NMKL Newsletter 77

NMKL - Nordic Committee on Food Analysis

www.nmkl.org · nmkl@vetinst.no · No. 77 April 2011

Contents

NMKL– Nordic Committee on Food Analysis

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Everything you ever wanted to know about reference materials!

NMKL is arranging courses in the Nordic countries

NMKL will be arranging courses in the five Nordic countries, based on the NMKL guides "Evaluation of method bias using certified reference materials" (NMKL Procedure No. 9) and Recovery information in analytical measurement" (NMKL Procedure in process). Lars Jorhem, the author of NMKL Procedure No. 9 and a key person in the elaboration of the recovery procedure, will be the lecturer of the courses. Both of the procedures are related to chemical analyses.

The courses are intended for analysts, quality assurance managers and purchasers of analytical services.

Date and location:

- 4 May 2011: National Food Administration, Uppsala, Sweden
- 10 May 2011: Norwegian Veterinary Insitute, Oslo, Norway
- 16 May 2011: Evira, Finland
- 18 May 2011: Danish Veterinary and Food Administration Copenhagen, Denmark
- 26 May 2011: Nýsköpunarmiðstöð Íslands / Innovation Center Iceland

Contents:

- Reference Materials (RMs) versus Certified Reference Materials (CRMs): What is the difference?
- ISO Guides and CRMs, EU legislation, CODEX requirements
- Interlaboratory studies: Differences and similarities between certification, validation and proficiency testing
- How are CRMs made?
- Recovery and bias: Relation to CRMs
- Selection, use and misuse of CRMs
- Estimation of bias using NMKL Procedure No. 9 (2007)
- A short introduction to measurement uncertainty
- Where to find CRMs and PT programmes

Language:
Swedish in Denmark, Norway and Sweden
English in Finland and Iceland

Fee: NOK 2000, covers lunch, course location and material
Registration: nmkl@vetinst.no
Deadline: 20 April 2011

Photo: Lars Jorhem, Lecturer and author/ project leader for NMKL Procedures

InterAgencyMeeting (IAM)

IAM held its 23rd meeting on 4 March 2011 at the Hungarian Food Safety Office in Budapest.

The main purpose of the IAM is to promote cooperation between international organisations, and to support the Codex Alimentarius Commission's and thus the governments' and industries' need for methods and quality assurance guidelines.

IAM Chairman Roger Wood, UK (ICUMSA)

IAM Secretary Richard Cantrill, USA (AOCS)

This is the forum where the regional and international organisations within methodology and quality assurance meet and discuss harmonisation and the policy of the organisations. Further, the organisations inform about ongoing activities which might be of relevance to the other organisations.

Present at this meeting were representatives from:

- AOAC International (The Scientific Association Dedicated to Analytical Excellence)
- AOCS (American Oil Chemists' Society),
- BIPM (Bureau International des Poids et Mesures)
- CEN (Comité Européen de Normalisation)
- Codex Alimentarius
- EURACHEM (A focus for analytical chemistry in Europe)
- ICUMSA (International Commission for Uniform Methods of Sugar Analysis)
- IDF (International Dairy Federation)
- ISO (International Organization for Standardization)
- IUPAC (International Union of Pure and Applied chemistry)
- NMKL (Nordic Committee on Food Analysis)

Roger Wood, United Kingdom, (ICUMSA), is the chairman of IAM. The secretariat is held by AOCS through Richard Cantrill, USA.

The IAM discussed whether the organisations are;

- quoting results from collaborative validation studies in the methods,
- publishing methods that are not collaboratively validated, and
- publishing methods with poor performance data.

All organisations are quoting validation data, if available. As NMKL has been the only organisation to state the validation status in the method header, NMKL will now abandon this practice. Furthermore, NMKL and AOAC International are the only organisations which employ acceptance criteria when reviewing validated methods for approval, and do not publish methods with poor performance. This is a practise NMKL will not abandon. *All laboratories should review the validation data carefully, whether or not the performance is satisfactory, especially when the method is published by an organisation without acceptance criteria.*

For Codex Committee on Methods of Analysis and Sampling (CCMAS), the IAM elaborated a discussion paper on the use of proprietary methods (test-kits) in Codex standards. IAM gave CCMAS the following recommendations:

- Proprietary methods should not be referred to in Codex standards, unless absolutely necessary
- Equivalent method criteria should be applied for proprietary methods as for conventional methods.
- In addition, proprietary methods should be evaluated by an independent party.

The CEN mandate for collaborative validation of microbiological ISO standards referred to in EC 2073/2005: "Microbiological criteria", has been signed! This means that the study of the ISO methods (approx 15) can start. The studies are to be carried out within a 3-year period. The European Union Reference Laboratories (EURL, previously known as CRL) will lead most of the projects.

NMKL has in the past collaboratively validated one of the methods; the ISO method for detection of *Yersinia enterocolitica.* The results were not satisfactory. The NMKL referee, Saija Hallanvuo, Evira Finland, will revise the method and is also the project leader of this method in CEN.

IAM/MoniQA workshop: "**Methods for Food Identification and Authenticity – Characterizing the Unknown",** with more than 70 participants, was held 6 March prior to the CCMAS meeting. The feedback was good.

IAM welcomes any topic suggestions for a possible workshop next year.

Codex Committee on Methods of Analysis and Sampling (CCMAS)

CCMAS aims to:

- define criteria appropriate to Codex Methods of Analysis and Sampling,
- be a coordinating body for Codex when it comes to analyses and quality assurance system,
- specify reference methods
- endorse analytical methods proposed by Codex commodity committees, and
- elaborate protocols, guidelines or related text for the assessment of food laboratory proficiency and quality assurance

CCMAS held its 32nd session on 7 - 11 March 2011 in Budapest, Hungary.

145 participants from 46 countries + 10 international organisations were present. From the Nordic countries the following participated: Harriet Wallin (FI), Taija Rissanen (FI), Astrid Nordbotten (NO), Ulla Edberg (SE), Leonardo Merino (SE) and Hilde Skår Norli (NMKL).

The session was chaired by Professor Arpad Ambrus, Hungary.

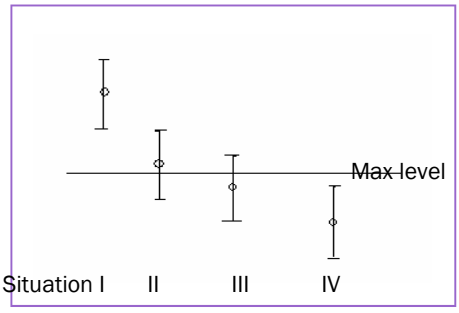
The committee discussed and approved a document giving explanatory notes on measurement uncertainty; what and how to estimate measurement uncertainty, which estimate values are expected and how to state and interpret measurement uncertainty in connection with given specifications (maximum limits).

Anticipated estimates of measurement uncertainty of chemical analyses (expressed as expanded uncertainty 2•u) are given in table 1. These numeric values are based on Horwitz/Thomsen's equation for expected standard deviation of the reproducibility.

Table 1: Anticipated estimates of measurement uncertainty values at different concentrations

Nominal concentration	Typical expanded uncertainty
100 g/ 100 g	4%
10 g/ 100 g	5%
1 g/ 100 g	8%
1 g/ kg	11%
100 mg / kg	16%
10 mg/ kg	22%
1 mg /kg	32%
< 100 µg / kg	44%

It is important to consider measurement uncertainty when deciding whether or not a sample meets a given threshold value/limit. This is illustrated in the diagram below, but does not necessarily apply if a direct health hazard is involved.



Situation I: The analytical result minus the expanded uncertainty exceeds the maximum level.

Situation II: The analytical result exceeds the maximum level by less than the expanded uncertainty.

Situation III:The analytical result is lower than the maximum level by less than the expanded uncertainty.

Situation IV: The analytical result is lower than the maximum level by more than the expanded uncertainty.

Only *situation I* indicates that the measured analyte in the test sample is above the given threshold/limit.

CCMAS discussed the IAM paper on proprietary methods. It was agreed to establish a project group which is to come up with a definition of proprietary methods (Norway and Sweden will participate). Further, it was agreed to elaborate a proposal for the Codex Manual regarding references to proprietary methods in Codex standards according to the IAM recommendations given on page 2.

A number of methods were up for endorsement, such as methods for dietary fiber in foods and contaminants in mineral water. NMKL received several questions from delegations regarding how to calculate method criteria, see page 4.

Photo: IDF's representatives, front from left: Aurélie Dubois, Jaab Evers Back from left: ISO representatives Ton Gersson and Sandrine Espeillac and Hilde Skår Norli (NMKL and AOAC International)

To ONLINE subscribers

New User IDs and Passwords are currently being forwarded to subscribers who have paid.

Should you enter an incorrect User ID and/or Password, please close your browser before trying again. Also remember to make sure that no blank spaces are included if you use copy/paste.

Method criteria: How to obtain the numeric values for method performance criteria for chemical food analyses

Both within EU legislation (2004/822/EEC, 2002/657/EEC, 2007/333/EEC) and in Codex, there is a tendency towards using method criteria rather than specifying specific methods. This gives the laboratories the freedom to choose appropriate collaboratively validated methods in accordance with certain method criteria, instead of using specifically selected methods.

Method criteria provide a more flexible system, which takes into account the development of new methods and techniques, thereby promoting development and ensuring better quality results as laboratories can use methods which they use routinely.

Where applicable, the methods should be characterised by the following criteria:

- applicability
- limit of detection
- limit of determination
- precision (repeatability and reproducibility)
- accuracy
- recovery

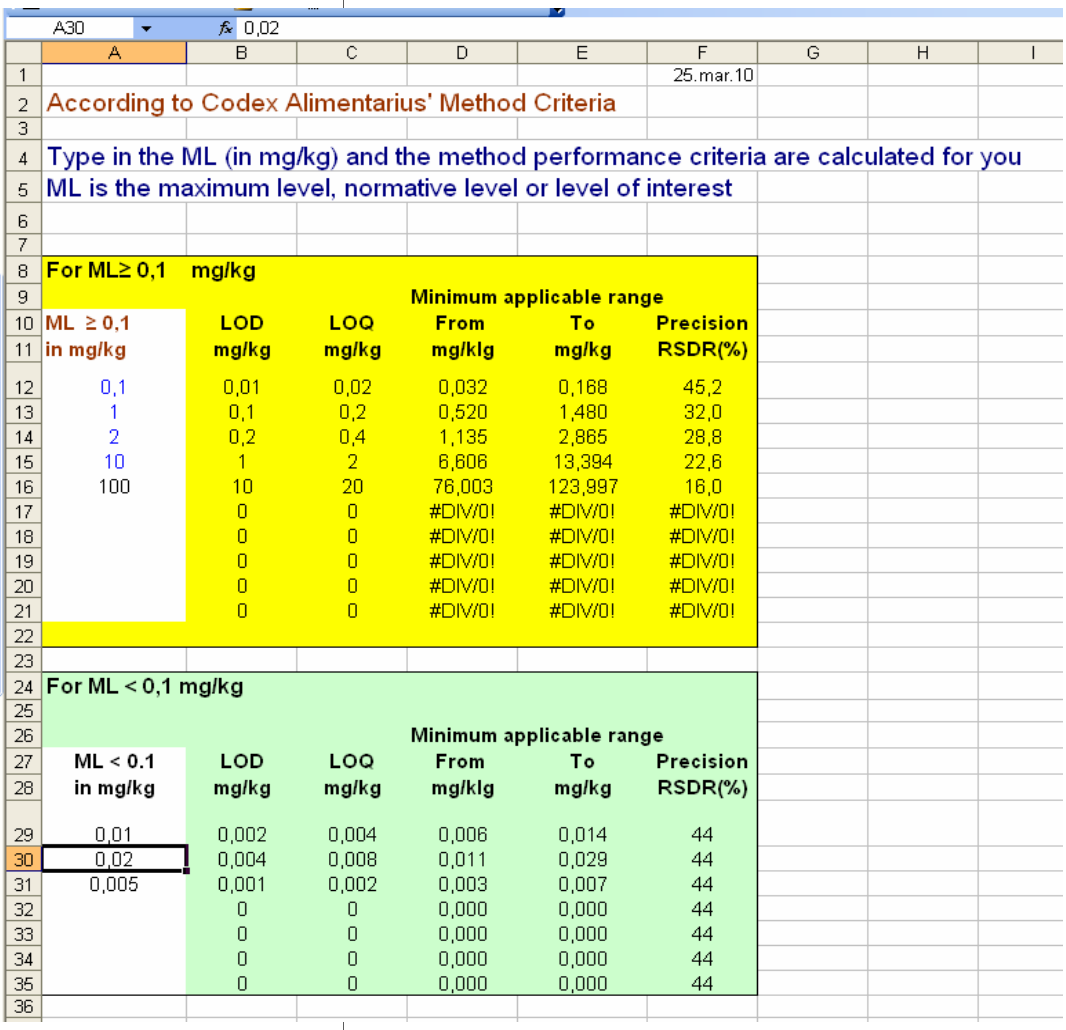
The methods should preferably be validated collaboratively (according to IUPAC International Harmonised Protocol (or ISO 5725:1994).

To check whether a method is suitable according to the criteria, one must consider the results from the collaborative study. The criteria depends on the level (maximum limit/threshold) of the analyte to be determined.

NMKL has elaborated a very simple Excel spreadsheet that calculates the minimum applicable range, limit of detection (LOD), limit of quantification (LOQ) and precision which the method must meet for that appropriate concentration level (maximum level).

The Excel spreadsheet may be downloaded at www.nmkl.org under "Download Excel spreadsheet".

Laboratories should use method criteria when selecting an appropriate method. Purchasers of analyses, method organisations and food authorities should use method criteria when considering methods for approval and reviewing their fitness for purpose.



A30 fx 0,02

	A	B	C	D	E	F	G	H	I
1						25.mar.10			
2	According to Codex Alimentarius' Method Criteria								
3									
4	Type in the ML (in mg/kg) and the method performance criteria are calculated for you								
5	ML is the maximum level, normative level or level of interest								
6									
7									
8	For ML≥ 0,1 mg/kg								
9				Minimum applicable range					
10	ML ≥ 0,1	LOD	LOQ	From	To	Precision			
11	in mg/kg	mg/kg	mg/kg	mg/klg	mg/kg	RSDR(%)			
12	0,1	0,01	0,02	0,032	0,168	45,2			
13	1	0,1	0,2	0,520	1,480	32,0			
14	2	0,2	0,4	1,135	2,865	28,8			
15	10	1	2	6,606	13,394	22,6			
16	100	10	20	76,003	123,997	16,0			
17		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
18		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
19		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
20		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
21		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
22									
23									
24	For ML < 0,1 mg/kg								
25									
26				Minimum applicable range					
27	ML < 0.1	LOD	LOQ	From	To	Precision			
28	in mg/kg	mg/kg	mg/kg	mg/klg	mg/kg	RSDR(%)			
29	0,01	0,002	0,004	0,006	0,014	44			
30	0,02	0,004	0,008	0,011	0,029	44			
31	0,005	0,001	0,002	0,003	0,007	44			
32		0	0	0,000	0,000	44			
33		0	0	0,000	0,000	44			
34		0	0	0,000	0,000	44			
35		0	0	0,000	0,000	44			
36									

New NMKL Method No. 68, 5. Ed., 2011: *Enterococcus*. Determination in Foods and Feeds.

Professor Niels Skovgaard, Denmark, has revised the NMKL method for determination of Enterococcus

This method can be used for quantitative determination of enterococci in foods, feeds and environmental samples.

As of today, the genus *Enterococcus* comprises 25 or more species. The two classical species, *E. faecalis* and *E. faecium*, are common in human and animal gut. Commonly encountered species are also *E. avium, E. gallinarum, E. cassileflavus, E. durans* among others.

Enterococci may occur in high numbers in many foods, particularly fermented products. They are further used as probiotics for humans and animals. They are fairly resistant organisms and grow at 10°C. Strains of *E. faecalis* and *E. faecium* grow at temperatures close to 0°C. Most of the enterococci grow at 45°C, and some, at least *E. faecalis* and *E. faecium*, grow at 50°C. Most species grow at pH 9.6, in 40% bile, and in 6.5% salt/water. Many strains, in particular *E. faecium*, are very heat resistant and may even survive 90°C or higher temperatures in certain foods.

The mentioned abilities make them important food spoilage organisms, especially in cured products kept for longer time at chilling temperatures. They are used as indicators for many purposes. *Detection of enterococci in heat-treated foods may thus indicate the use of raw materials of inferior microbiological quality, recontamination after heat treatment, insufficient heat- treatment, poor hygienic handling during processing, improper chilling and storage conditions, and in some cases also faecal contamination.*

Their implication in classical food poisoning is questionable, and has never really been proven. Some enterococcus species are important opportunistic pathogens and a major cause of nosocomial infections, i.e. endocarditis, caused by Vancomycin Resistant Enterococci (VRE).

The number of enterococci is determined by plating known amounts of the sample on the surface of a selective medium, *Enterococcus* agar (7.2.1), prepared according to the method of *Slanetz and Bartley.*

The sodium azide in the *Enterococcus* agar inhibits the Gram-negative bacteria, and the enterococci reduce the colourless triphenyltetrazolium to red formazan. It is a precondition for the formation of typical colonies that they appear as single, isolated colonies.

Typical colonies are raised, pink to dark red in the centre of the colony or throughout the colony.

If stressed enterococci are expected, i.e. in frozen foods, the number of bacteria may be determined by plating the samples on a non-selective media. After pre-incubation at 37°C for 2 hours, an overlay of the *Enterococcus* agar is poured on top.

After 48 hours of incubation at 44° C, typical colonies are counted. Weak reducers, having only a red centre of the colony, are confirmed by inoculation on Bile-Aesculin agar, pre-heated to 44°C. Enterococci are bile salt tolerant and hydrolyse aesculin in the medium in two hours. The end product, dihydroxy-coumarin, combines with iron (III) to give a tan-coloured to black compound which diffuses into the medium.

The method is elaborated by Prof. Niels Skovgaard, Denmark, in collaboration with Eeva Klemettilä-Kirjavainen, Finland, Laufey Karlsdottir, Iceland, Heidi Wikmark, Norway, and Anna Birgersson, Sweden.

Do you to subscribe to NMKL methods?

NMKL offers a subscription to its methods, as PDF files, hard copies or ONLINE (with user ID and password) for NOK 2500 (EUR 300).

If you have not subscribed to NMKL methods previously, the fee for first registration of an ONLINE subscription is NOK 5000 (EUR 600), which corresponds to the price of a method collection.

With an ONLINE subscription the latest version of the method is only a keystroke away.

Hygicult® TPC

NordVal Certificate No. 018

Hygicult TPC is a culture slide for rapid monitoring of microbiological hygiene on surfaces. Both sides of the slide is covered with Total Plate Count (TPC) agar which supports growth of the most common bacteria and fungi. Each plate is contained in a sterile tube.

Sampling is carried out by pressing the plates directly against the working surface.

The result is read after incubation, by counting the number of colonies or comparing with the accompanying reading manual.

Hygicult TPC was compared to NMKL Method No. 5, 2001: "Aerobic microorganisms and presumptive *Enterobacteraceae*. Enumeration on surfaces and utensils".

According to the collaborative validations it may be concluded that Hygicult ® TPC dip-slide does not differ in performance, yield or precision compared to the reference method. The lowest validated level tested was 1.41 cfu/cm^2.

Orion Diagnositica Oy is the manufacturer and supplier of Hygicult® TPC.

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 against a reference method.

Campylobacter real-time PCR

NordVal Certificate No. 017

This real-time PCR method is applicable to the detection of human pathogenic thermotolerant *Campylobacters* (*C. jejuni*, *C. coli* and *C. lari*) in raw chicken meat, cloacae swabs and faecal samples collected from disposable shoe covers in rearing houses.

The method is tested for detection of thermotolerant *Campylobacters* in levels from 1-10 cfu / 25 g in raw chicken meat and 100-1000 cfu / ml dilutions of cloacae swabs and faecal samples (shoe covers).

The samples of raw chicken are prepared according to NMKL Method No. 119. Cloacae swabs and samples from disposable shoe covers are transferred to tubes with 0.9% NaCl solution, homogenised and if needed lightly centrifuged. Specific volumes of the supernatant are transferred for extraction of DNA before real-time PCR-analysis.

The primers target a *C. jejuni* 16S rRNA sequence. An internal amplification control is analysed with the samples to detect false negative responses.

The comparison study and the collaborative validation study showed that the accuracy, sensitivity and specificity were satisfactory. The results are given in the Certificate.

The reference methods are: ISO/DIS 10272-4 and NMKL 119: Thermotolerant *Campylobacter.* Detection, semi-quantitative and quantitative determination in foods and drinking water.

Eurofins Steins, Denmark, requested the certificate.

To manufacturers and suppliers of test kits

NordVal would like to invite you to a user meeting 19 May 2011 in Uppsala, Sweden.
The purpose of the meeting is to inform about NordVal activities and procedures, but we would also like to start a dialogue with you and learn about your needs.

Would you like to participate? Please send an email to nmkl@vetinst.no by 1 May.

Salmonella ELISA test OPTIMA / RayAl *Salmonella* Optima

NordVal Certificate No. 010

Salmonella ELISA Test OPTIMA / RayAl *Salmonella* OPTIMA fulfils the requirements of the NordVal validation protocol. *Salmonella* ELISA Test OPTIMA is validated according to ISO 16140 against the reference methods ISO 6579:2002 and NMKL 71. The results document that there is no statistical difference in the performance of the methods.

Salmonella ELISA Test OPTIMA / RayAl *Salmonella* OPTIMA is an immuno-enzymatic test using a microtiter plate coated with specific antibodies directed against *Salmonella*, and ready-to-use reagents. The test allows the detection of *Salmonella* after enrichment steps (for about 40 hours) and a heat shock releasing any *Salmonella* antigens that might be present in the sample. The antigens are detected by a sandwich ELISA (Enzyme Linked Immuno Sorbent Assay).

The method is tested for detection of *Salmonella* in foods and feeds. The results are quoted in the Certificate.

Bioline Aps, Denmark, is the manufacturer and supplier of the *Salmonella* ELISA test OPTIMA. Rayal LtD, United Kingdom is the supplier of

Available NMKL Procedures (not included in the NMKL method subscription)

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, 2. Ed. 2010	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

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Invitation to Seminar on Sampling
26 August 2011, Bergen, Norway

Organiser: The Norwegian National Committee of NMKL
Date/ Location: 26 August 2011, Riksstuene, Bergen, Norway
Language: Scandinavian languages

Preliminary program:

09.30 - 10.00 Registration / Exhibition
10.00 - 10.10 Welcome
10.10 - 10.20 Sampling - Definitions
10.20 - 10.50 EU Project on Sampling, Baseline
10.50 - 11.15 Measurement Uncertainty in Sampling

11.15 - 11.45 Break / Exhibition

11.45 - 12.10 Sampling. Laws and Regulations for Fish and Fish Products
12.10 - 12.35 Sampling of Fish and Fish Products in the Fish Industry
12.35 - 13.00 Sampling of Fish for Scientific Purposes

13.00 - 14.00 Lunch / Exhibition

14.00 - 14.20 Marking of the International Year of Chemistry
14.20 - 15.15 Exhibitors present Sampling Equipment

15.15 - 15.45 Break / Exhibition

15.45 - 16.10 Sampling. Laws and Regulation for Vegetables
16.10 - 16.30 Sampling at a Primary Producer
16.30 - 16.50 Sampling in Imported vs Domestic Goods and in the Food Industry
16.50 - 17.00 Closure

Fee: NMKL subscriber: **NOK 2000** non-subscriber: **NOK 2500**
Registration to nmkl@vetinst.no by **15 June 2011**

Exhibitors of sampling equipment are also invited to give a presentation.
Fee for a stand: NOK 5000 including one participant.
Exhibitors are requested to register within 1 June 2011 to nmkl@vetinst.no.

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

- NMKL Methods
- NMKL Procedures
- PT schemes
- National reference laboratories in the Nordic countries
- NordVal protocols
- NordVal Certifi-cates
- List of certified NordVal methods
- Upcoming NMKL/ NordVal courses/ seminars