NMKL ·News 103
October 2020
NORDIC COMMITTEE ON FOOD ANALYSIS

CONTENTS

New secretary-general at NMKL:
Eystein Oveland, Institute of Marine Research, Norway

From 1 November, NMKL's general secretariat will move from the Technical University of Denmark to the Institute of Marine Research in Norway. Eystein Oveland becomes the new secretary-general and Susanna Marie Petrova the new secretary.

Eystein Oveland holds a BSc in Biotechnology, an MSc in Molecular Biology and a PhD I biomedicine. Eystein has been a postdoc and researcher at the University of Bergen and involved in research into various forms of cancer and multiple sclerosis. He has experience in establishing and applying a range of analytical methods, in particular HPLC and mass spectrometry, to the determination of various analyzers.

In 2016, Eystein became a researcher at the National Institute for Nutrition and Seafood Research (NIFES). Today he is a senior researcher and project manager at the new Institute of Marine Research and leads the development and establishment of analytical methods for determining vitamins in feed, fish and other foods. For almost 4 years, Eystein has been an expert member of the ISO / TC 34 / WG 14 and CEN / TC 275 / WG 9 working groups on vitamins and other nutrients and has actively participated in AOAC INTERNATIONAL and method testing under the auspices of SPIFAN.

Eystein got to know NMKL through participating in the NMKL method test No. 204 "Determination of Total Folate in Foods by LC-ESI-MS / MS", which is discussed in this newsletter. In addition to holding the position as secretary -general of NMKL, he will continue to lead the method development of vitamins at the Institute of Marine Research.

The internet address remains: www.nmkl.org

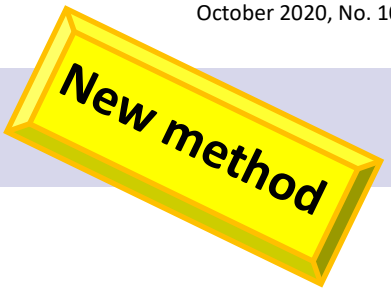
New email address: post@nmkl.org

The fact that the NMKL secretariat moves to the Institute of Marine Research does not result in any changes for subscriptions or the NordVal International certification process.

NMKL c/o National Food Institute, Denmark
Org. No. 30 06 09 46
Tel. +45 4019 1472

www.nmkl.org
email: nmkl@food.dtu.dk

NEW METHOD



NMKL method No. 204, 2020

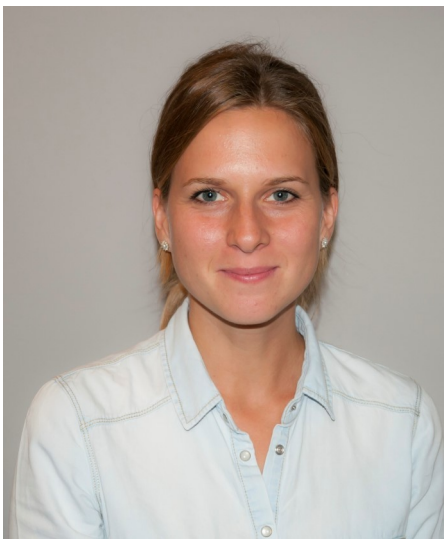
Total folate in food by LC-ESI-MS/MS. Quantification of 6 folate vitamers.

The essential part of the method is using a pure γ-glutamyl hydrolase, which enables efficient and fast break-down of the polyglutamates to monoglutamates. LC-ESI-MS/MS quantifies monoglutamates by use of $^{13}C_5$-labelled internal standards.

The standardized analytical method for folate in food has traditionally utilized quantification by a microbiological assay, a method that cannot distinguish between the different folate vitamers. This new NMKL method enables quantification of total folate based on quantification of six folate vitamers, contributing to the main folate activity. Besides providing the information about the content of the specific folate vitamers, the method includes fast extraction of folates from food that may be carried out within eight hours.

Jette Jakobsen, National Food Institute at Technical University of Denmark (DTU), has been responsible for the project. Method development and single-laboratory validation were performed by Petra Ložnjak Švarc, DTU, in her PhD study, and it served as a basis for collaborative study. The collaborative study was discussed with the contact persons in NMKL i.e. Hanna Sara Strandler (Sweden), Eystein Oveland (Norway) and Susanna Kariluoto (Finland). It was conducted in the summer of 2019 with participation of eight laboratories from seven European countries. The method has been successfully validated for total folate determination in strawberries, spinach, lentils, yogurt, liver and infant formula, providing acceptable Horwitz ratio values (0.60-1.94). The results are published in Food Chemistry (Ložnjak Švarc, P, Oveland, E, Strandler, HS, Kariluoto, S, Campos-Giménez, E, Ivarsen, E, Malaviole, I, Motta, C, Rychlik, M, Striegel, L, Jakobsen, J (2020): Collaborative study: Quantification of total folate in food using an efficient single-enzyme extaction combined with LC-MS/MS. Food Chemistry, 333, 127447).

An essential reagent for the new method is the pure γ-glutamyl hydrolase. In the collaborative study, the enzyme was of plant origin, and may be ordered from DTU (vitamin@food.dtu.dk).



Petra Ložnjak Švarc, photo: DTU

Jette Jakobsen, photo: DTU

UPDATED METHOD

NMKL method No. 67, 2020

Bacillus cereus group. Determination in foods.

has been updated

Bacillus cereus is a motile Gram-positive aerobic / facultative anaerobic spore-forming rod bacterium that loses motility once sporulation has begun. It is 1.0-1.2 µm wide and 3-5 µm long and occurs widely in nature, including soil, water, milk and in the gastrointestinal tract of animals and humans. *B. cereus* is able to produce an emetic toxin (cereulide) and three different cytotoxins (Hbl, Nhe and CytK) associated with diarrhea.

Most *B. cereus* strains produce a strong lecithinase response to egg yolk-containing substrate, and no strains produce acid from mannitol. Most *B. cereus* also produce phosphatidyl-inositol phospholipase C (PI-PLC).

B. cereus grows on blood agar with large irregular, grayish-white colonies with a well-defined hemolytic zone around the colonies. The colonies may vary somewhat in appearance. Presumed *B. cereus* is determined quantitatively by plating known amounts of samples on *Bacillus cereus* selective agar. Alternatively, parallel sowing is performed on blood agar and *Bacillus cereus* selective agar can be performed, especially when low counts are expected. In addition, it is possible to look at Cereus-Ident agar, where the *B. cereus* group forms blue-turquoise-typical colonies that may be surrounded by a blue halo.

The *Bacillus cereus* group comprises the species *Bacillus cereus*, *Bacillus weihenstephanensis*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides* and *Bacillus anthracis*. The method indicates different ways to distinguish the different species.

The method does not distinguish between *B. cereus*, *B. thuringiensis* and *B. weihenstephanensis*, while *B. mycoides*, *B. pseudomycoides* and *B. anthracis* can be distinguished. Since the species are very closely related and all can produce enterotoxin, it is often not necessary in food to distinguish between them.

The need for the revision of NMKL 67 arose from the wish of laboratories to use it as an alternative method for ISO 7932:2004 in analysis required in microbiological criteria (EC 2073/2005). In the revision, NMKL 67 was harmonized with ISO 7932:2004 by changing blood agar for selective agar as the primary plating medium. Colonies are then confirmed by haemolysis on blood agar. It is still possible to use blood agar in parallel with selective agar, especially, when low counts are expected.

In the title of the method, 'presumptive *Bacillus cereus*' was replaced by '*Bacillus cereus* group'. This group of several closely related *Bacillus* species shows similar characteristics, lecithinase reaction on egg yolk medium and no acid production from mannitol, in the conditions specified in this method.

The revision was done by Marjaana Hakkinen, Finnish Food Authority, Finland, Annie Kaalby, Arla Foods, Denmark, Cecilie From, Eurofins Food & Feed Testing Norway and Franklin Georgsson, Matis, Iceland. Ia Pudas og Christina Normark from Swedish Food Agency, Sweden, arranged the collaborative study. Determination of Cereus-Ident agar is not included in the overall study.

Marjaana Hakkinen is a senior scientist at the Finnish Food Authority. She is head of the Food and Feed Microbiology Laboratory Section and has long experience in the area of zoonotic microbial pathogens in food chain. She is also the NRL campylobacter contact person in Finland and member of the working group CEN 463 WG 3.

Marjaana Hakkinen

Cecilie From, private photo

Annie Kaalby, private photo

Franklin Georgsson, photo: NMKL

UPDATED METHOD

Coming soon!

NMKL method No. 117, 2020

Pathogenic *Yersinia enterocolitica*. Detection in food and animal feed and environmental samples

Yersinia enterocolitica is a foodborne bacterial pathogen causing infections in humans worldwide. *Y. enterocolitica* is widespread in nature and includes a broad spectrum of varieties, of which only a few are pathogenic to man. Clinical significance is associated with certain biotype and serotype combinations of which bioserotypes 4/O:3 and 2/O:9 are most frequently isolated in humans. Pigs represent an important source of human infection.

This method describes the qualitative detection of pathogenic *Y. enterocolitica* (associated with human disease) in food, feed and environmental samples in the area of food production and food handling.

The principle of the method has been completely revised compared to the previous version of the method. In addition to the general procedure, optional cold enrichment procedure described (in Annex 3) may be used, for example during foodborne outbreak investigations. The method is validated and harmonized with ISO 10273:2017 *Y. enterocolitica* standard method.

Saija is a microbiologist (PhD in Food Science) and works as senior research scientist at the Food and Feed Microbiology Laboratory Section at the Finnish Food Safety Authority. Her field of expertise covers foodborne pathogens, for example outbreak investigations along the food production chain using molecular microbiology methods, and method development and validation connected to food microbiology. She coordinates the reference laboratory activities for Finnish Food Microbiology laboratories. She takes part in working groups of ISO TC 34 SC 9 and CEN TC 463 and led the CEN project of the validation of Y. enterocolitica as one of the sub-projects mandated by EU commission for European and International validation of 15 main reference methods in the microbiology of the food chain, see

https://www.sciencedirect.com/journal/international-journal-of-food-microbiology/vol/288/suppl/C

Saija Hallanvuo
Photo: Finnish Food Authority

UPDATED PROCEDURE

NMKL procedure No. 19, 2020
Guidelines for sensory evaluation of food packaging
has been updated

When aiming to achieve a well-functioning food packaging a variety of highly important different factors of both the materials themselves and the nature and characteristics of the packaged food must be considered. The fundamental function of packaging is to protect the packaged food in many ways, such as mechanically, microbiologically, chemically and sensory, as well as design and appearance.

The packaging of food is also dealt with in official rules and regulations: Within the EU, food packaging is regulated by the Regulation, which states that materials which may come into contact with food shall not emit substances to the foods so that they cause an unacceptable change in e.g. their odour and taste.

The guidelines in this NMKL procedure provides several different procedures which may be applied in the sensory evaluation of food packaging and points out critical aspects and potential pitfalls of this work. It focuses mainly on the sensory aspects of packaged foods relating to odour and taste evaluation, and consequently does not deal with issues such as the design and practical usability of the packaging. The field of application is wide and covers issues such as continuous production control of incoming materials, evaluation of storage conditions for packaged goods, and controls to ensure compliance with applicable rules and regulations.

The nature and structure of the packaging and its relevant applications will, of course, govern the choice of method and how it is performed. The packaging may either be assessed in itself or it may be evaluated by assessing a test medium which has been in contact (indirectly or directly) with the packaging.

A sensory evaluation is then performed to assess odour and taste. Sometimes other factors are considered, such as visual appearance and consistency. Reference samples are used in practically all sensory evaluations, thus allowing comparison between the tested samples and the reference samples.

The main change in this new version of the procedure is an update of the list of references. Only minor editorial corrections are made on the main content.

Update was initiated by Leena Lilleberg

and finished by Liv Bente Strandos and Grethe Hyldig.

Liv Bente Strandos
Photo: Elopak, Norway

Grethe Hyldig
Photo: DTU

NordVal International - Renewed and extended certificates

Extension:

NordVal International Certificate 037 issued to Bio-Rad for "iQ-Check® Listeria monocytogenes II" has been extended. The method describes sample preparation according to one of three protocols followed by real-time PCR using flore-scent probes. An extension study has been performed for protocols for environmental samples, i.e. the Easy II lysis protocol, a new enrichment protocol and a protocol for use of Free DNA Removal Solution (FDRS) as well as a new ap-plication protocol, APF Fast. When reviewing the results, NordVal International concludes that "iQ-Check® Listeria mo-nocytogenes II" for detecting Listeria monocytogenes provides results equivalent to the results from the reference me-thod when applied for detection in a broad range of foods and environmental samples.

Renewals:

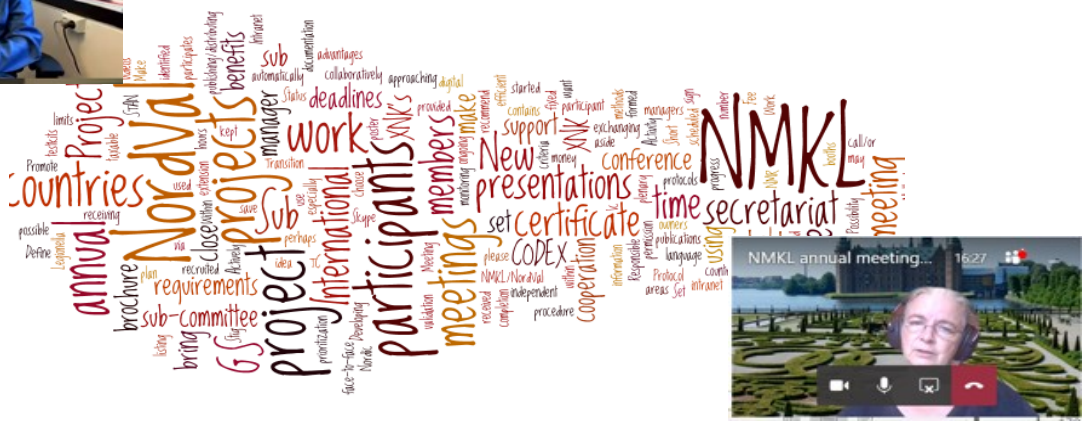
NordVal International Certificate 050 issued to HyServe for Compact Dry YM RAPID has been renewed. Compact Dry are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent. The Compact Dry YM RAPID method contains chromogenic medium and selective agents for the detection and enumeration of yeasts and moulds after 3 days of incubation. The method is applicable for a broad range of foods with an aw of >0.95. Like the reference method, it is not intended for mould spores or for heat resistant mould species.

The NordVal International certificate 049, issued to Bio-Rad for RAPID'*Staph*, has been renewed. The RAPID'*Staph* is based on an optimised Baird-Parker formula for enumeration of *S. aureus*. Some *Staphylococcus* coagulase positive strains can give colonies with a small or without halo after 24 hours of incubation. An additional incubation of 24 hours could be necessary to see the halo. Using RAPID'*Staph* the typical staphylococci produce gray to black colonies that are shiny and cover with a clear halo. The method is applicable for enumeration of coagulase-positive *Staphylococcus* (*Staphylococcus aureus* and other species) in a broad range of foods.

Certificates, validation protocols for microbiological and chemical certifications, workflow for certification process etc.

can be found on the website for NordVal at www.nmkl.org

NMKL's 74TH ANNUAL MEETING



NMKL's annual meeting was held in the period 28 August—1 September 2020.

The Danish national committee had prepared the meeting to take place in Hillerød, Denmark, however, due to restrictions concerning meetings and travels in connection with the COVID-19 pandemic the annual meeting was changed into a virtual meeting.

The meeting surpassed expectations and contained both plenary and parallel sessions with strategy work, academic presentations and work on specific projects. Furthermore, meeting participants enjoyed several virtual social breaks.

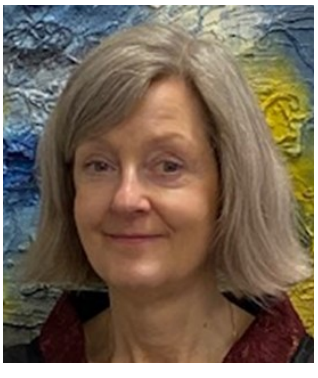
THANK YOU FOR YOUR KIND COOPERATION!

This is my last NMKL newsletter. As from 1 November I am no longer secretary-general of NMKL as DTU will not continue hosting the secretariat. For a little over four years I as Secretary-General and Kirsten Becker Hansen as Office Manager have had the pleasure of holding the incredible exciting job it is to run the secretariat. It was a huge pleasure to work together with so many skillful and committed people, both inside the NMKL organization and in connection with NMKL's many international cooperation projects.

Even though Kirsten and I will miss the work in the NMKL secretariat we are happy to notice that the Institute of Marine Research in Norway has expressed a big interest in hosting the NMKL secretariat; the new secretary-general has been introduced to NMKL work for some time now. Our best wishes go to the new secretariat staff, all NMKL members, cooperation partners and customers.

Thank you so much for your fantastic cooperation!

Nina Skall Nielsen, Secretary-General



Kirsten Becker Hansen, Office Manager

NMKL procedures available

No. 1, 2nd Ed. 2005 Kalibrering och kontroll av vågar på laboratorier. *Calibration and performance checking of laboratory balances*

No. 3, 1996 Kontrollkort och kontrollprov i den interna kvalitetskontrollen på kemiska livsmedelslaboratorier. *Control charts and control materials in internal quality control in food chemical laboratories*

No. 4, 3rd Ed., 2009 Validering av kjemiske analysemetoder. *Validation of chemical analytical methods*

No. 5, 2nd Ed. 2003 Skattning och angivande av mätosäkerhet vid kemiska analyser. *Estimation and expression of measurement uncertainty in chemical analysis (3rd Ed. 2019)*

No. 6, 2nd Ed. 2016 Generelle retningslinier for kvalitetssikring af sensoriske laboratorier. *(Yleiset ohjeet aistinvaraisten laboratorioiden laadunvarmistukseen)*

No. 7, 1998 Kontrol af UV/VIS spektrofotometre. *Checking of UV/VIS spectrophotometers*

No. 8, 4th Ed. 2008 Måleusikkerhet ved kvantitativ mikrobiologisk undersøkelse av næringsmidler. *Measurement of uncertainty in quantitative microbiological examination of foods*

No. 9, 2nd Ed., 2007 Utvärdering av det systematiska felet med användning av certifierade referensmaterial. *Evaluation of method bias using certified reference materials*

No. 10, 2nd Ed. 2017 Kvalitetskontroll av mikrobiologiske dyrkningsmedier. *Control of microbiological media*

No. 11, 2nd Ed. 2010 Sensorisk bedømmelse av drikkevann. *Procedure for sensory analysis of drinking water*
Juomaveden aistinvarainen arviointi.

No. 12, 2nd Ed., 2014 Håndbok i prøvetaking av næringsmidler. *Guide on sampling for analysis of foods*

No. 13, 2003 Volumentrisk kontrol. *Volumetric control*

No. 16, 2005 (2007) Sensorisk Kvalitetskontroll. *Sensory quality control.* Aistinvarainen laadunvalvonta

No. 17, 2006 Kravspesifikasjoner ved kjøp av analysetjenester. *Guidelines for requirement specifications for food analyses.*

No. 18, 2006 Bruk av referansematerialer, referansestammer og kontrollkort i mikrobiologiske næringsmiddellaboratorier. *The use of reference materials, reference strains and control charts in a food microbiological laboratory*

No. 19, 2007, 4th Ed. 2020 Riktlinjer för sensorisk bedömning av livsmedelsförpackningar. *Guideline for sensorial Analysis of Food containers/packages*

No. 20, 2007 Evaluering av resultater fra kvalitative metoder. *Evaluation of results from qualitative methods*

No. 21, 2nd Ed. 2016 *Guide for sensory analysis of fish and shellfish* (Available in English and Finnish)

No. 22, 2008 Anvisnigar för värdering av immunokemiska testkit för livsmedelsanalys. *Considerations regarding evaluation of immunochemical test kits for food analysis*

No. 23, 2008 Handledning i kvalitetssäkring för mikrobiologiska laboratorier. *Guide on quality assurance in microbiological laboratories*

No. 24, 2010 Veiledning i kvalitetssikring for kemiske levnedsmiddellaboratorier. *Guidelines for quality assurance for food chemical laboratories* (also available in Finnish)

No. 25, 2014 Utbyte (Recovery) vid kemiska analytiska mätningar. *Recovery information in analytical measurement*

No. 26, 2nd Ed., 2015 Kontroll och intern kalibrering av termometrar och temperaturkontroll på mikrobiologiska laboratorier. *Control and internal calibration of thermometers and temperature control on microbiological laboratories*

No. 27, 2013 Måleusikkerhet i sensoriske analyser. *Measurement uncertainty in sensory analysis*

No. 28, 2014 *Guidelines for reporting sensory data*

No. 29, 2014 *Guidelines for sensory analysis of meat and meat products (English and Finnish)*

No. 30, 2014 *Statistical Evaluation of Results from Quantitative Microbiological Methods (English)*

No. 31, 2015 *Guidelines for sensory evaluation of bread*

No. 32, 2017 Verifikation af mikrobiologiske metoder. *Verification of microbiological methods*