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NMKL Newsletter

NORDIC COMMITTEE ON FOOD ANALYSIS

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This is the last edition of the Newsletter in hard copy, see last page.

NMKL Secretary General
c/o Norwegian Veterinary Institute
P.O.Box 750, Sentrum, N-0106 Oslo, Norway
Tel. +47 23 21 62 50
Org.nr. 995 790 394

Email: nmkl@vetinst.no

www.nmkl.org

NMKL Secretary General: Hilde Skår Norli

Seminar on non-destructive analytical methods: Current insight into NIR and NMR technologies

21 November 2013

at the University of Copenhagen,
Department of Food Science, Rolighedsvej 30, Denmark

Several molecular spectroscopy techniques are able to provide information concerning the composition of various types of ingredients in food & feed, or biological samples. These methods are interesting due to being rapid, non-destructive, and requiring limited sample preparation. Calibration curves for specific compound classes must be established for proper measurement.

The seminar focuses on the use of NMR (Nuclear Magnetic Resonance) and NIR (Near Infrared Spectroscopy) for quantitative analysis of food and feed samples, and quality control of industrial processes. The seminar aims to bring together people from academia and industry to discuss current research, results, and problems of both a theoretical and practical nature.

Preliminary programme

9:30 – 10:00	Registration
10:00 – 10:10	Opening remarks: NMKL Secretary General Hilde Skår Norli, Norway
10:10-12:00	**Plenary session:** Chair: Dr. Päivi Teivainen-Lædre, Skretting ARC, Norway
	Lecture 1: Professor Søren Balling Engelsen, University of Copenhagen, Denmark
	Lecture 2 : Dr. Ida G. Aursand, SINTEF, Norway
12:00 – 13:30	Lunch & exhibition
13:30 – 15:00	**Session I**: Chair: Dr. Erik Nordkvist, National Veterinary Institute, Sweden
	Lecture 1: General Manager Peter Bom, MasterLab
	Lecture 2: Dr. Lars Nørgaard, Sr. Manager Foss
	Lecture 3: Dr. Frans van den Berg, University of Copenhagen, Denmark
15:00 – 15:30	Coffee break
15:30 – 16:50	**Session II:** Chair: Dr. Erik Nordkvist, National Veterinary Institute, Sweden
	Lecture 1: Dr. Vincent Baeten, Walloon Agricultural Research Centre, Belgium
	Lecture 2: Sr. Engineer Frank Lundby, Nofima, Norway
	Lecture 3: Arla?

Fee: Students: NOK 2000, NMKL subscribers: NOK 3000, Others: NOK 3500
Exhibition fee: NOK 8000 (Includes 2 persons)
Registration to: nmkl@vetinst.no
Deadline: 10 November 2013

The programme will be updated on www.nmkl.org

PATHOGENIC *YERSINIA ENTEROCOLITICA* AND *YERSINIA PSEUDOTUBERCULOSIS*

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are zoonotic bacterial pathogens causing food-borne infection (yersiniosis) in humans worldwide. The main reservoir for pathogenic *Y. enterocolitica* are domestic pigs, for *Y. pseudotuberculosis* a wide range of domestic and wild animals such as rodents, deer, birds and various farm animals serve as potential reservoirs. Some of the bio-serotype combinations of *Y. enterocolitica* are associated with human infection. In contrast, all *Y. pseudotuberculosis* are currently considered as potentially pathogenic to humans.

New revised NMKL Method: NMKL 163, 2nd Ed., 2013:

Pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis.* Real-time PCR methods for detection in foods, feeds and environmental samples

Dr. Susanne Thisted Lambertz

SCOPE AND FIELD OF APPLICATION:

This method describes two horizontal procedures for the detection of pathogenic bioserotypes of *Y. enterocolitica* and *Y. pseudotuberculosis,* by using real-time PCR. The methods detect the two pathogens by PCR and allow isolation of colonies. *Y. pestis*, the causative agent of bubonic and pneumonic plague, harbour a variant of the *ail* gene as well, and will be detected by the same primer/probe set as *Y. pseudotuberculosis*. However, *Y. pestis* is normally not associated with food. The method is applicable to products for human consumption, animal feeding stuffs and environmental samples.

DEFINITIONS:

Y. enterocolitica and *Y. pseudotuberculosis* are Gram negative, oxidase negative, catalase positive, nitrate reductase positive, facultatively anaerobic coccoid shaped bacilli. The size is 0.5 - 0.8 x 1.0 - 3.0 mm. They do not form capsules or spores, and are nonmotile when grown at 35-37 ^{o}C but motile below 30 ^{o}C. Further, the bacteria are urease positive and ferment glucose and other carbohydrates with acid production, but little or no gas production.

PRINCIPLE:

The number of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* bacteria cells is increased by growth in a non-selective or semi-selective liquid nutrient medium. Bacteria cells are separated from the nutrient broth, lysed and the nucleic acid is extracted for use in the PCR reaction. The extracted nucleic acid is amplified using a probe-based real-time PCR. Detection of the target sequence is achieved by monitoring a clear increase in the fluorescence signal above the cycle threshold, *Ct*.

Susanne Thisted Lambertz, National Food Agency, Sweden, has been the referee of this method. Flemming Hansen (DK), Saija Hallanvuo (FI), Viggó Þór Marteinsson (IS), Torkjel Bruheim (NO) and Charlotta E. Axelsson have been the contact persons.

PESTICIDES

New NMKL Method: NMKL 195, 2013:

Dr. Tuija Pihlström, National Food Agency, Sweden, has been the referee of this method. Pihlström was also the coordinator of the work with the SANCO document, SANCO/1245/2011, the "validation bible" for laboratories involved in official control of pesticide residues in food and feed in the EU.

Other experts involved:
Arvid Fromberg (Denmark), Kati Hakala (Finland), Vordís Baldursdóttir (Iceland) and Hans Ragnar Norli (Norway).

Pesticide residues. Analysis in foods with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric determination

SCOPE AND FIELD OF APPLICATION

The Swedish National Food Agency has since 1989 applied a multi residue method based on extraction with ethyl acetate for the analysis of pesticide residues in food. The method is used for the analysis of pesticide residues in fruit and vegetables, cereals and products of animal origin by means of LC-MS/MS and GC-MS/MS, comprising more than 400 different types of pesticides in one extraction. The method validation has been performed according to the guidelines in the SANCO document "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed", SANCO/12495/2011. The multi residue method (SweEt) has been revised continuously, resulting in a fast, robust and simplified methodology for analysis of pesticide residues in food. Therefore, the present multi residue method can be used for analysis of pesticide residues to ensure the quality and reliability of the measurements. The Limit of Quantification (LOQ) of the method is 0.01 mg/kg for most of the analytes. The method validation meets the method validation requirements for each representative commodity in the range of 70-120 % (mean recoveries) with an RSDr ≤ 20% (precision). The method has been evaluated in different proficiency tests organised by EU Reference Laboratories.

PRINCIPLE

Fruit and vegetables: The homogenised sample is extracted with ethyl acetate after addition of $NaHCO_3$. At the end of the extraction, Na_2SO_4 is added. Sample extract is centrifuged and filtered prior to injection on GC-MS/MS and LC-MS/MS.

Cereals: The grinded sample is extracted after water addition with acidified (1 % acetic acid) ethyl acetate and Na_2SO_4. The sample extract is centrifuged and filtered prior to injection on GC-MS/MS and LC-MS/MS.

Animal origin: The homogenised sample is extracted using ethyl acetate or ethyl acetate/cyclo hexane (1+1) with an addition of Na_2SO_4. The choice of extraction solvent depends on the fat content of the sample. Samples with a fat content ≤ 10% are purified with PSA/C_{18} , whereas GPC is used on samples with higher fat content. The sample extract is finally filtered prior to injection on GC-MS/MS and LC-MS/MS.

NMKL offers online access to NMKL Methods and NMKL Procedures.

PARALYTIC SHELLFISH TOXINS

Shellfish, such as blue mussels, scallops and oysters, live by filtering plankton. Certain algae can produce or contain toxins. Shellfish can take up and concentrate the toxins during blooms of such algae. Marine algal toxins can occur in shellfish in all seasons, and the shellfish show no apparent ill effects from even large amounts of toxins. The algal toxins do not affect the taste of the shellfish, and people therefore have no possibility of checking whether the shellfish are toxic on their own. The algal toxins are chemical compounds that are not destroyed by freezing or cooking.

The algal toxins are divided into groups based on their effects

- Paralytic shellfish toxins cause paralysis
- Diarrhetic shellfish toxins cause diarrhoea
- Amnesic shellfish toxins cause amnesia

In addition, there are three groups of compounds called azaspiracids, pectenotoxins, and yessotoxins.

Source: Facts on algal toxins and food poisoning, www.vetinst.no

Paralytic Shellfish Toxins consist of at least 18 structurally related toxins with varying toxicity. They are neurotoxins, and are mainly produced by the algae of the genus *Alexandrium*. The toxins accumulate in shellfish feeding on the algae with no apparent negative effect on the shellfish. However, humans and animals consuming the shellfish, experience neurological symptoms of poisoning and severe cases have to be treated in a respirator.

New NMKL Method 197, 2013:

Paralytic Shellfish Toxins. Determination in mussels, clams, oysters and scallops by post-column oxidation and HPLC/ fluorescence

This method corresponds to AOAC 2011.02.

SCOPE AND FIELD OF APPLICATION:

The method is intended for determination of paralytic shellfish toxins in mussels, soft shell clams, sea scallops, and oysters. The method quantifies 12 individual toxins, including saxitoxin (STX), neosaxitoxin (NEO), gonyautoxins-1 to -5 (GTX1-5), decarbamoyl-gonyautoxins-2 and -3, (dcGTX2-3), decarbamoyl-saxitoxin (dcSTX), and N-sulfocarbamoyl-gonyautoxin-2 and -3, (C1 and C2), in edible tissues, as well as the total toxicity of test samples with more than 0.10 mg STX dihydrochloride equivalents/kg of tissue (mg STX·diHCl/kg).

PRINCIPLE:

Homogenised samples are mixed with diluted hydrochloric acid and heated in a boiling water bath. Proteins are precipitated with trichloro acetic acid and the pH is adjusted. The extract is filtered and chromatographed on a C18 silica column with a step gradient using a heptane sulfonic acid/phosphoric acid buffer system for the analysis of gonyautoxins and STXs. The extract is also chromatographed on a C8 silica column using an isocratic tetrabutylammonium phosphate buffer system to determine the N-sulfocarbamoyl gonyautoxins. The toxins are derivatised by post-column oxidation at 85°C with a phosphoric acid, periodic acid buffer solution. This oxidised eluent is acidified, and the derivates are detected by fluorescence (excitation: 330 nm, emission: 390 nm).

Dr. John A. Aasen Bunæs, Norwegian School of Veterinary Science has elaborated this method for NMKL.

UPDATES FROM NORDVAL INTERNATIONAL

The following NordVal certificates have been renewed:

- No. 001: TRANSIA®PLATE *Salmonella* Gold from BioControl
- No. 014: 3M Petrifilm E.coli/Coliform Count Plate
- No. 016: 3M Petrifilm Yeast and Mould Count Plate
- No. 020: RAPID'E.Coli 2 from Bio-Rad
- No. 023: Foodproof Salmonella Detection Kit, Hydrization Probes and foodproof Salmonella Detection kit, 5' Nuclease in combination with foodproof ShortPrep I Kit from Biotecon Diagnostics
- No. 025: Foodproof Listeria monocytogenes Detection Kit, Hydrization Probes and foodproof Listeria monocytogenes Detection kit, 5' Nuclease in combination with foodproof ShortPrep I Kit from Biotecon Diagnostics
- No. 030: DuPont™ BAX® System PCR Assay for Salmonella (Classic + Q7 instruments) from Thermo Fisher
- No. 039: DuPont™ BAX® System Real-Time PCR Assay for *Campylobacter jenuni/coli* and *lari* from Thermo Fisher
- No. 042: HyServe Compact Dry XSA Method for the Enumeration of Staphylococcus aureus
- No. 043: HyServe Compact Dry YM Method for the Enumeration of Yeasts and Moulds

The certificates can be downloaded from "list of methods" under NordVal on www.nmkl.org.

The certificate specify the principle of the methods and the results of the validations. The certified methods are compared with reference methods, usually ISO methods.

The methods are validated according to ISO 16140 / the NordVal Protocol.

The following NordVal certificates have been withdrawn:

- No. 012: 3M Petrifilm Aerobic Count Plate
- No. 013: 3M Petrifilm Coliform Count Plate
- No. 019: 3M Petrifilm Staph Express Count System

3M has decided to no longer have NordVal certification for these methods, and hence the certificates can no longer be referred to. NordVal Certificate No. 012 was previously NMKL 146, which was withdrawn in 2004. NMKL 146 was not withdrawn because it was not working satisfactorily, but because NMKL decided not to have proprietary methods in its method collection.

UPDATES FROM NORDVAL INTERNATIONAL

New NordVal Certificate No. 045:

HyServe Compact Dry X-BC for determination of *Bacillus cereus* in foods

Compact Dry X-BC method contains a ready-to-use dry chromogenic medium, and selective agents for the detection and enumeration of *Bacillus cereus.* An aliquot of 1 ml of an appropriate dilution is plated onto a Compact Dry X-BC plate. The incubation conditions tested with satisfactory results in the study, were 30 ± 1°C for 48 ± 2h. *Bacillus cereus* forms blue colonies.

Source: www.hyserve.com

In 2012, the method was tested in two extensive comparison studies by CCFRA Technology Limited, Chipping Campden. In 2013, Campden arranged the collaborative validation trial with nine participating laboratories. The method has been compared with ISO 7932:2004: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony count technique at 30°C.

Over 300 samples of various food categories were analysed, including samples of meat, fruits, vegetables, dairy products, bakery products, and other products. Laboratories reported that it was easier to read the formed colonies on X-BC than on the agar described in ISO 7932:2004. The blue colonies on X-BC appeared more distinctly. The sample levels yielded satisfactory precision from 1.5 log cfu /g and higher.

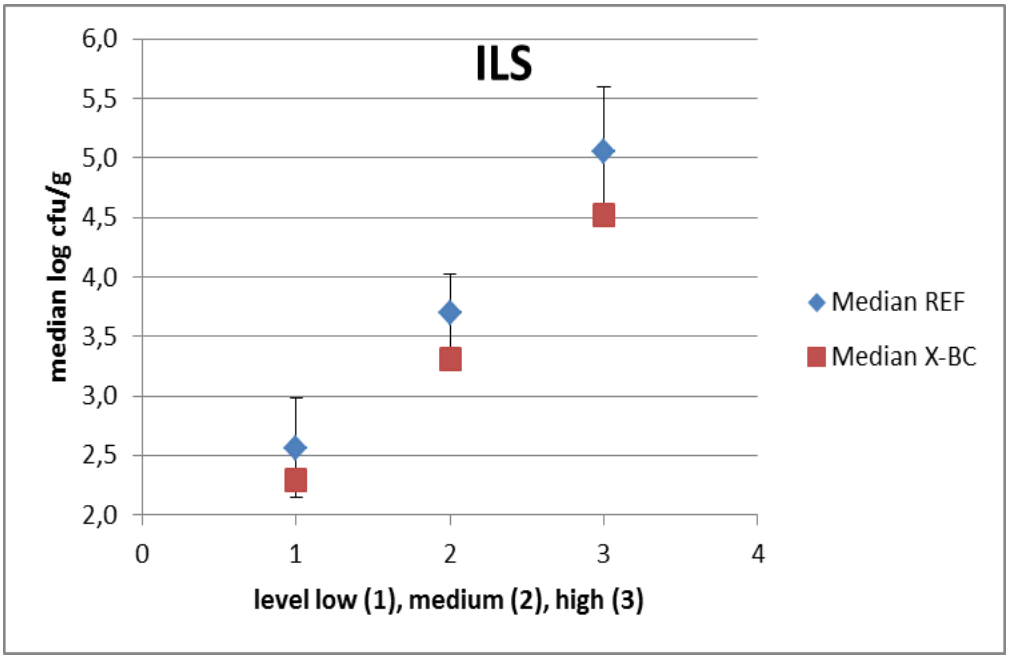
For the vast majority of the samples in the comparison studies, somewhat fewer colonies were counted on the X-BC than on the agar described in the reference method, i.e. it seemed that the alternative method had a systematic negative bias. This was also true in the collaborative validation study, where nine laboratories enumerated *Bacillus cereus* in dairy products using both the reference method and the X-BC. The results are given in the table and figure on page 8. The median of the samples of the low, medium and high level of *Bacillus cereus*, were slightly lower with X-BC (red squares in the figure) than the median of the results obtained by the reference method (blue squares in the figure). However, since the results of X-BC fall within the confidence interval of the results with the reference method, the negative bias is not statistically significant. Results obtained by the two methods will be overlapping, and hence it can be concluded that the alternative method provides results equivalent to those of the reference method.

Continued on page 8

Continued: HyServe Compact Dry X-BC

The table and figure below illustrate the results of the collaborative validation (Inter-Laboratory Study, ILS). This shows that the results obtained using the alternative method, X-BC, fall within the confidence interval (± $2s_R$) of the results obtained using the reference method. The results are given in log cfu/g. Nine laboratories participated in the validation.

	ISO 7932		XBC		Bias
Level	Median (log cfu/g)	$2s_R$ (log cfu/g)	Median (log cfu/g)	s_R (log cfu/g)	(log cfu/g)
Low	2.56	0.422	2.29	0.112	-0.27
Medium	3.70	0.322	3.31	0.140	-0.39
High	5.05	0.543	4.52	0.242	-0.53



NordVal Certificate No. 045 contains the results from all the validations of the X-BC, which form the basis of the NordVal approval. The certificate is available at www.nmkl.org.

Further information about HyServe and Compact Dry X-BC is available at www.hyserve.com.

NMKL 67TH ANNUAL MEETING HELD IN KALMAR

The NMKL 67th Annual Meeting was held on 31 August - 3 September 2013 in Kalmar, Sweden. The meeting was attended by members of the national committees, which are appointed experts from Denmark / Faroe Islands, Finland, Iceland, Norway and Sweden. The Swedish National Committee, under the leadership of Dr. Ulla Edberg, National Food Agency, hosted the event.

The Chair of NMKL, **Ulla Edberg,** opened the Annual Meeting by underpinning that the achievement of the organisation is based on the contributions of all of its members. Food laboratories are facing a stressful reality, the tight working situations and changes require hard priorities. Edberg expressed how pleased she was to see how many had been able to come to this important meeting for sharing knowledge and expertise. The fewer the laboratories there are within the Nordic countries, the more important it becomes to meet and exchange information about what is going on in the different Nordic countries, and with combined efforts work to ensure safe and healthy food for the Nordic consumers . "Your skills are NMKL's most important resource!"

Special thanks were extended to four experts who have contributed to NMKL's work through many years; **Franklin Georgsson**, Iceland, 30 years, **Sven Qvist**, Denmark 25 years, **Astrid Nordbotten**, Norway, 17 years and **Kristin Halldorsdottir**, Iceland, 10 years. **Urd Bente Andersen** was thanked for her contribution as chairman of the Norwegian National Committee.

Chair of NMKL
Ulla Edberg

Franklin Georgsson, Sven Qvist and Astrid Nordbotten are thanked for their outstanding efforts

Dr. Päivi Laakso, Eurofins Scientific, Finland was Referee of the Year, and was invited to present her experience with, and the results of, the method validation on plant stanols and sterols in phytosterol enriched foods using gas chromatography.

Päivi Laakso, the referee of the year

The Annual Meeting elected Dr. Ulla Edberg to be the Chair of NMKL, Special Scientist Liv Kukkonen to be the Chair of the Chemical Committee and M.Sc. Hilde Skår Norli to be the NMKL Secretary General, all for 4-year terms.

The terms for the NMKL Chairs and Secretary General:

Sub committee 1: Dr. Ulla Edberg, National Food Agency, Sweden (uled@slv.se)	2013 - 2015
Sub committee 2: Dr. Franklin Georgsson, Matis, Iceland (franklin.georgsson@matis.is)	2011 - 2015
Sub committee 3: Dr. Liv Kukkonen, Evira, Finland (liv.kukkonen@evira.fi)	2013 - 2017
Sub committee 4: Dr. Grethe Hyldig, DTU Food, Denmark (grhy@food.dtu.dk)	2012 - 2016
NordVal International: Dr. Sven Qvist, Denmark (sven@qvist.com)	2011 - 2015
Secretary General: Hilde Skår Norli, Norwegian Veterinary institute (nmkl@vetinst.no)	2013 - 2017

NMKL 67th Annual Meeting held in Kalmar

Most of the work at the NMKL Annual Meeting took place in the sub committees for microbiology, chemistry and sensory analyses, where all the projects on the NMKL working programme were discussed. In addition, NordVal International held a brief steering group meeting.

The working programme of NMKL

Microbiology:

- *Yersinia enterocolitica,* culture method
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis,* PCR method
- *Shigella,* culture method
- *Shigella,* PCR method
- *Clostridium difficilie*
- Aerobic microorganisms
- Aerobic or anaerobic plate count
- *Salmonella,* detection using MSRV
- *Giardia* and *Cryptosporidium* in drinking water
- Sulphite reducing clostridia
- Probiotics
- Halophilic and osmophilic bacteria
- Quality control of PCR analyses
- Verification of microbiological methods

Chair Franklin Georgsson and Secretary Gro S. Johannessen

The microbiological committee

Chemistry:

- Methyl mercury, determination with isotope dilution GC-ICPMS
- Histamine, HPLC determination in fish
- Sterols and stanols, GC determination
- Fat, NMR determination
- Banned colouring agents in spices
- Folate, HPLC determination
- Algae toxins , HPLC determination
- Pesticide resdiues, GC-MS/MS and LC-MS/MS
- Calibration of NIR and IR
- Control charts and control materials in internal quality control

Chair Liv Kukkonen and Secretary Ulf Bondesson

The chemical committee

NMKL 67th Annual meeting held in Kalmar

The working programme of NMKL

Sensory committee:

- Evaluation and reporting of sensory data
- Sensory analysis of meat and meat products
- Guidelines for sensory evaluation of bread
- Methods for discriminate tests (binomial tests)
- Recruitment/training and control of analytical panels, training of assessors

From the sensory committee, Chair Grethe Hyldig and Secretary Gunnar Forsgren to the right

Would you like to become a member of a

NMKL national committee?

Contact the Chairman:

Denmark: Arne Høygård Jensen (arho@fvst.dk)

Finland: Tuula Pirhonen (tuula.pirhonen@evira.fi)

Iceland: Franklin Georgsson (franklin.georgsson@matis.is)

Norway: Dag Grønningen (dag.gronningen@vetinst.no)

Sverige: Ulla Edberg (uled@slv.se)

User Survey 2013

In March 2013, NMKL conducted a survey on the use of NMKL Methods and Procedures. The subscribers of NMKL Methods were requested to respond to the survey, indicating how frequently they apply the methods and procedures. Three reply options were given for each method/procedure: "Never" = it has not been used for 5 years, "Seldom" = it is applied less than 1-3 times a year, and "Frequently" = it is applied more than 3 times a year.
The user survey showed that all the 41 microbiological NMKL Methods, the sensory method and all the NMKL Procedures (guidelines) are in regular use. It also showed that 71 of the 78 chemical methods are in use. The results of the user survey are very useful for the further work of NMKL, and is available at NMKL's website.

Thank you very much to all of you who took the time to respond to the user survey!

NMKL becomes more environmental - friendly!

This is the last number distributed in paper format. From now on NMKL Newsletter will only be published electronically in PDF format.

If you still wish to receive a hard copy, please notify the Secretariat.

For receiving the Newsletter by e-mail, please remember to send your e-mail address to NMKL, and to open up for receiving pdf-files.

E-mail: nmkl@vetinst.no