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Labinfo

Newsletter for the approved food safety laboratories

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NRL NATIONAL REFERENCE LABORATORIES

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Labinfo
Informatieblad voor de erkende laboratoria voedselveiligheid

Editorial

Dear Reader,

This is the third electronic newsletter on the activities and events in the world of Federal Food Safety Agency (FASFC) laboratories.
The economic crisis also had hard repercussions on laboratories: smaller margins, takeovers of small laboratories and harsher competition among labs. In our neighbouring countries the number of analyses to be performed also dropped. As a consequence, more and more foreign laboratories have their eye on the Belgian market.
Competition is the keystone of innovation and is necessary to develop the "entrepreneurship" that is needed so badly. Public authorities must bring about a legal framework within which the different players may operate. The Royal decree of 2003 on self-checking will be reconsidered and the amended version will include the requirement for laboratories performing analyses related to self-checking to take part in interlaboratory tests. The Belgian FASFC will make available a positive list of approved suppliers/organisers of interlaboratory tests. That will be the first step to improve the accuracy of test results used by operators of the agricultural and food business in relation with their HACCP system.
Moreover, in the first half-year of 2010 an internet based Dashboard will be made available to all approved laboratories and allow them to consult their own KPIs (including run times). This initiative is part of a larger concept known as the CRM (Customer Relation Management) which aims at further optimising the interaction between the Laboratories Administration of the Agency and the external laboratories by using state-of-the-art communication technology.
The procedure for approving external laboratories is still successful: the number of external laboratories that want to perform tests listed in the official control programme of the Agency is still increasing. Recently, applications for approval were submitted by laboratories in the Netherlands and in France. In all, their number now amounts to 59. The complete list is available on http://www.favv-afsca.fgov.be/laboratories/approved laboratories/generalities. However, an approval also includes certain requirements that must be met. I always bear in mind the principle that trusting is good but checking is even better. It is therefore logical that laboratories that break the rules of the game are penalized. Such an attitude can only be profitable to laboratories that do stick to the rules.
I hope you will appreciate this issue of Labinfo. I also send you my season's greetings, early as it may seem.

Geert De Poorter
Directeur-generaal Laboratoria

Proficiency tests

Interlaboratory tests on the physical-chemical properties of a pesticide formulation

In 2007, DG Laboratories decided to set up a Business Unit (BU) PT Schemes the task of which was to make a list of all needs of interlaboratory tests for analyses performed by both in-house and external laboratories. The aim was to conduct internally tests that were not proposed by the traditional suppliers. As it is, participation in interlaboratory tests is an absolute requirement with a view to accreditation.

The BU decided, therefore, to set up an interlaboratory test on the physical-chemical properties of a pesticide formulation. Currently, none of the usual suppliers offers this type of proficiency testing. The federal food safety laboratory in Liège was the first actor in this test. That laboratory is the only federal laboratory that has been accredited for controlling the quality of plant protection products.

For this first testing the Business Unit PT Schemes chose to keep the number of participants low and to confine the exercise strictly to Europe. The formulation that was chosen for the test (an aqueous solution) had the advantage of being perfectly homogenous and stable and contained a well-known active substance that could be analysed by means of an official method.

Several typical parameters of the formulation had to be analysed (active substance content, density, pH and foaming).

13 laboratories showed interest in taking part in the first test. The samples were sent in October 2008. Results had to be made known no later than 1 February 2009.

The samples were prepared in such a way as to keep matrix related variability at minimum level. Thus, this variability would have only an insignificant effect of the performance of laboratories.

Homogeneity tests (based upon the pH of the formulation) were done twice, before and after re-packing, on subsamples picked at random.

The statistical processing was done in accordance with the requirements laid down in the ISO 13528 : 2005 standard. The aim of this action was to help detect possible trends and to make it possible to compare results to one another. The Z-score as identified below, was used as a performance indicator :

Z-score : $(x-X)/s$

with : x = result communicated by the participating laboratory;

X = the robust mean calculated according to the A algorithm of ISO 13528 standard;

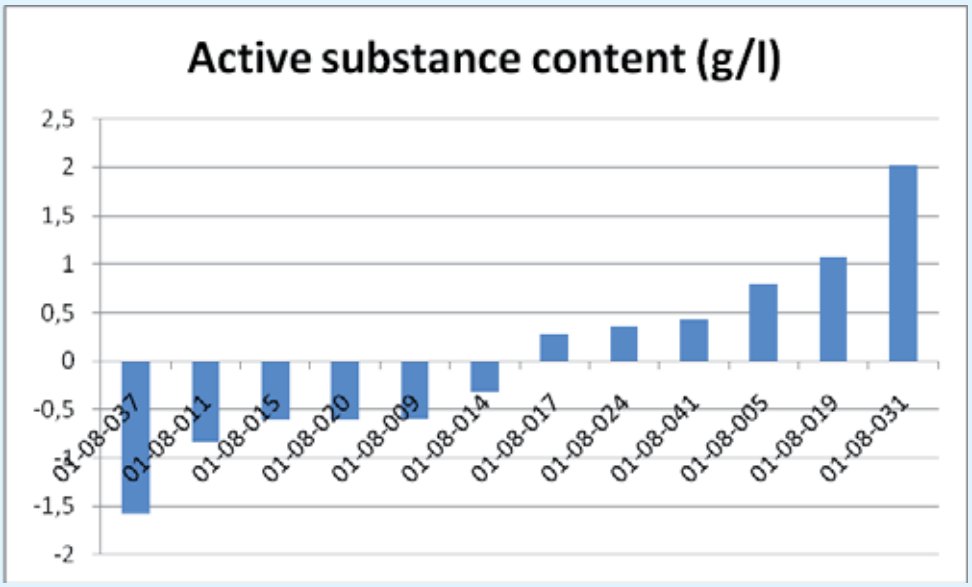
s = the standard deviation calculated from the results communicated by the participants.

Table: Results received and statistics

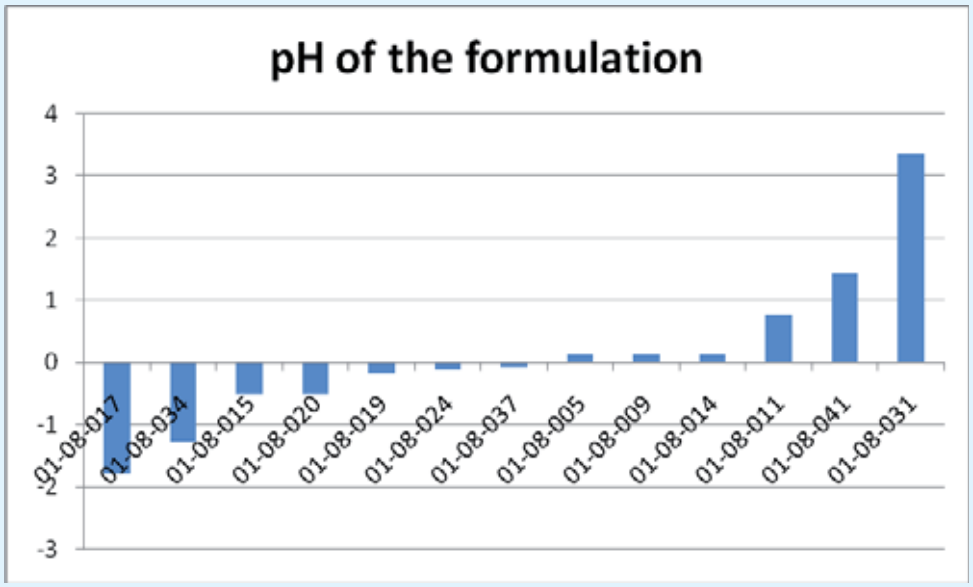
Parameter	Number of results communicated	Minimum	Median	Mean	Robust mean (according to A algorithm of ISO 13528)	Maximum	Standard deviation	Robust standard deviation (according to A algorithm of ISO13528)
a.s.* (g/kg)	12 (0)**	335,2	342,5	342,1	341,9	353,4	5,7	6
a.s. (g/l)	12 (0)	397,4	409,6	410	409,7	425,5	7,7	7,8
Density	13 (1)	1,1413	1,2044	1,1993	1,204	1,2061	0,017	0,0015
pH formulation	13 (1)	8,1	8,37	8,4	8,378	8,9	0,2	0,156
pH dil.2	13 (2)	6,6	7,8	7,8	7,8	9,1	0,55	0,26
Foam	13 (0)	0	-	-	-	-	-	-

* = active substance content
( )** = number of results with a Z-score > 3 for the test

Graph 1: Distribution of the Z-scores for determining the active substance content (g/l)



Graph 2: Distribution of the Z-scores for determining the pH of the undiluted formulation



This first interlaboratory test on the physical-chemical properties of a pesticide formulation appeared to be interesting in more than one respect. First, the reactions of the participating laboratories to the test were very positive. One of the participants could use this test in its audit for the ISO 17025 standard. Moreover, this test showed that some tests that are believed to be simple, such as measuring the pH, may, however, involve some problems. That finding proves that such tests are necessary.
In the future, the BU wants to extend the test by increasing the number of participants as well as the number of samples to be submitted. The second target is to obtain the ISO 17043 certification for setting up these tests.

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Microbiology

Microbiological Criteria for Food and Legislation

Microbiological hazards in food are an important cause of human diseases transmitted through food and may be divided into two large classes : food infections and food poisonings. A food infection is caused by the ingestion of pathogenic micro-organisms such as Salmonella spp., Campylobacter spp., Listeria monocytogenes, E. coli O157 whereas a food poisoning occurs following the ingestion of toxins that may be found in the product and were produced by bacteria (Staphylococcus, Bacillus cereus, Clostridium botulinum) or fungi (Aspergillus flavus, Fusarium spp.) that are present in the food.

In order to protect human health, microbiological criteria have been established at world, European and national level. In the 1960s the FAO and the WHO created the Codex Alimentarius, a body that develops both general and product specific standards for food safety, labelling and product composition on a worldwide scale. Although the standards of the Codex are not binding, they are at the basis of the European, national or regional regulations related to food safety. In 2000, the European authorities laid down their political priorities for food safety in the White paper on Food Safety intended to protect consumers' health. The main priorities were the creation of a European Food Authority, the improvement of regulations on several aspects of foods and official controls. For the first time, all aspects of food safety were taken into account, " from farm to fork". Within that context the Union European adopted the "General Food Law" laid down in EC Regulation 178/2002. This Regulation contains the basic principles of the European food law and was adopted in order to protect the health of humans and animals, to achieve free movement of food and feed in the European Community. Several other European regulations on hygiene, contamination and controls are based upon that regulation.

In 2006 came into force the following regulations related to food hygiene :

- ·Regulation (EC) No 852/2004 on the hygiene of foodstuffs and, consistently, Regulation(EC) No 2073/2005 on microbiological criteria;
- ·Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin ;
- ·Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption ;
- ·Regulation (EC) No 882/2004 on official controls.

As mentioned above, microbiological criteria for foodstuffs have been laid down in Regulation (EC) No 2073/2005. In 2007, this regulation was reviewed : new criteria were added and these amendments were laid down in Regulation (EC) No 1441/2007.
A microbiological criterion is a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch. All criteria consist of the following information : description of the food, the micro-organism or toxin, the stage where the criterion applies (processing, distribution, shelf-life, products placed on the market), the analytical reference method and the values n, m, M and c, with

- n: number of units comprising the samples
- m: accepted upper limit of the samples
- M: maximum upper limit for n samples
- c: the maximum number of the n samples giving parameter values between m and M.

It also describes the analytical method to be used for detecting the micro-organism and the measures needed to be taken when the criterion is exceeded.

E.g. for Listeria monocytogenes in ready-to-eat food placed on the market the criterion is : n=5, c=0, m=M=100 cfu/g, analytical method ISO 11290-2

When the criteria were laid down, this was done on the basis of a distinct strategy : all criteria must be relevant for the protection of public health, they must have practical interest and be based upon risk assessment or internationally adopted principles.
For each criterion is mentioned an analytical method that should be used for the quantitative and the qualitative determination of the micro-organism. The Laboratories Administration of the Belgian Federal Agency for the Safety of the Food Chain published a list of approved microbiological methods. The list mentions the methods that may be used in accordance with EC regulations for performing tests within the context of the control programme and self control.

There are 2 types of microbiological criteria. These are:

- food safety criteria : they define the acceptability of a product or a batch of foodstuff applicable to products placed on the market : e.g. for Listeria monocytogenes in ready-to-eat foods, Salmonella spp. in some ready-to-eat foods and in meat products intended to be eaten raw, Staphylococcal enterotoxins in some dairy products like cheese or milk powder, Cronobacter (Enterobacter) sakazakii in infant formulae;
- process hygiene criteria : they indicate the acceptable functioning of the production process, e.g. for Salmonella spp. on carcases, Enterobacteriaceae on carcases and in dried infant formulae: rather used as indicator organism, coagulase-positive Staphylococci in some dairy products.

These criteria are mandatory for each Member State of the EC. When there are no criteria, the Member State itself must take action in order to guarantee the safety of the product. It is within that context that the Federal Agency established action limits, following an opinion of the Scientific Committee.
It belongs to the operator to prove, by means of these criteria, that he puts on the market a product that is safe and that the criteria are met during the shelf-life of the product. Operators must also prove that their production process is fully under control by using an HACCP system that has been validated and is verified on the basis of these criteria. On the other hand, the authorities must carry out official controls to verify if the operators comply with the criteria.
These criteria are not absolute. They may be changed at any time in agreement with the progress made in the field of science, technology and methodology, following the emergence of new pathogens as well as new findings made in risk assessments. The criteria are permanently re-assessed and reviewed to make sure that the safety of our foodstuffs can be guaranteed.

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The various steps required to isolate and identify germs in food are based on biochemical characteristics. In general, the following steps are involved when isolating pathogenic bacteria : homogenization of the food, pre-enrichment, selective enrichment, inoculating on a selective medium and, finally, purification and confirmation of the germ. These classic methods are often labour intensive, time consuming (5 to 14 days) and sometimes, atypical germs are overlooked. Although these techniques are indispensable in microbiological food laboratories, their shortcomings may be corrected by using some molecular techniques such as PCR (Polymerase Chain Reaction).

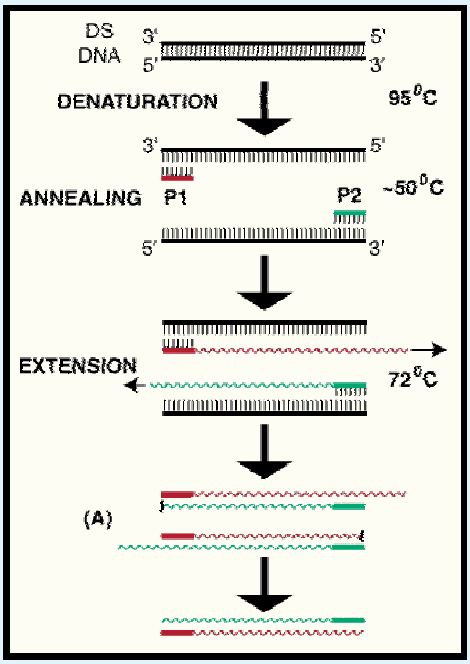
1) PCR

PCR for identifying bacteria is based upon the amplification of a specific region within the microorganisms genome. The components required to perform PCR are :

1. primers (one forward and reversed, 20bp oligonucleotides) that are complementary to a certain DNA fragment of the microorganism and result in the amplification of the entrapped DNA fragment;
2. target DNA (genomic DNA of the germ);
3. dNTPs (dATP, dTTP, dCTP and dGTP) DNA materials;
4. Taq DNA polymerase (enzyme) ;
5. MgCl2 for the working and the specificity of the enzyme, and
6. buffer for obtaining ideal salt concentration and pH during reaction.

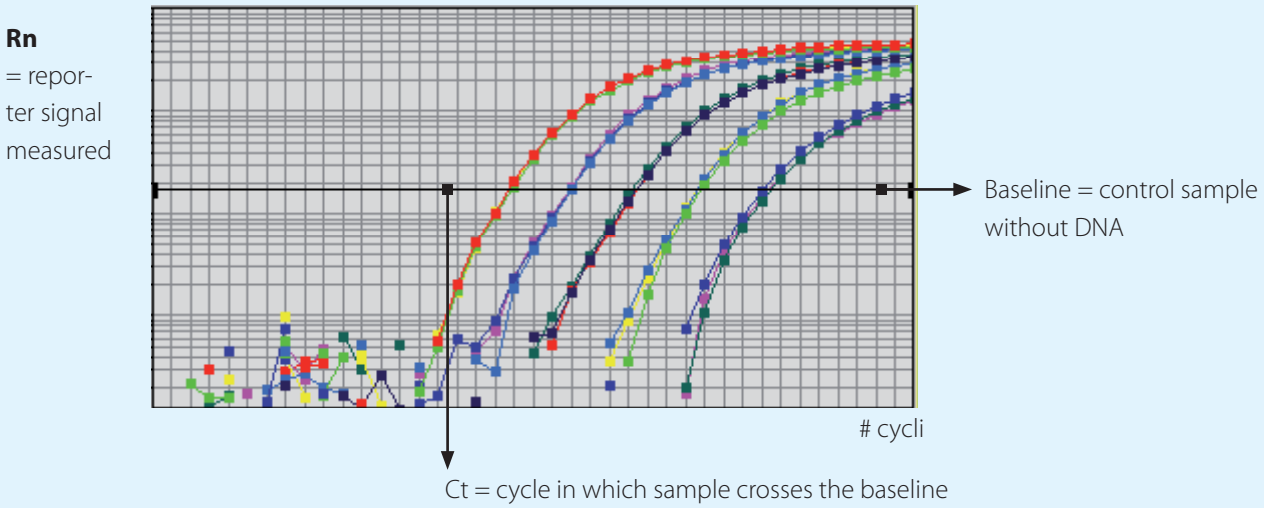
A PCR is a cyclical reaction in which after a first step consisting of the denaturation of the DNA at 95°C, the primers are able to bind (T ~ 50-60°C) during the step of annealing. Subsequently, the primers will extend during a elongation step at 72°C. Cyclically repeating (25-35 cycli) these 3 steps results in an exponential increase of the target DNA fragment.

Visualization of the PCR products obtained is done by separation of the fragments on agarosegel and coloration by ethidium bromide. In that case only the fragment length of a PCR product is a discriminating factor.



2) Real-time PCR (RT-PCR/qPCR)

A variation of this basic PCR method is the so-called 'Real-time PCR', in which the exponential increase of a fragment may be followed during every cycle of the PCR reaction .



This is possible because of the presence of a fluorescently labeled probe in the PCR mixture. These probes bind to a specific sequence of the target DNA, located between the forward and reverse primer. There are several kinds of probes but the TaqMan probe (Applied Biosystems) and the Molecular beacon (BioRad) are used most frequently. The principle of the TaqMan probe and the Molecular beacon is based upon the presence of a so-called 'reporter' and its 'quencher' at both ends of the probe. When both components are near to one another, i.e. when the probe is intact, the energy of the reporter will be transferred to the quencher when excited by light, so that no fluorescence may be measured. When the TaqMan probe is degraded by the 5' nuclease activity of the Taq polymerase, the distance between reporter and quencher increases. In that case, light excitement of the reporter will provoke fluorescence so that a signal may be detected. For Molecular Beacon the fluorescence is detected when the probe binds to the target DNA.

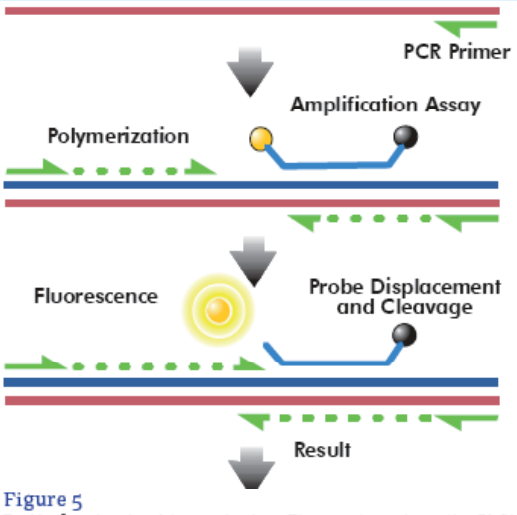
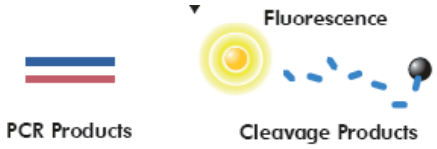


Figure 5
TaqMan® probe chemistry mechanism. These probes rely on the 5′–3′ nuclease activity of *Taq* DNA polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence.



TaqMan® probe chemistry mechanism. These probes rely on the 5′–3′ nuclease activity of *Taq* DNA polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence.

Molecular Beacon chemistry mechanism. The Molecular Beacon includes a hairpin loop structure, with the loop complementary to a target sequence and the stem formed by the addition of internal complementary sequences. When hybridized to the target, the fluorophore and quencher are far enough apart to allow fluorescence to be detected.

3) Applications

The PCR technique (PCR and RT-PCR) may be used at various levels for detecting pathogenic germs. The first level is directly in the food. It is important that the germ is present in sufficient numbers and that an effective extraction protocol is available. The advantage may be that it will quickly be known whether the pathogen is present and it is possible to obtain a semi-quantitative result. On the other hand, this technique has also some inconveniences at this level, e.g. the amplification of dead cells that are present in the food matrix. It should also be noted that the matrix may also contain components that inhibit the PCR reaction.

A second level at which the PCR technique may be used is after the first non selective enrichment. Important are the sensitivity of the PCR reaction to detect the pathogen as well as the specificity. Most of the commercial kits available on the market are used at this level. In addition to the detection of a specific pathogen, these kits use an internal control that follows the amplification process, showing whether inhibition due to the matrix or background flora is present. These kits may only be used as a routine in laboratories after exhaustive validation for various food matrices.

A third level at which the PCR technique may be used is that of the identification and confirmation of the isolated bacteria. The PCR will detect a specific gene that is characteristic for the bacteria. By using PCR in this context, a whole range of biochemical confirmations may be avoided and atypical strains may also be easily identified.

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Comparative study of the bacteriological quality and the shelf life of vacuum-wrapped Argentine and Belgian white-and-blue beef

Belgian farmers are concerned about the Argentine beef available on the Belgian market, as this meat is said to have a significantly extended shelf life compared to Belgian White-and-Blue beef. The NRL for Food Microbiology was asked to compare the bacteriological quality of both types of meat. In addition, possible specific treatments to attain the alleged superior shelf life of Argentine beef were investigated.

Argentine beef is imported vacuum-wrapped, cut into large pieces of 4 to 5 kg. The shelf life mentioned on the wrapping may amount to 6 months. For retail purposes in Belgium, the large pieces are cut in smaller portions and vacuum-wrapped once more with a new shelf life of 14 days. After slaughtering and immediate boning, Belgian White-and-Blue beef is also vacuum-wrapped for at least 2 weeks in order to let the meat ripen and to save cold store capacity.

Three different kinds of meat samples were tested : (i) vacuum-wrapped (not cut) Argentine back-piece with a shelf life of 6 months, (ii) Argentine beef cut and vacuum-rewrapped in Belgium with a shelf life of 2 weeks after rewrapping, and (iii) Belgian White-and-Blue vacuum-wrapped steak with a shelf life of 2 weeks. The general bacteriological quality was examined by quantitative determination of the total aerobic count at 30°C, E. coli, Enterobacteriaceae, lactic acid bacteria and Brochotrix thermosphacta. The samples were tested upon receipt and at the end of shelf life mentioned on the wrapping.
In the course of the period of shelf life of the vacuum-wrapped Belgian meat, an increase of the total microbial flora was observed, in particular a significant increase of the number of lactic acid bacteria (see Table 1). When first analysed (already two months after slaughtering), both types of Argentine beef showed bacteriological parameters of the same order of magnitude as those of Belgian beef at the end of shelf life (see Table 1). Therefore, upon arrival in Belgium Argentine beef had the same bacteriological quality as Belgian beef at the end of shelf life. At the end of shelf life, Argentine meat had, on average, a less satisfactory bacteriological quality than Belgian meat. Also, a clear difference in bacterial counts was observed between non-cut Argentine meat, and in Belgium cut and rewrapped Argentine meat with a new shelf life of 14 days. At the end of the shelf life, non-cut Argentine meat contained significantly larger numbers of Enterobacteriaceae and also showed definite organoleptic anomalies (unmistakable smell of rotting meat) as well as differences in texture.

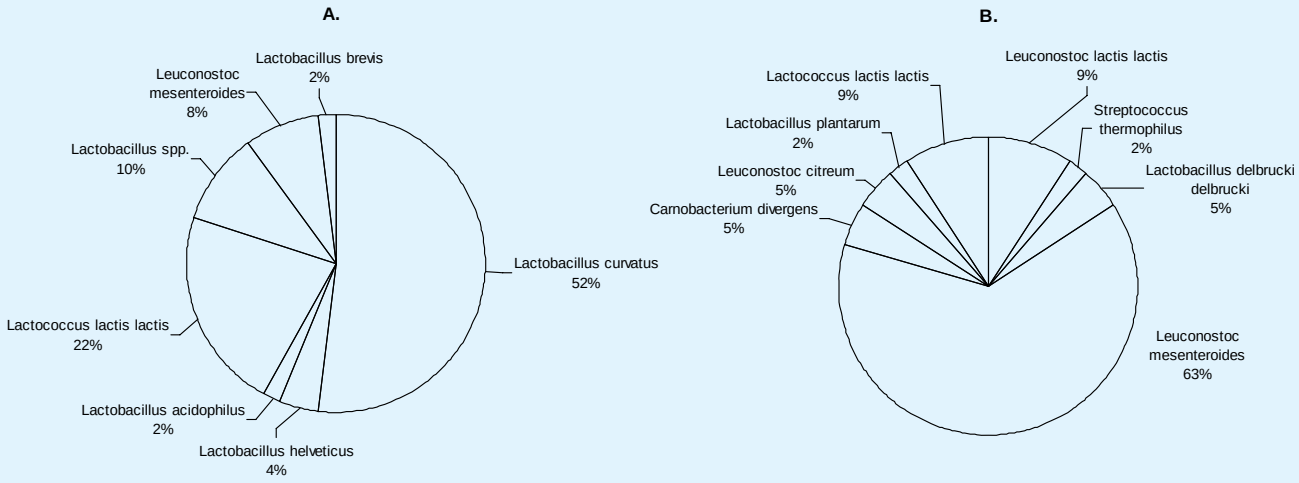
Table 1. Mean values of bacteriological parameters examined, in cfu/g. Results of quantitative determination of Brochotrix thermosphacta were all below the detection limit (< 100 cfu/g) and are not mentioned.

Sample	Analysis	Total aerobic count	E. coli	Enterobacteriaceae	Lactic acid bacteria
Belgian meat (cut)	First analysis	1,31E+04	<10	5,00E+01	2,38E+03
	End of shelf life	1,45E+07	<10	3,71E+03	1,10E+07
Argentine meat (cut)	First analysis	1,10E+07	<10	9,77E+02	1,06E+07
	End of shelf life	9,55E+07	<10	8,00E+03	7,80E+07
Argentine meat (non-cut)	First analysis	4,51E+07	6,50E+01	2,73E+03	5,27E+07
	End of shelf life	1,62E+08	<10	5,35E+05	1,24E+08

In addition to the bacteriological quality of both types of beef, irradiation was also examined as a possible reason for the supposed long shelf life of Argentine beef by lowering bacterial contamination. For this purpose, presence of alkylcyclobutanon was investigated by Eurofins (German company) in a sample of each analysed batch. Irradiation could be excluded as treatment for prolonging shelf life since no trace was found of the characteristic 2-dDCB marker (Milesi et al., 2008). This finding was also confirmed by the numbers and the variation of the microbial flora detected on the Argentine beef (see Table 1).

Another possibility for extending the shelf life of meat is to add bioprotective lactic acid bacteria, given their capacity to produce antimicrobial metabolites such as organic acids or bacteriocins (i.e. small antimicrobial peptides) (Castellano et al., 2008). Following the absence of irradiation markers, several indicative observations for addition of bioprotective cultures (Milesi et al., 2008) were examined.
(i) Presence of high numbers of lactic acid bacteria (>10^5 cfu/g) and Enterobacteriaceae (>10^5 cfu/g) compared to European meat. Argentine beef did indeed contain more lactic acid bacteria and Enterobacteriaceae than Belgian beef, both at the first analyses as at the end of shelf life (see Table 1). However, Argentine beef was already slaughtered for over two months.
(ii) Detection and identification of specific types of lactic acid bacteria known for their use as bioprotectants in foodstuffs, more specifically in meat. For that purpose, lactic acid bacteria from both Argentine and Belgian beef were isolated and identified ad random. Belgian meat was used as a reference. In total, 50 isolates of Argentine meat and 44 isolates of Belgian meat were confirmed as lactic acid bacteria through Gram staining and catalase test – lactic bacteria are Gram positive and catalase negative – and biochemically identified with API 50 CHL (incubation for 48 hours at 30°C). Both kinds of beef showed a clear predominance of one lactic acid species as well as an approximately equal distribution of isolates among the other prevailing species (see Figure 1). However, the predominating species are different, i.e. Lactobacillus curvatus in Argentine beef and Leuconostoc mesenteroides in Belgian beef; both are known as bacteriocin producers, as are many other lactic acid species.
The limited difference in lactic acid bacteria and Enterobacteriaceae, which was considered as normal given the experimental set-up (i.e. the difference in "age" of diiferent kinds of meat), as well as the occurrence and dominance of known bioprotective species in both types of meat make it impossible to establish whether a bioprotective lactic acid bacterium was intentionally added to Argentine beef.

Figure 1. Overview of the diversity of lactic acid bacteria isolated from A. Argentine beef and B. Belgian beef. Identification based upon biochemical API 50CH tests.



A comparison between the bacteriological quality of Argentine beef and Belgian beef reveals that the supposed differences in shelf life might be less important than was initially assumed. The potential maximum shelf life of Belgian meat is unknown because of the high turnover in shops but can be estimated at about two months. On the other hand, the results of this study justify questioning the supposed shelf life (of about six months) of Argentine beef, mainly given the large numbers of Enterobacteriaceae , the organoleptic anomalies at the end of the supposed shelf life and the absence of evidence for irradiation or addition of bioprotective cultures. Probably, the concern of Belgian farmers can be reduced to perception since Argentine beef has a market share of no more than 0.4 % (information supplied by Carrefour), is more expensive than Belgian beef (27 euro/kg against 20 euro/kg) and has a different taste and texture.

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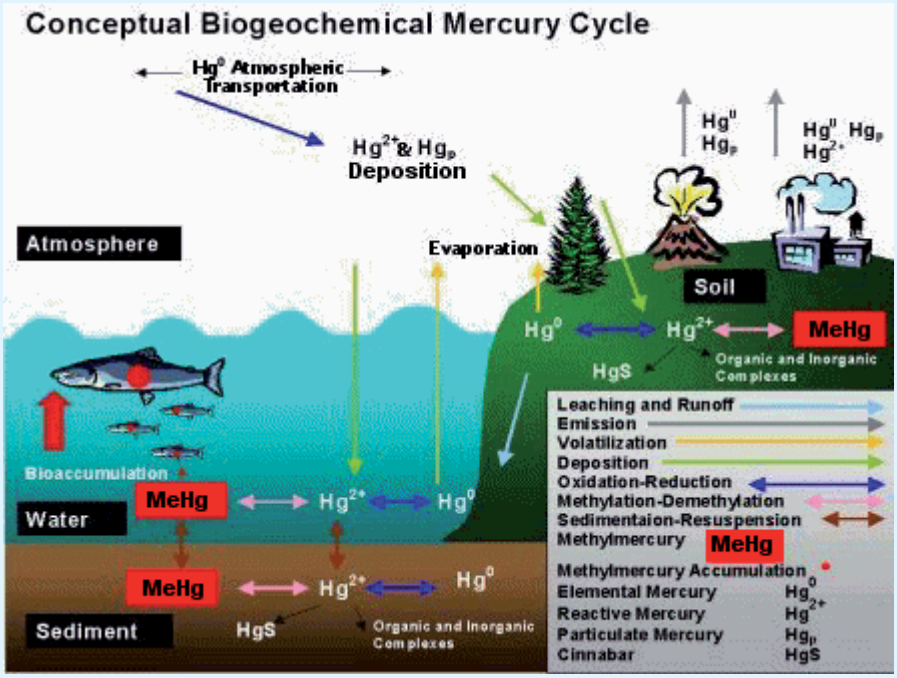
Heavy Metals

Methylmercury

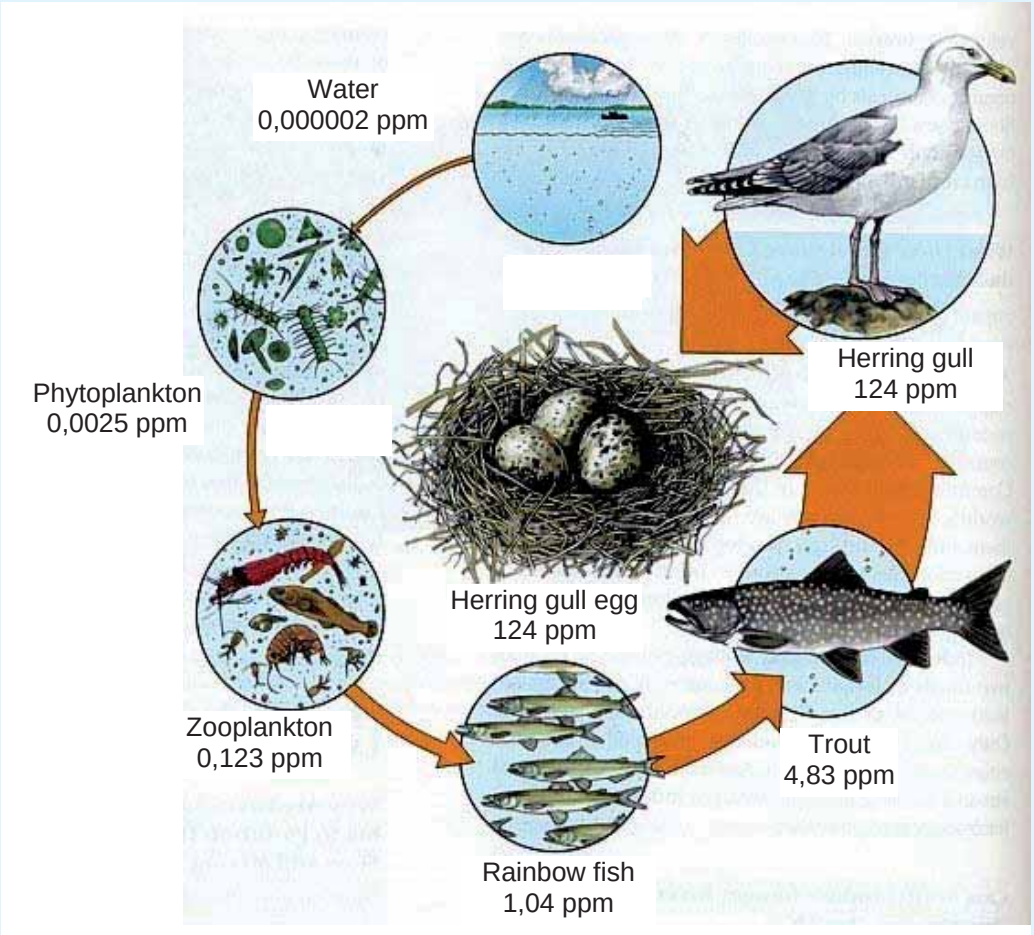
Mercury is a widespread and persistent element in the environment. It is naturally present in the form of mercury sulphide (HgS), also known as red cinnabar. Moreover, some human activities are responsible for the release of mercury into the atmosphere, the soil and the water. As soon as it is released into the environment, mercury undergoes a series of complex chemical and physical transformations (Figure 1).

Figure 1. The natural bio-geo-chemical cycle of mercury: degassing of the soil and the surface water, transport into the atmosphere; deposit of mercury on the land and on surface water; absorption of mercury by soil particles.



Mercury occurs as elementary mercury as well as in various organic and inorganic compounds and complexes. The inorganic forms of mercury are less toxic than the organic but in water they may be transformed into organic forms by bacteria. Of the inorganic forms, methylmercury is the most toxic. A wide range of toxic effects may be caused by exposure to methylmercury. Neurotoxicity is the most important effect of long-term exposure to high concentrations of methylmercury. Other effects are psychological problems, decrease in hearing, loss of acuity of vision, motor dysfunction.
For humans, the eating of marine animals seems to be the main mode of exposure to methylmercury. Mercury accumulates into the food chain. That is why the highest mercury concentrations are found in animals on top of the food chain and in older animals (Figure 2). The average mercury concentration in fish is estimated at 62 to 97 µg/kg in Europe. The share of methylmercury in the total mercury content may vary between 70 and 100 % according to the species.

Figure 2 : Bioaccumulation of mercury



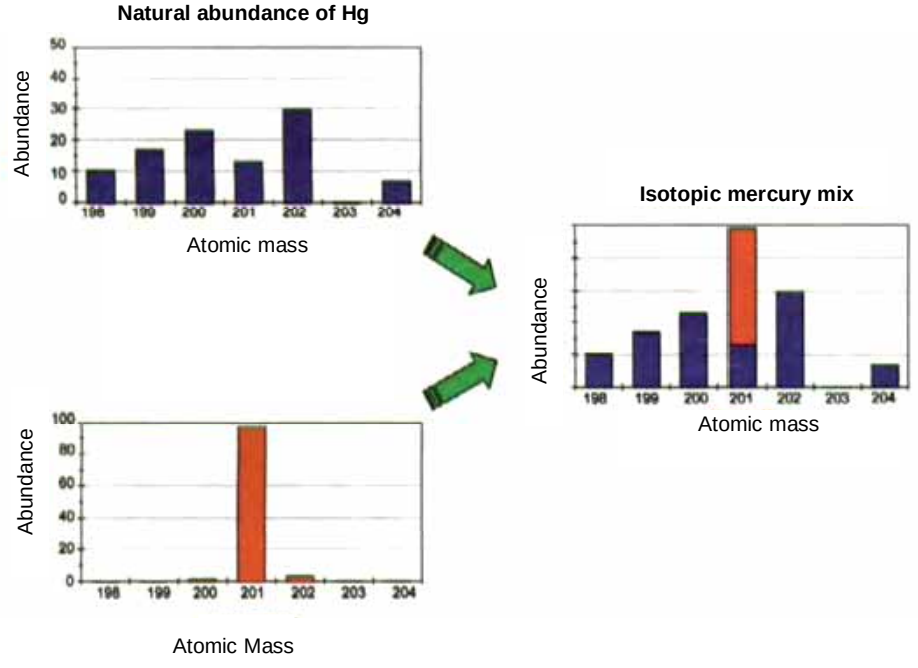
Given the toxicity of mercury, some international organisations decided to set standard values for mercury in aquaculture products. These standards apply to total mercury and were set at 0.5 and 1 mg/kg. No standards have as yet been set for methylmercury in food. The number of laboratories with a certain experience in determining the methylmercury content is limited. Yet, the determination of methylmercury has been the subject of research for many years. The oldest methods are based upon gas chromatography (GC) analysis of methylmercury chloride (CH_3HgCl). More recent methods use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) but also the more modern versions of GC substantially improved the analysis of methylmercury. In recent years, certified reference materials (CRM) are being manufactured which have guaranteed methylmercury contents and ring tests have been set up for analysing methylmercury in fish.

The specialist methods for determining methymercury include 5 characteristic steps :

1. Chemical digestion of the matrix and extraction of methylmercury in its original form.
2. Conversion of methylmercury by means of derivatisation.
3. Extraction of the analyte in a liquid phase, by cryogenic trapping or Solid Phase Micro Extraction (SPME).
4. Separation of methylmercury of the matrix and of other forms of mercury.
5. Detection and determination of the concentration of methylmercury.

For digesting mercury species the most frequent procedures are acid digestion, alkaline digestion, watery distillation, supercritical fluid extraction and extraction by microwave radiation.
Grignard reagents, sodium tetraethyl borate and sodium tetrapropyl borate are effective derivatisation reagents.
As for separation techniques, GC and LC are frequently used; on the other hand, capillary electrophoresis is also an appropriate technique. Faster methods use SPME before HPLC separation or combine digestion and derivatisation in a microwave method.
A whole range of detection techniques may be used for methylmercury detection after chromatographic separation, e.g. AFS, AAS, ICP-MS, ICP-OES, MIP-AES, FAPES.
The coupling of GC and ICP-MS is the most popular combination for mercury speciation because of the high resolution of GC and the high sensitivity, the wide dynamic range and the multi-element capacity of ICP-MS. Moreover, ICP-MS makes it possible to apply isotope dilution mass spectrometry which is the most accurate and precise calibration technique. This technique is more sensitive than external calibration, even than standard addition, because it is based on ratios and not on absolute intensities. Species-specific isotope dilution mass spectrometry (SS-IDMS or IDMS) is a recently developed technique that is based upon comparing isotope ratios. The principle of this technique consists of adding to the sample a standard material with known and stable isotope ratios of the element under detection (Figure 3).

Figure 3. Isotope dilution of mercury with a 201Hg enriched isotope



The transformation of species may be detected by enriching the measured isotope ratios by means of the natural isotope ratios. This technique may therefore be used only for determining elements with more than one isotope. Mercury has several isotopes, i.e. 196Hg, 198Hg, 199Hg, 200Hg, 201Hg, 202Hg en 204Hg.

SS-IDMS may also be used simultaneously on several elements. It is possible, e.g., to determine tin, mercury and lead species at the same time. This calibration technique is more precise and more accurate than external calibration and even than standard addition, because the determination of the concentration is based on ratios rather than on absolute intensities.

An advantage of this approach is that the loss of methylmercury during the distinct steps of the determination method does not affect the final result. In other words, this technique makes it possible to correct unwanted transformations (methylation and demethylation) of mercury species, and even drift in measuring instruments.

IDMS is a strong technique that allows obtaining SI-traceable results with a small combined measurement uncertainty. The "Comité Consultatif pour la Quantité de Matière (CCQM)" even recognises IDMS as a potential primary method for determining methylmercury, which means that the method has the highest metrological qualities, that the way to perform it is fully described and understood and that the measurement uncertainty of the method may be expressed in SI units.

Conclusion: Speciation methods are complex. Thanks to IDMS it is now possible to quantify methylmercury in an accurate and reasonably precise way down to ppb level.

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Trichinella

Feedback CRL – Workshops -Symposia

Report on the 4th Workshop of National Reference Laboratories for Parasites

This fourth workshop was set up by the Community Reference Laboratory (CRL) in Rome, and took place on 28 and 29 May 2009.
The workshop was not only attended by representatives of the distinct National Reference Laboratories (NRLs) of the Member States, but also by those of non-EU countries (such as Croatia, Norway, Serbia en Switzerland), by experts who had been invited to attend and by a delegation of the European Commission and the European Food Safety Authority (EFSA).
Mrs B. Janackova (DG SANCO, EU) emphasized that an important objective of this annual workshop is to bring about an effective network between the NRLs and the CRL. The recent 'pepsin crisis' and the co-operation between the distinct NRLs following the recovery of unexpected parasites in foodstuffs showed that there is already such a network. She also reported on the 'Working Group on Trichinella-matters' that took place on 21 April 2009. Some Member States perform their tests not quite in compliance with Regulation 2075/2005, but they nevertheless try to meet the standards ; other Member States perform 'risk based tests' without the official approval of the other Member States. This issue is still under discussion. The only Member State with the official 'Trichinella negligible risk status' is Denmark, none of the Member States has recognized Trichinella free holdings. Third countries must meet the same requirements when importing into the EU. With respect to the imminent requirement that all Trichinella tests should be performed only in accredited laboratories, it was pointed out that the transitional period expires at the end of 2009. The only subject for debate is the possible extension of this transitional period by 4 years, since accreditation has been considered as necessary by all Member States and adopted by vote in the European Parliament. An extension of the transitional period would only be relevant for laboratories which have already started the procedure for accreditation. The 'Working Group on Trichinella-matters' will soon hold a meeting on this issue and will draft a working document for the European Parliament if an agreement can be reached. Publication of the official decision by the European Commission is expected by the end of 2009.
The distinct Members States introduced their NRL very briefly and gave more details on the notification of some pathogens in their country. The full presentations are available on the CRLP website : http://www.iss.it/crlp.
Some remarkable facts : most of the Member States recently started ring tests on the recovery of Trichinella larvae, with variable results. In Denmark, 3 day trainings are set up twice a year. There was also a case of Trichinella pseudospiralis in a mink. The Danish still test about 99% of all pigs that are slaughtered, because of the massive exports to third countries. Latvia already reported 5 human Trichinella infections in 2009, all of which were caused by eating wild boar meat. Formerly, there were also infections caused by pork. Lithuania reported 41 human Trichinella infections in 2008 as well as 10 positive pigs and 61 infected wild boars. In Poland were found 69 infected pigs and 524 infected wild boars in 2008. That country also reported 270 human Trichinella infections in 2007 and 32 in 2008. The number of routine laboratories for Trichinella testing has decreased from more than one thousand to some eight hundred and ring tests are being set up. A special web application has been developed allowing the participating laboratories to record their results. Four staff members of the Polish NRL have worked fulltime for eight months on preparing the ring test samples. In Romania were found 1005 infected pigs, 27 wild boars and 22 bears in 2008, but no positive horses. There were also 268 human infections.
Slovenia reported 1 positive wild boar. In the United Kingdom a positive fox was found in April 2009. That animal originated from Northern Ireland and the type was confirmed as Trichinella spiralis. They now also validate Trichinella ELISA test kits. For the ring tests they use larvae fixated in digestion fluid and distribute them with a view to an evaluation of the second sedimentation liquid and reading.

Serbia reported that 0.06% of the pigs are infected with Trichinella and that some two hundred human Trichinella infections are diagnosed per year.
Germany reported 1 human infection in 2008, which was not of German origin. There was also a private German pig holding that was found positive : 3 pigs were infected by 1.2 to 299 larvae per gramme. The infection was probably caused by feeding waste material. In wild boars were found 13 isolates (12 T. spiralis and 1 T. pseudospiralis), in foxes were found 3 isolates (T. britovi) and in raccoon dogs (the population of which increases in Germany) were found 2 isolates (both Trichinella spiralis).

There were also some interesting presentations on Toxoplasma gondii in humans, animals and foodstuffs in Europe and on the development of new diagnosing tests for this parasite. Other speakers also focused on some other parasites, e.g. Giardia duodenalis, Cryptosporidium spp., Diphyllobothrium spp. and Opisthorchis felineus.
The results of the EFSA project "Development of harmonised schemes for monitoring and reporting of Echinococcus, Trichinella, Cysticercus and Sarcocystis in animals and foodstuffs in the European Union" were presented by the co-ordinator of the project and there was a contribution on parasites in imported frog legs.
The Belgian NRLT took part in both of these projects.
With respect to Trichinella, special attention was also given to the possible use of serology in the detection of infections and to the standardisation process of the test.
The interlaboratory tests set up in April 2009 by the CRL for the NRLs with a view to evaluating the detection of Trichinella larvae in pork and those on the detection of Anisakidae larvae in filets of fish were discussed.
Following the 'pepsin crisis', the current situation in the Member States was examined as well as the possible causes and efforts were made to find solutions.

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Genetically Modified Organisms

Developments

European project Co-Extra: GM and non-GM supply chains : their co-existence and traceability

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Co-Extra is a European project that was launched in April 2005 and will end in September 2009. More than 200 scientists take part in this project. They work in 51 multidisciplinary research teams and for some private companies spread over 18 countries (Europe, Brazil, Argentina, Russia). The total budget of the project amounts to 22 million euros, 13 millions of which are allocated by the European Commission through the financing of the 6th European framework programme for research.

The main objective of Co-Extra is to supply the tools that are required to implement co-existence and traceability with a view to the co-existence of supply chains of GM products, conventional products and organic products. This integrated project is related to two other European projects : SIGMEA that mainly deals with co-existence at farm level and Transcontainer that focuses on biocontainment.

Involvement of the NRL-GMO in Co-Extra
The Co-Extra consortium was structured in 8 work packages (WP), plus one WP for the overall management of the project.
The participants in the NRL-GMO (ISP, CRA-W, ILVO) were involved in 3 work packages : WP4 (development of testing and sampling approaches), WP5 (development and integration of analytical traceability tools) and WP6 (technical challenges of GMO detection). The ISP(IPH) also contributed to the development and the maintenance of the project website (WP8).

Main results of the project
The final results of the project were presented at an international conference held in Paris in June 2009. Co-Extra especially resulted in progress in the following fields:

- Traceability tools : sampling strategies, new methods for the detection, identification and quantification of GM ingredients, detection of unauthorised GMOs;
- Costs and benefits of co-existence;
- Legal liability and redress associated to co-existence;
- Development of decision support tools;
- Analysis of stakeholder views.

As for the participants in the NRL-GMO, the main results may be described as follows :

ISP/IPH (Scientific Institute of Public Health) : development of a semi-quantitative approach to the detection of GMOs in soybeans, maize and rapeseed by means of real-time SYBRGREEN PCR (patent registered) ; demonstration on the performance of dual target plasmids as a calibration tool for quantifying GMOs ; evaluation of storage conditions for materials used in PCR; development of a general approach for measuring the level of inhibition during PCR.

CRA-W : near infrared spectrometry was used to distinguish conventional soybeans from transgenic soybeans (event GTS40-3-2). One 800 mg sample of rapeseed is sufficient for a quantitative result with a satisfactory measurement uncertainty in the presence of 0.1 % GMO. The methods in use for preparing samples were identified by means of a questionnaire. A report was written on the detection of botanical impurities with the impact on the labelling of raw materials with transgenic contamination; the current technical restrictions were clearly identified.

The contribution of the ILVO – through scientific papers or the development of new methods – mainly related to : evaluating the sequence stability and the concordance between those sequences and assays on reference taxa ; evaluation of reference and calibrating materials for GMOs ; "gene stacks", the terminology used and detection methods ; multiplex screening of taxa, sampling, detection of "gene stacks" and of unknown GMOs.

More information is available :
- on the website of the Co-Extra project: http://www.coextra.eu
- at the National Reference Laboratory for GMOs (NRL-GMO) - email: NRL-GMO@sbb.ihe.be

Dioxins and DL-PCBs

Developments

New Combination Techniques in Dioxin Analysis

Context

In dioxin analysis the quest for the holy grail implies the development of reliable procedures producing congener specific results in a short time, at a reasonable cost. Each new procedure must of course also meet strict QA/QC requirements, such as those laid down e.g. in the (analysis) guidelines of Eurachem and of the EU as well as some standards, such as ISO17025 and/or the GLP procedures. Each step of such a procedure, especially extraction, purification, fragmentation, chromatographic separation and physio-chemical (or biological) measurements must be optimised to the utmost at maximum capacity.

Regardless of the method of measurement (physiochemical or biological), the sensitivity must be of ppq (part per quadrillon, 10-15) level. This corresponds to an extreme case of ultratrace analysis and is a true challenge for analytical chemistry. In his book "Our Stolen Future", T. Colborn illustrates the concept of ppt (part per trillion, 10 -12) by comparing it to a drop of gin in a train of tank wagons filled to the brim with tonic. Such a train should be 9 km long. In the case of ppq, the length of the train is over 90 km …

Target analytes being in fact ultratraces, the analysis must start from a large amount of sample and a wide range of matrix related interferences must be eliminated before measurement as such is even imaginable.

Preparing a sample

The greater the complexity of the sample, the greater the complexity of the method that should be applied... The extraction of target analytes from the matrix and the elimination of undesirable interferences are achieved by means of a time and money consuming multistep method. Although Soxhlet and liquid-liquid extraction are still used for solid and liquid matrices respectively, there are also more recent and more specific methods at hand. The most important of these are supercritical fluid extraction (SFE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), and solid phase extraction (SPE).

Regardless of the extraction method used, the extracts must be purified very thoroughly and the separation between dioxins, furans and planar PCBs and non planar types must be guaranteed so as to simplify gas chromatographic analysis.

An effective strategy for integrated extraction, purification and fragmentation is based upon the use of an automatic online system. One of the ways to achieve this, is to combine the SPE or the PLE extraction system with multicolumn LC purification. Figure 1 shows the proceedings of such a system for PLE extraction. Figure 2 shows the purification and fractionation of the system which is coupled to the PLE-system. The sample is inserted into an extraction cell and temperature and pressure are increased to allow extraction. The extract produced is immediately sent through the different purification columns which also allow fragmentation in compound subfamilies. The different fragments obtained in this way are then concentrated and injected into the GC-MS system.

Figure 1 : Flow chart of the online automatic extraction system (PLE type).

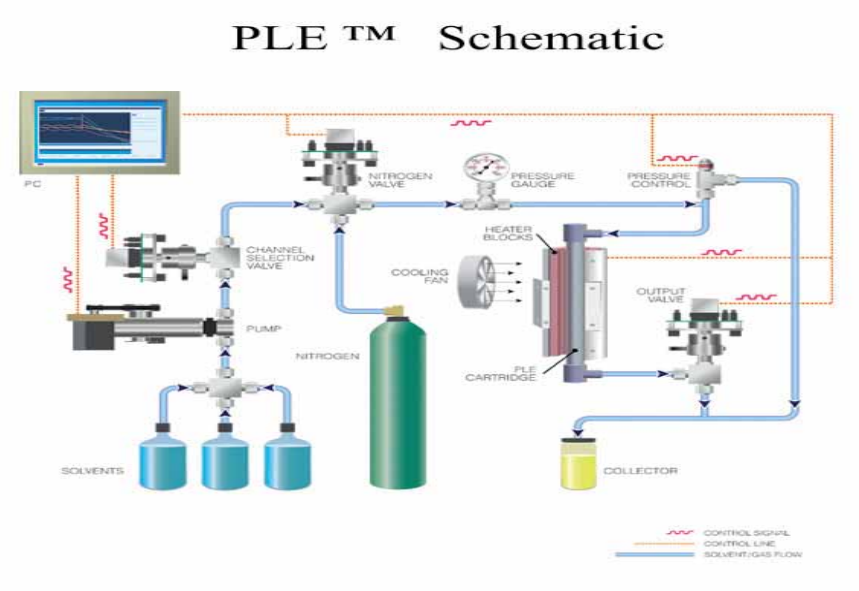
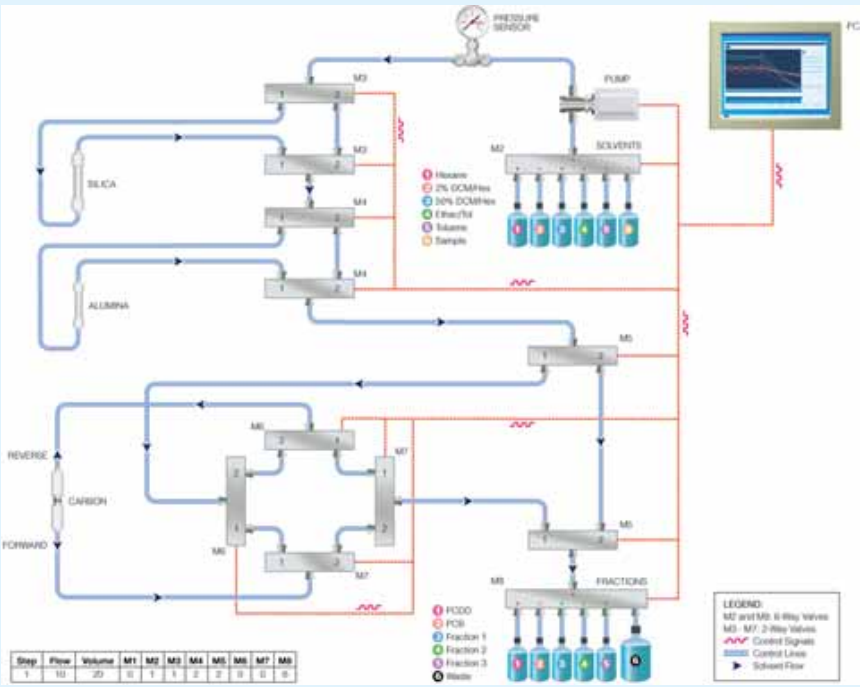


Figure 2 : Flow chart of the online automatic purification and fractionation system



Measurement

In the 70s, Baughman and Meselson performed measurements on ppt level, using high resolution mass spectrometry. From that point and with the advent of capillary gas chromatography, the availability of isotope-marked standards and the improvement of mass spectrometry appliances was developed the method known nowadays as GC-IDHRMS (ID=isotope dilution) for determining dioxins. This method has the sensitivity required to perform dioxin analyses as an instrumental detection limit of approximately ten injected femtogrammes may be obtained with it. Moreover, this sensitivity goes together with a very satisfactory specificity, thanks to the mass resolution force of this type of appliances (High Resolution Mass spectrometry). The technique has now become the golden standard and was made compulsory by the European and the American regulations for targeted analysis of dioxins and related molecules.

Thinking in terms of analytical dimensionality, the GC-HRMS technique may be divided into three dimensions: 1) gas chromatography to separate compounds, based essentially upon physicochemical properties, 2) mass spectrometry for separating in mass and 3) high resolution for molecule identification based upon correct mass. Hence, any other device should have at least the same dimensionality to be eligible as a valuable alternative.

With a view to simplification of the method, the main obstacle would be to replace the very complex HRMS by a more practical MS instrument. However, when low resolution mass spectrometry is chosen, the lacking dimension must be outweighed in order to observe the dimensionality of the GC-HRMS reference method and to maintain accurate and correct isotope diluted quantification. Several possibilities are open :

Gas chromatography coupled to ion trap tandem low resolution mass spectrometry (GC-QISTIDMS/MS)

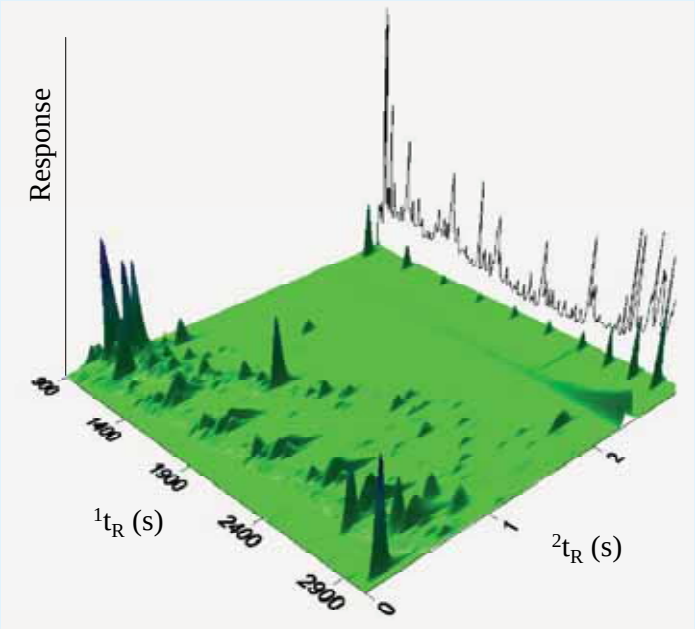
In this method the lacking dimension is replaced by the fact that the daughter ions formed after fragmentation of the parent ions (precursor ions) are followed, thus providing the specificity required. The choice of the tandem technique also produces the increase of sensitivity that is required to make the ion trap approach the sensitivity threshold values that have been defined. The additional use of a large volume injector (PTV) makes it possible to reach instrumental detection limits of ppt level.

Two-dimensional gas chromatography (GCxGC) coupled to time of flight low resolution mass spectrometry (GCxGC - TOFMS)

Instead of adding a dimension in MS, it is also possible to add a chromatographic dimension. In GCxGC, the sample comes successively into contact with two different chromatographic phases (two dimensions) linked to one another by means of a modulator. The main advantage of this technique is the significant increase of the number of individual peaks without extending the analysis time. The availability of two orthogonal retention times also results in an easier identification of the compounds (Figure 3). Hence, it is possible to separate several types of analytes with only one injection. The width of the peaks being however rather small (50 – 200 ms), this technique requires a high acquisition speed detector. The main inconvenient of this technique is that is has poor sensitivity as a result of the structure of the source. Comparative studies reveal however that the use of techniques involving the injection of large volumes might easily adjust LODs to the analysis of dioxins in food.

A major advantage of GCxGC – TOF MS lies in the exhaustiveness of the collecting of masses included in the range under observation. This makes it possible to detect all molecules that are present in the sample, without compromising the acquisition of information on the target compounds. As a result, the analyst has at his disposal a complete inventory of the contents of the sample which may possibly allow the detection of other contaminants. The combination of GCxGC and TOF is a good choice when it comes to solving complex analysis problems.

Figure 3 : GCxGC-TOFMS chromatogram of a human serum sample. Dioxins and PCBs are in the middle

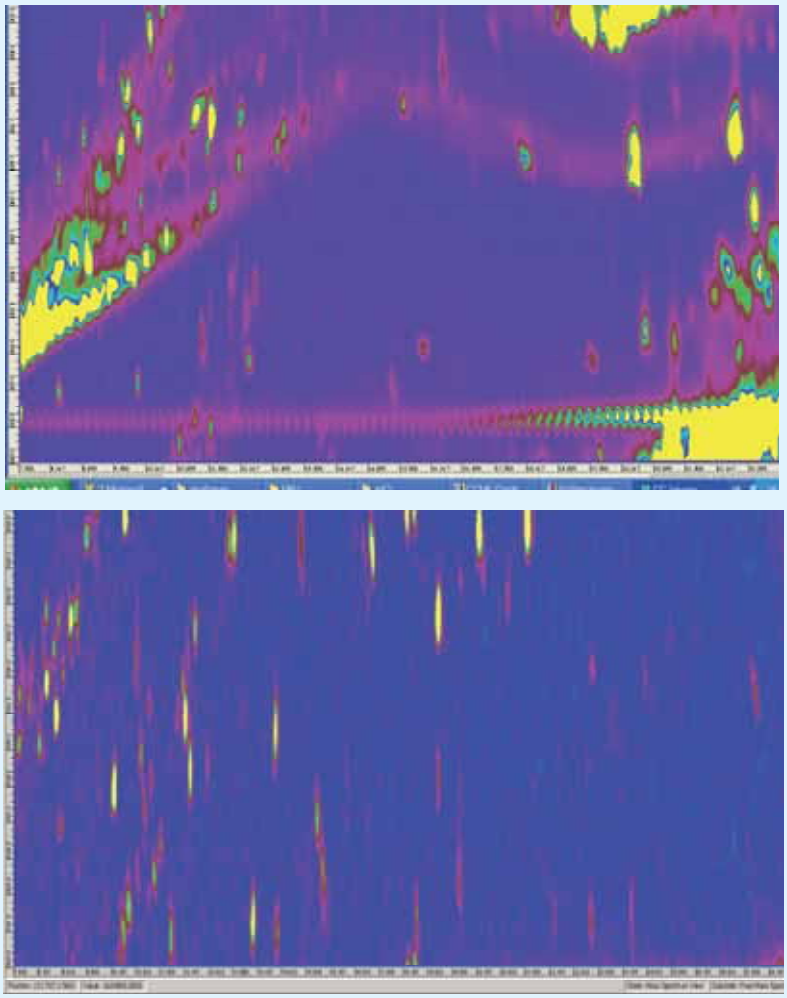


of the diagram.

Two-dimensional gas chromatography in combination with low resolution quadrupole mass spectrometry (GCxGC-qMS)

The quadrupole is a scanning mass spectrometer (with limited scanning speed) just like the high resolution spectrometer and is therefore not the best choice of detector for GC x GC. A combination of these two techniques would however allow another kind of ionisation, i.e. negative chemical ionisation (NCI) which goes smoother and produces less fragments from the parent ion. Hence, the intensity of the parent ion signal is amplified and sensitivity increases (Figure 4). Formerly, this ionisation method showed poor reproducibility and was therefore seldom used in dioxin analysis. Recent technical improvements made to this system resulted in higher stability so that it might become an alternative to electron impact ionisation (EI). The main challenge remains the development of robust quantification procedures based upon mass spectra containing few fragments and the signals of which are based on halogen atoms rather than on marked parent ions.

Figure 4 : GCxGC-qFMS chromatograms of a quality control sample of bovine fat. The upper chromatogram is obtained in EI mode, the lower in NCI mode.



Discussion

GC-HRMS is a ready-to-use analysis technique that has since long reached maturity for the analysis of organic traces. Because of all the knowledge that was acquired over the last twenty years, the effectiveness of this technique and the fact that it has been used intensively for determining dioxins it has become the reference analytical method in this field.

Recent developments of mass analysers resulted in the discovery of alternative methods to HRMS. Combinations of chromatography and new mass analysers opened certain possibilities that were unthinkable when the HRMS methods were put into use. The enumeration given in this document is not exhaustive. It only mentions the most valuable alternatives. Other recent studies based on a triple-stage quadrupole (TSQ) tandem LRMS also revealed the increased performances of this instrument in dioxin analysis.

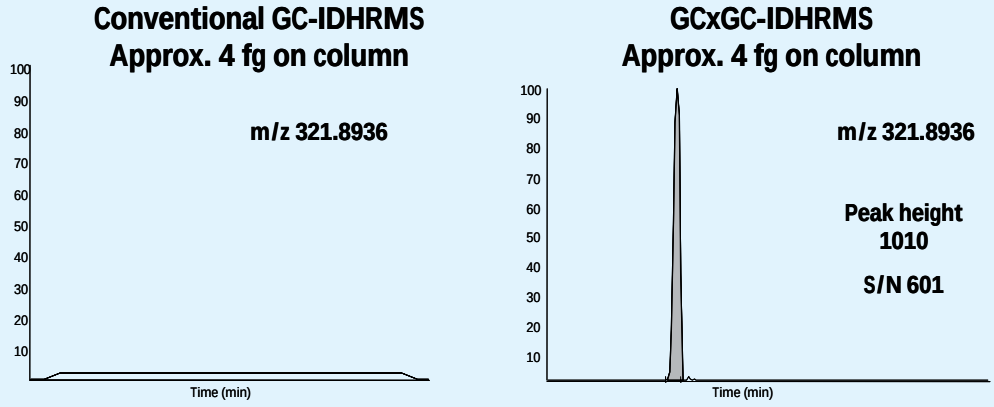
All these alternatives show that it is possible to achieve analytical performance levels that meet the strict requirements laid down in European standards without having recourse to high resolution mass. It will take some time but, in the long run, replacement of these "old" sector devices by more modern analysers will be inevitable. These new physio-chemical methods must be seen as possible techniques to replace HRMS and not as detection methods, such as biological methods.

Moreover, one of the main challenges to chemical analysts working in the field of trace contaminations is a trend that became more and more widespread in recent years and that consists of an increase in the number of compounds mentioned in surveillance plans for environment or food chain control as well as for biological surveillance of humans. SIM scanning (sector devices and ion traps) has shown its limitations in this particular respect since only a small number of ions can be analysed at the same time. Biological tests might help to solve these problems since they measure the overall toxicity of a sample in TEQ. However, this global toxicity, which is of the utmost importance for public health, does not allow an easy comparison with specific standards, such as those defining the maximum dioxin or PCB values that are admissible in food. This biological strategy makes it however possible to screen the samples and to focus further physio-chemical analysis on the most relevant of them.

With a view to solving these problems, GCxGC-TOFMS is probably the new technique that not only makes it possible to identify and quantify the target compounds, but to analyse them and to detect non-target compounds as well. GCxGC allows the identification of elution profiles, i.e. characteristic profiles linked to particular matrices or sources of contamination.
In this way, it is possible to draw up cartographies. Thanks to the use of a TOFMS as detector all spectral data will be available as long as the analysis goes on and the data may be processed afterwards. The price to be paid is that very large files must be managed in order to exploit the rough data material.

The second major problem analysts have to face lies in the ever decreasing contamination levels that these compounds have shown in recent years. The background noise for dioxin in food and human serum has decreased to a point where measuring is extremely difficult. It is by no means advisable to use large samples to avoid this problem, given the complexity of the matrices considered. Efforts must focus on improving the sensitivity of the methods. High resolution mass spectrometry is given new prospects here since a recent coupling of GCxGC and HRMS showed how very sensitive this technique is. In fact, an injection of no more than some ag (ag = attogram, 10-18 g) of 2,3,7,8 TCDD coming from a sample of human serum provokes signals with very high S/N ratios (Figure 5). This combination presents certain limitations, e.g. the limitation of the number of ions that may be screened and the fluctuations of the calculations of isotope ratios. These limitations are related to the very small number of molecules that actually enter the mass analyser. It is nevertheless evident that the use of GCxGC-HRMS concurrently with a more exhaustive method would lead to a decrease in the number of analyses in which no signal is being detected.

Figure 5 : GC-HRMS (left) and GCxGC-HRMS (right) chromatograms of a human serum sample (12C-2,3,7,8-TCDD).



The general trend that is noticeable in analytical trace chemistry is the development of increasingly inclusive and versatile analysis methods. Efforts are now being made towards an integrated and automated approach of the different steps of sample preparation and analysis by means of GC-MS. Our current procedures may already be improved and are being improved with a view to making analysis as cost-effective as possible.

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Milk and Milk Products

Feedback CRL – Workshops - Symposia

Report on the participation in the 12th Workshop of the EU Community Reference Laboratory for Milk and Milk Products (CRL MMP)

As National Reference Laboratories (NRL) for Milk and Milk Products, the ILVO-T&V and the CRA-DQPA attended on 28 and 29 May 2009 the two-yearly workshop set up by the European Reference Laboratory (CRL) for Milk and Milk Products.

This CRL activity is performed by the Laboratoire d'Etudes et de Recherches sur la Qualité Alimentaire et sur les Procédés Agroalimentaires – LERQAP in Maison Alfort and co-ordinated by Mr Bertrand Lombard. The CRL workshop was attended by representatives of the NRLs of 26 EU Member States. Véronique Ninane (CRA-DQPA) and Koen De Reu (ILVO-T&V) attended the workshop on behalf of the Belgian NRL.

After the introductory part, was given an overview of the core business of CRLs and NRLs. The tasks of these reference laboratories are described in EU Regulation 882/2004, under article 32 and 33, respectively. An NRL must, e.g., collaborate intensely with the CRL, ensure the dissemination to the competent national authorities of information that the CRL supplies, perform or coordinate the reference activities on the national level, take part in comparative studies and supply scientific reports to the competent authorities. The CRL must inform the EU whenever the NRL does not fully collaborate or when interlaboratory tests show poor results.

The next topic to be discussed was the requirement of accreditation for official controls. In accordance with Regulation 882/2004 official control must be carried out under accreditation (e.g. ISO 17025 or ISO 17011) (art. 12). In pursuance of Regulation 2076/2005 (articles 1 and 18) this requirement must be met as from 31 December 2009 for all laboratories performing official controls, including also the CRL and the NRL. As far as the CRL and the NRL are concerned, this requirement applies to the reference methods (mentioned in Regulations2073/2005 and 1664/2006) for which they are competent. The reference methods related to the scope of the NRLs Milk and Milk Products are listed in Regulation 1664/2006 and include the total bacterial plate count, the somatic cell count and alkaline phosphatase activity. Then was discussed the possibility of accrediting the reference method for the somatic cell count (EN ISO 13366-1), given the numerous problems related to this method and the limited use of the method in the field. The persons who attended the workshop agreed that the reference method for the somatic cell count should be the top issue of the CRL and the NRLs in the coming years.

Then were discussed the results of the 2007 ring tests on total bacterial plate count. The ring test showed that the NRL network is capable of performing this test in a satisfactory manner. As from 2009, the CRL will prepare the samples for this ring tests and therefore, it plans a previous study on the storage of these samples. Three types of preserving agents (sodium azide, boric acid and a mix of boric acid and glycerol) will be added to raw milk and the stability of the milk during preservation will be monitored. Since DG SANCO asked the CRL to examine the quality (total bacterial plate count) of colostrum (first milk, rich in antibodies) on a practical scale, the modus operandi of that research was explained and discussed. In addition, the effect of freezing on such samples will also be studied. If freezing appears to be possible, samples of other countries might be included in the study. The purpose is to have a general idea of the bacteriological quality of colostrum. Then, the CRL presented the preliminary results of the validation of the Bactocount (Bentley), which may become an equipment that might be used instead of the Bactoscan routine equipment used for total bacterial plate counts on raw milk. The principle of the Bactocount is comparable to that of the Bactoscan, the operating being based upon DNA staining of the bacteria

and on flow cytometry. The intermediate results show a too high residual standard deviation between the results obtained with the equipment and the reference results. The checklist for inspections of laboratories using the Basctosan equipment was then explained. This checklist was drawn up by the CRL in very close collaboration with the ILVO-T&V (Koen De Reu). It is known from the experiences of various NRL laboratories (such as the NRL of the UK) that the checklist is useful. The CRL therefore encourages the NRLs to use this checklist intensively.
Then attention was focused on the result of a questionnaire on the practical situation of the plate count criteria for milk payment. The replies to the questionnaire showed that in the EU dairy farms keep 49 cows on an average. Most of the milk is supplied every day or every two days to dairy industry. In 8 out of the 21 countries which sent a reply, sampling is fully automatic. In 9 cases sampling may be either automatic or manual. In the 4 remaining countries all sampling is manual. In 11 countries a preserving agent is added to the milk whereas in the other countries the analysis on milk is performed after cooled storage. Most countries also use only one conversion table for all Bactoscan devices installed on their territory.
The next topic on the agenda was the somatic cell count. In the first presentation, Mrs Alexandra Cauquil (CRL) discussed the results of the 2008 ring test. The count was carried out according to reference method EN ISO 13366-1. Both the ILVO-T&V and the CRA-DQPA took part in this ring test. As a whole, the results obtained by the NRLs were better than those of 2002. This presentation was followed by a thorough discussion of both a questionnaire on reference material for this parameter and the development of a reference system.
The second day was mainly devoted to the determination of alkaline phosphatase (ALP) in milk. A first presentation dealt with the ring test set up in 2007. A remarkable fact was that the repeatability and the reproducibility of the NRL network was substantially better than the target values mentioned in the ISO 118116-1 method. The purpose is to include the performance data achieved in an appendix to this ISO standard. The ILVO-T&V took part in the ring test and obtained good results. To prepare the ring test of 2009, the CRL will first carry out a study on homogeneity and stability. Samples will be stored at 4°C (for 3 weeks) and at -20°C (for 1 year). The stability and homogeneity will also be checked after the samples will have been transported.
Then Mrs Louisa Pellegrino (NRL-IT) and Mrs Marina Nicolas (CRL) gave an overview of the results found for ALP in various types of pasteurized cheese. Following these results, there is a tendency to propose 6 mU/g cheese as the legal limit to DG SANCO. But, it is emphasized once more that results of other Member States will also be welcome. ALP activity in pasteurized goat milk was also studied in 8 NRLs. Six indicated that the phophatase activity is less than 350 mU/l, the standard value for cow milk. Yet, in two countries, Romania and Cyprus, higher values have been recorded. A request is made to the Member States represented at the workshop to supply any other available research results. As for camel milk, ass milk and mare milk, various research groups, including the ILVO-T&V, showed that ALP is not an adequate parameter for checking the pasteurization or the contamination with raw milk.
Then, Mrs Marina Nicolas commented on the results of a comparativestudy of the alternative photometric method (Enzymatic photo-activated system – EPAS) NovaLum and the Fluorophos reference method. The alternative method showed a positive bias as opposed to the reference method. Given the linear correlation, it might be possible to use a conversion factor to adjust the results of the alternative method to those of the reference method. The deviation may be explained by the fact that these methods are based upon other principles and other substrates.
The following presentation was on the reactivation of ALP. The ALP is bound as a dimer to fat globules. Magnesium plays an important part in the development of this dimer. During pasteurization, the dimer is changed into a monomer and the magnesium is no longer bound. In products with a high fat content it may happen, however, that after pasteurization the freed magnesium will again bind to the monomer and that a dimer is formed once more, thus reactivating the ALP. This reactivation depends on the temperature.
The workshop was concluded with an overview of all CRL and NRL activities planned for 2010-2011.

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New Standards and Legislation

NewIDF- FIL (International Dairy Federation – Fédération International de Laiterie) standards in 2008 - 2009 (from 20 November 2008 until 12 May 2009):

Standards:
ISO 3356|IDF 063:2009 - Milk – Determination of alkaline phosphatase
ISO 22935-1|IDF 099-1:2009 – Milk and milk products – Sensory analysis – Part 1: General guidance for the recruitment, selection, training and monitoring of assessors
ISO 22935-2|IDF 099-2:2009 – Milk and milk products – Sensory analysis – Part 2: Recommended methods for sensory evaluation
ISO 22935-3|IDF 099-3:2009 – Milk and milk products – Sensory analysis – Part 3: Guidance on a method for evaluation of compliance
ISO 5764|IDF 108:2009 – Milk – Determination of freezing point
ISO 23065|IDF 211:2009 – Milk fat from enriched dairy products – Determination of omega-3 and omega-6 fatty acid content by gas-liquid chromatography

Other useful IDF publications:
IDF Bulletin nr 433/2009 'A revolution in food safety management – E-form'
IDF Bulletin nr 434/2009 'International collaborative study on the gas-liquid chromatographic method for the determination of milk fat purity in milk and milk products'
IDF Bulletin nr 435/2009 'Standardization of a chemiluminescent method to detect alkaline phosphatase in liquid dairy products'

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Workshops & Symposia

Datum	Onderwerp	Plaats	Meer informatie (website)
21/11/2009	Leven in de brouwerij!	Leuven	KVCV
11-13/11/2009	New Challenges in Food Preservation: Processing – Safety - Sustainability	Budapest	EFFoSTwww.effost-conference.elsevier.com
23-24/11/2009	New Trends in Food Analysis From LC-MS Technologies to UPLC	Paris, Espace Saint Martin, France	AOAC Europe, ASFILAB
24/11/2009	Symposium Chemische Veiligheid van de Voedselketen – Recente Wetenschappelijke Ontwikkelingen	Tervuren	FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieuhttps://portal.health.fgov.be
7-8/12/2009	Novel Food	Mainz, Germany	www.akademie-fresenius.com
26-27/01/2010	International Symposium on Hyphenated Techniques for Sample Preparation (HTSP)	Brugge	www.ordibo.be
27-29/01/2010	Eleventh International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analyzers (HTC-11)	Brugge	www.ordibo.be
22-26/03/2010	10th ASM Conference on Candida and Candidiasis	Miami, USA	
25/03/2010	Antibiotica en Alternatieven (AOAC Lowlands Symposium)	Breda, Nederland	http://aoaclowlands.nl/symposia.html
5-8/05/2010	ISOPOL XVII - International Symposium On Problems Of Listeriosis	Porto, Portugal	
1-4/06/2010	6th International Symposium on Hormone and Veterinary Drug Residue Analysis	Gent	Ugent, Faculty of Pharmaceutical Scienceshttp://www.vdra.ugent.be
06/2010	2nd ASM Conference on Antimicrobial Resistance in Zoonootic Bacteria and Foodborne Pathogens	Toronto, Canada	
21-26/08/2011	DIOXIN2011, 31st Symposium on Halogenated Persistent Organic Pollutants, 21-26 August 2011, Brussels, Belgium.	Brussels, Belgium	JF.Focant@ulg.ac.be

NRL NATIONALE REFERENTIE LABORATORIA

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