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Newsletter for
The Nordic committee on Food Analysis

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See:

www.nmkl.org

for information on

- → NMKL's publications. The WEB-SHOP provides descriptions of scope, field of application and principle for the various methods, as well as tables of contents of reports and procedures.
- → NMKL's working programme. Information about all method topics and where they are located in the NMKL system, is included in the working programme.
- → The expert laboratory network. Information on which laboratories to contact, and which laboratories have R&D activities within special analyses.
- → Courses/ Seminars/ Workshops.
- → Comparative laboratory studies. Links to laboratories/institutes with proficiency testing programs.

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NMKL welcomes any input or comments on the NMKL Newsletter and on NMKL's working programme.

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Chairman of NMKL: Ole Bjørn Jensen, Scanpharm A/S, Denmark.

New NMKL methods

Antibacterial substances. Microbiological examination of residues of antibiotics and chemotherapeutics in kidney and muscle tissue from carcasses (4 plate method).

NMKL method No. 121, 2nd edition, 2004

Mette Espersen and Niels Ladefoged Nielsen from the Danish Institute for Food and Veterinary Research (DFVF) elaborated this NMKL method.

The purpose of the method is to determine residues of antibiotics and chemotherapeutics in samples from slaughtered animals, which, by treatment or feeding, have received such substances close to the time of slaughter. Kidney tissue is used for the assay since it usually contains higher concentrations of residual antibiotics and chemotherapeutics than other organs and tissues. In special cases, muscle tissue can also be used. The method describes the detection, not identification, of bactericidal substances in carcasses.

A slice of tissue is placed on an agar substrate plate, which is inoculated with sensitive bacteria. Any antibacterial compounds present will diffuse out into the substrate. The growth of the bacteria will be inhibited in the diffusion zone, while obvious growth occurs outside this zone.

By performing the examination on substrates with different pH values and different bacterial cultures, the method is sensitive to tetracycline, sulphonamides, quinolones, aminoglucosides, macrolides, lincomycin, thiamulin and penicillin. Besides being more detailed than its previous version, this method also detects more compounds.

Listeria monocytogenes. Detection in foods and feeds.

NMKL method No. 136, 3rd edition 2004

Birgit Nørrung from the Danish Institute for Food and Veterinary Research (DFVF) elaborated this NMKL method.

At the annual meeting in 2003, the microbiological committee of NMKL decided to revise this method to allow an optional medium, for example ALOA that is frequently used, in addition to Palcam agar, which is obligatory. Oxford agar has been left out of the method, but can be used as the optional medium. Furthermore, feeds are included in the field of application.

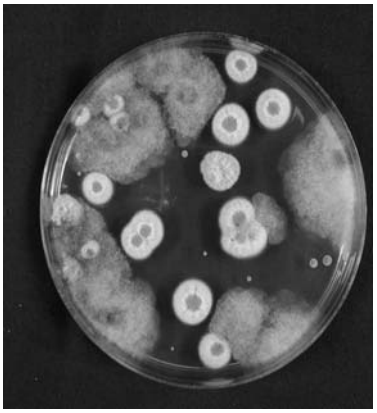
The method uses a two-stage enrichment procedure. The sample is homogenized and incubated in a low-level selective enrichment broth. After 24 hours, aliquots are transferred into a full-fledged selective enrichment broth for further incubation. Two optional selective enrichment broths are described for this enrichment process, and are considered to be equivalent to the first two. If necessary, the method can be used for semi-quantitative determination. Isolation is performed on two selective agar media (Palcam + an optional medium). The identification of the isolate is based on morphological, physiological and biochemical properties, or by using a validated commercial identification test.

The method has not been collaboratively studied. A performance study of a method for quantitative determination of *Listeria monocytogenes* is to be carried out some time during this year. This study is to be arranged by the National Veterinary Institute in Oslo. Laboratories wishing to participate in the study, please contact Semir Loncarevic (e-mail: semir.loncarevic@vetinst.no) or the NMKL Secretary General (e-mail: nmkl@vetinst.no).

Aspergillus flavus and *A. parasiticus*. Determination in foods and feeds.

NMKL method No. 177, 2004

Emma Frändberg from the National Food Administration of Sweden elaborated this NMKL method.



The method quantifies the possible aflatoxin producing mould species *Aspergillus flavus* and *A. Parasiticus*, and is suitable for routine analyses of foods and feeds which may contain aflatoxin, e.g. nuts, maize and spices.

.. excerpt from the method:

PRINCIPLE

The cfu count is obtained by a dilution plating technique, in which a known amount of sample is spread on Aspergillus flavus-parasiticus agar (AFPA). After incubation at 30.0 ± 1.0 °C for 45 ± 3 h, colonies with a characteristic appearance are counted.

The frequency of endogenous infection is determined by direct plating of surface-disinfected particles on AFPA. The plates are incubated at 30.0°C ± 1.0 °C for 45 ± 3 h, the number of particles from which at least one typical colony forms, is recorded, and the infection percentage is calculated…

New NMKL procedure

NMKL procedure No. 5, 2nd edition, 2003:

"Estimation and expression of measurement uncertainty in chemical analysis"

The procedure has been revised considerably and is now available in Swedish. An English version is under preparation.

The procedure has been elaborated in an EK-Livs project (Nordic Committee of Senior Officials for Food Issues), with representatives from all the Nordic countries. The project manager and author of the procedure is **Joakim Engman from the National Food Administration**, Sweden.
The other project group members are:

Denmark: Inge Meyland, The National Food Agency
Finland: Kirsti Nuotio, The Customs Laboratory
Norge: Per Lea, Norwegian Food Research Institute

The procedure has been significantly revised, and now harmonizes with the so-called GUM documentet (Guide to the Expression of Uncertainty in Measurement, ISO, 1993), and the new Eurachem document for measurement uncertainty.

The previous edition of the procedure was published in 1997, and was a user-friendly introduction into how to estimate measurement uncertainty. The previous version was criticized for not taking a broad enough perspective when dealing with the estimation of measurement uncertainty. This has lead to a more comprehensive approach to the subject of measurement uncertainty of quantitative analyses in this version. Previous experience and validation data are considered when estimating measurement uncertainty, thus simplifying the estimation of the total measurement uncertainty. It is important to try to identify all sources of uncertainty in the method. A thorough review of all the steps in a method, and all sources of uncertainty, can give the analyst useful information as to where to find the major sources of error.

The procedure describes simply and clearly how to achieve good estimates of the measurement uncertainty, by using among other things, data which have been obtained from validations and other quality controls. Joakim Engman has also created an Excel spreadsheet for calculating the combined measurement uncertainty.

The author of the procedure, Joakim Engman, has agreed to give a course in estimation and expression of measurement uncertainty in the Nordic countries some time during 2004 (latter half). Information about this will be published on the NMKL home page, and in the next edition of the NMKL Newsletter.

Withdrawal of methods.

NMKL has decided to withdraw the following methods:

- **No. 34, 1974, 2nd ed.:** Sampling of corn, grain mill and starch products.
The method is not used much, and since NMKL now has a procedure for sampling, NMKL procedure no. 12, 2002, users are referred to this.
- **No. 102, 1984:** Determination of saccharin, cyklamate and dulcin in soft drinks.
The method is considered to be outdated and therefore not in regular use. NMKL has more modern and practical methods for the determination of saccharine and cyklamate in NMKL methods no. 122 and 123, respectively.
- **No. 106, 1984:** Ochratoxin in barley. Determination through thin-layer chromatography.
This method is withdrawn because the detection limit does not comply with the given limit values for ochratoxin.

These methods should no longer be referred to as NMKL methods.

Laboratories wanted for collaborative method performance studies:

Does your laboratory have the capacity to participate in a collaborative validation of one or more NMKL methods?
NMKL plans to study the following methods this year:

- *Listeria monocytogenes*. Determination in foods and feeds.
- Thermotolerant campylobacters. Determination in foods and drinking water.
- *Clostridium perfringens*. Determination in foods and feeds.
- Fat content. Determination of fat content in whole milk using the Gerber method.
- Biogene amines. Determination with HPLC in foods.

Unfortunately, NMKL does not have the means to pay the laboratories that participate in these studies. The only reward is honour and glory, and the satisfaction of fulfilling many laboratories' need for a collaboratively validated method.

If you are interested, please notify the NMKL Secretary General: nmkl@vetinst.no.
PS: It is not necessary for laboratories which have already agreed to participate, to notify us again.

The following NMKL report is now also available English:
NMKL report no. 20, 2003: Guide for referees within microbiology. Elaboration of anlytical methods within NMKL.

News from NordVal

As of 1 January 2004, the secretariat of NordVal is no longer at the Danish Veterinary Food Administration. It now belongs to the new sector research institute called the Danish Institute for Food and Veterinary Research under the Danish Ministry of Food, Agriculture and Fisheries. This change involves a new e-mail address and a new fax and telephone number (se below).

The commissorium of NordVal remains unchanged as defined by the Nordic Committee of Senior Officials for Food Issues under the Nordic Council of Ministers. When NordVal has performed a method validation and drawn up a certificate, this is forwarded to the food administration authorities of the 5 Nordic countries. The authorities of each country decide individually how the information from NordVal should be communicated to the public and private laboratories which perform food control analyses.

On the NMKL home page there is a link to NordVal's documents, which are kept up to date at all times. The documents include a list of information on the most recently validated and certified methods. Two new methods have been included on the list since the previous edition of the NMKL Newsletter was published: BAX System - PCR Salmonella Assay from Dupont Qualicon, USA, and RAPID' L.MONO from Bio-Rad, France.

Other documents, such as application forms and the validation protocol have been moderately revised with changes which apply from 1 January 2004. In particular, the validation protocol is a core document for NordVal, and NordVal continuously keeps in touch with other international validation organisations aiming to harmonize elaboration, requirements and evaluation criteria. In connection with this work, NordVal will propose a revision of ISO 16140 – Protocol for the validation of alternative methods.

NordVal follows the development within the EU, which has been very restrictive when it comes to the approval of optional microbiological methods in public food control. In one specific area, there was a breakthrough in 2003, when the EU commission approved optional Salmonella methods in the control of meat and meat products for Finland and Sweden. The conditions were that the methods were validated by AFNOR, AOAC, or NordVal using the most recent version of ISO or NMKL Salmonella methods. Representatives of the Danish meat industry have expressed great appreciation of NordVal's contribution and active involvement in the solution of this issue.

The NordVal Board and the technical committees appointed by NordVal to deal with incoming applications for NordVal certificates, have had a high level of activity throughout 2003. There are indications that this trend will continue in 2004.

On behalf of the NordVal Steering group
Sven Qvist

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The withdrawal of the following methods:

- Aerobe microorganisms. Quantity at 30ºC. Determination in foods using petrifilm™ (NMKL no. 146, 1993)
- Coliform bacteria and *Escherichia coli* in foods. Determination using petrifilm™(NMKL no. 147, 1993)

which was announced in the previous number of the NMKL Newsletter, caused many reactions from the users.

These methods are not withdrawn from the NMKL method collection because NMKL find them unreliable or poor. They are withdrawn because it has been decided that NordVal shall evaluate test kits, proprietary methods (methods in which not all reagents are given), whereas NMKL shall concentrate on the traditional methods. NMKL recommended that the Nordic food administration authorities establish NordVal, as NMKL saw the need to validate test kits but had no resources and capacity of its own to actually perform the work. The members of NordVal are experts who are directly appointed by the food administration authorities in the Nordic countries. When NordVal approves a test kit, it should not be difficult to sustain or accreditate the method based on this approval. The method description in the above-mentioned methods can, if required, still be used, but they must be referred to with NordVal's reference numbers on the relevant test kits. For references, see the NMKL home page under NordVal and "Validation status".