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NMKL Newsletter

NORDIC COMMITTEE ON FOOD ANALYSIS

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Certified Test kits

- NordVal Certified methods that can be used according to microbiological criteria EC 2073/2005

Please note that the NMKL's Web page will soon appear in a new lay-out.

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New NMKL Method:

Plant stanols and plant sterols. Determination in phytosterol enriched foods with gas chromatograph (GC-FID)

NMKL 198, 2014

Ph.D. Päivi Laakso, Eurofins

This method describes a determination 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. Furthermore, to measure cholesterol content of phytosterol enriched foods. Naturally present phytosterol contents in vegetable oils and foods can be measured by applying this method with some limitations. E.g. purification of 4-desmethyl sterol fraction may be necessary if the sample contains 4-methyl and 4,4-dimethyl sterols. Also the conditions applied to extract phytosterols after saponification may require further optimization.

The term "plant sterols" is used to cover all unsaturated 4-desmethyl sterols existing in plants. In this method the term “total plant sterols” is defined to be the sum of brassicasterol, campesterol, stigmasterol, unidentified sterols eluting between stigmasterol and sitosterol in the chromatogram, sitosterol, sitostanol + delta-5-avenasterol (or only delta-5-avenasterol in cases when sitostanol elutes separately), stigmasta-5,24-dienol, stigmast-7-enol and delta-7-avenasterol. Plant materials may contain small proportions of cholesterol, which in this case is not included in plant sterols. The term "plant stanols" is used to cover all fully saturated 4-desmethyl sterols existing in plants, i.e. sitostanol and campestanol. The term "phytosterols" is used to cover both plant stanols and plant sterols.

A homogeneous test portion of a sample is saponified in the presence of an internal standard with ethanolic potassium hydroxide and unsaponifiable matter extracted with heptane (or hexane). The unsaponifiable matter containing plant stanols, plant sterols and cholesterol is analyzed as their trimethyl silyl derivatives with a gas chromatographic method utilizing flame ionization detection. Both esterified and unesterified plant stanols and plant sterols as well as cholesterol will be determined with this method. As a result, the total amount of plant stanols and/or plant sterols and/or phytosterols or individual plant stanol or plant sterol contents or cholesterol content in the sample are achieved. In some foods, plant stanols and plant sterols are tightly incorporated into the food matrix and an acid hydrolysis step prior to saponification is needed.

Nine laboratories participated in the collaborative study. The samples represented mainly commercially available foodstuffs being margarines, yoghurt drinks, soy and other drinks, vegetable oils and a biscuit sample. Nine of the test samples were enriched with phytosterols whereas three test samples contained only natural contents of phytosterols. The results were satisfactory, and are reproduced in the method.

Ph.D. Päivi Laakso, Eurofins Scientific Finland Oy, elaborated the method and arranged the collaborative study. Christina Bäckman (FI), Jette Jakobsen (DK), Gudjon Atli Audunsson (IS), Livar Frøyland (NO), Paresh Dutta (SE) were contact persons.

REVISED NMKL PROCEDURE

GUIDE ON SAMPLING FOR ANALYSIS OF FOODS

NMKL Procedure No. 12, 2. version, 2014

Considerable effort and resources are used on optimisation of methodology and in the consideration for minimalising the measurement uncertainty of food analyses. However, how samples are withdrawn and whether they are representative for the purpose are paid considerably less attention. Frequently, too few units are selected, or they are selected in such a manner that they are not representative of the lot under scrutiny. The consequences may be unreliable results and wasted time and effort, which may ultimately lead to wrong administrative decisions. Wrong results will also be obtained if test samples are incorrectly labelled, inappropriately stored or pre-treated in a manner not conforming to rules or regulations. The sample size should be appropriate to the purpose – either more or less samples would be a waste of money. It is therefore essential to give sampling procedures careful consideration when designing a project for surveillance, monitoring or inspection.
This guide is intended for everyone concerned with the analysis of food and decision-making based on such analyses.

There are many aspects to consider when designing a sampling procedure. In some foodstuffs, the parameters to be examined are evenly distributed (homogeneously) throughout the matrix, but frequently the compounds/microorganisms are unevenly (heterogeneously) distributed. It is, of course, also important to consider health hazards associated with the parameter, as well as the significance of the foodstuffs the parameter may be associated with, when determining the sampling plan.

Ideally, a simple set of rules should be applied to all sampling of foodstuffs. However, that is not possible, and would be just as inappropriate as having one single analytical method to analyse all types of compounds. Many sampling methods are available for certain foods, and should be applied where appropriate. Similarly, where sampling regulations already exist, they should be adhered to, in order that the results are legally valid.

To be cont. page 4

CONTENTS OF THE GUIDE ON SAMPLING

- Aim of sampling
- Definitions
- Project description including sampling procedure
- The character of the parameter and matrix to be examined
- Where to perform the sampling - location
- Equipment
- Sampling technique
- Number of samples taken
- Sealing and labelling the samples
- Sampling report
- Conditions for transport or shipping of samples
- Storage and pre-treatment of the samples at the laboratory
- Analytical results: interpretation
- References

Annex : Sampling Plans

- Attribute and variable sampling plans
- The choice between attribute and variable sampling plans
- Attribute sampling plans used within microbiology
- Attribute sampling plans used within inspection for chemical and physical parameters
- Sampling plans by variables
- Operating characteristics (OC) curve
- Statistical model of attribute sampling plans
- Definitions
- References

- Annex: Sampling methods available from international organizations

CONTINUED FROM PAGE 3 : GUIDE ON SAMPLING (NMKL PROCEDURE NO. 12)

This second version of the procedure has been elaborated in a project group consisting of:

Denmark: Majbritt Karlskov Moos and Carsten Theisen Pedersen, Eurofins
Iceland: Heida Palmadottir, Matis
Norway: Per Lea, Nofima, **Astrid Nordbotten**, Norwegian Food Safety Authority and **Per-Erik Clasen**, Norwegian Veterinary Institute (**Project leaders**).
Sweden: Kicki Granelli and Mats Lindblad, National Food Agency

Astrid Nordbotten, NFSA

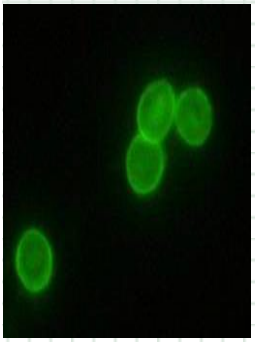
What is new compared to the previous version from 2002a?
The guide is updated with regard to references to standards and regulations. New information, and in particular on secondary sampling (sample reduction at the laboratory) for different matrices, and sampling for sensory analysis are included. Furthermore, the rational and a short illustration for estimating measurement uncertainty from sampling are added.

Per-Erik Clasen, NVI

The NMKL Procedure No. 12 is available at the NMKL web shop.

CRYPTOSPORIDIUM AND *GIARDIA*

In September 2013, the project group under the lead of **Karin Jacobsson, National Food Agency in Sweden**, arranged a Nordic workshop on *Cryptosporidium* and *Giardia* in water and on vegetables/berries.



The workshop aimed to:
- evaluate the need for an NMKL-procedure for detection of *Cryptosporidium* and *Giardia* in water.
- compile and evaluate the Nordic analytical capacity in relation to the need today.

The report of the workshop (Technical Report No. 4, 2014), is available for free under "Technical reports" at **www.nmkl.org.** The report includes information on the current situations on *Cryptosporidium* and *Giardia* in the Nordic countries and in the United Kingdom, alternative detection techniques and conclusions of the discussions.

WITHDRAWAL OR MODIFICATION

Do you use NMKL Method No. 103, or a modification thereof, for the determination of **benzoic aid and sorbic acid in foods by GC**?

According to the NMKL user survey in 2013, the NMKL method is in use. The method is collaboratively validated with satisfactory results. However, as the method is using chloroform as solvent, the method should be modified or else withdrawn.

Please contact NMKL if you are using the method or a modification thereof with respect to the solvent. NMKL would be very interested in the method modification.

NEW TECHNIQUE FOR IDENTIFICATION OF BACTERIA!

NMKL/SVA Workshop in MALDI-TOF analysis in foods

27 May 2014 at the National Veterinary Institute in Uppsala, Sweden

What is MALDI-TOF?

MALDI-TOF is a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), which offers the possibility of accurate, rapid, inexpensive identification of isolated bacteria.

Target group

Microbiologists and chemists, curious on this new technique and would like to see and learn about its applicability, and its pros and cons.

Preliminary Program

09:00 Registration

09:25 Welcome / Introduction

09:30 What is MALDI-TOF? How does MALDI-TOF work?
Field of application, procedures, databases..

Presentations:

- Changing Microbiology – An Introduction
- Rapid Microbial Method in Food and Product Safety
- Industrial Microbiology and Validation of a Rapid Microbial Method

12:00 Lunch

13:00 Practical examples on the use of MALDI-TOF

16:00 Closure

Language: English

Registration to **nmkl@vetinst.no** by 8 May 2014.

Registration fee: NOK 2000,-

NMKL / Eurachem Workshop on Measurement Uncertainty

29-30 September 2014 at IDA conference center in Copenhagen, Denmark

NMKL and Eurachem are arranging a joint workshop on measurement uncertainty (MU) for chemical food and feed analysis . The relation between MU and bias, target MU, MU in sampling, MU in non-destructive analytical methods and in multicomponent analytical methods will be discussed.

Preliminary Program

29 September

13.00 - 13.30	Registration / coffee
13.30 - 13.45	Opening remarks
13.45 - 14.15	What is MU, and what is the relation between MU, bias and precision? General aspects. *Joakim Engman, National Food Agency, Sweden*
14.15 - 15.00	MU and bias *Bertil Magnusson, SP, Sweden*
15.00 - 15.30	Coffee/tea break
16.30 - 16.00	MU in sampling *Astrid Nordbotten, National Food Authority, Norway*
16.00 - 16.30	MU in multi component methods *Tuija Philström, National Food Agency, Sweden*
16.30 - 17.00	Target MU, determining fitness for purpose – setting Standard Method Performance Requirements *Roger Wood, UK*
19.00	Dinner

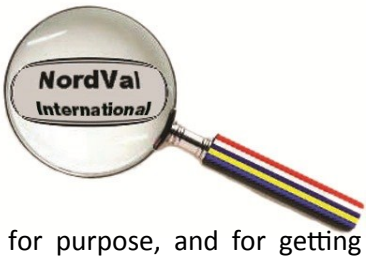
30 September

09.00 - 09.15	Uncertainty estimation from QC and validation data using the free software MUkit *Bertil Magnusson, SP, Sweden*
09.15 – 10.15	Practical examples on the following topics as introduction to round table discussions on: a) MU including bias, b) MU in sampling
10.15 - 10.30	Coffee/Tea break
10.30 - 11.45	Round table discussions
11.45 - 12.30	A sum up of the discussions
12.30 - 13.30	Lunch
13.30 - 14.00	MU in non-destructive analytical methods as NIR, IR *Wolfhard Wegscheider, University of Leoben, Austria*
14.00 - 14.30	On-line course "Estimation of measurement uncertainty in chemical analysis" *Ivo Leito, University of Tartu, Estonia*
14.30 - 14:45	Coffee /tea
14.45 - 15.15	Decision making (compliance assessment) based on results with MU *Lorens Sibbesen LabQuality International, Denmark*
15.15 - 15.45	Summing up

Registration fee:
NOK 4000 (before 15 July)
later: NOK 6000,

Registration to:
nmkl@vetinst.no

METHODS CERTIFIED BY NORDVAL INTERNATIONAL



Documentation of a method's performance is vital when reviewing its fitness for purpose, and for getting accreditation. According to the Microbiological Criteria in the EU regulation (EC) 2073/2005, specific methods are given as reference methods. In order to use alternative methods, these have to be validated against the reference methods in accordance with EN/ISO 16140 or other internationally accepted similar protocols, such as the NordVal Protocol.

THE FOLLOWING METHODS ARE CERTIFIED BY NORDVAL INTERNATIONAL

Aerobic Microorganisms

- Hygicult TPC for Hygiene monitoring (No. 018)
- HyServe Compact Dry TC Method for the enumeration of Total Viable Organisms for all foods (No. 033)

Bacillus cereus

- HyServe Compact Dry X-BC Method for the enumeration of *Bacillus cereus* in foods (No. 045)

Campylobacter

- *Campylobacter* real-time PCR in raw chicken meat, faeces on cloacae swabs and sock swabs (No. 017)
- BAX ® System Real-Time PCR Assay for *Campylobacter jejuni/coli* and *lari* on cloacae swabs (No. 039)

Coliforms/E. coli

- HyServe Compact Dry EC Method for the enumeration of Total Coliforms/*E.coli* in foods (No. 036)
- 3M Petrifilm *E.coli/*Coliform Count Plate in foods (No. 014)

Coliforms

- HyServe compact Dry CF Method for the Enumeration of Total Coliforms in foods (No. 035)

Cronobacter spp.

- iQ-Check™ *Cronobacter* spp. in infant formula and environmental samples in Infant formula and environmental samples (No. 044)

E.coli

- RAPID' E.Coli 2 (No. 020)
- 3M Petrifilm Select *E.coli* Count Plate (No. 027)

E.coli O157

- **food**proof® *E. coli* O 157 Detection Kit in combination with **food**proof® ShortPrep II Kit (No. 026)

Enterobacteriaceae

- 3M Petrifilm *Enterobacteriaceae* Count Plate (No. 029)
- HyServe Compact Dry ETB Method for the enumeration of *Enterobacteriaceae* (No.034)

Listeria

- Transia Plate *Listeria* (No. 002)

Listeria monocytogenes

- RAPID' L.MONO (No. 22)
- **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and **food**proof® *Listeria monocytogenes* Detection Kit, 5' Nuclease in combination with **food**proof® ShortPrep II Kit (No 025)
- iQ-Check TM *Listeria monocytogenes* II (No. 037)

METHODS CERTIFIED BY NORDVAL INTERNATIONAL

THE FOLLOWING METHODS ARE CERTIFIED BY NORDVAL INTERNATIONAL

Salmonella

- *Salmonella* ELISA Test Optima (No. 010)
- Bioline *Salmonella* ELISA test SELECTA (No. 028)
- Transia Plate *Salmonella* Gold (No. 001)
- **food**proof® *Salmonella* Detection Kit, Hybridization Probes and **food**proof® *Salmonella* Detection Kit, 5' Nuclease in combination with **food**prooof® ShortPrep I Kit (No. 023)
- DuPontTM BAX® System PCR Assay for *Salmonella* (Classic + Q7 instruments) (No. 030)
- Rapid'*Salmonella* method, short protocol, Rapid'*Salmonella* method, double enrichment protocol (24h) Rapid'*Salmonella* LATEX confirmation test (No. 032)
- iQ-Check TM *Salmonella* II kit (No. 038)
- *Salmonella* detection method by real-time PCR (No. 041)

Staphylococcus aureus

- HyServe Compact Dry X-SA Method for the Enumeration of *Staphylococcus aureus* in Foods (No. 042)

Yeast and Mould

- Compact Dry YM Method for the enumeration of Yeasts and Moulds in foods (No. 043)
- 3M Petrifilm Yeast and Mould Count Plate (No. 016)

The certificates are available under "list of methods" under NordVal at www.nmkl.org. The certificates are renewed every 2 years.

NEED FOR METHODS?

Do you need a new or improved analytical method and would you like to contribute in the elaboration and validation process, please do not hesitate to contact NMKL.