



• Newsletter ⁷⁵

NMKL – Nordic Committee on Food Analysis

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In this edition:

Workshops:

Quality assurance /
Measurement of uncertainty
**Finnish Food Safety Authority
(EVIRA) 14th October 2010
Uppsala, Sweden
28th September 2010.....2**

NordVal certificate is issued for
**Salmonella Dublin verification
by real-time PCR
(NordVal certificate No 040)...3**

NordVal certificate is issued for
**TRANSIA® PLATE Listeria
(NordVal certificate No 002).....4**

NordVal certificate is issued for
**Salmonella ELISA Test SELECTA /
RayAI Salmonella SELECTA
(NordVal certificate No 028).....5**

NordVal certificate is issued for
**Rapid'Salmonella double
enrichment protocol
Rapid'Salmonella short protocol
Rapid'Salmonella LATEX
confirmation test
(NordVal certificate No 032).....6**

**New NordVal Protocol in
Validation of Alternative
Proprietary Methods8**

NordVal 10 Year Anniversary Symposium Rapid Methods

Date: 20 August 2010
Place: Hotel Hilton at
Copenhagen airport
Language: English
Exhibition: Yes
Participation fee: DKK 1200
Registration: before 28 June
to nmkl@vetinst.no

Program

09.00 - 09.30

Registration / Coffee

09.30 - 09.45

Introduction – the history of NordVal
(Chairman of NordVal, Sven Qvist, Denmark)

09.45 - 10.15

Aspects on how and when to use rapid
methods. The value of standardisation and an
independent review (Mika Tuomola, Finland)

10.15 - 10.45

The NordVal validation protocols (Secretary
of NordVal Hilde S Norli, Norway)

10.45 - 11.15

The importance of using rapid methods,
microbiological and chemical methods, in the
food industry (John Marugg, Nestlé Research
Center, Switzerland)

11.15 - 12.45

Lunch and Exhibition

12.45 - 13.15

Custom-made, rapid methods in the Danish
meat industry (Flemming Hansen, Technologi-
cal Institute, Danish Meat Research Institute)

13.15 - 13.45

Validation and experience of rapid screening
methods for analyses of veterinary drug
residues (Kirsten Halkjær Lund, Danish
Veterinary and Food Administration, Region
East, Ringsted)

13.45 - 14.15

Experience of validation of rapid method for
NordVal approval (Majbritt Karlskov Moos,
Danish Veterinary Food Administration,
Region West, Aalborg)

14.15 - 14.45

Coffee and Exhibition

14.45 - 15.15

Experiences, good and bad, in the use of rapid
methods (allergens / mycotoxins / micro-
organisms) (Charlotta Engdahl Axelsson,
Eurofins, Sweden)

15.15 - 15.30

New rapid technology (chemistry) Producer I

15.30 - 15.45

New rapid technology (chemistry) Producer II

15.45 - 16.00

Conclusion (Arne Højgaard Jensen, Danish
Veterinary and Food Administration, Region
West, Århus)

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



NMKL Nordic Committee on Food Analysis

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Workshops:

Microbiological examinations: Quality assurance activities and measurement of uncertainty in particular

Target group: Laboratories performing microbiological analyses for food, animal feed, water and environmental samples.

14 October 2010

Finnish Food Safety Authority (EVIRA)
Lecture hall Kalevi, Mustialankatu 3, Helsinki



28 September 2010

Mariasalen, Stiftets Hus,
Dragarbrunnsgatan 71, Uppsala



Language: Finnish
Registration: to nmkl@vetinst.no
before 17 September 2010
Registration fee: 100 €

Language: Swedish
Registration: to nmkl@vetinst.no
before 1 September 2010
Registration fee: SEK 1200

Program

09.00 – 09.30 Registration and coffee

09.30 – 09.40 Introduction

09.40 – 10.05 Requirements from the accreditation bodies for estimating and reporting measurement of uncertainty in microbiological examinations. (Giselle Nick-Mäenpää, FINAS)

10.05 – 10.50 Measurement of Uncertainty according to ISO/TS 19036:2006/NMKL Proc. 8. An example: Campylobacter. (Marjaana Hakkinen, EVIRA)

10.50 – 10.55 Coffee break

10.55 – 11.40 Quality assurance activities and uncertainty of the measurement according to Niemelä, S. MIKES J04/2003. An example: water samples. (Tuula Laakso, HSY Helsingin seudun ympäristöpalvelut)

11.40 – 12.40 Lunch

12.40 – 13.00 Introduction to working groups Exercise I. Water samples. Tuula Laakso, HSY Helsingin seudun ympäristöpalvelut. Exercise II. Food samples. (Sanna Raunila, KVVY)

13.00 – 14.45 Working Groups with different themes (water, food, other samples) - dissolving the practical problems

14.45 – 15.00 Coffee break

15.00 – 16.00 Round table discussion, final conclusions and closure

Program

09.00 – 09.30 Registration and coffee

09.30 – 09.40 Introduction (Ulla Edberg, chairman of NMKL, National Food Administration, NFA)

09.40 – 10.15 Use of quality controls in the estimation of measurement uncertainty? (Tommy Šlapokas, NFA)

10.15 – 10.30 Requirements from the accreditation bodies for estimating and reporting measurement of uncertainty in microbiological examinations (Adia Groza, SWEDAC)

10.30 – 10.45 Coffee break

10.45 – 11.45 Some examples on estimation of measurement uncertainty in practise (Tommy Šlapokas, NFA)

11.45 – 13.00 Lunch

13.00 – 14.00 Some more examples for how to estimate the measurement uncertainty in practise (Representatives from different laboratories)

14.00 – 14.15 Introduction to working groups – discussion on practical aspects and experiences in the estimation of measurement uncertainty

14.15 – 15.30 Discussions in groups and coffee

15.30 – 16.30 Summing up of the discussions and closure

A renewed NordVal certificate is issued for **Salmonella Dublin verification by real-time PCR** (NordVal certificate No 040)

This method describes verification of *Salmonella* Dublin on solid media from samples containing *Salmonella* spp.

The method fulfils the requirements of the NordVal validation protocol. *Salmonella* Dublin verification by real-time PCR is an open method, tested in a comparison study and a collaborative study arranged by Danish Technological Institute – Danish Meat Research Institute. The applicant of the NordVal certificate was Ködbranchens Fællesråd, Copenhagen, Denmark.

The reference method was serotyping using slide agglutination according to Kauffmann-White Scheme performed by the National Reference Laboratory (Statens Serum Institut).

The verification method is a real-time PCR, based on TaqMan chemistry, targeting a sequence on the virulence plasmid specific for *S. Dublin*. The samples for the PCR analyse are taken as single colonies known to be *Salmonella*. A loop-full of colony material from cultures on solid media is resuspended in 1 ml 0.85% saline water. The suspension is centrifuged and the supernatant discharged. The pellet is resuspended in 200 µl of TE-buffer (pH 8.0). From this final cell suspension, DNA is released by lysis of the

cells at 96 °C for 10 minutes. PCR is performed using the lysate as template.

Comparison study

Inclusivity

50 isolates of *S. Dublin* were tested twice by the expert laboratory. Ct-values for the dye FAM below 36 indicated that the strains were identified as *S. Dublin*.

The strains were also tested according to the reference method: Kauffmann-White scheme and identified as *S. Dublin*.

Exclusivity

10 isolates of non-*Salmonella* bacteria which can be found in meat and 20 isolates of *Salmonella* other strains than *S. Dublin* were tested twice. For the 20 *Salmonella* strains, no Ct values were found for the FAM dye. The Ct-values were below 36 for the HEX dye, indicating *Salmonella* strains. For the 10 non-*Salmonella*, no Ct-values were obtained for either HEX or FAM.

Collaborative study

Eight laboratories participated in the collaborative study. 20 cultures of *Salmonella* (10 *S. Dublin* and 10 non-Dublin isolates), a positive control and a NTC (no template control) were tested.

No false negative results were obtained. One laboratory obtained one false positive result. Ten of the 80 non-*S. Dublin* samples gave Ct-values above 36 and thus were not *S. Dublin* positive, but must be considered as doubtful results. The reason for these results is unknown, but five of the doubtful results were obtained by one single laboratory, which might be due to inexperience with PCR analysis, and could be a result of cross contamination between samples.

According to the studies no differences were found between the reference method and the real-time PCR verification, however, experience is required for carrying out PCR analysis.



Salmonella Dublin is a host adapted serotype associated with cattle and are rare in other species. The bacteria can cause serious symptoms in humans, occasionally death.

Call for laboratories to take part in a collaborative study on Methyl Mercury. Determination by isotope dilution GC-ICPMS in foodstuffs.

The method is approved by NMKL for a collaborative study, and is intended for quantitative determination of mono-methyl mercury (MMHg) (10-5000 µg/kg dry weight) in mainly marine biota samples.

Samples are spiked with Hg-isotope enriched MMHg and extracted using tetramethylammonium hydroxide (TMAH). After pH adjustment, deri-

vation and extraction, the organic phase is analysed using GC-ICPMS. The GC separates the different mercury species before MMHg is atomised and ionised by the high temperature of the ICP. The ions are extracted from the plasma and transferred to a MS where the ions are determined by a pulse-count and/or analogue detector. Calculation of the result is done

using the isotope dilution equation.

The study will take place Autumn 2010. Fourteen samples are to be analysed at each laboratory.

Please contact Stig Valdersnes (stig.valdersnes@nifes.no) for further information and participation in the collaborative study.

A renewed NordVal certificate is issued for **TRANSIA® PLATE Listeria** (NordVal certificate No 002)

TRANSIA® PLATE Listeria is an Enzyme Linked Immuno Sorbent Assay (ELISA) based on a two step sandwich-type reaction.

The method describes

- enrichment on ½ Fraser broth for 20-26 h at 30°C ± 1°C, then
- inoculation of 0.25 mL of the ½ Fraser broth in 10 mL Fraser broth, incubated at 22-26 h at 30°C ± 1°C, followed by
- TRANSIA® PLATE Listeria test after heating of 1 to 2 mL of the enrichment Fraser broth at 95-100°C (boiling water) for 20 minutes.

The reading of the microtitre plate is carried out using a spectrophotometer at a wavelength of 450 nm.

The method has been tested on foods and environmental samples and compared with the following reference method:

- EN ISO 11290-1:2004:
Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method.

Comparison study – extensive study carried out at the expert laboratory

The latest study of the method was conducted in 2007 by Institut Pasteur de Lille, France.

Accuracy, sensitivity, specificity

A total of 325 samples were analysed, 165 positives and 16 negatives, representing the following categories: meat products, dairy products, seafood products, vegetables and environmental samples.

A total of 325 samples were analysed, 165 positives and 16 negatives, representing the following categories: meat products, dairy products, seafood products, vegetables and environmental samples.

The following results were obtained:

- ✓ Relative accuracy: 98%
- ✓ Relative specificity: 99%
- ✓ Relative sensitivity: 97%
- ✓ Kappa > 0.80

Detection level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The detection levels were 1-10 cfu per 25 g or 25 ml for all matrices.

Inclusivity /exclusivity

Inclusivity: 55 strains of *Listeria* (25 strains of *Listeria monocytogenes* and 25 strains of other *Listeria*) gave all positive results.

Exclusivity: The study of the 30 non-*Listeria* strains by the TRANSIA® PLATE Listeria test did not detect the presence of any cross-reaction.

Collaborative study

The collaborative study was conducted in 2007.

Number of laboratories: 14

Valid results were obtained from 10 of the 14 laboratories. Exclusion of four laboratories was necessary due to shipment and delivery problems.

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Listeria innocua* at the following levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative method and the reference method. The following results were obtained:

- Relative sensitivity: 99%
- Relative specificity: 100%
- Relative accuracy: 100%
- Kappa: 0.99

Conclusion

According to the comparison and the collaborative study no statistical differences were found between the TRANSIA® PLATE Listeria and the reference method, EN ISO 11290-1:2004, for the detection of *Listeria* spp in foods and environmental samples. The validation is performed according to, and fulfil the criteria of the NordVal protocol.

The test kit is manufactured and supplied by BioControl, USA.
www.rapidmethods.com



The relative accuracy;

the degree of correspondence between the response obtained by the alternative method and the reference method.

The relative sensitivity;

the ability of the alternative method to detect the analyte compared to the reference method.

The relative specificity;

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

Kappa; the degree of agreement between the alternative method and the reference method. Kappa of 0.80 or higher is considered to be very good agreement.

A renewed NordVal certificate is issued for **Salmonella ELISA Test SELECTA / RayAI Salmonella SELECTA** (NordVal certificate No 028)



Salmonella ELISA Test SELECTA / RayAI *Salmonella* SELECTA 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 in buffered peptone water for 6-10h at 37°C ± 1°C, and then
- incubation in SELECTA broth for 18-24h at 41.5°C ± 0.5°C followed by an
- immuno-enzymatic test after heating of an aliquot of the SELECTA broth.

Confirmation of positive samples is not necessary, i.e. if it is not required according to the legislation.

The method has been tested on food and animal feed.

Comparison study

The *Salmonella* ELISA Test SELECTA / RayAI *Salmonella* SELECTA is compared against ISO 6579:2002, for the detection of *Salmonella*, in validation studies carried out in 2004 and 2008.

Accuracy, sensitivity, specificity

A total of 528 samples were tested in the two studies, whereof 225 samples were *Salmonella* positive, 72% of these were artificial contaminated and 28% naturally contaminated. The following results were obtained:

- ✓ Relative accuracy: 98%
- ✓ Relative specificity: 100%
- ✓ Relative sensitivity: 97%
- ✓ Kappa > 0.80

Detection level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The detection levels were 1-10 cfu per 25 g or 25 ml for all matrices.

Inclusivity /exclusivity

Inclusivity: 55 strains of *Salmonella* were detected out of 55 tested.

Exclusivity: 30 strains not belonging to genus *Salmonella* was all negative; i.e. no cross-reaction was identified.

Collaborative study

The collaborative study was conducted in 2008.

Number of laboratories: 15

The analyses were performed on samples of pasteurized milk, artifi-

cially contaminated with a strain of *Salmonella typhimurium* at the following levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative method and the reference method. The following results were obtained:

- Sensitivity: 99%
- Specificity: 97%
- Relative accuracy: 99%
- Kappa: > 0.80

Conclusion

According to the comparison and the collaborative study no statistical differences were found between the *Salmonella* ELISA Test SELECTA/ RayAI *Salmonella* SELECTA and the reference method, ISO 6579:2002, for the detection of *Salmonella* in foods and animal feeds. The test kit fulfils the requirements of the NordVal Validation Protocol.

The test kit is manufactured and supplied by Bioline Aps, Denmark and supplied by RayAI Ltd, United Kingdom (www.bioline.dk and www.rayal.com).

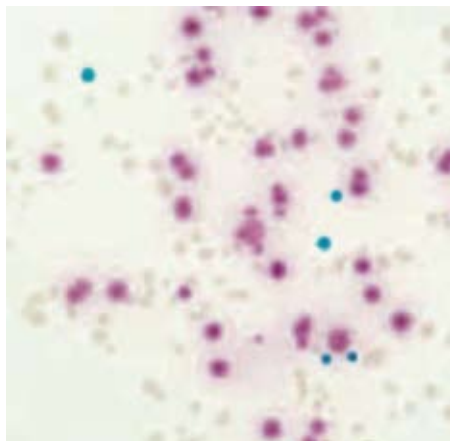
The 64th NMKL Annual Meeting

The Danish National Committee of NMKL has invited its members to the 64th NMKL Annual Meeting to be held in Ebeltøft, Denmark, 21-24 August 2010. About 60 appointed experts, 10-15 representatives from each Nordic country, will be participating.

A renewed NordVal certificate is issued for

- **Rapid' Salmonella double enrichment protocol**
- **Rapid' Salmonella short protocol**
- **Rapid' Salmonella LATEX confirmation test**

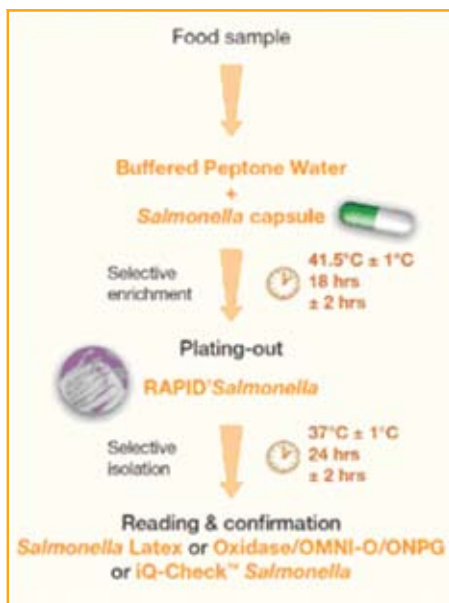
(NordVal certificate No 032)



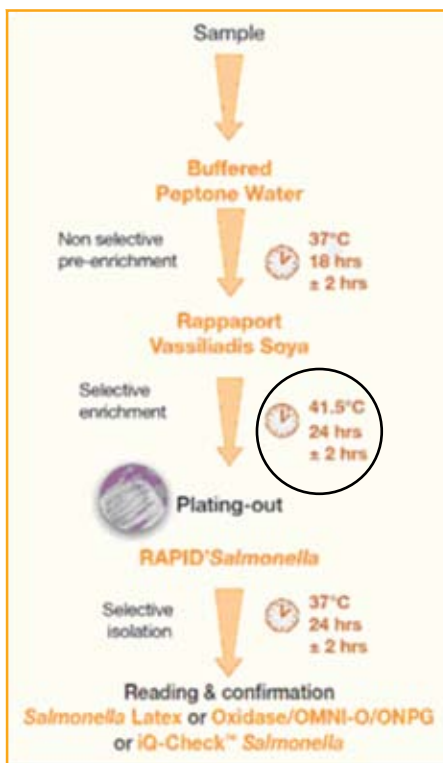
Bio-Rad Rapid' Salmonella.

RAPID' Salmonella is a chromogenic agar medium, the principle of which relies on demonstration of two enzymatic activities. The RAPID' Salmonella test methods approved by NordVal are:

- **RAPID' Salmonella method – Short protocol:**



- **RAPID' Salmonella method – Double enrichment protocol:**



Salmonella spp present appear as typical magenta colonies. The methods are applicable to foods and animal feeds.

- **RAPID' Salmonella LATEX confirmation test** on isolated colonies is applicable for confirmation of Salmonella of groups B to E and G.

Comparison study

The comparison study of the double enrichment protocol was carried out in 2005. The validation study of the short protocol and the Salmonella LATEX confirmation test were carried out in 2009. The comparisons were conducted against the reference method EN ISO 6579:2002: Microbiology of food and animal feeding stuffs -- Horizontal

method for the detection of Salmonella spp.

Accuracy, sensitivity, specificity

RAPID' Salmonella method – Double enrichment protocol

A total of 408 samples of meat products, vegetables and seafood products, dairy products, ovo products, and animal food products were analysed both with the RAPID' Salmonella method and the reference method.

The following overall results were obtained:

	After screening	After confirmation
Relative accuracy	84%	94%
Relative specificity	75%	80%
Relative sensitivity	96%	101%

- Kappa: 0.69
- False negatives: 6%
- False positives: 20%

The relative sensitivity is satisfactory for all products. The agreement between the methods, kappa, is not satisfactory. The reason for the non-agreement is due to the relatively high number of false positives, poor specificity, with the alternative method. The total number of false positive is quite high: 20%.

RAPID' Salmonella method – Short protocol

A total of 324 samples on meat products, vegetables and seafood products, dairy products, ovo products, and animal food products were analysed both with the RAPID' Salmonella

method and the reference method.

The following overall results were obtained:

	After screening	After confirmation
Relative accuracy	91%	91%
Relative specificity	91%	99%
Relative sensitivity	91%	101%

- Kappa: 0.82
- False negatives: 9%
- False positives: 15%

The agreement between the methods, kappa, is satisfactory, except for meat products. In the screening, the relative sensitivity is low due to poorer sensitivity by the reference method. There are some false negatives obtained by the alternative method, however, the reference method does not perform any better.

Confirmation with *Salmonella* LATEX test and the conventional test using double enrichment protocol

118 samples of meat products, dairy products, ovo products and animal feed, were analysed using the double enrichment protocol.

The agreement between the methods is satisfactory (kappa > 0.80). This *Salmonella* LATEX test presents about 5% false negatives.

Detection level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The detection levels were 1-10 cfu per 25 g or 25 ml for all matrices.

Inclusivity /exclusivity

RAPID'*Salmonella* method – Double enrichment protocol

The study was conducted in 2005.

Inclusivity: 51 strains of *Salmonella* were detected out of 52 tested. The non-identified strain is a strain of *paratyphi* A. Two other strains of *Salmonella paratyphi* A were tested and found positive. All target strains

show an Omni-0 positive/ONPG negative profile with the exception of *Salmonella arizonae* (lactose –positive phenotype) presenting a positive ONPG test.

Exclusivity: The study of 30 non-*Salmonella* strains revealed typical colonies on RAPID'*Salmonella* agar in the case of a single strain of *Enterobacter sakazakii*. However, this latter presents a negative Omni-0-test, non-characteristics of *Salmonella*.

Certain strains of *Escherichia hermanii* isolated during the course of the study demonstrate magenta colonies. Consequently 12 strains of this species were tested: 8 yielded positive reaction to Omini-0 test, but present a positive ONPG test, non-characteristic of *Salmonella*.

RAPID'*Salmonella* method – Short protocol

The study was conducted in 2009.

Inclusivity: 47 strains of *Salmonella* were detected out of 51 tested. Three strains of *Salmonella* (*Salmonella paratyphi* A ATCC 9150, *Salmonella paratyphi* B Ad 301 and *Salmonella paratyphi* C ATCC 13428) showed difficulty to grow, as well as *Salmonella gallinarium* Ad 300. Five strains of *Salmonella* gave a negative latex test: *Salmonella arizonae* Ad 450, *Salmonella bongori* Ad 599, *Salmonella cerro* Ad 689, *Salmonella houtenae* Ad 596 and *Salmonella veneziana adria* 233.

Exclusivity: 42 non-*Salmonella* strains, of which 12 strains of *Escherichia hermanii*, were studied. 11 of the *Escherichia hermanii* strains tested, 1 strain of *Citrobacter diversus adria* 140 and 1 strain of *Serratia Salmonella*. All these strains gave a negative latex test.

Collaborative study

RAPID'*Salmonella* method – Double enrichment protocol

The collaborative study was conducted in 2005.

Number of participating laboratories: 15

The analyses were performed on

samples of half-cream pasteurized milk, artificially contaminated with a strain of *Salmonella typhimurium* at the following levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative method and the reference method. Results from five laboratories were excluded to abnormal results apparently resulting from internal contamination and /or discordance in identification test. The following results were obtained:

- Sensitivity: 100%
- Specificity: 98%
- Relative accuracy: 99%
- Kappa: > 0.80

No collaborative study has been carried out on the RAPID'*Salmonella* method – Short protocol.

Conclusion

The studies show that RAPID'*Salmonella* double enrichment protocol and short protocol perform with a satisfactory sensitivity. Some false positives might occur and hence confirmation is necessary.

The test kit is manufactured and supplied by Bio-Rad Laboratories, France. www.bio-rad.com

Do you want a subscription for NMKL methods?

NMKL offers method subscription either by PDF files, hard copies or online (using user ID and password) for NOK 2500 (EUR 300) annually.

If you are a new subscriber of NMKL methods, the first time fee for online subscription is NOK 5000 (EUR 600), which corresponds to the price of a compiled method collection.

With access to the online method collection, the newest version of the NMKL methods are only a click away. The subscribers are notified by email when a method is updated / issued.



New NordVal Protocol:

Guide in Validation of Alternative Proprietary Chemical Methods

This NordVal Protocol describes validation of proprietary chemical methods (test kits). The objective is to guide expert laboratories, NordVal technical committees and the NordVal steering group in the validation, evaluation and certification of test kits. The protocol consists of the following two parts, describing

- ✓ validation and evaluation of qualitative proprietary methods
- ✓ validation and evaluation of quantitative proprietary methods

Preferably, the alternative method should be validated against a reference method. However, when no such method is available the validation can be carried out using certified reference materials, control materials and/or spiked samples at different levels in various matrixes.

The validation consists of two studies: Method comparison study and an intermediate study.

The method comparison study

The method comparison study is carried out by the expert laboratory only. The proprietary method is tested against the reference method on a number of method performance characteristics. When no reference method is available, the proprietary method can be compared against “expected results” of certified reference materials (CRM), control materials and/or various spiked samples.

The method comparison should be carried out on real incurred samples when possible obtaining in the levels of interest. Otherwise, it should be carried out on artificially contaminated samples. If the validation is requested for all food matrices, at least 5 relevant food matrices are selected. For other categories of matrices, select a relevant number of matrices. The concentration levels tested should be low, medium and high in addition to a blind sample, i.e. a sample matrix free of the analyte of interest. The number of replicates should be about 10.

The intermediate study

The aim of the intermediate study is to confirm the obtained results on at least one additional laboratory.

At least three relevant food materials, artificially contaminated at three levels (low, medium and high) and a negative control should be used. The lowest level should be about the detection/screening level. If the intermediate study is conducted at only one additional laboratory, the number of replicates for each matrix has to be at least five. The levels of the samples should be concealed to the laboratory.

The NordVal Protocol is available for downloading at www.nmkl.org under NordVal.

Call for laboratories to take part in a NMKL collaborative study on

Plant stanols and plant sterols

Determination in phytosterol enriched foods with a gas chromatographic method

This method describes a gas chromatographic determination of the contents and compositions 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. These two sample categories are the main application fields of this method. The method can also be applied to measure cholesterol content of phytosterol enriched foods.

Please contact Päivi Laakso, Raisio Nutrition Ltd., Eurofins, Finland (e-mail:PaiviLaakso@eurofins.fi) for further information and participation in the collaborative study.

The following NMKL method is made available in Finnish: **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.

Please visit NMKL's homepage www.nmkl.org for information on

- NMKL Methods
- NMKL Procedures
- Proficiency testing schemes
- National reference laboratories in the Nordic countries
- NordVal Protocols
- NordVal Certificates
- List of certified NordVal Methods
- Upcoming NMKL/NordVal courses/seminars