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Newsletter for Nordic Committee on Food Analysis

NMKL wishes you all a great summer!

Contents

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The secretariat is closed in July.

www.nmkl.org

The Swedish National Committee of NMKL invites to a Nordic Seminar on

MEASUREMENT UNCERTAINTY in analysis of foodstuffs – international guidelines, requirements and adoption

What kind of requirements and guidelines are given with respect to expression of measurement uncertainty (MU)? How should MU be estimated? How should it be expressed? Should MU be considered when reviewing results? If yes, how? These questions will be discussed at the seminar, that will be arranged on **22. August 2008 at Ryttargårdskyrkan, Djurgårdsgatan 97, Linköping, Sweden.**

The seminar will be held in Scandinavian languages.

Programme:

09.00 – 09.30	Registration & coffee
09.30 – 09.40	Introduction
09.40 – 10.00	Measurement uncertainty (MU), what's that?
10.00 – 10.30	What MU estimation requirements are available in regulations and directives?
10.40 – 11.00	Determination of MU within microbiological analysis of foodstuffs.
11.00 – 11.30	Determination of MU within chemical analysis of foodstuffs including ccα and ccβ.
11.30 – 13.00	Lunch
13.00 – 13.30	The Accreditation bodies' requirements for the expression of MU.
13.30 – 14.00	How is MU used by "the customers", e.g. food control authorities?
14.00 – 14.30	How is MU expressed from different laboratories?
14.30 – 15.00	Coffee / Fruit
15.00 – 16.00	Discussion: How are we to become better and more harmonised with regard to MU? Does NMKL have a role in this harmonisation?

Programme including speakers will be posted on www.nmkl.org when available.

Fee: 1 500 SEK excl. tax. Lunch and coffee are included. The National Food Administration (NFA) will confirm the registration and the invoice is forwarded to the participants.

Registration: Binding registration before **1 August 2008** to Gunilla Everitt, NFA, Box 622, SE-751 26 Uppsala, Sweden. Fax: +46 18 10 58 48. E-mail: guev@slv.se.

New NMKL method: No. 189, 2008

Aerobic or anaerobic microorganisms or bacterial spores. Enumeration on Blood Agar.

This method is a routine method for the enumeration of aerobic or anaerobic microorganisms on Blood Agar. The method may also be used for the enumeration of bacterial spores (spore count).

The method is applicable for monitoring bacteria in the production or control of all kinds of food, feed or water, for instance mineral water. It can also be used as a supplementary analysis when investigating the causes of suspected infections or poisoning through food or water. The method does not replace Aerobic Plate Count (NMKL 86), but may be used as a supplement. For instance surface inoculation on Bood Agar may give valuable information regarding the colony morphology.

In cases of preliminary analyses for the presence of pathogenic bacteria, the number of haemolytic bacteria can be stated separately, before further identification is carried out. Selective substances may be added to the Blood Agar to prevent overgrowth of unwanted Gram-negative bacteria.

Both pour plate inoculation and surface inoculation may be used, depending on, whether the plates are only used for counting, or further subcultivation and evaluation of the colony morphology. For evaluation of colony morphology directly, surface inoculation should be used. Surface inoculation is also used for direct plating of swab samples. When examining samples with an expected high content of microaerophilic microorganisms, use either pour plate inoculation or surface inoculation both aerobic and anaerobic.

The temperature of incubation may be adjusted to the temperature used for production or storage of the food in question, or in relation to the purpose of the analysis. If the method is used for screening of potentially pathogenic bacteria, the plates shall be incubated at 37 °C.

Depending on the purpose and the product examined, the plates are incubated under aerobic or anaerobic conditions.

For quantification of spores, the sample is heat-treated at 80 °C in a water bath before inoculation. Through this treatment the vegetative cells are inactivated, and the spore count can be determined following ordinary principles for inoculation and reading of plates.

Quantitative determination of bacteria on Blood Agar is performed by inoculating a known amount of the sample, if necessary, after preparing a serial dilution of the sample on Blood Agar, either on the surface or pour plate. If the purpose of the analysis is the investigation of possible food poisoning by Gram-positive bacteria, Polymyxin may be added to the media to inhibit an accompanying Gram-negative flora. Other substances may also be used in qualitative determinations to prevent overgrowth of other unwanted bacteria.

The plates are incubated under aerobic or anaerobic conditions in an incubator at the selected temperature (30.0 or 37.0 °C for 24 - 48 hours).

Haemolytic colonies may be counted separately. After the subcultivation of colonies, further identification may be carried out following normal principles for microbiological identification.

The numbers of spores on Blood Agar are determined quantitatively by inoculating a known amount of the sample in a suitable dilution, after inactivating the vegetative cells by heating at 80 °C. The sample material is spread on the surface of the plates. The plates are incubated under aerobic or anaerobic conditions as requested.

This NMKL method is elaborated by **Lis Nielsen** (photo above), Dianova, INCUBA Science Park Skejby, Århus, Denmark and **Vibeke From Jeppesen** (photo below), Chr. Hansen A/S, Denmark.
In addition, the following persons have contributed: Janne Lunden, Helsingfors University, Finland, Páll Steinþórsson, Matis, Iceland, Øyvin Østensvik, Norwegian School of Veterinary Science and Erik Eriksson National Veterinary Institute, Sweden.

Note:

- Please remember to notify NMKL about new e-mail addresses.
- In order to receive methods, NMKL must have received payment for the subscription.

New NMKL Procedure:

NMKL PROCEDURE NO. 22, 2008:
GUIDELINES FOR EVALUATION OF IMMUNOCHEMICAL TEST KITS FOR FOOD ANALYSIS

Simple and reliable methods of analysis are needed within the food control authorities as well as in the food industry for the purpose of internal control. A number of methods which are based on antibodies, usually in complete test kits, are marketed as fast, reliable, sensitive, specific and relatively easy to perform and assess, compared to other methods of analysis such as gas chromatography, liquid chromatography and electrophoresis. The test kits are based solely on reagents provided by the manufacturer. Primarily, the manufacturer also conducts the validation of the test kit, and states its performance characteristics. Organizations such as NordVal, AFNOR, AOAC RI and MicroVal offer producers an impartial review of the validation, as a step in the certification of the test kits.

The purpose of this procedure is to assist analysts who are considering using immunochemical test kits, to evaluate the information provided by the manufacturer.

Originally these instructions were elaborated in 1996 (NMKL Report No 17, Guidelines for evaluation of immunochemical test kits for food analysis). In the original report of 1996, all the then current test kits from different manufacturers were listed, filling more than 20 pages. Today, with access to Internet, this information is easily searchable and, moreover, readily updated. Therefore, information on current test kits from different manufacturers is omitted. However, other parts of the report were considered important, such as identifying requirements, definition of quality, method performance characteristics and indication of statistical methods. The revision of the report was started in January 2007 by an NMKL working group consisting of the following persons:

Denmark: Anne Dragsbæk Rasmussen, National Food Institute, Technical University of Denmark, Copenhagen,
Norway: Marianne Theodorsen Werner, National Veterinary Institute, Oslo,
Finland: Mika Tuomola, Raisio Diagnostics, Turku
Sweden: **Ingrid Malmheden Yman**, (photo) National Food Administration, Uppsala, (project leader)

This procedure includes all types of commercial test kits for food analysis, based on antibody technique, both polyclonal and monoclonal antibodies. It also includes immunoaffinity columns (IAC), which operate under the same principle. The procedure does not include test kits based on DNA / RNA probes.

The currently available immunochemical methods are agglutination, enzyme immunoassay (EIA) and fluorescence immunoassay (FIA). Antibodies are also used in "strip" technologies, such as lateral flow, in different kinds of "chip" technologies and optical biosensors. In some bioaffinity assays, something other than antibodies are used for detection, purification and concentration. Previously, immunoassays were often based on radioactive antibodies (RIA). This type of test kit is rarely used within food, agriculture and environmental analysis. However, it is sometimes used for the quantification of progesterone from dairy cows.

Antibody-based methods have an extensive application area, ranging from species control to the detection of allergens (protein ingredients), hormones, antibiotics and other drugs, pesticides, bacteria and bacterial toxins, algae toxins, mould and mould toxins and vitamins. An evaluation of these methods require attention to a number of parameters such as sensitivity, precision, specificity, interference between added and naturally occurring substances, repeatability and recovery. It also requires knowledge of the sample preparation and matrix effects, as well as access to reference materials. When assessing the methods, factors such as costs, instrumentation and time should also be considered.

Possible problems that might arise when using antibody-based tests are, for example, biological changes in the analyte, cauing the formation of metabolites, which may give rise to differences between the immunological and biological activity. The tests may also be sensitive to non-specific reactions, which can give false positive results. An example of this, is protein A from *Staphylococcus aureus* and certain extracts of shrimp and mussels, which can bind antibodies. Both papain and lysozyme can modify the antibodies. Some matrixes can inhibit the reactions between the enzyme activity and the substrate. Some matrixes may also contain endogenous enzymes that can react with the test substrate, such as peroxidase in plants.

The procedure will be made available in English within a few months.

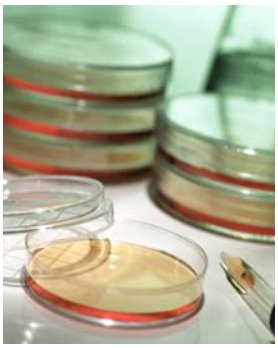
New NMKL Procedure:

NMKL PROCEDURE NO. 23, 2008:

GUIDE ON QUALITY ASSURANCE IN MICROBIOLOGICAL LABORATORIES

CONTENTS

The NMKL Report No. 5 "Quality Assurance Guidelines for Microbiological Laboratories" has been, and still is, a very important guide for microbiological laboratories, when it comes to quality assurance. The first edition was published in 1987 and the second in 1994. A lot has appended in this area, including accreditation and the establishment of the international standard [EN-ISO/IEC 17025:2005] for the competence of testing and calibration, and hence the NMKL report No. 5 was revised again. This time, the guide is published as a NMKL Procedure, which is NMKL's publication series within quality assurance. Most of the microbiological NMKL methods refer to NMKL Report No. 5. This report will be now replaced by NMKL Procedure No. 23.



Under the leadership of Lennart Larsson, LaVet, Sweden, the procedure has been elaborated by the following individuals:

- Vibeke From Jeppesen. Chr. Hansen A / S, Denmark
- Sigrid Gregusson. LabNett (Eurofins), Norway
- Franklin Georgsson, Matis, Iceland
- Lennart Larsson (project leader) LaVet, Sweden
- Seija Kalso, City of Helsinki Environment Institute, Finland

The quality of the analyses carried out in a microbiological laboratory is influenced by many factors, both external and internal. The procedure is general and describes different relevant factors (see the content on the left). The routines described in the procedure have to be adapted by the individual laboratory, their surroundings and activities.

The procedure will also be made available in English.

Correction of NMKL Method No. 56, 4 Ed., 2008: Anaerobic sulphite-reducing bacteria. Determination in foods.

In NMKL Newsletter, No. 68, April 2008, NMKL Method No 56 gives the impression that it is also possible to determine sulphite-reducing *Clostridia* and *C. perfringens* after confirmation.

The following is stated in the NMKL Method No. 56, under 8.5 Confirmation: **If sulfite-reducing *Clostridia* or *C. perfringens* are to be determined, further verification should be performed using i.e. NMKL Method No. 95.** (*Clostridium perfringens*. Determination in heat-treated foods.)

NMKL Method No. 95 **describes only how to verify *C. perfringens*, not how the rest of the clostridia are confirmed**. No 95 is selective for *C. perfringens,* and thus other clostridia are not detected.

The wording in No. 56, as quoted, is therefore not correct. Instead it should say the following:
If the determination of sulphite-reducing clostridia is requested, further confirmation is necessary.

The reference to NMKL Method No. 95 should be deleted as it is only applicable to *C. perfringens.*

Prof. Niels Skovgaard

Courses in:

Requirement specifications for food analysis

- Checklist for considerations in connection with tendering procedures

Whether you are calling for tenders or providing analytical services, it is important to learn about factors that should be considered in connection with the tendering process. NMKL has compiled guidelines for this (NMKL Procedure No. 17) and is arranging courses in order to assist the authorities, laboratories and food industry to specify their needs regarding this topic. Courses have been held in Norway and Sweden, and were very well received.

Target group:

- Food control authorities and other authorities – buyers of external analyses
- Laboratories buying internal/external analyses
- Food producers buying analyses for internal control
- Laboratory personnel – to learn about the requirements set by the authorities

Time and place:

Denmark: In the beginning of November (the date will be announced later), Technical University of Denmark, Food Institute, Mørkhøj Bygade 19, Søborg

Iceland: 7 or 14 November, Matís ohf, Skúlagata 4, Reykjavík

Program:

09.30 - 10.00	Registration & coffee
10.00 - 10.15	Practical information and general information on the NMKL and the guide
10.15 - 11.00	General requirements for tendering procedures of services, review and possible rejection of an offer based on general requirements
11.00 - 11.45	Sampling, transport & receipt of the samples in the laboratory
11.45 - 12.45	Lunch
12.45 - 13.45	Sample preparation, analysis, reporting & quality assurance
	Review of relevant laboratories with the example from:
13.45 - 14.30	a) Microbiology
14.30 - 14.45	Coffee break
14.45 - 15.30	b) Chemistry
15.30 - 16.15	c) Sensory analysis
16.15 - 16.45	d) Economical aspect

Programme including speakers will be published on www.nmkl.org when available.

Fee: NOK 2500,- incl. course material (NMKL Procedure No. 17) + lunch and refreshments.

Languages: Scandinavian languages in Denmark. English in Iceland.
Please register to: nmkl@vetinst.no, see web page for form.
Deadline: 10 October 2008.

NMKL Procedures will be presented at a session at the

Wednesday 24 September, 10.15 - 11.45

Session:
Quality requirements for conducting reliable analysis

Chair: Hilde Skaar Norli
NMKL Secretary General, National Veterinary Institute, Oslo, Norway

Guidelines for preparing specifications in connection with call for tenders for conducting food analysis (NMKL Procedure No 17)
by Astrid Nordbotten, Norwegian Food Safety Authority, Head Office - Section for Control Strategies, Oslo, Norway

Guidelines for preparing specifications, application of EURACHEM/CITAC Guide: Use of uncertainty information in compliance assessment
by M. L. Jane Weitzel, Watson Pharmaceuticals, Corona, California, USA

Guidelines for the evaluation of results from certified reference materials (NMKL Procedure No 9)
by Lars Jorhem, National Food Administration, Uppsala, Sweden

Guidelines for estimation of measurement uncertainty within microbiological analysis. (NMKL Procedure no 8)
by Hilde Skaar Norli, NMKL Secretary General National Veterinary Institute
Oslo, Norway

NMKL's. 62nd Annual Meeting will be held in August 2008 in Vadstena, Sweden.

The Swedish National Committee of NMKL is inviting NMKL members to the NMKL Annual Meeting in Vadstena Klosterhotel, Vadstena, located by the lake Vätteren. The hotel is largely located in the same medieval buildings that were once the Holy Bridget (Birgitta) monastery, now adapted to meet the requirements of modern living.

A little history: In 1370, the Holy Bridget received the right to found a female and male monastery in Vadstena - the foundation of the Bridgettine Order. She died in Rome in 1373 and was buried in Vadstena in 1374. She was canonised in 1391. Her daughter Catharina, who was an abbess in Vadstena monastery, was also regarded as a saint after her death. In 1999, Holy Bridget was made the patron saint of Europe.

In the seat of the patron saint, within these historical walls and venerable buildings, NMKLs experts will meet to discuss all the projects (and perhaps establish new ones) on NMKL's working programme and perform networking.

The members of NMKL (10-15 in each of the Nordic countries) are nominated experts in microbiology, chemistry, sensorial analysis and statistics. They are appointed by the respective Nordic food safety authorities, or their nominated institution. The following institutions are represented in NMKL:

Denmark:

- Danish Meat,
- The Technical University of Denmark, DTU, National Food Institute,
- DTU, DIANOVA
- DTU, National Institute of Aquatic Resources
- The Danish Accreditation and Metrology Fund – DANAK
- Eurofins Steins Laboratory A/S
- Danish Veterinary and Food Administration, Århus
- University of Copenhagen
- Scanpharm A/S
- The State Serum Institute

Finland:

- Finnish Food Safety Authority, Evira
- Lihateollisuuden tutkimuskeskus, LTK
- Finnish Customs laboratory

Faroe Islands:

- Food, Veterinary and Environmental Agency

Iceland:

- Matis ohf
- Norðurmjólk
- Innovation Center Iceland
- Syni Laboratory Service

Norway:

- The Wine Monopoly
- Elopak
- Eurofins Norwegian Institute for Food and Environmental Analysis,
- Norwegian Food Safety Authority,
- NIFES, National Institute of Nutrition and Seafood Research
- Nofima Matforsk, Norwegian Food Research Institute
- Norwegian School of Veterinary Science
- The Norwegian Institute of Public Health
- National Veterinary Institute

Sweden:

- Arla Foods Innovation
- Eurofins Foods/Agro Sweden AB
- Iggesund Paperboard,
- Kristianstad University
- LaVet
- Lantmännen Food R&D AB
- National Food Administration
- National Veterinary Institute
- SWEDAC, the Swedish Board for Accreditation and Conformity Assessment

NordVal will be arranging a session at

Food Micro 2008,

Tuesday 2 September at 14.15-17.15
in Aberdeen. The title of the session is:

Validation of alternative methods - why, how and who?

Why take the trouble of validating alternative methods / test kits? This will be discussed from a legislative, a food producer and a test-kit manufacturer's point of view. How these methods should be validated will be presented by test-kit producers, expert laboratories and the certification bodies AFNOR, Microval and NordVal.

Chair: Sven Qvist, NMKL/ NordVal, Denmark
Co-chair: Åsa Rosengren, National Food Administration, Sweden

Program:

- **Introduction** by Sven Qvist, NMKL, Denmark
- **Why validate – from a legislator's point of view**
  by Maija Hatakka, Evira, Finland
- **Validation of same-day PCR method for Salmonella in meat**
  by Charlotta Löfström, National Food Institute, DTU, Denmark
- **The importance of validated methods within the meat industry**
  by Flemming Hansen, Danish Meat, Denmark
- **Why and how – RAPID' L.MONO, an example from a kit producer**
  by Frederic Martinez, Bio-Rad Food Science Division, France
- Tea Break: 15.30 – 16.00
- **AFNOR Validation: Certifying commercial methods performance**
  by Valentine Digonnet, AFAC AFNOR Certification, France
- **MicroVal: A European validation and certification organisation**
  by Pauline Kalkman, MicroVal, The Netherlands Standardisation Institute
- **NordVal: A Nordic system for validation of alternative methods**
  by Sven Qvist, NordVal/NMKL, Denmark
- **The revision of ISO Standard 16140 and its impact on laboratories and control of foodstuffs in Europe**
  by Paul in't Veld, Food and Consumer Product Safety Authority, the Netherlands
- **Panel discussion**

www.foodmicro2008.org

Renewed NordVal Certificate for *Salmonella* ELISA Test SELECTA from Bioline ApS

NordVal Certifikate No. 28

Bioline *Salmonella* ELISA Test SELECTA uses a simple and fast two-step, specific enrichment procedure for 24 hours. A part of the enriched sample is heat-treated and tested with the sandwich ELISA method. The method is applicable for food products and animal feeds. A well described method procedure is provided in the Bioline *Salmonella* ELISA Kit. The method is validated against ISO 6579:2002: Microbiology of food and animal feedingstuffs - horizontal method for the detection of *Salmonella* spp. *Salmonella* ELISA Test SELECTA has excellent sensitivity, accuracy and specificity (100%). The limit of detection is 1-10 cfu/25g. Bioline *Salmonella* ELISA Test SELECTA can be used without further confirmation. However, it is important to note that national legislations may require that positive results are confirmed.

The products of Bioline are produced in Denmark, but available worldwide. For further information about the products and their availability, see www.bioline.dk or email Bioline at info@bioline.dk.

Renewed and extended NordVal Certificate for TRANSIA PLATE *Listeria* from BioControl Systems Inc.

NordVal Certificate No. 2

TRANSIA PLATE *Listeria* is an ELISA kit for the detection of *Listeria* spp. The method is based on a two-step enrichment procedure (enrichment with half-Fraser Broth and a selective enrichment in Fraser Broth). The detection is carried out by a sandwich ELISA test on a microtiterplate. The method was previously only certified for foodstuffs. New collaborative and comparative tests have been carried out on both foods and environmental samples, with satisfactory results. The method is validated against ISO 11290-1:2004. The relative accuracy is 98%, the relative sensitivity 97% and the relatively specificity 99%. The method can detect between 1 - 10 cfu/25 g.

BioControl Systems Inc. acquired Raisio Diagnostics in September 2007, thus Raisio's development, production and marketing of test kits and analytical systems for the analysis of foods and animal feeds are taken over by BioControl. BioControl Systems Inc. is a US company based in Bellevue, WA. The company is recognized for the development of microbiological tests for the food industry since 1985. BioControl offers an extensive line of proprietary, rapid tests for pathogen detection, quality control, and hygiene monitoring. (www.biocontrolsys.com).

Extended NordVal Certificate No. 31 for Lantmännen Danpo's *Salmonella* PCR method

The previous certificate for the PCR method for the detection of *Salmonella* in meat within 14 hours, is expanded to include detection of *Salmonella* in swabs of carcases after 16 hours and swab boots (poultry faeces socks) after 20 hours. The method is a rapid *Salmonella* method based on 10-16 hours of enrichment followed by detection with real-time PCR. For food samples and swabs of carcases, the limit of detection is 1-10 cfu / 25 g, whilst for swab boots the limit of detection is 10-100 cfu/25g. The method is open, i.e. users can purchase and make the necessary ingredients themselves. Unfortunately, NordVal / NMKL has not been given permission to publish the method. However, the method is described in the following publication:
Burkhard M, Paccassoni E, Fach P, Bunge C, Martin A, and Helmuth R. 2004 Diagnostic Real-Time PCR for the Detection of Salmonella in Food. Appl. Environ. Microbiol., 70:7046-7052.

Please note:

- NordVal certified methods are evaluated by an independent third party (the only validation organisation where manufacturers of test kits are not represented in neither technical committees nor the board).
- NordVal validate according to an internationally recognised protocol (at present the only protocol with acceptance criteria).
- NordVal certified methods can be used (according to EC 20073/2005 on microbiological criteria) also outside the Nordic countries.

A list of NordVal approved methods is available at www.nmkl.org under NordVal.