NMKL Newsletter

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Newsletter for Nordic Committee on Food Analysis

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NMKL Procedure No. 4 (2009), Version 3: Validation of chemical analytical methods

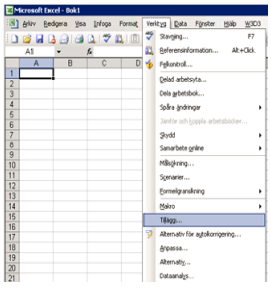
Authorities who have chemical analyses carried out in connection with the control of food, drinking water or animal feed, scientists performing analyses in conjunction with research projects or assignments, and individual chemical laboratories all need to know whether an analysis result is "correct" or "good". In connection with accreditation, it is an obvious requirement that the method of analysis is up to standard.

Validation of a method means examination and determination of the parameters of the method. This can either be done by a single laboratory (internal validation), or by several laboratories (collaborative study). Verification of a method is an examination of a single laboratory's ability to perform the analysis in accordance with the method parameters established in the validation.

Key method parameters are:
- Field of application
- Trueness
- Precision
- HorRat value
- Limit of quantification

The extent of the verification performed locally in each laboratory depends on how thoroughly the method has been validated externally; A thorough external validation simplifies the internal verification. How to validate and verify methods is described in NMKL Procedure No. 4.

The first version of the procedure was issued in 1996. The procedure was revised in 2005, and in 2006, NMKL arranged courses in the procedure in all the Nordic countries. The evaluation reports of the courses were positive. However, a request was made to include a practical example in the procedure. This has been taken into account in the 3rd version. The example (chapter 6) is elaborated by the lecturers of the courses: **Torben Leth,** the National Food Institute, Technical University of Denmark, and **Joakim Engman,** National Food Administration, Sweden. Chapter 6 gives a detailed description through an example of how to carry out a full internal method validation. It is described how to use Excel (both the 2003 and the 2007 version) for the statistical calculations. Torben Leth has elaborated Excel spreadsheets with the example data, which are made available for downloading at NMKL's homepage.



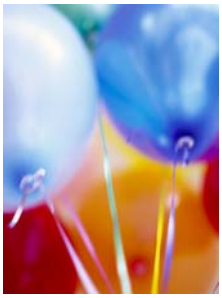
Chapter 6 illustrates how to use Excel for the calculations.

NordVal - 10th years anniversary

NordVal - an independent third-party, reviewing alternative/proprietary methods (test-kits). A proprietary method means a method for which a party, or proprietor, exercises private ownership. The reagents included are not necessarily described in detail.

NordVal was established in 1999, by recommendation of NMKL, and has since then reviewed a number (about 30) of microbiological methods. NordVal has focused on alternative microbiological methods for the analysis of foods, water, feeds, animal faeces and food relevant environmental samples.

Previously, the food control authorities in each of the Nordic countries appointed the board members of NordVal. The Danish Veterinary and Food Administration had the secretariat of NordVal from 1999 to 2007. The obvious chairperson, a key person in the establishment of NordVal, was Dr. Sven Qvist of the Danish Veterinary and Food Administration. Dr. Qvist held the chair until his retirement in 2006. Niels Ladefoged Nielsen, Danish Veterinary and Food Administration, then took over the chairmanship and secretariat.



In February 2007, the Danish Veterinary and Food Administration wished to withdraw from all NordVal activities. NordVal was included as a project in NMKL, and its activities were continued under the NMKL framework. Approved test-kits were followed up, and new applicants were welcomed. The validations were carried out according to the same validation protocol as before.

Sven Qvist accepted to become the chair again, and the other members of the board continued. We were pleased to learn that the test-kit producers continued to see the advantage of having their products certified by NordVal. Already the year after NordVal was included in NMKL, it was obvious that there was a need for its activities. Hence NordVal went from being an NMKL project to become a committee of NMKL with the NMKL Secretary General as the secretary.

NordVal offers

- A user-friendly validation protocol
- Scientific confirmation policies
- Specified acceptance criteria
- Independent and rapid approval procedures
- Guidance in the validation process

The members of the NordVal steering group

The members of the steering group of NordVal are appointed by NMKL, with one representative from each of the Nordic countries.

Chairman: Sven Qvist, Denmark, (photo to the right) E-mail: sven@qvist.com

The other board representatives:

Maija Hatakka,
Evira, Finland

Franklin Georgsson,
Matis, Iceland

Kjell Hauge,
Norwegian Food Safety Authority

Åsa Rosengren
National Food Administration, Sweden

The steering group has board meetings 3-4 times a year, or when necessary.

How to obtain a NordVal Certificate

Fill in the NordVal application form, which is available at NordVal's homepage under www.nmkl.org.

↓

NordVal reviews the application form and nominates a technical committee of experts and an expert laboratory.

↓

The expert laboratory elaborates a test protocol in accordance with the NordVal protocol at this stage. Consultation with the applicant is appropriate on the extent of work, supply of test materials, choice of bacteria strains, etc.

↓

NordVal considers the test protocol for approval.

↓

The expert laboratory carries out a method comparison study in which the alternative method is compared to a reference method using several materials and levels. The expert laboratory organises the collaborative study of the alternative method.

↓

The technical committee reviews the reports of the studies. The committee forwards its conclusions and advice to NordVal.

↓

NordVal informs the applicant of the results, and issues the certificate if the alternative method has proved to be satisfactory.

↓

NordVal announces the approval in the NMKL Newsletter and on its homepage.

Alternative methods are reviewed every 2 years.

For information about NordVal, please contact the chairman of NordVal Sven Qvist (sven@qvist.com) or the NMKL Secretary General Hilde Skaar Norli (nmkl@vetinst.no).

NordVal certification of chemical test-kits

There is also a need for validation of proprietary methods within chemical analysis. Such methods include analyses of mycotoxins, algae toxins, veterinary drugs etc. Laboratories have reported that not all kits are "good". Hence, there is a need to validate the methods and give recommendations based on documented results about the performance of the various test-kits. A protocol for how to perform the validation is under elaboration by a Nordic project group.

If you are a manufacturer of a test kit and would like an independent review of your product, please contact NMKL/NordVal.

Status of NordVal accepted microbiological alternative methods

EU Commission Regulation (EC) 2073/2005 of 15 November 2005, on microbiological criteria for foodstuffs, Article 5: Specific rules for testing and sampling, says:

The use of alternative analytical methods when the methods are validated against the reference method in Annex I, and if a proprietary method, certified by a third party in accordance with the protocol set out in EN /ISO Standard 16140 or other internationally accepted similar protocols is used.

NordVal's protocol is one of these internationally accepted protocols, and is referred to in the ISO 16140 as well as in the EU Regulation for the *Salmonella* control programme.

Where to find information about NordVal-certified methods

- NordVal has approved about 30 alternative microbiological methods.
- A list of approved methods is available at www.nmkl.org. Click on the NordVal logo and visit NordVal's Web pages.
- "List of methods" is the list of approved methods.
- "Certificates" are pdf files of the certificates. In the newer editions of the certificates, the results from the method validation are also given, which might be useful in the establishment of measurement uncertainty and when seeking accreditation for the method.

Renewed NordVal certificates from 3M

3M™Petrifilm™ are small, flat, ready-to-use plates that enable food and beverage manufacturers to perform on-site microbial testing easily and efficiently. The plates consist of a film with nutrients and a gelling agent. The plates may be used to test raw materials, in-process products, finished products, and the production environment. Petrifilm Plates may also be used as part of a production's Hazard Analysis and Critical Control Point (HACCP) program.

3M™ Petrifilm™ is produced by 3M Health Care, Microbiology Products, St. Paul, Minnesota, USA.

3M Deutschland GmbH has received renewed certificates for the following products:

- **3M™Petrifilm™ Aerobic Count Plate**
- **3M™Petrifilm™ Coliform Count Plate**
- **3M™Petrifilm™ E.coli / Coliform Count Plate**
- **3M™Petrifilm™ Staph Express Count System**
- **3M™Petrifilm™ Yeast and Mould Count Plate**

3M™Petrifilm™ Aerobic Count Plate – NordVal Certificate No. 12

3M™Petrifilm™ Aerobic Count Plate has been validated against ISO 4833, 2003: Horizontal method for the enumeration of microorganisms – Colony count technique at 30 °C. There was no statistically significant difference between the results of the Petrifilm method and those of the reference method.

On Petrifilm Aerobic Count Plates, aerobic bacteria colonies will appear as red colonies.

Comparison studies have been performed twice, the latest in 2007, in which 48 hours incubation was tested in addition to the 72 hours incubation test. 66 naturally contaminated samples (1.3 - 7.9 log cfu/g) of meat products, egg products and pastries, vegetables, fish and sea food were analysed both with 3M™Petrifilm™ Aerobic Count Plate at 48 hours and with the reference method (72 hours).

> Using the Petrifilm method, the results can be read after 48 hours

219 naturally contaminated samples were tested with Petrifilm and the reference method after 72 hours. The results show that Petrifilm may also be read after 48 hours, with satisfactory result.

In 2001, a collaborative study was conducted on the Petrifilm method. 15 laboratories participated. All the studies showed satisfactory results both for selectivity, relative sensitivity and specificity.

3M™Petrifilm™ Coliform Count Plate NordVal Certificate No. 13

3M™ Petrifilm™ Coliform Count Plate was tested against ISO 4831, ISO 4832 and NF V08-060, which describe horizontal methods for the detection and enumeration of coliforms by MPN and plating techniques, respectively.

Coliform will appear as red colonies with or without gas.

The method is tested on foods with satisfactory results both in comparison studies and in a collaborative validation study with 12 participating laboratories. The lowest validated level is 0.5 log cfu/g.

Compared with the MPN technique, some differences in the inclusivity can be observed. However, some species show lack of reproducibility in gas production. The validation showed that the selectivity was equivalent for the other reference methods and the 3M™ Petrifilm™ Coliform Count Plate.

The selectivity is a measure of
a) **the inclusivity:** detection of the target microorganism from a wide range of strains, and
b) **the exclusivity**: the lack of interference from a relevant range of non-target microorganisms.

The relative sensitivity is the ability of the alternative method to detect the analyte compared to the reference method.

The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

3M™Petrifilm™ *E.coli* / Coliform Count Plate - NordVal Certificate No. 14

3M™ Petrifilm™ E.coli/Coliform Count Plate has been compared to ISO 16649- 2, 2001: Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -- Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide.

E. coli: All blue colonies with and without gas, after 48 hours of incubation at 37°C.

Coliforms: Red colonies with gas and all blue colonies with or without gas, after 24 hours of incubation at 37°C.

This method is the former NMKL Method No. 147.

In 2005, ADRIA (Association pour le Développement de la Recherche Agro-Alimentaire) carried out a comparison study with several foodstuffs. No statistically significant differences in the results were obtained.

The method was also validated in a full collaborative study. The study was carried out on samples of turkey, mushrooms, beef with gravy, cheese, wheat flour and nut meal with levels ranging from 0.7 - 3.6 log cfu/g. The samples were analysed for *E.coli* and total coliform bacteria count.

The results showed good precision for all samples except for wheat flour, where the repeatability and reproducibility were too high for *E.coli* as well as for the total coliform count. The study resulted in NMKL Method No. 147 and AOAC 991.14: Coliform and *Escherichia coli* Counts in Foods Dry Rehydratable Film. The study report is published in J.AOAC **74**, 635 (1991).

As it is NMKL's policy not to include proprietary methods in its collection, NMKL Method No.147 was withdrawn when NordVal issued the certificate for this method. The method was not withdrawn due to poor performance. The collaborative study shows quite the opposite. The study results are given in the certificate.

3M™Petrifilm™ Yeast and Mould Count Plate - NordVal Certificate No. 16

3M™ Petrifilm™ Yeast and Mold Count Plate was tested against Bacteriology & Applied Microbiology Division (BAM), 18 Chapt., 8th Ed., 2001: Enumeration of Yeasts and Moulds in Food--Dilution Plating Technique.

The results from the study of yeast and mould showed that there were no statistically significance differences between the reference method and the Petrifilm method.

In this case, however, no statistical difference between the alternative method and the reference method does not mean that the results are satisfactory.

The methods were tested on orange juice, hot dog, yogurt, ketchup, corn meal and cake mix. The repeatability (the variation within laboratories) for the analysis of mould is satisfactory, but not the reproducibility (the variation between laboratories). This applies to both the reference method and the alternative method. For yeast, the repeatability is only satisfactory for juice, and the reproducibility is too high. *Obviously, it is equally hard to obtain reproducible results for mould and yeast with the reference method as with the alternative method.*

3M™Petrifilm™ Staph Express Count System - NordVal Certificate No. 19

3M™Petrifilm™ Staph Express Count System was tested against:

- ISO 6888-1:1999/Amd 1:2003: Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) -- Part 1: Technique using Baird-Parker agar medium
- ISO 6888-2:1999/Amd1:2003: -- Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) -- Part 2: Technique using rabbit plasma fibrinogen agar medium

The method was tested on meat, dairy products, sea food, vegetables and other products. There was no statistical difference between the results obtained with Petrifilm and those obtained using the reference method.

If no colonies or only red-violet colonies are present after the incubation, red-violet colonies are counted as *S. aureus*; and the test is complete. If colony colors beside red-violet are present, use a Petrifilm Staph Express Disk. Count all pink zones whether or not colonies are present. Pink zones are usually associated with *S. aureus* but may indicate *S. hyicus* or *S. intermedius*.

Extensive validations are carried out on this method as well. The results show that the selectivity is good (the method detects the bacteria of interest without interferences). A collaborative validation was conducted in 2007, with 13 participating laboratories. The validations studies showed that the precision for both the reference method and the 3M™Petrifilm™ Staph Express Count System was highly satisfactory.

BAX® System for detection of *Salmonella* - NordVal Certificate No. 30

OXOID A/S Thermo Fisher Scientific, Denmark, has renewed its NordVal certificate for the BAX® System for detection of *Salmonella* in foods, feeds and environmental samples. The method is validated against ISO 6579:2002; a horisontal method for detection of *Salmonella.* Bax *Salmonella* PCR is also an AOAC method (AOAC 2003.09).

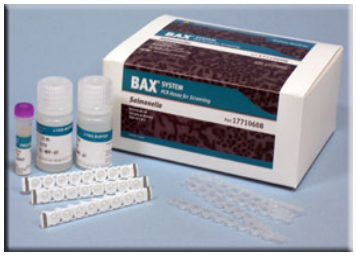
The BAX® System for detection of *Salmonella* is a detection kit using PCR (Polymerase Chain Reaction) technology. The method procedure consists of the following four steps:

- enrichment
- preparation of DNA
- amplification
- detection

The Bax® system for detection of Salmonella targets a specific bacterial DNA fragment, which is specific for *Salmonella* and not found in any other bacteria, and therefore is an indicator of the presence of *Salmonella.*

The BAX Salmonella PCR System (BAX Classic and BAX Q7) can be used without further confirmation.

NordVal has reviewed the results of the validation studies carried out by AFNOR in 2002, 2004 and 2006. The validation is conducted according to ISO 16140. A number of matrixes are tested. The results show that BAX *Salmonella* PCR corresponds to the specifications from the producer, and that the method is equivalent to the reference method.



Bax® System for detection of Salmonella.

The Bax System is applicable to the detection of *Salmonella* in foods, feeds and environmental samples.

The sensitivity is above 95%. The specificity is around 100%. The limit of detection is 1-10 cfu/25g.

The selectivity is satisfactory. No cross reaction was detected.

TRANSIA™ PLATE *Salmonella* Gold - NordVal Certificate No. 1

BioControl, USA,has renewed the certificate for TRANSIA™ PLATE *Salmonella* Gold. This method was validated against ISO 6579:2002, which is a horisontal method for detection of *Salmonella*. TRANSIA™ PLATE *Salmonella* Gold has the following features**:**

- Short, flexible enrichment procedure
- No M-broth required
- No second selective enrichment broth
- Maximised sensitivity through LPS detection
- No cross-reactions with other enterobacteria
- Ready-to-use reagents
- Single and multiple testing

The limit of detection is 1-10 cfu/25g, the specificity is 100% and the sensitivity is above 95%. The method is applicable to foods, feeds and environmental samples.

Salmonella detection using real-time PCR - NordVal Certificate No. 31

Danish Meat, Denmark, has renewed the certificate for detection of *Salmonella* using Real-Time PCR. The method was elaborated by the National Food Institute of the Technical University of Denmark, and is very fast.

The method was validated on raw meat (including poultry neck skin), swabs from beef and pork carcases and poultry faeces on sock samples/ boot swabs. The performance of the method, such as selectivity and precision was tested against the following methods:

- NMKL 71, 5th Ed, 1999: *Salmonella.* Detection in foods.
- NMKL 187, 2007: *Salmonella.* Detection in foods, faeces and materials from primary animal production using MSRV.

The method is based on a shortened pre-enrichment in buffered peptone water for 10-16 hours. DNA is extracted by use of the KingFisher system and the "MagneSil KF Genomic System". Subsequently, *Salmonella* is detected by a novel real time PCR assay developed for the specific detection of *Salmonella* spp.

The results from the validation is satisfactory, with a sensitivity of no less than 95%.

The method is fast:
- 14 h for detection of *Salmonella* in raw meat,
- 16 h for detection of *Salmonella* in swabs of carcases,
- 20 h for detection of *Salmonella* on sock samples of poultry faeces.

NMKL TrainMiC Workshop
Measurement Quality in Analytical Chemistry: With a Focus on Food
Uppsala 17-18 September 2009

The aim of this NMKL/TrainMiC workshop is to give those performing chemical measurements in food, practice in the main issues, but also a new perspective on the quality requirements in ISO 17025 – traceability, method validation, measurement uncertainty and quality control.

Another important aim is to harmonise the way we work with quality, and to support laboratories in improving quality systems, making them more effective, based on competence and acceptance by all personnel.

Target group
The workshop is directed at analytical chemists, technical assessors, and laboratory and quality managers in laboratories working with the analysis of food.

We will invite participants from the Nordic countries, as well as from other countries. All presentations will be held in English.

This NMKL TrainMiC Workshop is organised by the National Food Administration of Sweden and SP Technical Research Institute of Sweden in cooperation with NMKL and IRMM.

Thursday 17 September 2009

09:00 Registration and coffee
09:30 Introduction and presentation of participants (Magnusson)
09:45 Presentation of NMKL (Engman)
10:00 Statistics and terminology TrainMiC (Mangusson)
11:00 Presentation of example; Pesticide residues TrainMiC (Engman)
Exercise: Traceability of measurement results (Magnusson)
12:00 Lunch
13:00 Single laboratory validation of measurement procedures TrainMiC (Engman)
Exercise: Recovery and bias estimation (Magnusson)
15:00 Coffee
15:20-17:00 Uncertainty of measurement - Principles and the Nordtest approach (Magnusson)

19:00 Dinner

Friday 18 September 2009

08:30 Internal quality control – QC TrainMiC (Magnusson)
Exercise: Evaluation over time (Magnusson)
09:30 Inter-laboratory comparisons TrainMiC (Engman)
10:30 Coffee
11:00 Selection and use of reference materials © TrainMiC (Magnusson)
12:00 Lunch
13:00 Exercise: Pesticide residues TrainMiC (Engman)
14:20 Learning evaluation (Magnusson)
14:40 Conclusion of the learning evaluation, and coffee
15:00 Closure of workshop

Evaluation of your own method validation and measurement uncertainty
After this workshop, you will have the opportunity to send one of your methods for an evaluation by the speakers. They will advise and suggest improvements, or just comment on your good work.

Speakers

Bertil Magnusson, SP, Sweden, National TrainMiC Team Leader, industrial analytical chemist with focus on measurement quality and Swedish representative in metrology organisations such as CCQM, Eurachem and Euromet.

Joakim Engman, National Food Administration, Sweden. Analytical chemist working with control of heavy metals in foods. Works with validation and standardisation of methods within NMKL and CEN.

General Information

Location: National Food Administration (**www.slv.se**), Hamnesplanaden 5, Uppsala, Sweden.

Fee: SEK 6 000 excl. VAT, including documentation, coffee, lunch and dinner.

Registration: On the SP home page **www.sp.se/conf** (mätteknik, kemisk mätteknik) or by contacting Johan Seger; johan.seger@sp.se, phone: + 46 (0)10 – 516 52 67 **before 14 August**.

Accommodation: Please make your own room reservation by e-mail to **uplandia@scandichotels.com before 10 August**.

We have reserved rooms for 16-18 September at SEK 971 per night (incl. VAT) at Hotel Scandic Uplandia. The booking code is **LIV160909.**

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Nordic Committee on Food Analysis

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NMKL aims to

- *elaborate and publish relevant and well documented analytical methods for foods and feeds*
- *evaluate and certify alternative analytical methods for foods, feeds and environmental samples under the framework of NordVal*
- *elaborate guidelines for laboratories and users of analytical results*
- *organise relevant courses, workshops and seminars*
- *advance Nordic interests internationally on matters concerning food analysis*
- *be a network and a cooperation forum for Nordic scientists and analysts within food analysis*
- *be an independent third party in reviewing methods and results for instance in disagreements between parties (laboratories / governments / industries)*

NMKL's activities shall help to safeguard quality and traceability all the way from sampling and analysis to reporting of results, thus contributing to ensuring safe food and providing valid studies, surveys and management decisions.

The 63rd NMKL Annual Meeting, Iceland

The Icelandic NMKL National Committee has invited NMKL members to the 63rd NMKL Annual Meeting to be held at Selfoss, Iceland in August.

About 60 experts, including 10-15 representatives from each Nordic country, will be participating.

Photo from Selfoss

In each of the Nordic countries NMKL has a national committee consisting of microbiologists, chemists and sensory analysts representing food laboratories, food control authorities, food industries and relevant scientific research institutions.

As each of the Nordic countries does not have the capacity to perform the validation work carried out by NMKL, of their own account, the Nordic cooperation is of vital importance. NMKL serves the food control authorities in the Nordic countries and therefore is linked to the Nordic Council of Ministers through EK-FJLS (Food).

Each national committee has a chairperson and a secretary:

Denmark:
Chair: Ole Bjørn Jensen, Scanpharm
Secretaries: Arne Højgård Jensen and Else Buskov, Food Control Office, Aarhus

Finland:
Chair: Harriet Wallin, Evira
Secretary: Taija Rissanen, Evira (on maternity leave)

Iceland:
Chair: Franklin Georgsson, Matis
Secretary: Margret Geirsdóttir, Matis

Norway:
Chair: Urd Bente Andersen, Vinmonopolet
Secretary: Dag Grønningen, National Veterinary Institute

Sweden:
Chair: Ulla Edberg, National Food Administration (NFA)
Secretary: Gunilla Everitt, NFA

The office of the NMKL Secretary General wishes you all a great summer!