NMKL Newsletter

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Dr. Ulla Edberg, the new NMKL chairman

Dr. Ulla Edberg, National Food Administration (NFA), Sweden, is elected as the new chairman of NMKL. Edberg has been a member of NMKL for 25 years, and has for the past 22 years been the chairman of the Swedish national committee.

Her PhD is from the medical faculty at the University of Uppsala, where she also has a position as Assistant professor in medicinal and physiological chemistry.

In 1982, Edberg started working at NFA, and has for more than 20 years been the Director of the Department for Research and Development. Her department develops analytical methods for the analysis of foods within various fields such as allergens, metals, vitamins and GMO. The Department is a national reference laboratory (NRL) for 8 different areas. For the past 20 years, Edberg has been responsible for the NFA's allergy information. NFA has been very active in method development for the analysis of allergens, inspection projects and information to the industry, as well as in influencing the regulation formation for allergens in Europe and Codex. For this work Ulla Edberg and Ingrid Malmheden Yman received the Swedish Food Award "Livsmedelspriset" in 2003.

Internationally, Ulla Edberg represents Sweden in CEN/TC 275 Horizontal Methods and in Codex Committee on Methods of Analysis and Sampling. Edberg is also NMKL's representative in CEN/TC 275.

Ulla Edberg has the following comment to the Newsletter: "I regard the Nordic cooperation in NMKL as very important for Sweden, which is a small country with few food laboratories in order to be able to influence the development effectively. NMKL is an excellent network for the Nordic laboratories".

Dr. Ulla Edberg, new chairman of NMKL

IN THIS EDITION:

Dr. Ole Bjørn Jensen, chair of NMKL for the past 20 years

Dr. Ole Bjørn Jensen

Dr. Ole Bjørn Jensen, Managing Director of Scanpharm AS, has been an NMKL member since 1976. He has been the chairman of NMKL since 1989. Jensen has been an outstanding leader with clear visions, and has lead NMKL through many different phases in the Nordic cooperation.

Since1984, NMKL has been linked to the Nordic Council of Ministers' food cooperation. In his very last Chairman's Report given at the NMKL 63rd Annual Meeting, Ole Bjørn Jensen stated: "After having participated in the work of NMKL since 1976, I have reason to assert that NMKL is the most successful example of what can be achieved through Nordic cooperation within the food area, in terms of Nordic influence as well as making the Nordic countries known. Hence, it has often been a source of wonder to me why NMKL at times has been given second-class treatment in the allocation of the available limited Nordic resources."

To be continued at page 3

From NMKL's 63rd Annual Meeting

NMKL's 63rd Annual Meeting was held at Hotel Selfoss, at Selfoss, Iceland. About 60 members from the national committees of Denmark, Finland, Iceland, Norway and Sweden participated. The annual meeting is indeed an important meeting forum for the Nordic experts.

Also this year there were social events and excursions to sites such as Geysir and Gullfoss (pictured). Iceland is fantastic!

We would like to thank the Icelandic National Committee of NMKL, Matis, MAST (Icelandic Food and Veterinary Authority) and the Ministry of Fishery and Agriculture for arranging such a technically and socially successful annual meeting.

Working program - chemistry, microbiology and sensory

The discussions at the NMKL Annual Meeting take place partly in Plenary sessions and partly in Sub committee meetings. In the Plenary sessions, the participants get information from NMKL's officials and from invited partners. NMKL's working program is discussed in the expert committees. The discussions were lively in the professionally strong, but relatively informal, sub committees on microbiology, chemistry and sensory analysis.

- Sub committee 2: Microbiology with chairman Flemming Hansen, Danish Technological Institute, Denmark
- Sub committee 3: Chemistry with chairman Kaare Julshamn, National Institute of nutrition and seafood research, Norway
- Sub committee 4: Sensory analysis with chairman Gunnar Forsgren, Iggesund Paperboard, Sweden

MICROBIOLOGY

Topics on the working program for the Micriobiological Committee:

- Sampling and pre-treatment of foods and animal feedstuffs for quantitative microbiological examination.
- *Enterococcus*. Determination in foods and feeds.
- *Bacillus cereus.* Determination in foods.
- *Clostridium botulinum* & botulinum toxin.
- Pathogenic *Yersinia enterocolitica*. Culture and PCR methods for detection in foods.
- *Shigella.* Detection in foods.
- *Listeria monocotygenes*. Detection and determination in foods.
- *Brochothrix thermosphacta*. Determination in meat and meat products.
- Detection of gram negative bacteria in pasteurised milk and cream.
- Quality assurance of PCR analyses.

CHEMISTRY

Topics discussed at the Sub Committe 3 meeting included:

- Methylene mercury. Determination by isotope dilution-GCICPMS
- Histamine. HPLC determination in fish.
- Plant sterols and stanols: GC determination of phytosterol-enriched foods.
- PAH. Determination in foods.
- Vitamin K_1 and K_2. HPLC determination in foods.
- Nitrate and / or nitrite. Determination in food and water after reduction with zinc and Griess reaction.
- Fat in foods by NMR.
- Recovery. Estimation and expression.
- Quality assurance.

SENSORY ANALYSIS

This year, the Committee of Sensory Analysis had its 15th anniversary. The committee was established in 1994 in Iceland. The sensory analysts are keen and in addition to national meetings, they have regular Nordic telephone conferences. Their working program includes:

- Evaluation and reporting of sensorial data including measurement uncertainty.
- Quality control test of drinking water.
- Sensorial analysis of meat products.
- Sensorial analysis of cereals.
- Procedures for recruiting /training and control of analytical panels and judges.
- Method for discriminating tests (bionomical tests).

Sub committee3
Photo: Ulf Bondesson

Cont. from page 1: Dr. Ole Bjørn Jensen, chair of NMKL for the past 20 years

Ole Bjørn Jensen is a visionary leader, and when accreditation was introduced in the 80's, it was obvious that NMKL should elaborate guidelines for laboratories, and that documentation of method performances should be included in NMKL methods. Ole Bjørn Jensen has performed the final review of all the elaborated NMKL procedures listed on page 12, and all the NMKL methods, which means more than 120 methods during his chairmanship. Today, NMKL methods are used world-wide, not only within the Nordic countries.

Ole Bjørn Jensen regarded it as important to meet the food industry's need for rapid methods, and during his chairmanship, NordVal has been included in NMKL.

Ole Bjørn Jensen ended his last Chairman Report by the following greeting:

Ole Bjørn Jensen pictured with his NMKL order, the award he received at the banquet

"It has always been inspiring and stimulating and a great privilege for me to work with all you gifted people, as it has been rewarding and sometimes also quite challenging to contribute to NMKL's development and well-being and sometimes survival. I am proud of the highly professional and international respected organisation NMKL is today "

Harriet Wallin, new chairman of the Chemical Committee

Harriet Wallin, Senior Officier at the Food Safety Authority, Evira, Finland, is well known in NMKL.

Harriet Wallin was the NMKL Secretary General in the period 1985-1997; an exceptionally talented professional and efficient administrator. We were all sorry when she had to resign from the position. Fortunately, she continued in NMKL and in the NMKL's executive committee as the chairman of the Finnish National Committee. Now, Harriet Wallin is also taking over the chairmanship of the Chemical Committee of NMKL. In addition to chairing the meetings, the chair is obliged to approve study plans and review methods before publishing.

Harriet Wallin is also involved in EK-FJLS under the Nordic Council of Ministers, and in the Nordic working group for food management and consumer information. Wallin is further active in EU Scoop projects and in committees of Codex Alimentarius.

Harriet Wallin, Chairman of Sub Committee 3

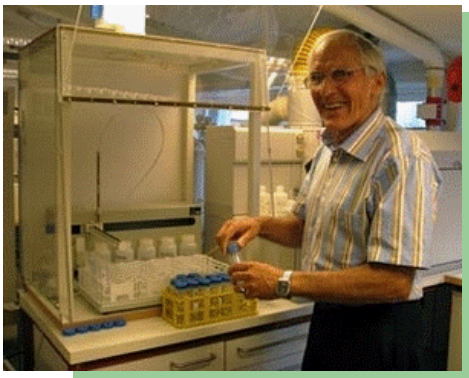
Kaare Julshamn, 24 years as chairman of the Chemical Committee, 30 year as active NMKL member

Kaare Julshamn, National Institute of Nutrition and Seafood Research, NIFES, Norway, has been a member of NMKL since 1977, Chairman of the Norwegian National Committee from 1990-2000 and Chairman of the Chemical Committee for 24 years. He is one of the chemists who has elaborated most methods. Some of his methods have received considerable international recognition, and some of them have been adopted by CEN, AOAC International and Codex Alimentarius.

"It all started in 1975 when I published a method related to metals in seafood. This was the reason I joined a working group of NMKL in 1977 for the determination of metals in foods," says Julshamn to NIFES's web editor, who has written a comprehensive article about Julshamn, see www.nifes.no.

The latest published method elaborated by Julshamn, was approved in 2008; a method for the determination of arsenic, mercury, cadmium and lead in foods by inductively coupled plasma mass spectrometry (ICPMS).

For many years, Julshamn has been the NMKL representative of the heavy metal group in the European Organisation for Standardisation, CEN / TC 275. Julshamn has also been active in elaborating various NMKL procedures within quality assurance.



Kare Julshamn in front of an ICPMS. Photo: NIFES

A thank you for years of considerable contribution

The members of NMKL are appointed by the national committees in their respective countries. Each member puts a great deal of effort into the Nordic cooperation. It is the members and their institutions who run and make up NMKL. Many of the experts have been members of NMKL for years. This year, in addition to the retiring NMKL chairs Ole Bjørn Jensen and Kaare Julshamn, the following people were thanked for their long-standing and valuable efforts in NMKL:

- **Per Lea**, Nofima Mat AS, Norway, has been an active member of NMKL for 20 years. He has led a number of NMKL projects, assisted with statistical expertise, led the statistical group and been the secretary of the Sub Committee 4.
- **Lisbeth Tillge Lund**, DANAK, Denmark, has been a member for about 20 years, and has led and participated in different projects. Lund has been an important link between NMKL and the accreditation organizations. Lund has retired, but is willing to finish the project she is currently leading.
- **Haakan Johnsson,** National Food Administration, Sweden, has been a member for 20 years. He has been a referee and project leader in NMKL, and the secretary of sub committee 3 for more than a decade.

From left: Per Lea, Lisbeth T. Lund, Haakan Johnsson

Nordic cooperation on national reference laboratories (NRLs)

A working group in the Food Department of EK-FJLS under the leadership of Finland, has obtained information from the Nordic countries regarding appointed national reference laboratories (NRLs) and the Community reference laboratory (CRLs) where required according to the Control regulation (EC) 882/2004. A list of the NRLs in the Nordic countries and the CRLs in the different areas will be made available on NMKL's home page.

Each of the Nordic countries are relatively small, and within the respective reference laboratory fields of competence, the responsible analysts do not necessarily have a national network for the exchanges of administrative or technical issues. Thus a Nordic network is useful.

There has also been elaborated a Nordic standard (in a Scandinavian language) regarding national reference laboratories. This standard describes the requirements for appointing NRLs, and how the NRLs are appointed in the Nordic countries. It also describes what is required of the NRLs and how to take advantage of the Nordic cooperation within this area. The following table lists tasks described in the Control Regulation, Article 33.

Tasks according to Article 33	Examples of what the tasks mean in practice
Collaborating with the Community reference laboratory in their area of competence	Participate in CRL organised, relevant workshops and meetings, and follow-up decisions and recommendations
Coordinate, for their area of competence, the activities of laboratories responsible for the analysis of samples within the official control	Provide advise and guidance regarding the use of methods and /or method criteria for official control analysis; develop and validate analytical methods; keep up-to-date on the analytical technical development and, where appropriate, arrange courses and workshops for laboratories analysing samples for the official control
Where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing	Inform the official laboratories of appropriate national and international proficiency tests. If necessary, arrange proficiency testing schemes and evaluate the laboratories performance in the trials
Ensure the dissemination to the competent authority and official national laboratories of information that the Community reference laboratory supplies	Regularly inform the authorities, NMKL and the official laboratories about CRL decisions and recommendations as given in correspondence, at workshops or in meetings
Provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans	Participate in the development of government projects and assist the official laboratories when having technical challenges; make concrete risk assessments

Two new NMKL methods for the determination of tin, Sn, in foods

In most unprocessed foods, tin levels are generally less than 1 mg/kg. Higher concentrations are found in canned foods due to dissolution of the tin coating or tin plate. The concentration of tin in canned foods depends on a number of factors, including the type and acidity of the food, time and temperature of storage, and the presence of air in the can headspace. Oxidising agents such as nitrates, iron and copper salts, certain pigments, and sulphur compounds all accelerate the dissolution of tin. Other substances, such as tin salts, sugars, and colloid like gelatine, retard detinning. Lacquering of cans also reduce corrosion and prevent detinning. Tin concentrations in foodstuffs in unlacquered cans frequently exceed 100 mg/kg, while in lacquered cans, tin levels are generally below 25 mg/kg. Storing foods in opened unlacquered cans results in substantial increases in the tin concentration of the food.

In Europe, the maximum levels of tin are regulated in Commission Regulation (EC) No. 1881/2006 Setting maximum levels for certain contaminants in foodstuffs. For canned foods, except beverages, the maximum level is 200 mg/kg wet weight. For beverages, including juice, the maximum level is 100 mg/kg wet weight. For canned food intended for kids, the maximum level is 50 mg/kg wet weight.

NMKL Method No.190, 2009: Tin (Sn). Determination in foods by flame or graphite furnace atomic absorption spectrometry (AAS).

This NMKL method describes the determination of tin in foodstuffs and canned foods by flame or graphite furnace atomic absorption spectrometry (AAS) after pressurised digestion. The method is applicable to the determination of tin using flame AAS (FAAS) from about 5 mg Sn/kg and graphite furnace AAS (GFAAS) from about 0.1 mg Sn/kg.

The sample is mineralised through pressurised digestion with nitric acid and hydrochloric acid. In the resulting digestion solution, tin is quantified by FAAS or GFAAS depending on the concentration range. The method was validated in a collaborative study in 2006. The study was arranged by Peter Fecher, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Germany.

Eight laboratories analysed carrot puree, tomato puree, pineapple, mixed fruit and powder of peach, tomato, beans and fruit yoghurt by FAAS and GFAAS, respectively. In addition, white wine was analysed by GFAAS. For FAAS the samples varied from 43 to 260 mg tin/kg. For GFAAS the samples varied from 2.5 to 269 mg tin/kg. The precision; repeatability and reproducibility, as well as the trueness were satisfactory.

Both tin methods are elaborated and validated by **Peter Fecher,** Germany

NMKL Method No. 191, 2009: Tin (Sn). Determination in foods by ICPMS.

This method describes how to quantify tin in foodstuffs and canned foods by inductively coupled plasma mass spectrometry (ICPMS) after pressurized digestion.

The sample is mineralised through pressurised digestion with nitric acid and hydrochloric acid. The digestion solution obtained thereby is diluted and then atomised and ionised in an inductive coupled argon plasma. After extraction from the plasma, the positive (charged) tin ions are separated according to their mass charge ratio in a mass spectrometer and quantified in a detector system. The method was validated in a collaborative study in 2006. The study was arranged by Peter Fecher, Bayerisches LGL, Germany.

15 laboratories participated, analysing carrot puree, tomato puree, pineapple, mixed fruit, white wine and powder of peach, tomato, beans, fruit yoghurt. The lowest validated level in the study was 2.5 mg/kg. The limit of quantification depends on the amount of sample used for digestion, the final volume of digestion and the minimum dilution applied. The ICPMS instrument should be able to quantify 1 µg/L tin in the diluted digestion solution. For the example in this method, the limit of quantification is 0.4 mg/kg. The results obtained were satisfactory as regards precision and the trueness.

These tin methods are also published as CEN methods. The tin methods are rewritten to NMKL format by Hilde Skår Norli, Lars Jorhem, National Food Administration, Sweden, and Kaare Julshamn, NIFES, Norway.

New NMKL method No. 96, 4th Ed. 2009: Coliform bacteria, thermotolerant coliform bacteria and E. coli, two MPN methods for Fresh and Frozen Seafood

This method can be used for the determination of coliform bacteria, thermotolerant coliform bacteria and *E. coli*. Two different methods are described, one for all kinds of fresh and frozen seafood, and one for raw shellfish only. The examinations provide information on the coliform bacteria, and thereby the hygienic quality of fresh and frozen seafood and hygienic standards maintained during processing.

All coliform bacteria are able to grow on lactose forming gas and acid. Gas production is in this method detected by adding a Durham tube to a liquid medium, which might be filled with gas and float up in the liquid growth medium. Acid production is detected as colour change of a pH indicator in the growth medium. Most growth media for analysis of coliform bacteria contain nutrition components which allow many bacteria to grow. However, bile salt in the growth media partly inhibits the background flora. Mineral modified glutamate medium contains only a few amino acids, vitamins and minerals, so that bacteria with higher nutrition needs are not able to grow. In case of thermotolerant coliforms and *E.coli*, the background flora is partly inhibited by the high incubation temperature (44°C).

In this method, quantitative examinations for coliform bacteria, thermotolerant coliform bacteria and *E. coli*, are carried out using two different tube methods (MPN methods) depending on the matrix. The pour-plate method (NMKL 44 and 125) can also be used, however the tube method gives better estimation when the level of coliform bacteria is below about 300 cfu/g (mL) sample.

The principle of the tube method is that several replicates of 10-fold dilutions of the sample are inoculated in test tubes containing suitable medium, incubated and read for positive reactions after completed incubation. Positive reactions are either gas or acid production, depending on which growth medium used. The number of positive tubes in each dilution is registered and the most probable number of bacteria per mass/volume unit is read from an MPN table.

Typical and suspicious colonies are counted and confirmed on a suitable diagnostic media. *E. coli* is confirmed if the indole reaction is positive. ß-glucuronidase positive *E-coli* are confirmed if they form blue or blue-green colonies on tryptone-bile-glucuronide agar.

Referee of the method: Taran Skjerdal

The method has been revised by Taran Skjerdal as referee and Gro S. Johannessen as coo-referee. Both are employed at the National Veterinary Institute, Norway. Birna Mørkøre, the Food and Veterinary Agency, the Faeroe island, and Sanna Raunila, the Food and Environmental Laboratory of the Oulu Region, Finland, have been contact persons for the method.

NMKL Technical Report No. 3, 2009: *Clostridium difficile* - a potentially foodborne zoonosis? Significance in humans, animals and food.

Clostridium difficile is a Gram positive, anaerobic, spore forming and toxin producing bacteria, which is widely distributed in the environment. It is present as part of the flora in the large intestine of only approx. 2% of healthy adults, but in 10-20% among the elderly population The spores are resistant to heating, drying and chemical reagents including alcohol based disinfectants.

Since 2003, outbreaks of severe *Clostridium difficile*-associated diarrhoea (CDAD) with increased mortality rate have been caused by the emergence of a hypervirulent *C. difficile* strain in North America and Europe.

Flemming Hansen, Danish Technological Institute (former: Danish Meat Association), and **Katharina E. P. Olsen,** Statens Serum Institut, have elaborated a report regarding emergences of a hypervirulent variant of *Clostridium difficile* in Europe and incidence of *Clostridium difficile* in meat, other foodstuffs and in the environment.

The report is available in English for downloading on NMKL's home page: www.nmkl.org.

Authors of the report: Flemming Hansen and Katharina E. P. Olsen

NordVal Certificate No. 38 for iQ-Check™ *Salmonella* II kit

The iQ-Check *Salmonella* II is a qualitative method allowing the detection of *Salmonella* spp specific DNA sequences after enrichment by culture in buffered peptone water. It is based upon polymerase chain reaction and real time detection using fluorescent probes.

iQ-Check *Salmonella* II describes the following four procedures, differing from each other in preliminary enrichment and lysis steps:

- Standard Protocol I: 18h ± 2h enrichment in buffered peptone water at 37°C ± 1°C, followed by the standard lysis protocol.
- Easy Protocol I on micro plates: 21 h ± 1h enrichment in buffered peptone water at 37°C ± 1°C, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.
- Standard Protocol II: specific for raw meat: 10h ± 2h enrichment in buffered peptone water at 37°C ± 1°C, followed by the standard lysis protocol.
- Easy Protocol II on microplates, specific for raw beef: 21 h ± 1h enrichment in buffered peptone water at 37°C ± 1°C, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.

The method is applicable to the detection of *Salmonella* spp in food, animal feed and environmental samples.

The method has been tested in extensive validations against the reference method EN ISO 6579:2002 – Food microbiology-Horizontal method for the detection of *Salmonella* spp. The comparative and collaborative studies have been arranged by l'Institut Pasteur de Lille and ADRIA Développement, France. The validations are carried out according to ISO 16140.

The results for all 4 procedures showed that the method is satisfactory both with regards to sensitivity, specificity, selectivity and agreement between the results obtained by iQ-Check *Salmonella* II kit and the reference method.

The manufacturer and supplier of the iQ-Check *Salmonella* II kit is Bio-Rad Laboratories, France. For further information please visit www.bio-rad.com.

iQ-Check products from Bio-Rad

NordVal Certificate No. 37 for iQ-Check™ *Listeria monocytogenes* II kit

The iQ-Check *Listeria monocytogenes* II kit is a qualitative method allowing the detection of *Listeria monocytogenes* in environmental samples and food products.

The iQ-Check *Listeria monocytogenes* II kit is a culture method, by enrichment either in a Listeria Specific Broth (LSB) for 23 h ± 1h at 30°C ± 2°C or in half Fraser broth for 25h ± 1h at 30°C ± 2°C. The method is based upon polymerase chain reaction and real time detection using fluorescent probes.

iQ-Check *Listeria monocytogenes* II describes the following three procedures, differing from each other in preliminary enrichment and lysis steps:

- Enrichment in half Fraser followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by a simplified extraction protocol, no longer requiring the first centrifugation step

The method has been validated extensively against the reference method ISO 11290-1(1996/ amendment 2004) Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method. The comparison and the collaborative study were arranged by the expert laboratory l'Institut Pasteur de Lille France. The validation was conducted according to ISO 16140.

The results document no statistical difference in the performances between the iQ-Check™ *Listeria monocytogenes* II for enrichment by half Fraser followed by the standard lysis protocol and the reference method. For enrichment in the *Listeria* Specific Broth, however, the sensitivity is better than obtained by the reference method.

The manufacturer and supplier of the iQ-Check *Listeria monocytogenes* II kit is Bio-Rad Laboratories, France.

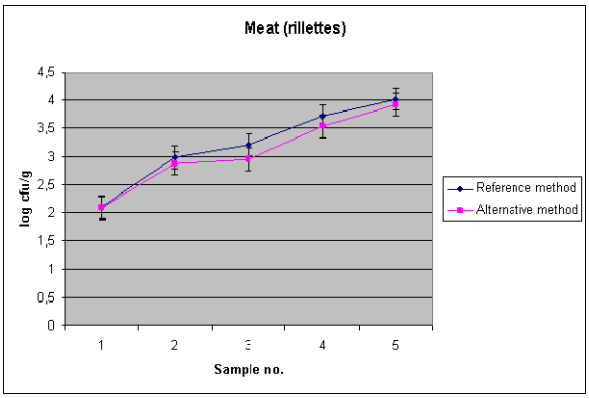
NordVal Certificate No. 22: RAPID' *L. mono*

NordVal has renewed the certificate for the Bio-Rad product RAPID'*L.mono*. The method is applicable to the detection and the enumeration of *Listeria monocytogenes* and to the detection of other *Listeria* species in food and environmental samples.

The principle of the RAPID'*L. Mono* medium relies on the chromogenic detection of the *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C and on the inability of these species to metabolise xylose. After 24 ± 2 hours of incubation, *Listeria monocytogenes* forms characteristic blue (pale blue, grey blue to dark blue) colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. The selective mixture in the medium allow the inhibition of most interfering flora (Gram-positive and Gram-negative bacteria, yeast and mould). Thus RAPID' L mono detects *Listeria monocytogenes* in 24 hours and other *Listeria* species in 24 and 48 hours. RAPID'*L. mono* is compared and validated against EN ISO 11290-1(1996/ amendment 2004): Horizontal method for the detection and enumeration of *Listeria monocytogenes*.

The validation is conducted by l'Institut Pasteur de Lille, France, in accordance with EN ISO 16140.

NordVal has concluded that it has been satisfactorily demonstrated that there are no statistical differences in the performances of *RAPID' L. mono* and the reference method.



Quantification of L. monocytogenes with RAPID' L. mono and the reference method, respectively. The results are overlapping when the measurement uncertainty is included.

NordVal Certificate No. 25: foodproof® *Listeria monocytogenes* Detection Kit in combination with foodproof® ShortPrep II Kit

Biotecon Diagnostics GmbH, Germany, has NordVal certified the **food**proof® *Listeria monocytogenes* Detection Kit in combination with the **food**proof® ShortPrep II Kit for detection of *Listeria monocytogenes* in foods by PCR.

The method detects *Listeria monocytogenes* DNA isolated from enrichment cultures prepared by various valid methods. **food**proof® *Listeria monocytogenes* Detection Kit in combination with **food**proof® ShortPrep II Kit is validated and compared against EN ISO 11290:1996/Amd 1:2004: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method.

NordVal has reviewed the methods, and concluded that **food**proof® *Listeria monocytogenes* Detection Kit in combination with **food**proof® ShortPrep II Kit is just as fit for its purpose as the reference method for the detection of *Listeria monocytogenes*.

Method performance characteristics:
Selectivity: No cross reaction was obtained and all the tested *Listeria monocytogenes* strains were positive.
Relative sensitivity: 100%
Relative specificity: 96.0%
Relative accuracy: 99,2%
Limit of Detection: 1-10 cells per 25 g

For further information about the product, please look at the NordVal Certificate or visit the homepage of Bitecon Diagnostic: www.bc-diagnostics.com

Listeria monocytogenes is a Gram-positive, coccoid, non-spore forming rod bacteria belonging to the genus *Listeria*. In foods, *L. monocytogenes* often appears together with other listeria species, especially the non-pathogenic *L. innocua*. The bacteria grow both aerobically and anaerobic. *L. monocytogenes* in foods can cause serious systemic bacterial infections such as sepsis and meningitis, and can also cause abortion at a late stage.

The bacteria are found naturally in soil and the environment, both humans and animals can be healthy carriers of infection. *L. monocytogenes* can form bio films on production equipment and thus contaminate food products during the manufacturing process.

The bacteria can grow at temperatures from 0 - 45°C, at pH between 4.4 and 9.6, aw down to 0.90 and in the presence of 10-12% sodium chloride. *L.. monocytogenes* grows well in vacuum packs and in modified atmosphere. It will not survive common pasteurisation conditions, and heat treatment of meat products to 70°C for two minutes is sufficient to inactivate the bacteria.

(source: www.veths.no)

NordVal Certificate No. 26: foodproof® *E.coli* O157 Detection Kit in combination with foodproof® ShortPrep II Kit

NordVal has reviewed the results from tests and a collaborative study of **food**proof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit for PCR analysis, and concluded that there are no statistical differences in the results obtained by this method and the compared reference method.

The reference method is EN ISO 16654:2001: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Escherichia coli* O157**.**

foodproof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit is applicable to the detection of *E.coli* O157 DNA isolated from enrichment cultures prepared by various valid methods. *E.coli* O157 is determined by PCR. The method is applicable to foods.

Method performance characteristics:
Inclusivity: 100%. 60 different strains of *E.coli* O157 tested were positive.
Exclusivity: 100% 73 non-*E.coli* O157 serovars and 47 non-*E.coli* strains tested were negative.
Relative sensitivity: 100%
Relative specificity: 96.2%
Relative accuracy: 98.8%
Limit of detection: 1-10 cells/25 g

foodproof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit is manufactured and supplied by Biotecon Diagnostics GmbH, Germany

The selectivity is a measure of a) **the inclusivity:** detection of the target microorganism from a wide range of strains, and
b) **the exclusivity**: the lack of interference from a relevant range of non-target microorganisms.

The relative sensitivity is the ability of the alternative method to detect the analyte compared to the reference method.

The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

The relative accuracy is the degree of correspondence between the response obtained by the alternative method and the reference method on artificially inoculated samples.

The limit of detection is the lowest level the method can detect the analyte with a certain probability.

NordVal Certificate No. 23 foodproof® *Salmonella* Detection Kit in combination with foodproof® ShortPrep I Kit and Roche Diagnostics MagNA Pure LC DNA Isolation Kit III

Biotecon Diagnostics Gmbh, Germany, has received a NordVal certificate for the **food**proof® *Salmonella* Detection Kit in combination with **food**proof® ShortPrep II Kit and also in combination with the MagNA Pure LC DNA Isolation Kit III which is manufacured by Roche Diagnostics. This last mentioned kit is used for meat and meat products.

food**proof®** *Salmonella* Detection Kit is applicable to PCR analysis for *Salmonella* in foods, animal feeds and environmental samples.

NordVal has reviewed the validation studies, the method documentation and the information provided to the customers, and found it satisfactory. The method is as fit for the purpose as the reference method, EN ISO 6579:2002: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella spp*.

There is no statistically significant difference between the obtained results between food**proof®** *Salmonella* Detection Kit and the reference method for the detection of *Salmonella spp.*

Method performance characteristics:
Inclusivity: ≈100%
Of the 126 *Salmonella* strains tested all were positive except for *Salmonella* group I *Thyphi* (which also was the case for ISO 6579).
Exclusivity: 100%
All 56 tested non-*Salmonella* strains were negative.
Relative sensitivity: 100%
Relative specificity: 100%
Relative accuracy: 100%
Limit of detection: 1-10 cells/25 g

NordVal Certificate No. 39: BAX Q7 *Campylobacter jejuni, coli* and *lari* in chicken cloacae swabs

BAX® Q7 *Campylobacter jejuni, coli* and *lari* is manufactured by DuPoint Qualicon, USA. Oxoid AS, Thermo Fisher Scientific, Denmark, is a supplier and has applied for the certification of this method.

BAX® Q7 *Campylobacter jejuni, coli* and *lari* is compared against the reference method EN ISO:10272-1:2006: Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 1: Detection method.

Validation studies have been carried out by the expert laboratories Danish Veterinary and Food Administration, Denmark, and Cherney Microbiological Services, Green Bay, Wisconsin, USA. The method was first validated according to the AOAC RI protocol and then further studied according to the NordVal protocol.

- Detects and quantifies all three species in the same sample
- Less than 90 minutes processing time
- Same-day results for highly contaminated samples without enrichment
- Next-day results for enriched samples

Method performance characteristics:
The detection level is higher for the Bax Q7 method than for the reference method. This is due to the small sample volume required, only 50 µL. The limit of detection for Bax Q7 for this matrix is about 100 cfu/g, whilst the limit of detection for the reference method is 1- 10 cfu/g. As the matrix is cloacae swabs it is of no significance whether the limit of detection is 100 cfu/g or 1 cfu/g, as the levels will be considerably higher if *Campylbacter* is present.

For levels above 100 cfu/g, the sensitivity is satisfactory and there are no significant differences in the results obtained by Bax Q7 and the reference method. Both the sensitivity, selectivity and accuracy is 100%.

NordVal certification of chemical test kits

NordVal is expanding its field of work to include the validation of test kits / proprietary methods within chemical analysis. Such methods include analyses of mycotoxins, algae toxins, veterinary drugs, etc.

The steering group of NordVal will be expanded with chemists, one representative from each Nordic country. The representatives of NordVal have to be completely independent, and cannot have special interests in the production / sales of test kits, special reagents or apparatus that might raise doubt about their independency.

There certainly is a need to validate proprietary methods, that an independent third party can give recommendations based on documented results about the performance of the various test-kits. The first task of the new steering group will be to approve the protocol for the validation of chemical test kits, which has been elaborated by a Nordic project group.

If you are a manufacturer of a test kit and would like an independent review of your product, may be even obtain the first chemical NordVal validated product, please contact NMKL/NordVal.

Where to find information about NordVal certified methods

- NordVal has approved about 30 alternative microbiological methods.
- A list of approved methods is available at www.nmkl.org. Click on the NordVal logo and visit NordVal's Web pages.
- "List of methods" is the list of approved methods.
- "Certificates" are pdf files of the certificates. In the newer editions of the certificates, the results from the method validation are also given, which might be useful in the establishment of measurement uncertainty and when seeking accreditation for the method.

MoniQA Network of Excellence

MoniQA (Monitoring and Quality Assurance) is a European Network *of Excellence* (NoE) project working internationally for the harmonisation of analytical methods within food quality and food safety, as well as control strategies.

MoniQA is a 5-year EU funded project which started in 2007 with 33 partners from 20 different countries. The project is part of the EU's 6th Framework Program, and the coordinator is ICC in Vienna. The activities consist of a total of 10 work packages that include internal and external integration activities, research and communication of knowledge in the form of international conferences, educational programs and training. The funding of this project is based on the following factors:

- The implementation of new EU regulations in the food sector (e.g. food allergens, mycotoxins and food additives)
- Lack of communication between research and standardisation organisations
- Limited validity of standardisation / validation certificates for analytical methods, especially for rapid methods, alternative methods and test kits
- Increased demand for and development of rapid methods of measurement in the food sector
- Lack of proper validation protocols for new and rapid methods, including qualitative methods
- Lack of reference methods and reference materials for some analytes
- Lack of adequate tools for calculating the cost/benefit effect in relation to the introduction of regulations and directives in the food industry

MoniQA works actively in the field of food quality and food safety in a number of sectors and interest groups such as research, public administration, both nationally and internationally (EU), food industry, consumer organisations, manufacturers and suppliers of rapid analytical methods and international standardisation bodies. The project's steering committee includes representatives from international standardisation organisations (AOAC International, CEN), and a number of other organisations are associated partners.

One of the main activities of the project deals with the harmonisation and standardisation of analytical methods for monitoring and control of quality and safety in the food chain (WP4), and is coordinated by Nofima Mat, Norway. This constitutes a central part of the project's research program which aims to elaborate guidelines for validation and method performance criteria, with special emphasis on the new rapid methods and their applicability for routine use in the food sector. Five thematic working groups have been established, including microbiology, mycotoxins, chemical contaminants, allergens, additives and process contaminants and authenticity. The working groups contribute to the implementation of the working program of WP4 including the following tasks:

- Prioritising the issues (analytes - working groups)
- Interacting with stakeholders
- Identifying differences in practices related to the regulations (sampling, measurement uncertainty, interpretation of results and reporting) of the EU, and between EU and international trading partners
- Mapping and detecting deficiencies in validation protocols, validation criteria and validated methods
- Harmonising the validation criteria and terminology
- Elaborating a validation protocol for qualitative methods
- Conducting specific validation studies
- Harmonising bulk sampling protocols for loose commodities
- Developing harmonisation strategies and guidelines for the validation of new rapid methods

Some of the results already achieved in WP4 are:

- A first draft of harmonisation strategies and evaluation of new rapid analytical methods in food quality and food safety
- Establishment of the ICC / MoniQA journal QAS: Quality Assurance and Safety of Crops and Foods, published by Wiley Blackwell (first issue published in March 2009)
- First harmonised validation protocol for allergens in collaboration with AOAC: Guidance on a Harmonized Protocol Validation for Quantitative Food Allergen ELISA Methods
- Joint IUPAC / MoniQA protocol for validation of qualitative methods
- ISO International Workshop Agreement on the harmonisation of the "bulk" sampling procedures in cooperation with AOCS, ICC, ISO and CEN, and MoniQA.
- MoniQA's first international conference, Rome, 8-10 October. 2008
- Socio-economic MoniQA-EC Workshop "Recent developments on the methodology assessing the socio-economic impact of European food safety regulations, October 20, 2009"

Ongoing activities:

- Validation studies on selected allergens
- Proficiency tests of allergens in milk products
- Development of reference material for gliadin as a food allergen using ELISA kits
- Collaborative study for the validation of rapid methods for the determination of selected mycotoxins in foodstuffs
- Organization of MoniQA's second international conference in Krakow, Poland, May 2010.

For more information, see:
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Available NMKL Procedures (Guides)

(sorted by year of publication)

No. 23, 2008	Guide on quality assurance in microbiological laboratories.
No. 22, 2008	Considerations regarding evaluation of immunochemical test kits for food analysis.
No. 8, 2008, 4th Ed.	Measurement uncertainty in quantitative microbiological examination of foods.
No. 21, 2008	Guide for sensory analysis of fish and shellfish.
No. 20, 2007	Evaluation of results from qualitative methods.
No. 19, 2007	Guideline for sensorial analysis of food containers/packages.
No. 9, 2007, 2nd Ed.	Evaluation of method bias using certified reference materials.
No. 18, 2006	The use of reference materials, reference strains and control charts in a food microbiological laboratory.
No. 17, 2006	Guidelines for requirement specifications for food analyses.
No. 16, 2005	Sensory quality control. (Update of references in 2007.)
No. 4, 2005, 2nd Ed.	Validation of chemical analytical methods.
No. 1, 2005, 2nd Ed.	Calibration and performance checking of laboratory balances.
No. 15, 2004	Temperature control in microbiological laboratories.
No. 14, 2004	SENSVAL: Guidelines for internal control in sensory analysis laboratories.
No. 5, 2003, 2nd Ed.	Estimation and expression of measurement uncertainty in chemical analysis.
No. 13, 2003	Volumetric control.
No. 12, 2002	Guide on sampling for analysis of foods.
No. 11, 2002	Procedure for sensory analysis of drinking water.
No. 10, 2001	Control of Microbiological Media.
No. 7, 1998	Checking of UV/VIS spectrophotometers.
No. 6, 1998	General guidelines in quality assurance for laboratories performing sensory analysis.
No. 3, 1996	Control charts and control materials in internal quality control in food chemical laboratories.
No. 2, 1995	Performance check and in-house calibration of thermometers.