NMKL • Newsletter 76

NMKL - Nordic Committee on Food Analysis

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NMKL– Nordic Committee on Food Analysis

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NMKL
Secretary General
Hilde Skår Norli

New NMKL Procedure

NMKL Procedure No. 24, 2010: Guidelines for quality assurance for food chemical laboratories

The purpose of this procedure is to provide a guide which may be read and understood by all categories of personnel working with laboratory analysis.

These days, most laboratories are accredited and have established quality control systems. However, from time to time, it may be necessary for personnel to be updated and new co-workers to be given a general introduction to quality assurance.

The comprehensive procedure addresses:

- Quality systems and quality models
- Requirements for the laboratory, from management and organisation to locations
- Samples; sampling, registration, preparation and storage
- Methods: methods requirements, selection and use of methods
- Equipment and chemicals
- Calibration, standardisation and adjustment
- Requirements for documentation of the work
- Quality control in the analytical work
- Subcontractors
- Complaints
- Abnormal/deviations in tests

NMKL Procedure No. 24 is a revision and replacement of NMKL Report No. 8, 1997.

This procedure has been elaborated by a project group, funded by NMKL, consisting of:

Lisbeth Tillge Lund, DANAK, Denmark (Project leader)
Christina Bäckman, Finnish Food Safety Authority (Evira), Finland
Dag Grønningen, National Veterinary Institute, Norway
Ulf Bondesson, National Veterinary Institute, Sweden.

Lisbeth T. Lund, Project leader, former employed by DANAK, the national accreditation organisation of Denmark

THE 64th NMKL ANNUAL MEETING

The 64th NMKL Annual Meeting was held at Hotel Ebeltoft Strand, Ebeltoft, Denmark, on 21-24 August 2010. About 40 members of the NMKL National Committees in Denmark, Finland, Iceland, Norway and Sweden participated.

"We are NMKL, and NMKL will only be as good as we make it"

NMKL's chairman Ulla Edberg,

Ulla Edberg, the Chairman of NMKL opened her report with the following statement: "It feels great to be standing here as your Chairman. When I took over the chairmanship last year, I received congratulations from all around the world, not only from Nordic colleagues. This shows once again, that the NMKL's field of work extends far beyond the Nordic region. But then NMKL has stakeholders in more than 40 countries worldwide.

Measured in years, NMKL is certainly an old organisation. It began its work in 1947, aiming to support the Nordic governments' commitment to ensure safe and good quality of food by developing analytical methods for food analysis. This was needed as the trading of food from different countries increased after the Second World War.

Today, the food production is global and the trade in natural resources is worldwide in an extent far beyond our dreams back then when NMKL was established. Consequently, this means that the quality of analytical methods, and the entire process from sampling, analysis and reporting of results is as important as, or even more important today, than it was 63 years ago. A very big difference between back then and now is that there are fewer laboratories analysing food in the Nordic countries. This means that a network organisation like NMKL has an important role in the Nordic countries' authorities, food industries and laboratories. "

Edberg closed her speech by stating: "When I look back at my first year as the Chair of NMKL, I am very pleased to note that NMKL has accomplished its mission in every way. Through NMKL's expertise and sound reputation, the Nordic countries have a significant influence, and are involved in the development of the whole chain from sampling and analysis to reporting results.

I extend my warm thanks to all of you for your dedication and skilled work in the past year. We are NMKL, and NMKL will only be as good as we make it. So thank you to the referees, the project managers, the chairmen of the sub committees and their secretaries, and the national chairmen and their secretaries. And of course, many thanks to all your employers."

Focus on politically important issues

Organisationally, NMKL is related to the Nordic Council of Ministers (NMR) in the area of food cooperation (Nordic Committee of Senior Officials for Fisheries and Aquaculture, Agriculture, Food and Forestry, EK-FJLS).

The contact person at NMR, Lene Breum Larsen, informed the participants about the work in NMR on foods.

NMR is a political organization, and there is an increasing focus on politically important areas within the Nordic cooperation.

The Nordic countries collaborate on the following matters:

- protecting consumers from health risks through good food safety
- improving animal health and welfare
- promoting informative labeling and marketing of food produce
- ensuring that foodstuffs can be traced from the consumers back to the refrigerated display and, ultimately, the producer, farmer or fishing company

NMR contact person Lene Breum Larsen

- encouraging healthier eating habits and lifestyles
- promoting co-operation between industry, consumers and official bodies
- building networks that collate, use and communicate knowledge of food issues.

Lene Breum Larsen also informed about ongoing activities in the three working groups of the food committee of NMR, respectively:

• **Nordic Working Group for Food, Nutrition and Toxicology (NKMT)** is responsible for the implementation of the Nordic Action Plan for improved health and quality of life through food and physical activity. NKMT has included a key-hole label as a brand for products that represent healthier alternatives. NKMT is involved in the update of the Nordic Nutrient Recommendations.

• **Nordic Working Group for Microbiology and Animal Health/Animal Welfare (NMDD)** is responsible for ensuring that the Nordic authorities for animal welfare and feeds work together in an effective way.
NMDD is working on establishing national and international preparedness efforts against serious infectious animal diseases.

• **Nordic Working Group for food and consumer information (NMF)** is responsible for activities within legislation, inspection and control, labeling and consumer information. NMF works to achieve equivalent control systems in the Nordic countries, and to strengthen the competence of the control authorities, which strengthens the Nordic voice in the EU cooperation.

For further information please visit www.norden.org.

Updates from NMKL's Partners

CEN - the European Standardisation Organisation

Lars Jorhem, Chair of WG 9, represented NMKL in addition to Sweden at the CEN/TC 275

Lars Jorhem, National Food Administration, Sweden, reported from the CEN Technical Committee meeting on Foods, CEN / TC 275, which is held annually at DIN (Deutsches Institut für Normung).

CEN / TC 275 has 14 working groups. In each of the working groups, NMKL has a representative, who participates actively, ensuring the interests of the Nordic countries and is the link between the working group and NMKL. Thus, several NMKL methods have been forwarded to CEN and have become CEN methods (EN standards), or have been made out in collaboration between the organisations. At the CEN/TC 275 meeting, the chairs of the working groups give reports of their work and overall decisions are made.

The working groups of CEN/TC 275

- WG 1 Sulphite
- WG 2 Sweeteners
- WG 3 Pesticides / fatty
- WG 4 Pesticides / not fatty
- WG 5 Biotoxins
- WG 6 Microbiology
- WG 7 Nitrate and nitrite
- WG 8 Irradiation
- WG 9 Vitamins
- WG 10 Heavy Metals
- WG 11 Genetically modified organisms
- WG 12 Allergens
- WG 13 Neoformed contaminants
- WG 0 Policy Guides

A report from this meeting is available from NMKL.

AOAC International

Hilde Skår Norli, NMKL Secretary General and Director at the AOAC International Board of Directors

In addition to giving updates on NMKL and NordVal activities, the NMKL Secretary General Hilde Skår Norli, also informed about some ongoing activities in AOAC International, where she is a Director in the Board of Directors.

For many years, NMKL has had a good collaboration with AOAC International, and again NMKL contributes with a method in the AOAC's "Ten Methods Project". AOAC want to strengthen their brand of high-tech methods by selecting 10 methods of high quality to be validated. It comes as no surprise that an NMKL method is among these, namely NMKL Method No. 186: "Heavy metals - As, Cd, Hg and Pb. Determination by ICP-MS after acid digestion under pressure."

From a meeting in Sub committee 1

Due to the oil catastrophe in the Gulf of Mexico, AOAC has called for relevant PAH methods. The experts have reviewed the forwarded methods and chosen the "best method" to be studied collaboratively. The study is already in process.

Moreover, AOAC has started projects for renewing the methods for vitamin A, vitamin B12, vitamin D, Folate and Inositol in infant formula and adult nutritional formula, and are calling for methods for this. When the experts have selected the best method that meets the established criteria, the methods will be collaboratively validated. This was also discussed at the AOAC International 126th Annual Meeting, which was held in September in Orlando, Florida.
Please visit www.aoac.org for further information.

NMKL's working programme

Eurofins, Steins sponsored an excursion for the participants

At the NMKL Annual Meeting, analytical methods, guidelines and the planning of courses/seminars/workshops are discussed in the scientific committees; microbiology, chemistry and sensory analyses, which are chaired by:

- Flemming Hansen, Technological Institute, Denmark (microbiology)
- Harriet Wallin, Evira, Finland (chemistry)
- Gunnar Forsgren, Iggesund Paperboard, Sweden (sensory)

COMMON PROJECTS

• **Revision of NMKL Procedure No. 2, 1995: Performance check and in-house calibration of thermometers** Project leader: Håkan Johnsson, Sweden

• **Revision of NMKL Procedure No. 12, 2002: Guide on sampling for analysis of foods** Project leader: Per Erik Clasen, National Veterinary Institute, Norway

MICROBIOLOGICAL PROJECTS

- ***Enterococcus*. Determination in foods and animal feeding stuffs**
Referee: Niels Skovgaard, Denmark

- ***Bacillus cereus*. Determination in foods**
Referee: Cecilie From, Norwegian School of Veterinary Science, Norway

- **Botulinum toxin. Detection in foods, blood and other samples**
Referee: Miia Lindström, the University of Helsingfors, Finland

- ***Yersinia enterocolitica*. Detection in foods**
Referee: Saija Hallanvuo, EVIRA, Finland

- **Patogenic *Yersinia enterocolitica*. PCR methods for detection in foods**
Referee: Susanne Thisted Lambertz, National Food Administration, Sweden

- ***Shigella*. Detection in foods (culture method)**
Referees: Gro S. Johannessen and Tone M. Fagereng, National Veterinary Institute, Norway

- ***Shigella*. Detection in foods (PCR method)**
Referee: Catarina Nilsson, National Food Administration, Sweden

- ***Listeria monocytogenes*. Detection in foods and animal feeding stuffs and enumeration in foods**
Referee: Semir Loncarevic, National Veterinary Institue, Norway

- **Verocytotoxin producing *E. coli*. Detection in foods**
Referee: to be appointed

- **Gram negative bacteria in milk and cream. Detection of re-contamination.**
Referee: Anders Christiansson, Swedish Milk, Sweden

- **Quality assurance of PCR analyses**
Project leader: Lisa Lundberg, National Food Administration, Sweden

- ***C. difficile*. Detection in foods**
Referee: to be appointed

NMKL calls for laboratories to participate in a collaborative study on:

Plant stanols and plant sterols. Determination in phytosterol enriched foods with a gas chromatographic method

This method describes a gas chromatographic determination of the contents and compositions of plant stanols and plant sterols in phytosterol enriched foods. The method can also be applied to phytosterol fatty acid ester ingredients being either plant stanol or plant sterol fatty acid esters. The method can also be applied to measure cholesterol content of phytosterol enriched foods.

For more information and If you are able to participate, please contact Päivi Laakso, Raisio Nutrition Ltd., Eurofins, Finland (e-mail:PaiviLaakso@eurofins.fi)

CHEMICAL PROJECTS:

- **Methylmercury. Determination in foods by using Isotope Dilution GC-ICPMS**
  Referee: Stig Valdersnes, NIFES, Norway
- **Histamine. Determination in fish and fish products by HPLC**
  Referee: Jarle Wang Andersen, Nofima (BioLab Analyses), Norway
- **Plant sterols and stanols: Determination in phytosterol enriched foods by GC**
  Referee: Päivi Laakso, Ravintoraisio Ab, Finland
- **Nitrate and nitrite. Spectrophotometric determination of nitrate and / or nitrite in foods and water after reduction with zinc and Griess reaction**
  Referee: Leonardo Merino, National Food Administration, Sweden

SENSORIAL PROJECTS:

- **Sensory quality control test of drinking water**
  Referee: Leena Lilleberg, Evira, Finland
- **Procedure: Measurement uncertainty in sensory analysis**
  Project leader: Per Lea, Nofima Mat, Norway
- **Procedure: Reporting of sensorial data**
  Project leader: Per Lea, Nofima Mat, Norway
- **Procedure: Sensorial analyses of meat and meat products**
  Project leader: Camilla Bejerholm, Technological Institute, Denmark
- **Guidelines for sensory evaluation of bread**
  Project leader: Mari Sandell, the University of Åbo, Finland
- **Procedure: Recruit/ training and inspection of analytical panels. Training of assessors**
  Project leader: Grethe Hyldig DTU Aqua, Denmark
- **Fat. Determination in foods by NMR (Nuclear Magnetic Resonance)**
  Referee**:** Päivi A. Teivainen-Lædre, Skretting, Norway
- **Banned dies in spices**
  Referee: to be appointed
- **Determination of net weight and drained solids in canned fruits, vegetables and other products**
  Referee: Astrid Nordbotten, Norwegian Food Safety Authority, Norway
- **Folat in milk and milk products**
  Referee**:** Hanna Sara Strandler, National Food Administration, Sweden
- **Calibration of NIR and IR for analyses of nutritionals in foods**
  Project leader: Erik Nordkvist, National Veterinary Institute, Sweden
- **Estimation and expression of recovery**
  Project leaders**:** Arne Højgård Jensen, Danish Veterinary and Food Administration, Håkan Johnsson and Lars Jorhem, National Food Administration, Sweden

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- **Methods for binomial tests**
  Project leader: Gunnar Forsgren, Iggesund Paperboard, Sweden
- **Sensorial analyses of fruits and vegetables**
  Project leader: To be appointed

The sensory committee arranged sensorial tests for the participants

THE 10 YEAR ANNIVERSARY OF NORDVAL

The official programme of NordVal started 1 January 2000, and thus NordVal celebrates its 10th anniversary this year.

Sven Qvist, the Chair of NordVal and initiator

The background for establishing NordVal dates back to 1993, when there was an extensive *Salmonella* outbreak in Denmark. There was a need for rapid methods, both within the food industry and the food control authorities, and it was soon realised that it was a need for a third party for reviewing these methods. Therefore, the Danish Veterinary and Food Administration established DanVal in 1995.

Sven Qvist was the initiator and chairman of the organisation. Several of the members of DanVal were also members of NMKL, and it did not take long before it was discussed whether DanVal methods also should become NMKL methods, and whether NMKL would consider evaluating test kits. The national committees found that the NMKL did not have the capacity for undertaking this work. NMKL requested the food committee of the Nordic Council of Ministers to establish a Nordic system for reviewing and approving alternative methods. So they did, and DanVal became NordVal. The secretariat was kept in Denmark until 2007. Then NordVal was incorporated into NMKL, and the secretariat of NordVal was moved to Norway.

The NordVal's 10 Year Anniversary Symposium 20 August 2010 was a successful event with 71 participants and 5 exhibitors from 13 countries.

The Chairman of NordVal, Sven Qvist, opened the meeting by giving an account of the background of NordVal. Representatives from research institutions, food laboratories, food and test kits manufacturers contributed to the symposium.

This year, NordVal has expanded its programme to include chemical methods, and hence, the steering committee has been extended with chemical experts. The chemical steering group has worked on the completion of the protocol for how to validate rapid chemical methods / test kits / proprietary methods. This protocol and the protocol for how to validate the microbiological alternative methods are available on NordVal's website.

This year, the NordVal steering has consisted of the following persons:
Denmark:
Sven Qvist (Chairman)
Arne Højgaard
Finland:
Maria Sjöman
Maija Hatakka
Mika Tuomola
Iceland:
Franklin Georgsson
Heida Palmadottir
Norway:
Kjell Hauge
Marianne T. Werner
• Sweden:
Åsa Rosengren
Ylva Sjögren
The NMKL Secretary General is the secretary of the group.

The NordVal certificates include the scope and the principle of the method, as well as the results from the validations studies. The methods are reviewed every 2 years.

On the same day as the NordVal symposium, **NordVal signed an agreement with the MicroVal.** The organisations agreed to intensify the cooperation concerning the validation of rapid methods. This would be beneficial for the applicants as the study requirements from both organisations can be considered at the same time and the studies been done simultaneously. Both organisations' procedures are followed, and the organisations are carrying out their own reviewing process.

NMKL calls for laboratories to participate in a collaborative study on:

Methyl Mercury. Determination by isotope dilution GC-ICPMS in foodstuffs.

The method is intended for quantitative determination of monomethyl mercury (MMHg) (10-5000 µg/kg dry weight) in mainly marine biota samples.

Samples are spiked with Hg-isotope enriched with MMHg and extracted using tetramethylammonium hydroxide. After pH adjustment, derivatisation and extraction, the organic phase is analysed using GC-ICPMS. The GC separates the different mercury species before MMHg is atomised and ionised by the high temperature of the ICP. The ions are extracted from the plasma and transferred to a MS where the ions are determined by a pulse-count and/or analogue detector. Calculation of the result is done using the isotope dilution equation. Fourteen samples are to be analysed at each laboratory.

Please contact Stig Valdersnes (e-mail: stig.valdersnes@nifes.no) for further information and participation in the collaborative study.

NEW NMKL METHODS

NMKL Method No. 10, 4. Ed, 2010: Gravimetric determination of fat in milk, cream and milk powder.

This method is based on the ISO|IDF standards:

- ISO 1211:2010 | IDF 1:2010
- ISO 2450:2008 | IDF 16:2008
- ISO 1736:2008 | IDF 9:2008

These methods are endorsed in Codex Alimentarius.

This method describes a reference method for the determination of fat content in raw and processed liquid milk, partly skimmed milk, skimmed milk and buttermilk with not less than 0.1% fat, cream, milk powder and milk powder products.

Samples where separation or splitting of fat has occurred cannot be analysed by this method. Such samples may, if necessary, be analysed using a SBR method such as ISO 1735:2004|IDF 5:2004; however, the reliability may be reduced.

Principle: An ammoniacal ethanolic solution of the test sample is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation, and the weight of the extracted substances determined.

The changes in this edition of the method consist of an update of the references of the IDF standards and inclusion of the corresponding ISO standards.

NMKL Method No. 94, 3. Ed. 2010: Lead and cadmium, release from ceramic articles

This method describes the quantitative determination of the release of lead and cadmium from ceramic articles intended to or otherwise suitable to be used in contact with food.

The surface of the ceramic article which would normally come into contact with food is extracted with a 4 % solution of acetic acid at 22 ± 2 °C for 24 ± 0.5 hours. Determination of lead and cadmium in the extraction solution is made by atomic absorption spectrometry.

This method has not been validated in a collaborative study. The main reason for this is the difficulties in producing sufficiently homogenous test items. In a proficiency test (PT) arranged in 2009 by the National Food Administration (NFA), Sweden, ceramic mugs, prepared with sufficient amounts of lead and cadmium so that they released relevant amounts of the metals, were used as test samples. The laboratories used NMKL 94 or an equivalent method. The results from this PT give an indication of the method performance at one level of lead and cadmium. The whole procedure, including the extraction process and homogeneity of the test material, shows a satisfactory precision.

Lars Jorhem, NFA, has included the results from the PT-scheme in the method.

NMKL Method No. 136, 5. Ed., 2010: *Listeria monocytogenes.* Detection in foods and feeding stuffs and enumeration in foods

The method is intended for the detection of *Listeria monocytogenes* in foods and feedstuffs and for the enumeration of *L. monocytogenes* in foods.

The method describes both the detection and the enumeration procedure of *L. monocytogenes*.

In the **detection** procedure, two-step enrichment is used. The primary enrichment is carried out in an enrichment broth with reduced selectivity (Half-Fraser broth) at 30 °C for 24 h. The primary enrichment culture is further enriched in a secondary enrichment broth with full selectivity (Fraser broth) at 37 °C for 48 h. The cultures obtained from both the enrichments are plated out on two selective solid isolation media; first either Agar Listeria according to Agosti and Ottaviani (ALOA) or Listeria Chromogenic Agar (LCA) or Listeria monocytogenes blood agar medium (LMBA) or a chromogenic Listeria agar medium with equal operating principle as ALOA, and second on a solid selective isolation medium with another diagnostic principle than the first one, after the choice of the user of the method. After incubation, presumptive *L monocytogenes* colonies are confirmed by appropriate biochemical and morphological tests.

In the **enumeration** procedure, the initial suspension and/or its dilutions, carried out either in Saline peptone water (SPW), Buffered peptone water (BPW) or base medium for Half-Fraser broth, are surface plated on a *L. monocytogenes* specific isolation medium, ALOA, LCA or LMBA or chromogenic Listeria Agar with equal operating principle as ALOA. After incubation, presumptive *L. monocytogenes* colonies are counted and confirmed using appropriate morphological and biochemical tests.

The collaborative study showed that there were no statistical differences, neither in the qualitative nor the quantitative part, in the results by use of the different selective media. The media included were ALOA, OCLA, LMBA and LCA.

NMKL finds the method comparable with ISO 11290 provided ALOA is used as the first selective medium in the detection procedure and as the only medium in the enumeration procedure. ISO 11290 is given as reference method in the Microbiological criteria, EU 2073/2005.

From NMKL Method No.136, 4th ed., 2007, the following revisions have been done:

1. it is described how the qualitative and quantitative part can be carried out simultaneously,
2. a flow diagram has been provided,
3. the number of colonies for verification have been clarified.

The revision has been carried out by
Semir Loncarevic and Gro S. Johannessen, National Veterinary Institute, Oslo, Norway, Sven Qvist, Denmark, Margret Geirsdottir, Matis, Iceland, Tuula Johansson, Evira, Finland, Charlotta Engdahl Axelsson, Eurofins, Sweden.

Referee: Semir Loncarevic, National Veterinary Institute, Oslo, Norway

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	NOK	EUR
Subscription fee (annually) PDF-files, hardcopies, online for 1-3 user	2.500	300
New online subscription (first year)/ Compiled collection	5.000	600
Method (single)	500	60
Procedures (less than 30 pages)	400	50
Procedures (above 30 pages)	600	70
Reports	300	40
Technical Reports	Free of charge	
Protocols	Free of charge	

NMKL Method No. 67, 6. Ed., 2010: Presumptive *Bacillus cereus*. Determination in foods

Referee: ***Cecilie From****, the Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Norway*

This method describes the enumeration of presumptive *Bacillus cereus* in foods.

Presumptive *B. cereus* is determined quantitatively by plating known amounts of sample on blood agar, or alternatively by replicate plating on blood agar and *Bacillus cereus* selective agar or the chromogenic plating medium Cereus-Ident-Agar. After incubation at 30 °C for 24 ± 3 hours typical colonies with a hemolytic zone are counted.

The colonies are confirmed on *Bacillus cereus* selective agar or Cereus-Ident-Agar. Presumptive *B. cereus* forms bluish colonies surrounded by egg yolk reaction (lecithinase) on *B. cereus* selective agar. On Cereus-Ident-Agar presumptive *B. cereus* grows as blue-turquoise colonies with species-specific morphology, sometimes surrounded by a blue halo.

The *Bacillus cereus* group comprises the species *B. cereus*, *Bacillus weihenstephanensis*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides* and *Bacillus anthracis*.

The method does not distinguish between *B. cereus*, *B. thuringiensis* and *B. weihenstephanesis*, whilst *B. mycoides, B. pseudomycoides* and *B. anthracis* can be distinguished morphologically on blood agar and Cereus-Ident-Agar. For *B. thuringiensis* this distinction must be performed microscopically by examining whether the bacteria produce para-sporal crystals.

B. weihenstephanensis can be distinguished from *B. cereus* by growth temperature (growth below 7 ºC, but not above 43 ºC) and PCR methods in an expert/reference laboratory. All three species form spores after 1-2 days on most media. Because they are very closely related and all three may produce enterotoxins, differentiation in foods is rarely necessary.

B. cereus is able to produce an emetic toxin (cereulide) and three cytotoxins (Hbl, Nhe and CytK) involved in the *B. cereus* diarrhoeal syndrome. Isolates of *B. cereus* can be investigated for toxin production in an expert/ reference laboratory.

This method has been validated in a collaborative study arranged by Ia Pudas and Christina Normark at the National Food Administration, Sweden.

13 laboratories participated in the study. Each laboratory analysed 16 samples. The study did not include Cereus-Ident-Agar, but assessment of this medium has been performed in the scientific article which is described in the method. The collaborative study organised in 2002 was conducted on rice, porridge, pea soup and cous-cous. The results were satisfactory with a reproducibility of 0.15 – 0.35 log cfu/g.

It is brought to your attention that the highly toxic, cytK-producing *B. cereus* NVH 0391-98 involved in a fatal food poisoning outbreak in France in 1998 fails to identify on Cereus-Ident-Agar. The isolate grows with species-specific morphology on blood agar and *B. cereus* selective agar.

The main changes made in this edition of the method are a more precise description and the inclusion of the Cereus-Ident-Agar as an alternative media.

The method has been audited by Cecilie From the Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Norway.

Other experts who have contributed are: Kari Dommersnes Eurofins, Norway, Niels Skovgaard, Denmark, Seija Kalso, Helsinki Environment Center, Finland, Franklin Georgsson, Matis, Iceland, Christina Normark, National Food Administration, Sweden.

IMPORTANT!

Correction to NMKL Method No. 91, 5. Ed., 2010:

"Preparation of the test sample and initial supension of food and animal feeding stuffs for quantitative microbiological examination"

clause 4.1.2:

Buffered Peptone Water (BPW). The weight of Pepton should be **10.0 g** and not 20.0 g.

The receipt should be:

Peptone	10.0 g
Sodium chloride	5.0 g
Na_2HPO_4 x12 H_2O	9.0 g
KH_2PO_4	1.5 g
Distilled water or equivalent	1000 mL

Renewal of NordVal Certificate No. 031: *Salmonella* detection method by Real-Time PCR

For a method to receive a NordVal certification, it is required that the method is extensively validated by an expert laboratory (in a comparison study), and then validated in a collaborative study, organised by the expert laboratory. The method is compared to a reference method.

The Danish Meat Research Institute at the Danish Technological Institute, has received a renewed certificate for the enumeration of *Salmonella* using Real-Time PCR. The method is tested on raw meat, including poultry neck skin, swabs from cattle and pig carcasses, and fecal samples (poultry boot swabs).

The method's performance, such as selectivity, sensitivity, specificity and accuracy, was tested against the following reference method:

- NMKL 71, 5th Ed., 1999: *Salmonella* spp.. Detection in foods. NMKL 187, 2007: *Salmonella* spp.. Detection in foods, faeces and materials from primary animal production using MSRV.

The method is based on a shortened pre-enrichment in buffered peptone water for 10-16 hours. DNA is extracted by use of the KingFisher system and the "MagneSil KF Genomic System". Subsequently, *Salmonella* is detected by a novel Real-Time PCR assay, developed for the specific detection of *Salmonella*.

The results of the validations were acceptable with a sensitivity of 95% and higher. There were no statistical differences in the performances between the methods.

New NordVal Certificate No. 041: *Salmonella* detection method; DNA extraction by boiling followed by real-time PCR

The Danish Meat Research Institute at the Danish Technological Institute, Danish Meat Research Institute applied for an extension of NordVal certificate No. 031, a modification of the method described above. However, as the original method is still in use, it was decided to issue a new NordVal certificate for the new method.

The modification consists of a DNA extraction by boiling as an alternative to the KingFisher system. This makes the method easier and cheaper to perform. Method comparisons have been conducted on the different extraction techniqes, by comparing this method (extraction by boiling) with the method described in NordVal 031. Moreover, the method is compared with NMKL 187, 2007: "*Salmonella*. Detection in foods, faeces and materials from primary animal production using MSRV."

The method is tested on raw meat and swabs from beef and pork carcasses.

This year (2010) a comparison study was carried out on 60 samples of ground beef, veal and swab samples from pigs. 30 of the samples were left uninoculated, 15 samples were artificially inoculated with 1-10 CFU *Salmonella Typhimurium* and 15 samples were inoculated with 10-100 CFU *Salmonella Typhimurium*.

There were no statistically significant differences in the results obtained with the three methods; sensitivity, specificity, accuracy and agreement between the methods were acceptable.

This year, a collaborative validation of the method was also organized. 12 laboratories analyzed in duplicate three samples of ground beef and swabs of animal carcasses. The results were satisfactory, except for the results obtained by one laboratory which reported positive results for all the negative controls. This laboratory was therefore excluded from the evaluation of the results.

The selectivity is a measure of how well the method manages to detect the target organisms from a wide range of strains (the inclusivity), and the lack of interference from a relevant range of non-target microorganisms (the exclusivity).

For quantitative analysis, the inclusivity is determined by selecting at least 30 pure cultures of target microorganisms relevant to the method and the food matrices.

For quantitative analysis, the exclusivity is determined by selecting at least 20 pure cultures of non-target microorganisms chosen from both the strains known to cause interference with the target microorganism, and from strains naturally present in the food matrices.

The compliance and precision are examined by the expert laboratory by analysing a minimum of five replicates at five different levels (low, medium and high) of the target microorganisms in relevant food samples using both methods. For horizontal methods, five food categories are required.

Renewing of the NordVal certificate from HyServe GmbH & Co. KG, Uffing, Germany, on the following products:

Compact Dry TC Method for the enumeration of total viable organisms
Compact Dry ETB Method for the enumeration of *Enterobacteriaceae*
Compact Dry CF Method for the enumeration of total coliforms
Compact Dry EC Method for the enumeration of *Escherichia coli* and total coliforms

The products are produced by Nissui Pharmaceutical, Japan.

CCFRA Technology Limited, Chipping Campden, England was the expert laboratory in the testing of the Compact Dry methods. The validation was carried out in accordance with ISO 16140.

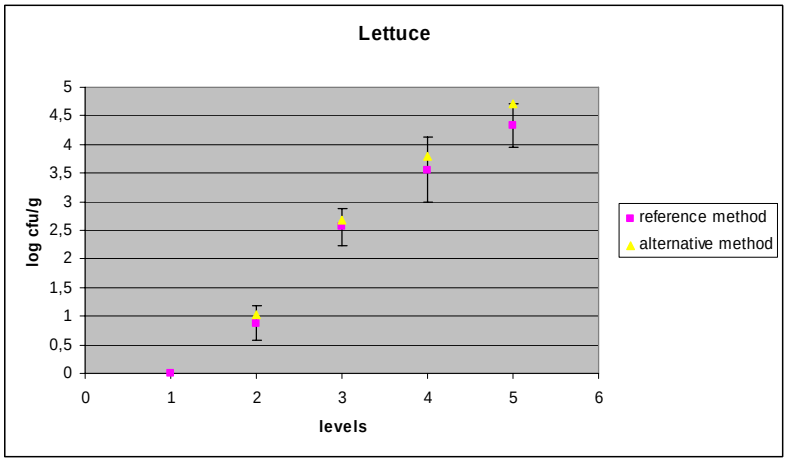
Comparison of the Dry Compact methods against reference methods

The Compact Dry methods (alternative methods) were compared with ISO methods (reference methods).

The expert laboratory examined the selectivity of the method. For the Compact Dry methods, the selectivity was satisfactory.

The method's reliability/precision: The method studies carried out by CCFRA on the Compact Dry Methods in 2007, included cooked chicken, frozen fish, lettuce, milk powder and raw meat. Five levels of contamination were used for each food matrix. For all foods, except the milk powder, naturally contaminated samples were tested. Five replicates were analysed at each level.

The mean and the standard deviation of the results obtained by both the reference method and the alternative method were calculated. The obtained mean values were plotted in a graph as shown below. In addition to the mean, the confidence levels (± 2 times the standard deviations) of the results obtained by the reference method were also plotted. Thus, it is easy to illustrate whether the alternative method gives the same results as the reference method:



According to NMKL Procedure No. 8, the precision (standard deviation) should not be above 0.4 log cfu/g.

When the results obtained by the alternative method fall within the confidence levels of the results obtained by the reference method, there are no significant differences in the results obtained by the two methods. Further, the precisions (the standard deviations) have to be satisfactory for both methods.

Collaborative studies of the Compact Dry Methods

The collaborative studies of the five Compact Dry Methods involved more than 10 laboratories. Each Compact Dry Method was compared with a current ISO Standard. The laboratories analysed duplicates of milk samples on four levels including blind samples with the respective methods.

The mean values and standard deviations were calculated. The precisions, both repeatability and reproducibility, were satisfactory for both the Compact Dry methods and the reference methods.

All the results of the Compact Dry methods were included in the confidence levels of the reference method. Thus, the collaborative study also showed that we will obtain comparable results with Compact Dry methods and the respective ISO methods.

Compact Dry TC Method for the Enumeration of Total Viable Organisms in Foods.

NordVal Certificate No. 033

Compact Dry TC is a ready-to-use, dry, chromogenic plate for the enumeration of total viable organisms count.

An aliquot of 1 ml of an appropriate dilution is plated onto a Compact Dry TC plate. The incubation conditions tested in the study were 30 ± 1°C for 48 ± 3h and 72 ± 3h, respectively.

Compact Dry TC was compared to ISO 4833:2003: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count techniques at 30°C."

In the comparison study, the lowest validated levels with satisfactory results were:

chicken:	2.6 log cfu/g
frozen fish:	3.0 log cfu/g
lettuce:	3.4 log cfu/g
milk powder:	2.1 log cfu/g
raw meat:	3.4 log cfu/g

Compact Dry ETB Method for the Enumeration of *Enterobacteriaceae* in Foods.

NordVal Certificate No. 034

Compact Dry ETB is a ready-to-use, selective plate containing glucose for the enumeration of *Enterobacteriaceae*.

An aliquot of 1 ml of an appropriate dilution is plated onto a Compact Dry ETB plate. The plate is incubated at 37 ± 1°C and colonies (red/purple) are counted after 24 ± 2h.

Compact Dry ETB was compared to ISO 21528-2:2004: "Microbiology of foods and animal feeding stuffs. Horizontal method for the detection and enumeration of *Enterobacteriaceae* - part 2: Colony Count Method."

In the comparison study, the lowest validated levels with satisfactory results were:

chicken:	0.8 log cfu/g
frozen fish:	2.0 log cfu/g
lettuce:	2.6 log cfu/g
milk powder:	2.0 log cfu/g
raw meat:	3.4 log cfu/g

Compact Dry CF Method for the Enumeration of Total Coliforms in Foods.

NordVal Certificate No. 035

Compact Dry CF is a ready-to-use, dry, chromogenic plate for the enumeration of coliforms.

An aliquot of 1ml of an appropriate dilution is plated onto a Compact Dry CF plate. The plate is inverted and incubated at 37 ± 1°C and colonies (blue/blue green) were counted after 24 ± 2h.

Compact Dry CF Method was compared to ISO 4832:2006: "Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of coliforms -- Colony-count technique."

In the comparison study, the lowest validated levels with satisfactory results were:

chicken:	0.9 log cfu/g
frozen fish:	1.2 log cfu/g
lettuce:	2.0 log cfu/g
milk powder:	2.0 log cfu/g
raw meat:	3.4 log cfu/g

As for the method selectivity, the Compact Dry CF is somewhat more selective than ISO 4832. For exclusivity 3 strains interfere on Compact Dry CF while 9 strains interfere in the ISO 4832, giving false positives.

In the collaborative study there were no significant differences in the results obtained by the reference method and Compact Dry CF method.

Please visit NMKL's homepage, www.nmkl.org, for information on

- NMKL Methods
- NMKL Procedures
- NMKL Reports
- PT- schemes
- National reference laboratories in the Nordic countries
- NordVal Protocols
- NordVal Certificates
- List of certified NordVal Methods
- Upcoming NMKL/ NordVal courses/ seminars

Compact Dry EC Method for the Enumeration of *Escherichia coli* and total coliforms in foods.

NordVal Certificate No. 036

Compact Dry EC is applicable for the enumeration of *E. coli* and/or determination of the number of total coliform bacteria in foods.

An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry EC plate. The incubation conditions tested in the study were 37 ± 1°C for 24 ± 2h. For the deterimination of *E.coli* count the blue colonies after 24 ± 2 hours. For the determination of the total coliforms red colonies are counted in addition to the blue ones.

The Compact Dry EC method was compared with ISO 16649-2:2001: "Horizontal method for the enumeration of beta-glucuroni-dase-positive *Escherichia coli* -- Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide."

For the determination of total coliforms, the Dry Compact EC method was compared with the ISO 4832:2006: Microbiology of foods and animal feeding stuffs. Horizontal method for the enumeration of coliforms -- Colony-count technique.

In the comparison study, the lowest validated levels with satisfactory results were respectively:

For the enumeration of *E.coli*:	For the enumeration of the total coliforms:
cooked chicken: 0.8 log cfu/g frozen fish: 1.9 log cfu/g lettuce: 1.0 log cfu/g milk powder: 2.8 log cfu/g raw meat: 3.4 log cfu/g	coocked chicken: 0.9 log cfu/g frozen fish: 1.0 log cfu/g lettuce: 2.0 log cfu/g milk powder: 2.7 log cfu/g raw meat: 3.5 log cfu/g

Are chemical test kits validated?

For more than 10 years, microbiological test kits have been evaluated by an independent third party to obtain documentation on the methods' performance and the compliance of the manufacturers' information about the methods' applicability and performance.

What is the status of chemical test kits? Are they validated? Can we rely on the validation data and that the validations are carried out in a satisfactory manner?

Have they been reviewed by an independent third party, or do the laboratories have to make extensive validation studies themselves?

Without doubt, it would be extremely resource-efficient and confidence –inspiring for laboratories to use test kits that are reviewed by an independent third party. Test kit manufacturers using independent evaluation/certification of their products, will seem highly professional and thereby achieve favorable market shares.

NordVal can offer this type of evaluation. NordVal has recently started a program for evaluation / certification of chemical test kits, and would be happy to cooperate with manufacturers to evaluate chemical methods as well.

Please contact the NordVal / NMKL Secretariat for further information.

Quality Management for Sanitary Laboratories involved in Food Surveillance

Workshop, Havana, Cuba, 22 – 24 November 2010

Photo: Lars Jorhem
From the streets of Havana

Photo: Lars Jorhem
Coming home from sunny Havana at 25°C to –15°C must have been quite a shock.

This workshop was held by the National Food Administration (NFA), Sweden, and the NMKL in coordination with the Cuban Technical Committee of Standardisation N^{o} 46 on Methods of Analysis and Sampling and the Cuban National Network for Food Analysis Laboratories.

About 35 persons from Cuba, Brazil, Costa Rica and Nicaragua participated.

The workshop included

- An overview of Quality Assurance (ISO 17025)
- Basic statistics
- Validation (NMKL Procedure No. 4)
  - The strategy for validating an analytical method
  - Experimental design
  - How to handle precision, sources of variability
  - Control charts
  - Recovery
- Sampling (NMKL Procedure No. 12)
- Measurement uncertainty (NMKL procedure No.5)
- Traceability - use of reference materials (NMKL Procedure No. 9)

The NMKL Procedures have been translated into Spanish by the Cuban Technical Committee of Standardisation.

The workshop was organised by Nelson Fernandez (Cuba), Leonardo Merino (Sweden), Lars Jorhem (Sweden) and Ulla Edberg (Sweden).

In the evaluation of the workshop, the participants were very pleased with the workshop and gave top score (in a scale from 1-10) to the workshop and the NMKL Procedures' practicability and applicability.

The NMKL / NFA representatives were warmly welcomed, and enjoyed the friendly hospitality of their hosts.

Workshops in Quality Assurance in Microbiological Analyses with Main Focus on Measurement Uncertainty

NMKL has now arranged the workshop in Norway (27 May at the Veterinary Institute in Oslo), in Sweden (28 September, Stiftets Hus, Uppsala, Sweden) and in Finland (14 October 2010 on Evira, Helsinki).

More than 150 people have participated. The feedback has been positive. Microbiological laboratories are now in the process of estimating the measurement uncertainty for quantitative analyses.

NMKL would like to extend their thanks to the project group:
ISL: Margrét Geirsdóttir
FIN: Sanna Raunila,
NO: Marianne Økland
SE: Åsa Rosengren
for their extensive contribution. Thanks are also forwarded to the local organisers and speakers in the respective countries for having made the workshops so successful. The evaluation forms also indicated that many of the participants would like follow-up courses / workshops.

A new corrected Excel file for the estimation of measurement uncertainty is made available at www.nmkl.org under Excel sheet for download under NMKL Procedure No. 8.

A new corrected Excel file for the estimation of measurement uncertainty is made available at www.nmkl.org under Excel sheet for downloading under NMKL Procedure No. 8.

IAM/MoniQA CCMAS Workshop on rapid methods /proprietary methods (test kits) for official purposes and food authenticity

In connection with the meeting in the Codex Committee on Methods of Analysis and Sampling (CCMAS), the InterAgency (IAM), which is a forum for international organisations working within analytical methodology and quality assurance, will be arranging a symposium together with MoniQA, (Monitoring and Quality Assurance in the Food Supply Chain) – an initiative within the 6th frame program financed by the EU Commission.

Time and place: Sunday 6th March, 2011 at Hotel Ramada Plaza Budapest, Hungary

Workshop Agenda (tentative)

9.30 Registration, coffee
10.00 Opening/welcome
Introduction to today's program
10.30 Proprietary methods – introduction
11.00 Evaluation of proprietary methods

11.30 Break

11.45 Proprietary methods – manufacturer´s and industry´s point of view
12.15 Proprietary methods and certified reference materials

12.45 Lunch

14.00 Proprietary methods – a regulators point of view
14.30 Proprietary methods, Codex and consequences
The panel's point of view

15.00 Break

15.30 Authenticity in food – what does this mean?
16.00 Regional perspectives (15 minutes each)
European Union
North America
South America
Asia
Africa
17.30 Panel discussion
18.00 Close of meeting

Further information will be given when available at www.nmkl.org. Mark the date on your calendar if you find the topic interesting.

A seminar on sampling

will be held at Clarion Hotel Royal Christiania, Oslo, Norway, 25 August 2011. Mark the date on your calendar if you consider sampling an important topic.

The seminar will be arranged by the NMKL Norwegian National Committee, which consists of representatives from the State Wine and Liquor Monopoly (Vinmonopolet), Nofima, National Institute of Nutrition and Seafood Research (NIFES), Norwegian Food Safety Authority, Eurofins, Standard Norway, the Norwegian School of Veterinary Science and the National Veterinary Institute.

Do you have expertise in the area and would like to contribute, or want to exhibit sampling equipment etc, please contact the Chairman of the Norwegian National Committee Urd Bente Andersen (urd.bente.andersen @ vinmonopolet.no) or NMKL at (nmkl@vetinst.no)

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Available NMKL Procedures

No 1, 2. Ed. 2005	**Calibration and performance checking of laboratory balances**
No 2, 1995	**Performance check and in-house calibration of thermometers**
No 3, 1996	**Control charts and control materials in internal quality control in food chemical laboratories**
No 4, 3. Ed. 2009	**Validation of chemical analytical methods**
No 5, 2. Ed. 2003	**Estimation and expression of measurement uncertainty in chemical analysis**
No 6, 1998	**Yleiset ohjeet aistinvaraisten laboratorioiden laadunvarmistukseen** (avail. Danish/Finnish)
No 7, 1998	**Checking of UV/VIS spectrophotometers**
No 8, 4. Ed. 2008	**Measurement of uncertainty in quantitative microbiological examination of foods**
No 9, 2. Ed. 2007	**Evaluation of method bias using certified reference materials.**
No 10, 2001	**Control of Microbiological Media**
No 11, 2002	**Procedure for sensory analysis of drinking water**
No 12, 2002	**Guide on sampling for analysis of foods**
No 13, 2003	**Volumetric control**
No 14, 2004	**SENSVAL: Guidelines for internal control in sensory analysis laboratories**
No 15, 2004	**Temperature control in microbiological laboratories**
No 16, 2005	**Sensory quality control**
No 17, 2006	**Guidelines for requirement specifications for food analyses**
No 18, 2006	**The use of reference materials, reference strains and control charts in a food microbiological laboratory**
No 19, 2007	**Guideline for sensorial Analysis of Food containers/packages**
No 20, 2007	**Evaluation of results from qualitative methods**
No 21, 2008	**Guide for sensory analysis of fish and shellfish**
No 22, 2008	**Considerations regarding evaluation of immunochemical test kits for food analysis**
No 23, 2008	**Guide on quality assurance in microbiological laboratories** (replacing NMKL Report No. 5)
No 24, 2010	**Guidelines for quality assurance for food chemical laboratories**

The Secretariat of NMKL wishes all of you
a Merry Christmas and a Happy New Year 2011