the one that is probably most relevant to readers of this article, is the detection of pathogenic bacteria and their toxins, and also fungal toxins. Although not strictly a microbiological problem, animal disease caused by prion proteins has similarities to microbial infections, and advances in diagnostics here were also presented.

The thirty scientific talks were complemented by over sixty posters covering a very wide range of topics; these were given in dedicated sessions, thus allowing presenters plenty of time for personal discussion of their work. One talk and three posters describing work in our *Listeria* project were presented.

I will now mention some of the talks which were, in my opinion, the highlights of the conference. The opening lecture, by **Jean Daussant** of Paris, described historical aspects of the development of antibody technology; the talk was given, of course, in Jean's usual polymath style, and was probably quite unusual at a scientific conference in that he quoted extensively from classical literature!

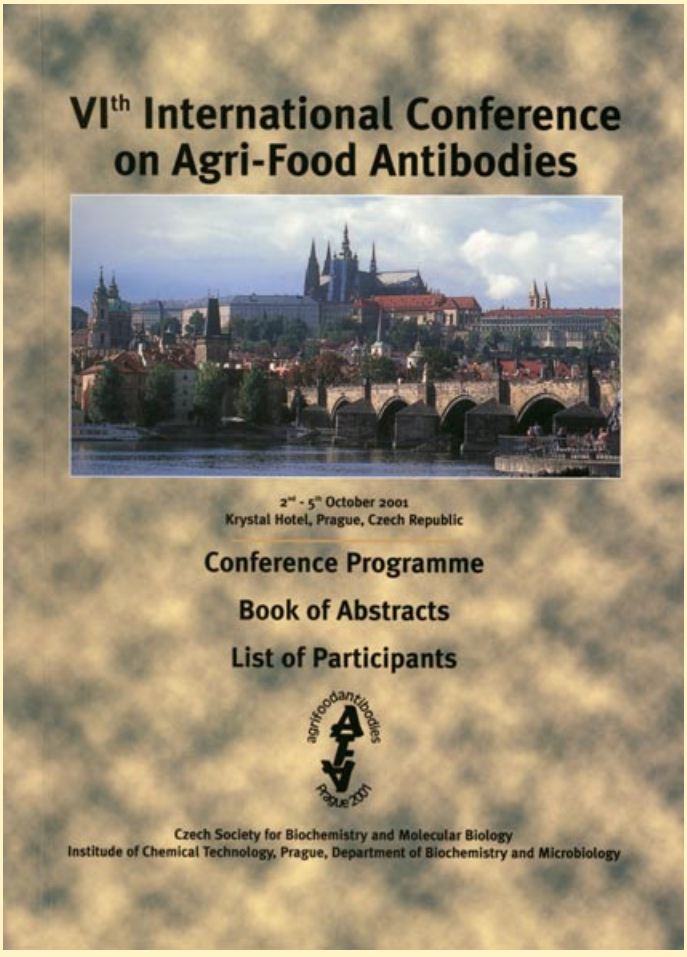
A quality concern that has had a major impact in the food industry in recent years is the, sometimes severe, allergic reaction to common food proteins seen in some people, especially children; peanut (groundnut) allergy is probably the best known example. **Sue Hefle** from Nebraska, USA, gave a very comprehensive plenary lecture on the reaction of the food industry and regulatory bodies to this problem, and the development of *in vitro* immunochemical methods; she was careful to emphasise the fact that detection limits for these assays can now approach analyte levels that have no clinical relevance, and discussed how this gives concern to the food industry.

A major area of public health concern relevant to food, especially in the UK, is the group of disease known as Transmissible Spongiform Encephalopathies (TSEs), caused by an "infectious" agent that is thought to be entirely protein, containing no nucleic acid. Excellent talks on this subject were given by **Sally Everest** and **Roy Jackman** from the UK Central Veterinary Laboratory. The protein agents, known as prions, appear to exist in two conformations with the abnormal form, responsible for the disease state, able to induce change from normal to abnormal confirmation in the brains of "infected" animals. Roy described antibody-based histological techniques for detection of prions in *post mortem* brain tissues, and progress that is being made towards a diagnostic test that can detect pre-clinical disease in the live animal.

Of course, I must mention my colleague in Norwich, **Clare Aldus** who, together with **Aart van Amerongen** (Wageningen, The Netherlands) gave talks describing our work on development of antibody-based dipsticks for detection of verotoxigenic *E. coli*, a rapidly increasing cause of bacterial food poisoning.

My final highlight was, in fact, the last talk at the conference; this was given by **Petr Skládal** (Brno, Czech Republic) with whom I have collaborated in a previous EU-funded project. Petr is developing electrochemical and piezoelectric immunosensors that are much smaller and much cheaper than the kind of surface-plasmon resonance biosensor instrumentation under development by the large manufacturers. If immunosensors are to become much more widely used in the food industry, then this is the kind of device that will be necessary; it is, of course, also one of the aims of our *Listeria* project to produce such simple sensors.

On a personal note, I was pleased to meet again with many old friends and former colleagues, and have the opportunity to discuss further collaborations. In my view, the conference was a great success and was superbly organised by **Pavel Rauch** and **Pavel Jenč** and their team of very willing and hard-working helpers from ICT, Prague.



Summary of results and achievements of project PL 979012

1st January 1999—31st December 2001

The aim of the project was to develop rapid, specific immunoassays for the detection of *Listeria monocytogenes* (*Lm)* in foods, with the aim of introducing appropriate quality control into food production processes of the CCE partners. The methods developed would be used both to assess the quality of raw materials and the final food product, including on-line sensor technology, and be incorporated into advanced quality control procedures, such as the HACCP system.

Antisera have been raised against: synthetic multiple antigenic particles (MAPs) which contain peptides from listeria proteins, virulence *Lm* protein Internalin B, *Lm* protein extract, *Lm* dead cells. From the totally 38 antisera, which have been raised during the duration of the project, the antibody against Internalin B was found specific to *Lm.* By using this antibody direct sandwich format of ELISA was developed.

Antiserum against Internalin B was used as capture antibody. The same antibody was used for preparation of conjugate with horseradish peroxidase and served as a detector molecule. The specific dipstick assay of *Lm,* by using the same antibody and conjugate, was developed. The detection limit for dipstick is slightly higher (4.5×10^7 CFU/ml) than for microplate ELISA (1.7×10^7 CFU/ml). Developed ELISA and dipstick was used for confirmation of listeria collection. All *Lm* strains interact with anti-Internalin B antibody, while other listeria species not bind at all. **The developed ELISA and dipstick technology were found specific for *Lm.***

These polyclonal antibodies were used in the development of an assay on the BIAcore 1000™ instrument (a surface plasmon resonance-based optical biosensor capable monitoring biomolecular interactions in "real-time"). A commercial goat anti-rabbit Fab antibody was immobilised on the BIAcore and an assay for the detection of *Lm* created using anti-*Lm* cell IgG. In this case, the BIAcore assay developed compares favourably with many other methods of detection for *Lm*. Two miniature SPR based sensors were developed too. To obtain a specific assay of *Lm* was necessary to determine the growth parameters (specific growth rate, lag time, asymptotic amount of growth, generation time, time for max growth rate) of *Lm* in different broths by standard cultivation method. It was found that the best condition for the production of surface proteins (internalin B) can be reached in Fraser broth. Under these conditions there is possibility **to detect *Lm* in the concentration from 10^0 to 10^4 cells per mL of milk sample after 42 hours cultivation by ELISA and dipsticks.** It was confirmed that ELISA and dipsticks techniques developed are applicable for *Lm* determination in 25 mL of milk test samples, contaminated with few cells (approx.. 5) of still not tested *Lm* strain. In all 5 capsules were detected positive reactions on the presence of *Lm* by ELISA method and dipsticks confirmed by plate count method on Oxford agar.

Using developed test in the identified control system in dairies should be oriented on service control laboratories providing special tests, in Slovak Republic and the Czech Republic. As in the new procedure is desirable to involve pre-enrichment step, it is not possible to do it in normal production factories that are usually not furnished with special room for handling with pathogens. From practical point of view it is not a problem because all producers are depended on such laboratories, as a part of their quality management systems, mainly for purpose of verifications their HACCP plans. On the other hand in modern well-equipped food factories, the tests can be used "in-house way".

Training of the industrial workers in ELISA and dipstick technology in assays of model, artificially contaminated and real samples was realised. Finally, the proposal of the application for funding from Accompanying Measures to hold workshop for reporting/discussion of the complete project with other potential industrial users, which is planned to Žilina (Slovak Republic) in May 2002, was applied.

Pavel Rauch

Project posters:

Wyatt G.M., Karasová L., Brett G., Rauch P.: Specific detection of *Listeria monocytogenes* in foods: a novel approach using antibodies to peptides representing virulence proteins, Society for Industrial Microbiology meeting, Washington, April 16—19, 2000.

Karasová L., Fukal L., Rauch P., Wyatt G., Brett G., Morgan M.R.A.: Perspectives of *Listeria monocytogenes* immunoassay detection, Chemical reactions in foods IV, September 20—22, Prague 2000

Wyatt G., Aldus C., Peck M., Brett G., Karasová L., Rauch P.: Antibody-based detection of food-borne pathogens and toxins, Federal Food Safety and Nutrition Research Meeting, Otawa, Canada, 24—26 September 2000.

Wyatt G., Aldus C., Peck M., Brett G., Karasová L., Rauch P.: Antibody-based detection of food-borne pathogens and toxins, Agriculture and Agri-Food Canada Food Network 2000, Saint Hyacinthe, Canada, 28—29 September 2000.

Karasová L., Doležal J., Fukal L., Brett G., Rauch P., Wyatt G.M.: Characteristaion of antibodies to peptides representing virulence proteins of *Listeria monocytogenes*, XVII. Czech and Slovak Biochemical Congress, Praha, 7—10th September 2000.

Karamonová L., Rauch P., Wyatt G. M., Greifová M., Horáková K.: Production of antibodies to *Listeria* virulence proteins and development of an ELISA for *L. monocytogenes*, Book of Abstracts, VIth International Conference on Agri-Food Antibodies,115, October 2—5, 2001, Prague, CZ

Urbančíková M., Greifová M., Seemannová Z., Rauch P., Karasová L., Wyatt G.M., Horáková K.: Fluorescence detection *of Listeria monocytogenes* using new antibodies as well as FITC-phalloidin, Book of Abstracts, VIth International Conference on Agri-Food Antibodies, 104, October 2—5, 2001, Prague, CZ

Karasová L, Greifová M., Novák P., Horáková K., Rauch P., Wyatt G.: Vliv kultivačních medií na imunochemickou detekci buněk *Listeria monocytogenes*, Sborník souhrnů sdělení, XXXII.Symposium o nových směrech výroby a hodnocení potravin, 32, květen 2001, Skalský dvůr, ČR, ISBN 80–902671–3–0

Horáková K., Greifová M., Seemannová Z., Gondová B., Wyatt G.M.: A microplate method for monitoring of *Listeria monocytogenes* growth kinetics and the infuence on the expression of p60 in selected enrichment media. In: VIth International Conference on Agri-Food Antibodies, Prague 2nd—5th October 2001. Czech Republic. p. 75. ISBN 80–7080–441–6 (En)

Horáková K., Greifová M., Seemannová Z., Gondová B., Wyatt G.M.: Development of a microplate method for characterization of *Listeria* species growth kinetics and the influence of selected media on p60 expression. In: 22nd Congress of the Czechoslovak Society for Microbiology — Healt and Microorganisms, Košice 5th—9th September 2001. Slovakia. p. 174. (En).

Urbančíková M., Greifová M., Seemannová Z., Karasová L., Wyatt G.M., Rauch P., Horáková K.: New polyclonal antibodies to *Listeria monocytogenes* give strong reaction by fluorescence microscopy. In: 22nd Congress of the Czechoslovak Society for Microbiology — Healt and Microorganisms, Košice 5th—9th September 2001. Slovakia. p. 324. (En).

Workshops of Partners P1, P3, P5, P6

Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology Bratislava, Slovak Republics

In September and November 2001 there were two workshops held in laboratory of Partner P3 focussed on demonstration of the procedure, the training of detection of *Lm* by ELISA and dipstick techniques and to optimise the sample precultivation before use of ELISA or dipstick. These workshops meant the "cream of the project" since after the final optimisation they confirmed the practical applicability and selectivity of the method demonstrated on real samples.

Ludmila Karamonová

"I would like to thank to Professor Horáková and to Mária Greifová for perfect organisation of these workshops. This is not the first time I visited the laboratory in STU and I always appreciate to find there a creative and cooperative atmosphere so important for successful work. And I had an opportunity to have my favourite Slovak dish — bryndzové halušky!"

Anna Slottová

"It Is always a great motivation to work in such constructive and collaborative collective. This is my imagine of teamwork and it shows the sense of international projects like that."

Martin Tomáška

"Results of these two hectic weeks had confirmed the success of all the project partners involved in development and optimisation of the ELISA and the dipstick methods. It was possible thanks to really precise planning and preparation of both stays."

September 2001 — welcoming the first participant (Z. Seemannová, L. Karamonová, K. Horáková, A. Šovčíková, M. Greifová)

Working hard…

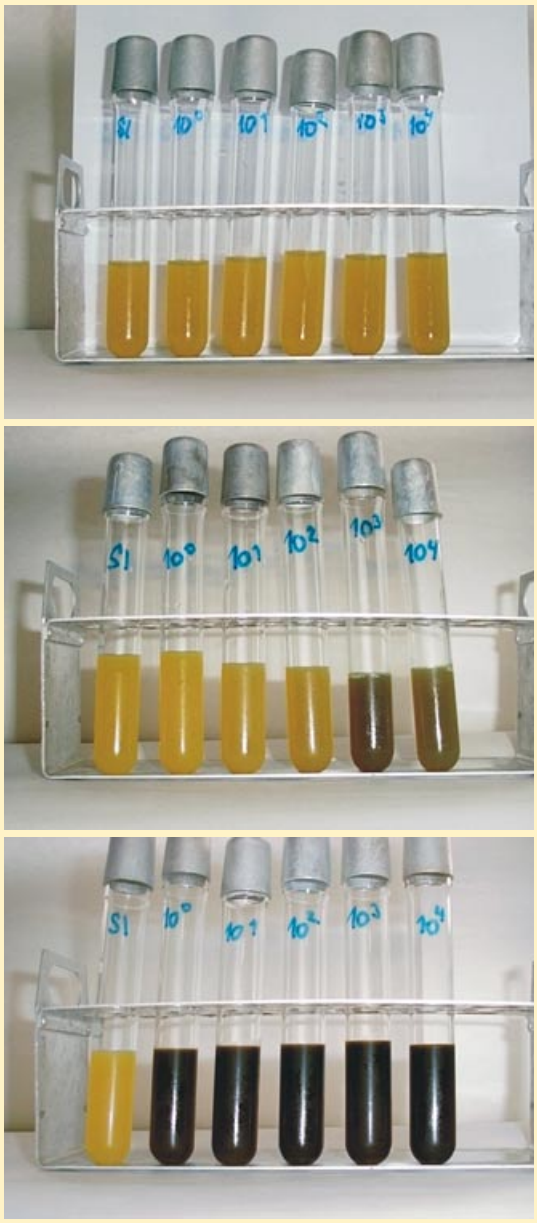
…and hard…

…and the first results are here!

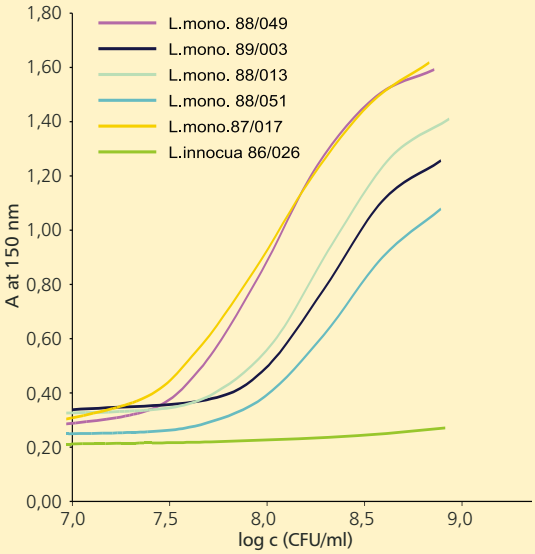
November 2001 — P. Roubal, A. Slottová, L. Karamonová, M. Greifová

Discussion during break

Petr and Lída



Incubation of *L. monocytogenes* as observed in 18 hours (upper line of tubes) 24 hours (middle line) and 42 hours (bottom line). Starting concentration of *Lm* from left 0, 10^0, 10^1, 10^2, 10^3, 10^4 (coloration of media is dependent on time and concentration).



Immunoreactivity of *Listeria monocytogenes* and *Listeria innocua* strains cultivated in Fraser medium

Dipstick method. Calibration curve for *Lm*. From left: 0, 0.23, 0.45, 0.9, 1.8, 3.6, 7.2 c (CFU/ml) × 10^8 (coloration is dependent on concentration).

Dipstick method. Specifity for *Listeria*. From left: *L. grayi* 88/044, *L. seeligeri* 89/017, *L. welshimeri* 88/043, *L. ivanovii* 88/039, *L. innocua* 88/025, *L.monocytogenes* 88/049 (the only positive result for this strain)

Petr Roubal

"It was two weeks of hard working day and night finished by a happyend. We fulfilled all planed aims and results were more than satisfactory"

Mária Greifová

"We had to do many experiments in relatively short time and such situation always needs careful timing and high working discipline. Guys were really nice, and especially Martin and Peter were still keeping us in good temper. Although this time there was only short time for socialising outside the laboratory, we had many opportunities for fruitful discussions and I hope all of us were enjoing the work."

Photos by
K. Horáková, P. Chovanec

Summarisation of workshops results

- ✔ There is possibility to detect *Lm* (pure strain) in the concentration 10^0 per mL of milk sample after 42 hours cultivation by ELISA and dipsticks as confirmed also by plate count of *Lm*.
- ✔ 24 hours cultivation of milk sample does not provide sufficient response of ELISA up to initial concentration 10^2 cells/ml of milk sample. After this cultivation period are ELISA responses in correlation with initial concentration.
- ✔ Dipsticks method allows to detect the presence of *Lm* after 24 hours cultivation only upon initial concentrations 10^3 and 10^4 cells/mL sample.
- ✔ After 42 hours of cultivation are responses of ELISA and dipsticks sufficient and comparable for all used initial concentrations of cells in milk — from 10^0 to 10^4 cells per mL of sample. This is confirmed also by results of other parameters — the same dark colour and plate count comparable in all samples
- ✔ In the same way were treated control samples of sterile UHT milk without inoculation with *Lm.* The value A_{450} of this sample was as low as 0.23 after 42 hour incubation, dipstick method also showed no colour change
- ✔ Results from presented experiment indicate that cultivation time 42 hours is sufficient for detection *Lm* in concentration 10^0/mL in artificially contaminated milk.
- ✔ It was possible to detect *Lm* by developed ELISA and dipstick method in acceptable time interval, less than 48 hours, making ELISA more advantageous for detection *Lm* than standard methods.
- ✔ ELISA and dipsticks techniques developed are applicable for *Lm* determination in 25 mL of milk test samples, contaminated with low counts of *Lm* (approx. 5).
- ✔ Adopted method with inoculation of 1 mL sample to 9 mL of Full Fraser is applicable for detection of *Lm* in concentrations 10^0 per tested sample.

Project papers

Karasová L., Fukal L., Rauch P., Wyatt G., Brett G., Morgan M.R.A.: Perspectives of *Listeria monocytogenes* immunoassay detection, Czech J. Food Sci. 18 (2000) 268—269. ISSN 1212–1800.

Greifová M., Melišová D., Horáková K.: Occurrence and surviving of *Listeria monocytogenes* in milk and milk products. Dairiing, 31(2), 2000, p. 40—43 (in Slovak language).

Tomáška M., Slottová A., Kontová M., Gondová B.: *Listeria* in cheese — occurrence and monitoring, in: *Proceedings of lectures and posters from Syrotech 2000, 3rd—5th May 2000 Žilina* (ed.: K. Herian), 170—177, Dairy Research Institute, Žilina, 2000

Horáková K.: Report from INCOCOPERNICUS meeting in Žilina. Dairiing, 31(2), 2000, p. 36 (in Slovak language);

Horáková K, Greifová M, Seemannová Z, Gondová B and Wyatt G M: A comparison of the traditional method of counting viable cells and a quick microplate method for monitoring the growth characteristics of *Listeria* species. *Journal of Applied Microbiology* (in preparation)

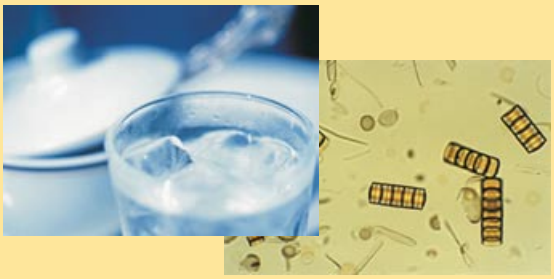
Karamonová L, Rauch P and Wyatt G M: Development of an ELISA specific for *Listeria monocytogenes*, using a polyclonal antibody raised against Internalin B protein. *Applied and Environmental Microbiology* (in preparation)

Plenary lecture

Wyatt G.M., Karasová L., Rauch P.: Towards a Listeria monocytogenes-specific ELISA: some experiences with production of antibodies to *Listeria* virulence proteins, Book of Abstracts, VIth International Conference on Agri-Food Antibodies, 28, October 2—5, 2001, Prague, CZ

Project publications

All partners cooperate and prepared issue of COPERNICUS NEWS No. 1, 2 and 3 (editor Partner 3), see Annexes. The new and last issue of COPERNICUS News No. 4 is in press.



TL Examinala, Dairy Research Institute, Žilina, the Slovak Republic
with kind support of the European Union
are pleased to invite you to

a training workshop for the food industry focused on

New methods for detection of *Listeria monocytogenes*

13th—14th May 2002
Dairy Research Institute
Žilina, Slovak Republic

The workshop will be for representatives from:

- ✓ Food industry
- ✓ Control authorities
- ✓ QA supporting organizations
- ✓ Universities and R&D organizations

Presented topics:

- ✓ Session on the importance of *L. monocytogenes* in the food chain
- ✓ Session on the current methodology for detection of *L. monocytogenes*
- ✓ Session on the purpose and results from the EU-supported project
- ✓ Demonstration of the new techniques
- ✓ Round-table discussion on possible applications of the new tests, with special emphasis on HACCP

The speakers will include:

- ✓ **Pavel Rauch**
  Professor on the Institute of Chemical Technology, Prague, CZ
- ✓ **Gary Wyatt**
  Senior research worker on the Food Research Institute, Norwich, UK
- ✓ **Katarína Horáková**
  Professor on the Slovak University of Technology, Bratislava, SK
- ✓ **Richard O'Kennedy**
  Professor on the Dublin City University, Dublin, RI
- ✓ **Petr Roubal**
  Senior research worker on the Dairy Research Institute, Prague, CZ
- ✓ **Karol Herian**
  Director on the Dairy Research Institute, Žilina, SK

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Lectures from 4th, 5th and 6th internal meetings of INCO Copernicus project PL 979012 and summarisation of project results for project participants and research community on the field of *L. monocytogenes* detection. • Published by Slovak University of Technology, Bratislava, February 2002. **ISBN 80–227–1674–X**