

NMKL Newsletter

No. 74 April 2010

www.nmkl.org nmkl@vetinst.no

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New NMKL Method No. 141, 3rd. Ed, 2010: Brochotrix spp. Enumeration in meat and meat products

NMKL Method No. 141 is intended for enumeration of Brochothrix spp. in meat and meat products. Former editions of this method described determination of Brochothrix thermosphacta in various meat products including refrigerated, fresh and heatproducts. processed However, the method does not differentiate Brochothrix between spp., and thus, Brochothrix campestris, if present in the meat, is also determined by this method.

Brochothrix thermosphacta is common in packaged, refrigerated meat and meat products, and may cause meat spoilage.

Typically, meat spoilage due to *B. thermosphacta* is associated with an offensive odour described as "cheesy" or "sweaty socks". *B. campestris* is common in soil and vegetation. It is not currently clear whether *B. campestris* plays a role in the spoilage of meat or processed meat products.

Brochothrix SPD. are Gram-positive, catalasepositive. oxidasenegative, non sporeforming, facultative rod-shaped anaerobic bacteria. In Gram-staining of older cultures (48 h), B. thermosphacta often appears coccoid.

Brochothrix SDD. are quantitatively determined by surface plating appropriate dilutions on streptomycin to sulphate-thallium acetate (STA) agar medium. Plates are incubated at $25 \pm 1.0^{\circ}$ C for 48 ± 4 h. Colonies are confirmed from STA medium by testing for catalase and oxidase reactions.

The method has been evaluated in a collaborative study. Fifteen Nordic laboratories analysed ten samples of freeze-dried *Brochothrix thermosphacta* cultures mixed with unspecified bacterial flora from meat or fish. The results were satisfactory.



Elina Vihavainen (above), Dept. of Food and Environmental Hygiene, University of Helsinki, Finland, has elaborated this new version of NMKL Method No. 141.

New NMKL Method No. 91, 5th Ed., 2010: Preparation of the test sample and initial suspension of food and animal feeding stuffs for quantitative microbiological examination

The objective of this method is to ensure that preparation of test samples and initial suspensions of food and feeding stuffs for quantitative microbiological examination are performed correctly and in a uniform way. Unless specified, animal feeding stuffs are included in the term 'food'.

This method is intended for analysis of all solid, semi-solid and liquid food products. Further, it describes how surface and depth sampling, respectively, are carried out in solid foods. NMKL 91 is an important method, and is referred to in almost all microbiological NMKL methods; for analysis of bacteria, yeast and moulds.

General principles for sampling and pretreatment in connection to quantitative microbiological analyses are described. If other sample sizes, dilutions etc. are given in vertical standards/methods for specific food products or microorganisms, those descriptions should be followed.

• The main changes of the method from the previous editions are:

- § I) Specific references to certain commodities, such as "milk", have been removed and replaced by a general statement in § I Scope and field of application, allowing other specific standards to overrule NMKL 91 for certain products/microorganisms.
- § 2) References: IDF 122C was removed and ISO 6887-5 is included.
- § 4) Reagents: Buffered Peptone Water has been included as a possible diluent. The footnotes have been removed.
- § 5) Apparatus and Glassware: More utensils have been added.
- § 6) Sampling: A reference to NMKL Procedure No. 12 is included Frozen foods can be stored (and analysed) until expiry date.
- § 7) Procedure: The sample size is changed from the fixed "10 gram" to the more flexible "10 - 25 gram".

A remark considering the sampling of "composite" samples is included.

§ 7.3.4 Dried, ground food items, a 30 min. soaking has been included in order to slowly rehydrate the sample to avoid cell lysis.



(and analysed) until expiryFlemmingHansen (above), Danishdate.Technological Institute, DMRI, has re-
vised this method.

NMKL Methods now available in Finnish:No 95, 5th Ed., 2009:

- Clostridium perfringens. Määrittäminen
- elintarvikkeista, rehuista ja
- ympäristönäytteistä.

• No 56, 4th Ed., 2008:

Anaerobiset sulfiittia pelkistävät bakteerit. Määrittäminen elintarvikkeista. An update of the references in NMKL Procedure No. 19 2007: Guidelines for sensory evaluation of food packaging, is made available for downloading under publications at www.nmkl.org

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New NMKL Procedure No. 11, Version 2, 2010: Sensory analysis of drinking water

It is a well known fact that drinking water can be of very poor quality, and that sensorial parameters are important to be able to reveal this. The EU Drinking Water Directive outlines requirements for sensorial quality.

NMKL has elaborated this procedure in order to assist laboratories starting up analysis of drinking water. NMKL has also elaborated a sensorial method for analysis of drinking water, NMKL Method No. 183: Quality control test for drinking water. It is recommended to use this procedure along with that method.

This NMKL procedure describes the selection and training of assessors, preparations for the analyses, instructions for the as-

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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. sessors, how to treat and prepare samples, requirements for the testing area and equipment, and how to serve the samples.

This procedure is meant to serve as a reference guide for water works and laboratories who want to use sensory analysis, or require more knowledge about these techniques. It is mainly written for analysis performed in laboratories. But it may also give examples for those who perform analyses in the field, e.g. at a tapping point in the main system.

The procedure is based on international recommendations and standards, as well as experiences from laboratories or others that perform sensory analysis of drinking water. More general procedures for quality assurance of sensory laboratories can be found in NMKL Procedure No. 6.



Urd Bente Andersen, (above) Vinmonopolet, The Norwegian Monopoly for sales of liquors and wine, has elaborated and revised the procedure.

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 μ g/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, derivatisation 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 in autumn 2010. The number of samples analysed at each laboratory will be 14. Contact Stig Valdersnes (stig.valdersnes@nifes.no) for participation.

New NMKL Protocol No. 4, 2010: Manual for NMKL Peer-Verification (Interlaboratory verification) of methods.

The centralising of laboratory facilities and the use of more sophisticated, expensive and specialised analytical equipment have created a need for a procedure to verify analytical methods in situations where it is not possible to perform a full collaborative study due to lack of (participating) laboratories.

The purpose of the NMKL peerverification programme is to provide a class of tested methods which have not been the subject of a full collaborative study. Verification of a method includes describing, validating and testing it for performance according to international recognised standards, as well as

> Establishing acceptable performance parameters within a laboratory

Demonstrating acceptable performance in at least one more independent laboratory The programme is intended for chemical methods.

The protocol is elaborated by an NMKL working group chaired by Torben Leth, The National Food Institute, Technical University of Denmark.

The other project members have been Flemming Hansen, Denmark, Lenna Sari, Finland, Guðjon Atli Auðunsson, Iceland, Per Lea, Norway, Lars Jorhem, Åsa Rosengren and Gunnar Forsgren, Sweden.

The process of NMKL Peer-Verification of methods

The process involves a method referee, two independent laboratories. a technical referee, two technical experts in addition to NMKL members.

The method referee

- writes the method in the NMKL format according to ISO 78-2
- conducts a full validation of the method in compliance with the NMKL Procedure No. 4, for validation of chemical methods or the NMKL Procedure No. 20 for evaluation of results from qualitative methods
- designs a protocol for second laboratory testing of method performance according to the NMKL Protocol No. I for chemical analysis
- if appropriate, selects a third laboratory

- prepares necessary samples and sends the method description, samples, protocol and report form to the second laboratory
- analyses the data from the second laboratory and sends the method validation report and the independent laboratory report to the technical referee

The independent laboratory

- conducts the method performance test according to the method and the protocol
- submits the results to the method referee according to the report form

The technical referee

 selects at least 2 experts to provide technical reviews within 3 weeks, and informs the Secretary General of names and addresses

- acts as follows:
- if both reviews are negative, the technical referee notifies the method referee that the method is not acceptable
- if both reviews are positive, with minor comments, the technical referee notifies the method referee that the method is acceptable with minor revisions
- if the reviews are split, the technical referee can seek the advice of one or more additional experts to obtain other reviews
- if both reviews are positive, with moderate revisions, the technical referee requests the necessary revisions from the method referee before acceptance of the method

When the method has been approved by NMKL, the method is published as an NMKL verified method.

The following method performance characteristics are established in NMKL Protocol No. 4

- trueness
- recovery
- standardisation curve, linearity
- limit of detection, LOD
- decision limit, $CC\alpha$
- limit of quantification, LOQ
- detection capability. CCβ
- precision: repeatability, reproducibility
- specificity
- cross-reactivity
- false positive and false negative, sensitivity, kappa (qualitative methods)

Available NMKL Methods

Lists of NMKL publications are available at www.nmkl.org.

For information about specific methods, please use the Web Shop and search for the particular method or analyte of interest. You will then find information about the method's scope, field of application, principle and performance.

NordVal Certificates

List of proprietary methods (test kits) certified by NordVal is available under

NordVal at www.nmkl.org.

The certificates inform about the principle and the results of the comparison and collaborative studies of the methods.

NMKL in Hong Kong



Photo: R. Wood

The Hong Kong Laboratory Accreditation Scheme, HOKLAS, an accreditation scheme operated by Hong Kong Accreditation Service (HKAS) invited NMKL to participate in arranging a seminar in Hong Kong, 4 and 5 February 2010.

The main purpose of this seminar was to provide HOKLAS technical assessors and technical personnel of testing laboratories with information on the legislative requirements for food imported into European Union and Australia, with particular focus on testing requirements. Further, to provide information on quality assurance practices such as method validation, verification, measurement uncertainty, use of control charts, certified reference materials, i.e. areas for which NMKL has elaborated guidelines. Mr Wang Wah Wong, HKAS was the organizer in Hong Kong. Further participated Dr Roger Wood, Food Standards Agency, UK, Mr Shyam Kumaran, National Measurement Institute, Australia, and Hilde Skaar Norli, NMKL.

The participants, mainly from public and private chemical food laboratories in Hong Kong participated actively. Several of the laboratories were accredited or in the accreditation process. The feedback from the evaluation of the seminar was positive.



From left: Hilde Skaar Norli, Roger Wood, Shyam Kumaran, Wang Wah Wong

Standardisation of proprietary methods of analysis



A joint JRC/CEN workshop was held 29 January 2010 in Brussels to discuss possibilities and requirements for the standardisation of proprietary methods of analysis.

A proprietary method is a method that requires either specific reagents and/or instrumentation, is mostly available from only one supplier, does not disclose fully composition and nature of chemicals and may be protected by patents and/or a registered trademark, which is owned by a commercial company.

Proprietary methods (test kits) are of considerable importance for routine control of food and feed safety and quality. Usually, these methods offer simplicity and speed of use, the possibility of using them in the field, cost advantage over reference methods, etc. In some areas, for instance in allergens, there are no other available methods than proprietary methods.

How should a standardization organization like CEN deal with proprietary methods?

In general methods adopted in CEN (as in NMKL) have to be "open", which means describing in detail all components (chemicals, consumables, etc) of the method. The items used for carrying out the analysis shall be widely available, and the mandatory use of items that are only available from one commercial supplier is discouraged.

Inclusion of proprietary elements
in standardised methods are
possible when the chemical identity of the reagents are de-

scribed. Thus, in principles, end users could synthesize the substances and would not have to rely exclusively on their commercial availability.



European Committee for Standardization Comité Européen de Normalisation Europäisches Komitee für Normung

Validation of alternative methods in comparison to a (standardised) reference method is an option that has been applied successfully in microbiological testing. The availability of recognised protocols and independent organisations (MicroVal, NordVal, AFNOR) have proven to be effective in ensuring the validity and acceptance of results obtained by alternative methods.

Participants agreed that whenever possible proprietary methods shall be validated on the basis of recognised protocols, and that the validation shall be organised by an independent organisation. Further, that it is important to have acceptance criteria of the method performance characteristics (like established in Nord-Val).

Possibilities and requirements for the standardisation of proprietary methods of analysis is also a topic of relevance to the Codex Committee on Methods of Analysis and Sampling (CCMAS). CCMAS had requested Inter AgencyMeeting (IAM) to elaborate a policy document on this issue. The discussions and conclusions from this JRC/CEN workshop, including comments from IAM members, resulted in the first IAM draft paper for CCMAS.



Organiser of the workshop Franz Ulberth European Commission Joint Research Centre Institute for Reference Materials and Measurements

Photo: Roland Poms

NordVal certification of chemical test kits

NordVal is expanding its field of work to include the validation of test kits / proprietary methods in chemical analyses.

Contact NMKL/NordVal if you would like an independent party review.

NMKL at InterAgencyMeeting, IAM

NMKL is a member of IAM, which is a forum for international organisations working on methods of analysis and sampling and associated quality assurance measures.

The main purpose of IAM is to promote cooperation between international organisations, and to support Codex Alimentarius and thus the governments' and industries' need for methods and quality assurance guidelines.

IAM held its 22nd meeting in Budapest on 5 March 2010. Ten international organisations were represented.

IAM discussed the criteria approach (see page 9) and how the organisations deal with the HorRat

Chairman of IAM: Roger Wood, ICUMSA

Photo: Roland Poms



values in determining the acceptability of methods of analysis containing precision data (see below). IAM had prepared a first draft policy paper presented to CCMAS regarding use of references to proprietary methods in Codex standards. The paper highlighted some concerns that could arise from quoting references to specific proprietary methods in standards:

- prevention of further development of new and better techniques,
- distort competition between companies producing the reagents, and
- create difficulties for government authorities if particular reagents were not readily available for official methods.

The minutes from the meeting and information about IAM is given at the homepage of IAM: http://www.aocs.org/meetings/iam/index.asp



Secretary of IAM: Richard Cantrill, AOCS

Photo: Roland Poms

The amazing HorRat value

Dr William Horwitz (1918-2006) studied results from thousands of collaborative validated methods. He noticed a striking pattern in the relative standard deviations, RSD, which gave rise to the famous 'Horwitz Trumpet'. The results of collaborative studies seemed to follow the same pattern regardless of the nature of the analyte and the test material, or the principle of the method. Based on the results Dr. Horwitz came up with a mathematical function, for predicting the relative standard deviation at a given concentration: RSD(theoretical) =2C^{-0,150 5}. where C is the concentration ratio.

The Horwitz ratio, HorRat value, is the ratio between the obtained and the predicted RSD (RSD/RSD (theoretical)). HorRat is applicable to most chemical methods, however not to physical properties (viscosity, RI, density, pH, absorbance, etc.) and empirical methods (e.g. fiber, enzymes, moisture, methods with indefinite analytes (e.g. polymers and quality measurements, e.g. drained weight.). NMKL recommends to use the following guidelines when considering the obtained precision in a study:

- HorRat ≤ 0.5 Method reproducibility may be in question due to lack of study independence, unreported averaging, or consultations. However, for some methods using advanced techniques (LCMSMS), the HorRat values might be less than 0.5.
- 0.5 < HorRat ≤ 2 Method reproducibility as normally would be expected
- HorRat > 2 Method reproducibility is problematic, as it indicates major variation in the analytical results of equivalent samples analysed by different laboratories.

IAM/MoniQA Workshop:

"Codex Methods of Analysis: What, When, Why, How to Use?"

Prior to the Codex Committee meeting on Methods of Analysis and Sampling, members of IAM and project members of MoniQA (www.moniqa.org) arranged a workshop, 7 March in Budapest. There were 80 participants from all over the world. The topics discussed were:

- the endorsement procedures for methods of analysis within the Codex system
- the method performance characteristics required for methods of analysis within the Codex system
- the rationale for the adoption of the criteria approach within the Codex system and information on how it is to be applied
- the Codex's and the standard development organisations challenges by adoption of proprietary methods of analysis
- international protocols for
 - recovery
 - single laboratory validation
 - proficiency testing and collaborative studies
 - method verification
 - use of certified reference materials
 - measurement uncertainty in sampling
- compliance an measurement uncertainty in analysis

Presentations and photos taken from the workshop are available at http://www.moniqa.org/budapest2010.

The organisers of the workshop were requested to arrange a new workshop in 2011, in connection with the CCMAS meeting.

NMKL contributed with the following speakers :

Astrid Nordbotten, Norwegian Food Safety Authority





Lars Jorhem, National Food Administration, Sweden



Hilde Skaar Norli, NMKL Secretariat, National Veterinary Institute, Norway

Photo: Roland Poms

Codex Committee on Methods of Analysis and Sampling (CCMAS)

CCMAS held its 31st Session in Budapest, Hungary, from 8 to 12 March 2010, by courtesy of the Government of Hungary. The session was chaired by Professor Árpád Ambrus, Deputy Director General, Hungarian Food Safety Office. Dr Béla Kovacs, Professor, University of Debrecen, acted as the Vice-Chairperson. The session was attended by 162 delegates and observers representing 46 member countries, one member organisation (EU) and 15 international organizations, including NMKL.

CCMAS is a committee of Codex Alimentarius Commission, which was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts. such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this programme are protecting health of the consumers and ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations.

CCMAS aims among other to:

• consider, amend and endorse, as appropriate, methods of

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analysis proposed by Codex (commodity) Committees

elaborate, consider and define procedures and guidelines for the assessment of food laboratory proficiency, as well as QA systems for laboratories.

This year a number of AOAC/ • AACC methods (including a couple of NMKL methods) for dietary fibres were considered for endorsement. Further a number of ISO|IDF methods for milk and milk products were updated and en- • dorsed.

For the very first time the criteria approach (see below) where taken into use in Codex. The criteria were endorsed for contaminants in min- New topics for the next session eral water.

Furthermore the committee discussed:

- ods for detection, identification proprietary methods in Codex. and quantification of specific DNA sequences and specific For further information about proteins in foods
- ment uncertainty from the

method analysis and from sampling.

would be procedures for conformity assessment and resolution • of disputes, taking into account * measurement uncertainty, samdraft guidelines on performance pling uncertainty and other rele-. criteria and validation of meth- vant issues, and a policy paper on '

Codex and CCMAS, please visit draft guidelines on measure- www.codexalimentarius.net.

Establishing method criteria for the identification of relevant analytical methods

The Criteria Approach is adopted in Codex Alimentarius Commission and included in Codex Procedural Manual.

The criteria, which NMKL has been involved in the elaboration of, give the laboratories freedom to choose any appropriate, collaboratively validated method, as long as it meets the method performance criteria.

The method criteria are based on the experience and knowledge of results of method performance characteristics gained from method performance studies. Guidelines for how to establish numeric values for the criteria are given as follows:

Applicability:

The method has to be applicable for the food, analyte and level(s) (maximum and/or minimum/ Precision: normative level; here mentioned as ML) of interest. The minimum applicable range of the method For ML < 0.1 mg/kg, depends on the specified level (ML) to be assessed, and can either be expressed in terms of the $\ensuremath{\,\mbox{Trueness:}}$ reproducibility standard deviation For the evaluation of trueness check the data of the study before (s_R) or in terms of limit of detec- preferably certified reference ma- taking a method into use.

tion (LOD) and limit of quantifica- Recovery: tion (LOQ).

Minimum applicable range:

For ML \geq 0.1 mg/kg, the minimum range should be:

 $[ML - 3 s_R, ML + 3 s_R]$ For ML < 0.1 mg/kg, the minimum range should be:

 $[ML - 2 s_R, ML + 2 s_R]$ The s_{R} should be calculated from the Horwitz / Thompson equation.

Limit of Detection, LOD:

For ML \geq 0.1 mg/kg: $LOD \leq ML \cdot I/I0$ For ML < 0.1 mg/kg, $LOD \leq ML \cdot 1/5$

Limit of Quantification, LOQ:

For ML \geq 0.1 mg/kg, $LOO \leq ML \cdot 1/5$ For ML < 0.1 mg/kg, $LOQ \leq ML \cdot 2/5$

For ML \geq 0.1 mg/kg, HorRat value ≤ 2 the $RSD_R < 44\%$

terial should be used.

A guideline for recovery ranges is given below:

Recovery (%)
98 - 102
98 – 102
97 – 103
95 – 105
90 – 107
80 – 110
80 – 110
80 – 110
60 – 115
40 – 120

The laboratories should consider the criteria approach when taking methods into use. The standard development organisations should apply the criteria when approving methods.

Be aware that standard development organisations (not NMKL) publish methods even though the method precision obtained in the collaborative study is poor, hence

A tool for establishing numeric values of the criteria

If you have a maximum, minimum or a normative level, for which the method has to be applicable to, NMKL has made a simple Excel spreadsheet that calculates the numeric values of the method performance criteria for you. You only need to insert the level of interest, and the minimum applicable range, LOD, LOQ and the precision are calculated for you. The Excel spreadsheet is available for downloading at www.nmkl.org under "Download Excel spreadsheet".

	A30 🔻	∱ 0,02							
	A	В	С	D	E	F	G	Н	
1						25.mar.10			
2	According to	o Codex A	Alimentari	us' Metho	d Criteria				
3	l l								
4	Type in the	ML (in mg	/kg) and t	he metho	d performa	nce criteria	are calc	ulated for	r you
5	ML is the ma	aximum le	vel, norm	ative leve	or level of	interest			
6									
7									
8	For ML≥ 0,1	mg/kg							
9				Minimum a	pplicable ran	ge			
10	ML ≥ 0,1	LOD	LOQ	From	То	Precision			
11	in mg/kg	mg/kg	mg/kg	mg/klg	mg/kg	RSDR(%)			
12	0,1	0,01	0,02	0,032	0,168	45,2			
13	1	0,1	0,2	0,520	1,480	32,0			
14	2	0,2	0,4	1,135	2,865	28,8			
15	10	1	2	6,606	13,394	22,6			
16	100	10	20	76,003	123,997	16,0			
17		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
18		0	0	#DIV/01	#DIV/0!	#DIV/0!			
19		0	0	#DIV/01	#DIV/0!	#DIV/0!			
20		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
21		0	0	#DIV/0!	#DIV/0!	#DIV/0!			
22			1						
23									
24	For ML < 0,1 i	ng/kg							
25									
26				iviinimum a	pplicable ran	ge			

Microbiological examinations: NMKL Workshops Quality assurance with focus on measurement of uncertainty

LOQ

mg/kg

0.004

0.008

0.002

From

mg/klg

0.006

0.011

0,003

0,000

0,000

0.000

0.000

Тο

mg/kg

0.014

0.029

0,007

0,000

0.000

0.000

0.000

Precision

RSDR(%)

44

44

44

44

44

44

Aims: To discuss quality assurance activities and practical aspects on the estimation of measurement uncertainty based on different approaches.

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

Time and place:

- 27 May 2010 at the National Veterinary Institute Oslo, Norway
- 28 September 2010 at "Stiftets Hus", Uppsala, Sweden
- 14 October 2010 at Evira, Finland

27

28

31 32

33 34 ML < 0.1

in mg/kg

0.01

0.02

0.005

LOD

mg/kg

0,002

0 004

0.001

0

Π

• Iceland: To be determined. The workshop will not be held in Denmark.

Language: Nordic languages

The workshops will be held in cooperation with the accreditation organisations in the Nordic countries.

Members of the organising committee:

Finland: Sanna Raunila (sanna.raunila@kvvy.fi)

Iceland: Margrét Geirsdóttir (margretg@matis.is)

Sweden: Åsa Rosengren (asa.rosengren@slv.se)

Norway: Marianne Økland (marianne.okland@vetinst.no) and Hilde Skaar Norli (nmkl@vetinst.no).

The program are available at the NMKL homepage, and distributed on emails to NMKL interests.

Registration to NMKL: nmkl@vetinst.no

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Renewed NordVal certificate No 027 for: 3MTMPetrifilmTM Select *E. coli* Count Plate

The 3M Petrifilm Select *E. coli* Count Plate is a sample-ready culture medium which allows selective enumeration of *B*glucuronidase producing *E. coli*. This enzyme reacts with BCIG (5-bromo-4-chloro-3-indolyl-B-D-glucuronide) in the Petrifilm Select *E. coli* Count plates to produce dark green to bluegreen colonies.

The procedure consists of three steps :

- Inoculation of 3M Petrifilm count plate with Iml of sample
- Incubation: 24h ± 2h at 42° C ± 1°C
- Interpretation: E.coli colonies appear as dark green to light blue-green colonies, regardless of gas production. Colonies other than E. coli are difficult to see because they are colourless

to a light grey-beige.



The method has been tested on foods. The method should not be used for the enumeration of β -glucuronidase negative *E. coli*, such as *E.coli* O157:H7.

3M[™]Petrifilm[™] Select *E. coli* Count Plate has been validated by AFNOR. The expert laboratory ADRIA Développement, Quimper, France carried out the validation according to ISO 16140, using the following reference method: ISO 16649 part-2, 2001: -Horizontal method for the enumeration of betaglucuronidase-positive Escherichia coli -- Part 2: Colony-count technique at 44 degrees C using 5bromo-4-chloro-3-indolyl beta-D-glucuronide.

According to the studies no statistical differences were found between the $3M^{TM}$ PetrifilmTM Select *E. coli* Count Plate and the reference method ISO 16649 part-2, 2001.

The results are given in the certificate No 027, which is available for downloading at www.nmkl.org.

Renewed NordVal certificate No. 029 for: 3MTMPetrifilmTM *Enterobacteriaceae* Count Plate

The 3M Petrifilm Entrobacteriaceae Count Plate is a sampleready culture medium system which contains modified Violet Red Bile (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration.

The method procedure consists of three steps:

- Inoculation of 3M Petrifilm count plate with Iml of sample
- Incubation: 24h ± 2h at 30°C or 37°C ± 1°C.
- Interpretation: After incubation, *Enterobacteriaceae* will appear as red colonies with

yellow zones and/or red colonies with gas bubbles

The method has been tested on foods. Maximum colonies to be enumerated in an oplate is 100 colonies.

The method has been tested in studies according to ISO 16140. The method is also certified by AFNOR and is validated by AOAC International, AOAC 2003.01.

NordVal has not found any statistical differences between the 3M Petrifilm *Entrobacteriaceae* Count Plate and the reference method ISO 21528: 2004.

The 3M Petrifilm products are manufactured by: 3M Health Care,

> Microbiology Products, St. Paul, Minnesota 551144-1000 USA

The applicant of the certificates

is:

3M MEDICA Hammfelddamm 11 D-41453 NEUSS GERMANY

Postal address: Laboratoires 3M Santé, Boulevard de l'Oise 95029 CERGY CEDEX, FRANCE Page 11



Available NMKL Procedures (Guides)

(in chronological order) /
No I, 2. Ed. 2005	Calibration and performance checking of laboratory balances
No 2, 1995	Performance check and in-house calibration of thermometers
No 3, 1996	Control charts and control materials in internal quality control in food chemical laboratories
No 4, 3. Ed. 2009	Validation of chemical analytical methods
No 5, 2. Ed. 2003	Estimation and expression of measurement uncertainty in chemical analysis
No 6, 1998	General guidelines in quality assurance for laboratories perform- ing sensory analysis
No 7, 1998	Checking of UV/VIS spectrophotometers
No 8, 4. Ed. 2008	Measurement of uncertainty in quantitative microbiological examination of foods
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NMKL Nordic Committee on Food Analysis

Address: NMKL / NordVal National Veterinary Institute PB 750 Sentrum N-0106 Oslo Norway

nmkl@vetinst.no

Secretary General Hilde Skaar Norli

Tel: +47 2321 6249 +47 46 8888 07

www.nmkl.org

Number of copies: 1500 ISSN 100-5386