NMKL Newsletter 81

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May we use NMKL and NordVal methods for microbiological examinations, or do we have to apply EN/ISO methods?

Many have asked that question.

The hygiene regulation, which is an implementation of EU regulation*, specifies EN/ISO methods as reference methods under the article on specific rules for testing and sampling. In an article (No. 5 in (EF) 2073/2005) the following is stated:

Food business operators may use other sampling and testing procedures, if they can demonstrate to the satisfaction of the competent authority that these procedures provide at least equivalent guarantees.

… The use of alternative analytical methods is acceptable when the methods are validated against the reference method 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.

If the food business operator wishes to use analytical methods other than those validated and certified as described in (the regulation) the methods shall be validated according to internationally accepted protocols and their use authorised by the competent authority.

Comprehensive method validations have been and are being carried out by institutions, organisations and test-kit manufacturers, in order to facilitate the possibility of choosing other methods that provide at least equivalent guarantees - methods for which also the performance characteristics, the fitness for use, are well documented.

* Commission Regulation (EC) No. 852/2004 on the hygiene of foodstuffs, and Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs

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Comparison between NMKL methods and EN/ISO methods using data from Proficiency Testing schemes

For several years, the National Food Agency (NFA), Uppsala, Sweden, has frequently arranged PT schemes for microbiological examinations. Many laboratories from the Nordic countries, Europe and countries worldwide have participated. In two recent schemes, NFA subjected the conditions so that the PT data could be used in comparing methods. The laboratories have described the principle of the methods in more detail, in order to disclose any modification of the methods. NMKL would like to express great gratitude to Christina Normark and NFA for making the comparison possible, and for giving NMKL access to the results.

Based on the results from more than 200 laboratories (anonymous to NMKL), NMKL has compared results obtained by different analytical methods. Comparisons have been made between:

- *Listeria monocytogenes*: qualitative methods: ISO 11290-1:2004 and NMKL 136, 2007
- *Listeria monocytogenes*: quantitative methods: ISO 11290-2:2004 and NMKL 136, 2007
- *Salmonella* spp.: ISO 6579:2002/Corr 1:4 and NMKL 71, 1999
- *Enterobacteriaceae:* ISO 21528-2:2004 and NMKL 144:2005
- Aerobic microorganisms: ISO 4833:2003 and NMKL 86, 2006
- *E. coli:* ISO 16649-2:2001 and NMKL 125, 2005
- Coagulase positive *Staphylococcus:* ISO 6888-2:2004 and NMKL 66, 2003
- *E. coli O157*: ISO 16654:2001 and NMKL 164, 2005

Other methods of analysis than the NMKL and ISO methods used in the PT schemes, are not included in the evaluation. Nevertheless, the amount of data was sufficient to make a statistical evaluation of the results of the methods. The purpose of the evaluation was to provide a basis for assessing whether the methods provide equivalent results.

Table 1: Microorganisms in the solutions of the PT scheme January 2012

Solution	Microorganisms	Strain labelled
A	*Escherichia coli*	SLV-165
	Campylobacter coli	SLV-271
	Listeria monocytogenes	SLV-361
	Salmonella agona	SLV-318
B and C	*Klebsiella pneumoniae*	SLV-537
	Campylobacter jejuni	SLV-540
	Listeria monocytogenes	SLV-444
	Listeria innocua	SLV-312
	Salmonella bovismorbificans	SLV-443
	Escherichia coli O157	SLV-515

Table 2: Microorganisms in the solutions of the PT scheme October 2011

Solution	Microorganisms	Strain labelled
A	*Citrobacterfreundii*	SLV-424
	Enterococcus faecalis	SLV-051
	Bacillus thurigensis	SLV-478
	Klebsiellapneumoniae	SLV-564
B	*Proteus vulgaris*	SLV-476
	Bacillus cereus	SLV-556
	Escherichia coli	SLV-523
C	*Micrococcus sp.*	SLV-055
	Staphylococcus aureus	SLV-280
	Bacillus cereus group	SLV-518
	Escherichia coli	SLV-524

Each laboratory analysed three freeze-dried microorganism solutions; A, B and C, given in the tables to the right. The test materials were carefully tested by NFA before shipment. The results from the PT schemes of October 2011 and January 2012, were further evaluated by a project group consisting of Hilde Skaar Norli (project leader, NMKL), Marianne Halberg Larsen (Denmark), Marjaana Hakkinen (Finland), Margret Geirsdóttir (Iceland), Gro S. Johannessen (Norway) and Asa Rosengren (Sweden). The results were evaluated statistically, based on ISO/ DIS 16140 (doc N120, March 2012) and NordVal's validation protocol. The confidence interval for the probability of detection (POD) was calculated according to the Wilson score confidence intervals.

Listeria monocytogenes – comparison of results obtained with ISO 11290-1:2004 and NMKL 136, 2007

Listeria monocytogenes - Qualitative method

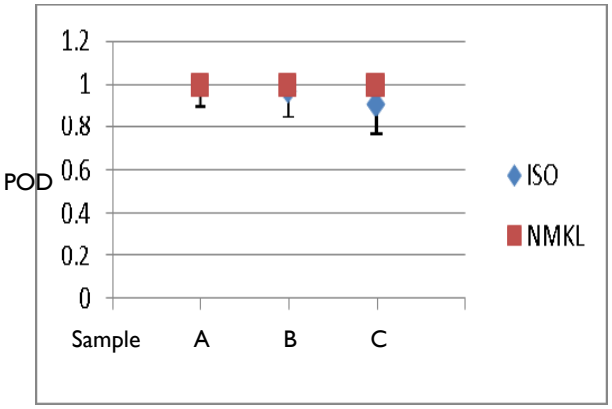
In the PT scheme of January 2012, 33 laboratories had applied ISO 11290-1, and 20 laboratories had applied NMKL 136, in the analyses of three sample solutions A, B and C (Table 1, page 2). The results are given in Table 3, and illustrated in Figure 1.

Table 3: Qualitative results of Listeria monocytogenes with ISO 11290-1:2004 and NMKL 136, 2007

Method	**ISO 11290-1:2004**			**NMKL 136, 2007**		
No of labs	33			20		
Solutions	A	B	C	A	B	C
No of positives	33	32	30	20	20	20
No of negatives	0	1	2	0	0	0
POD*	1	0,97	0,91	1	1	1
Lower limit**	0.90	0.85	0.76	0.84	0.84	0.84
Upper limit**	1	0.99	0.97	1	1	1

*POD = probability of detection = number of positives/ number of laboratories
** Lower and upper limit of 95% confidence interval according to E.B Wilson

Figure 1: A *plot of the probability of detection (POD) for results obtained by the methods, including the confidence interval of the ISO method.*



The principle of the methods for detection of *Listeria monocytogenes*.

Both the ISO and the NMKL method describe a two-step enrichment procedure, Half-Fraser broth at 30 °C for 24 h + Fraser broth at 37 °C for 48 h. The cultures obtained from both enrichment steps are plated out on a *L. monocytogenes* specific isolation medium. The NMKL method gives the option of using ALOA, LMBA or Chromogenic Listeria Agar medium basically alike ALOA and one other optional solid selective isolation medium. In the ISO method, LMBA is not described. [The NMKL collaborative study showed, however, that there is no statistical difference in the results obtained by ALOA, LMBA, OCLA or LCA.]

Both methods confirm *L. monocytogenes* colonies by using the same morphological and biochemical tests.

Both methods are collaboratively validated.

All the (20x3) 60 results were positive with the NMKL method. Three out of (33x3) 99 samples were false negative with the ISO method. According to the draft ISO/DIS 16140 (doc N120), the acceptance limit for 60 test samples is 3, and for 99 samples the acceptance limit is 5. This means that it is acceptable to have up to 3 negative deviations (false negatives) when analysing 60 samples, and no more than 5 negative deviations when analysing 99 samples. Subsequently, both methods obtained acceptable results.

The figure shows a plot of the POD, the probability of detecting positive samples, including the confidence interval, which is the range between lower and upper limits of the results obtained by the ISO method. The results obtained using NMKL 136, fall within the confidence level of the results obtained with ISO 11290-1. This illustrates that the two methods give equivalent results for the samples in the PT scheme.

Listeria monocytogenes - Quantitative method

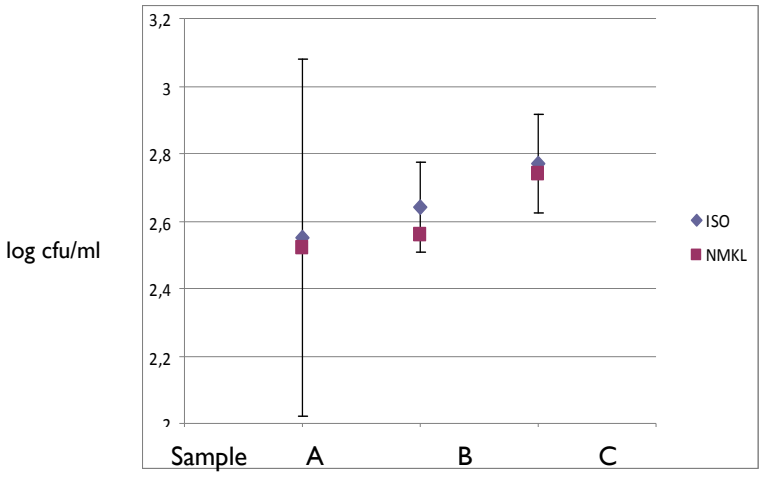
34 laboratories used ISO 11290-2, and 21 laboratories used NMKL 136, for the enumeration of *Listeria monocytogenes* in the PT scheme of January 2012 method (Table 1, page 2). The following results were reported:

Table 4: Quantitative results for Listera monocytogenes with ISO 11290-2:2004 and NMKL 136, 2007

Method	**ISO 11290-2:2004**			**NMKL 136, 2007**		
No of labs	34			21		
Solution	A	B	C	A	B	C
No of outliers	7	5	4	3	1	1
Mean (log cfu/ml)	2.77	2.55	2.64	2.75	2.53	2.56
Standard deviation (log cfu/ml)	0.53	0.13	0.15	0.10	0.16	0.16

16 of the (34x3) 102 results were outliers with the ISO method, while 5 of the (21x3) 63 results were outliers with the NMKL method. The mean values were approximately equal for all samples. The results obtained with the NMKL method falls within the confidence interval of the standard deviation of the ISO method, which shows that the NMKL method gives equivalent results to the ISO method. The standard deviation of sample A obtained with the ISO method is not satisfactory. The standard deviation should not be more than 0.4 log cfu/g (NMKL Procedure No 8). The results are also illustrated in the figure below.

Figure 2: Results (in log cfu/ml) *for Listera monocytogenes with ISO 11290-2:2004 and NMKL 136 for solutions A, B and C*



The results show that there are no significant differences in the results obtained the ISO and the NMKL method. However, the precision for sample A is not satisfactory for the ISO method.

The principle of the ISO and NMKL methods for the enumeration of *Listeria monocytogenes*.

The initial suspension is incubated for 1h at 20 °C either in buffered peptone water or in the Half-Fraser base, to resuscitate stressed microorganisms. After the resuscitation period, the initial suspension and/or its dilutions are surface plated on a *L. monocytogenes* specific isolation medium, ALOA, LMBA or Chromogenic Listeria Agar medium, basically alike ALOA. In the ISO method, only ALOA is described. [The NMKL collaborative study showed, however, no difference in the results between the media tested.]

After incubation, presumptive *L. monocytogenes* colonies are counted and confirmed using appropriate morphological and biochemical tests.

The NMKL method is tested in an extensive collaborative method validation study, the ISO method is not tested collaboratively.

Salmonella spp. - Comparison of results obtained using the ISO 6579:2002/Corr 1:2004 and NMKL 71, 1999

32 laboratories applied ISO 6579, and 39 laboratories applied NMKL 71, in the PT scheme of January 2012 (Table 1, page 2). The following results were obtained:

Table 5: Results for Salmonella spp. with ISO 6579 and NMKL 71

Method	ISO 6579			NMKL 71		
No of labs	32			39		
Solutions	A	B	C	A	B	C
No of positives	29	32	32	34	39	38
No of negatives	3	0	0	5	0	1
POD*	0.9	1	1	0.85	1	0.97
Lower limit**	0.74	0.89	0.89	0.70	0.91	0.87
Upper limit**	0.96	1	1	0.94	1	1

*POD = probability of detection = number of positives/number of laboratories
** Upper and lower limit of 95% confidence interval according to E.B Wilson

The principle of the methods:

The ISO method includes two selective enrichment procedures (RVS and MKTTn), while the NMKL method describes one procedure (RVS).

Three of (32x3) 96 samples were negative with the ISO method and 6 of (39x3) 117 samples were negative with the NMKL method. According to the draft ISO/DIS 16140 (doc N120), the acceptance limits for 96 and 117 samples are 5 and 6, respectively. This means that for 96 samples, 5 negative deviations (false negatives) are acceptable, and for 117 samples, 6 negative deviations are acceptable. **Both methods obtained acceptable results for these samples. As the results obtained with the NMKL method fall within the confidence level of the probability of detection, POD, of the ISO method, the methods do provide equivalent results**, see Figure 3.

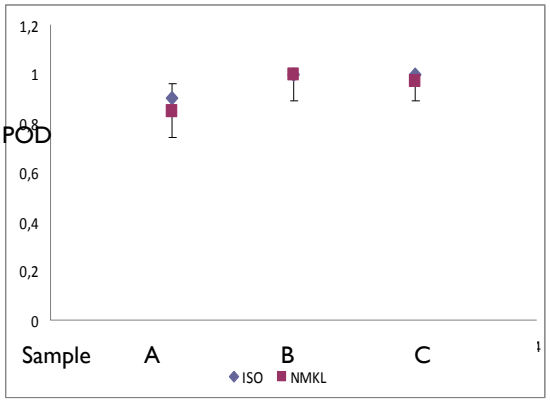


Figure 3: POD, including confidence interval, for samples A, B and C, using the ISO and NMKL methods for detection of Salmonella spp.

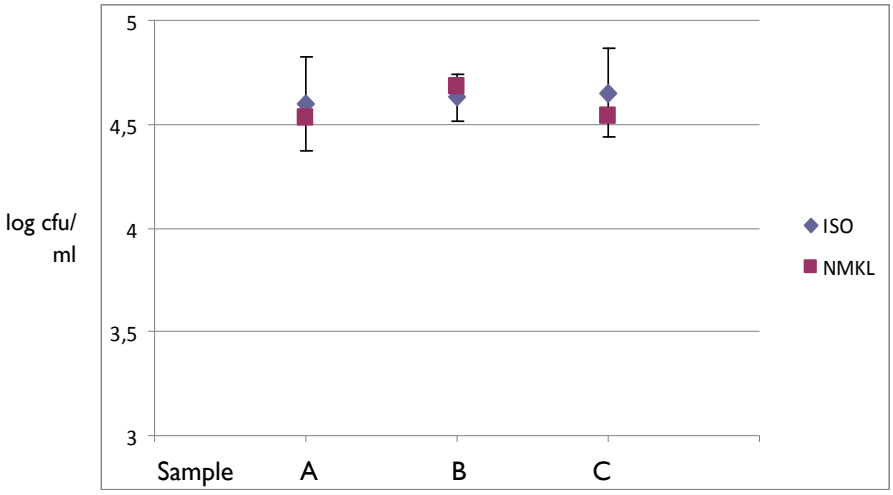
Enterobacteriaceae – Comparison of the results obtained using the ISO 21528-2:2004 and NMKL 144:2005

19 laboratories applied ISO 21528-2, and 71 laboratories applied NMKL 144, in the PT scheme of January 2012 (Table 1, page 2). Of the (19x3) 57 samples analysed with the ISO method, 11 results were outliers. Of the (71x3) 213 samples analysed using the NMKL method, only three results were outliers. The results are given in the table below.

Table 6: Results of Enterobacteriaceae with ISO 21528-2 and NMKL 144

Method	ISO 21528-2:2004			NMKL 144:2005		
No of labs	19			71		
Solution	A	B	C	A	B	C
No of outliers	5	4	2	1	2	0
Mean (log cfu/ml)	4.63	4.60	4.65	4.68	4.53	4.54
Standard deviation (log cfu/ml)	0.23	0.11	0.21	0.13	0.19	0.22

Figure 4: Mean values of results of Enterobacteriaceae with ISO 21528-2 and NMKL 144, including the confidence interval of the ISO method



The methods give equivalent results, and may be considered as equal in their method description. There are, however, two minor differences:

1) The ISO method prescribes two plates of VRBG-agar per dilution. The NMKL method prescribes one plate.

2) The ISO method prescribes confirmation by tests for fermentation of glucose and the presence of oxidase while NMKL uses the oxidase test only.

The NMKL method is collaboratively validated, the ISO method is not.

The figure above shows that the results of the samples analysed with the two methods are overlapping. The standard deviation is satisfactory when the outliers are omitted.

Aerobic microorganisms – Comparison of results using the ISO 4833:2003 and NMKL 86, 2006

40 laboratories applied ISO 4833:2003, and 55 laboratories applied NMKL 86, for the enumeration of aerobic microorganisms in the PT scheme January 2012 (Table 1, page 2). Six of the (40x3) 120 samples analysed with the ISO method, were outliers, and six of the (55x3) 165 samples analysed with the NMKL method were outliers. The results are given in Table 7.

Table 7: Results of aerobic microorganisms with ISO 4833:2003 and NMKL 86, 2006

Method	ISO 4833:2003			NMKL 86, 2006		
No of labs	40			55		
No of outliers	2	2	2	3	1	2
Solutions	A	B	C	A	B	C
Mean (log cfu/g)	4.76	4.57	4.57	4.82	4.61	4.60
Standard deviation (log cfu/g)	0.12	0.19	0.22	0.12	0.24	0.22

The results are overlapping, and the precision of the samples is satisfactory, which indicates that the methods provide equivalent results for these samples. This is also illustrated in Figure 5.

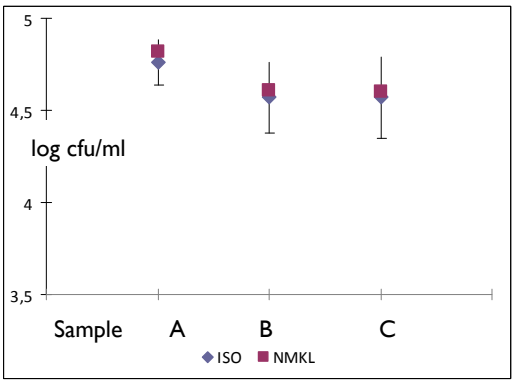


Figure 5: Results (in log cfu/ml) of aerobic microorganisms with ISO 4833 and NMKL 86

The method descriptions for the enumeration of aerobic microorganisms in foods are equivalent for ISO 4833:2003 and NMKL 86, 2006. None of the methods are collaboratively validated.

Coagulase positive Staphylococcus – Comparison of results using the ISO 6888-2: 2004 and NMKL 66, 2003

28 laboratories applied ISO 6888-2, and 48 laboratories applied NMKL 66, for the enumeration of coagulase positive *Staphylococcus* in the PT scheme October 2011 (Table 2, page 2). The median of sample solutions A and B were both zero. A few laboratories (less than five) had detected *Staphyloccocus* in these samples. The results for solution C is given in Table 8, and illustrated in Figure 6.

Table 8: Results of coagulase positive Staphylococcus with ISO 6888-2:2004 and NMKL 66, 2003

Method	ISO 6888-2:2004	NMKL 66:2003
No of labs	28	65
Solution	C	C
No of outliers	4	2
Mean (log cfu/g)	4.20	4.21
Standard deviation (log cfu/g)	0.82	0.11

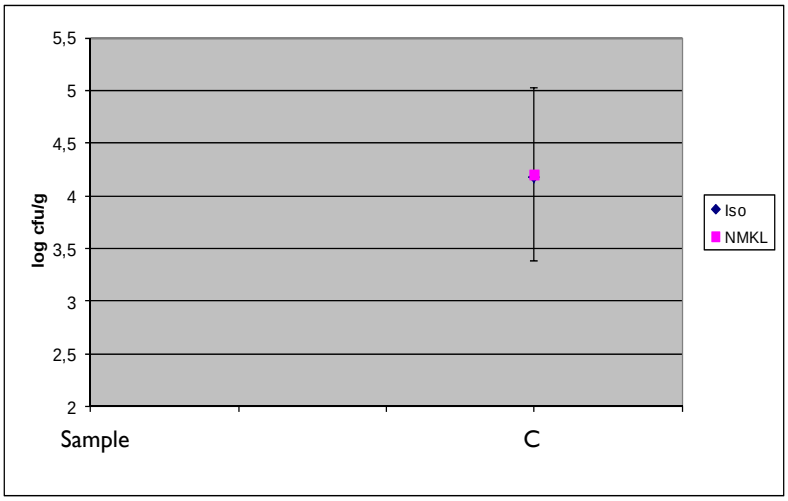


Figure 6: Results of coagualse positive staphylococcus with ISO 6888-2 and NMKL 66

The mean value is 4.2 log cfu /g for both methods.

The standard deviation obtained with the ISO method (0.82) is too high. The ISO method and the NMKL method give equivalent results for sample solutions A, B and C.

Principle of the methods

The method description of NMKL 66 and ISO 6888-2 may be considered as equal. Coagulase positive staphylococci are enumerated by surface inoculation of decimal dilutions of a specified quantity of the food sample on the selective agar-medium Baird-Parker (BP) and/or Baird-Parker agar with a Rabbit-plasma fibrinogen supplement (BP+RPF). The NMKL method includes the possibility of including Blood agar, but does not recommend using this as the only medium, but more as an extra plate. The ISO method states that the pour-plate method is used with BP-RPF agar, thereby lowering the detection limit.

After incubation, typical and atypical colonies are counted. By the use of BP, a selection of colonies must be confirmed by the coagulase test. By using BP+RPF, the production of coagulase is tested directly on the agar plate.

The results of the samples included in the PT scheme is overlapping. However, samples at different levels would be desirable.

E. coli – Comparison of results with ISO 16649-2: 2001 and NMKL 125, 2005

16 laboratories applied ISO 16649-2, and 40 laboratories applied NMKL 125, for the enumeration of *E. coli* in the PT scheme arranged in October 2011 (Table 2, page 2). Of the (16x3) 48 samples analysed with the ISO method, five results were outliers. Of the (40x3) 120 samples analysed with the NMKL method, nine samples were outliers. The results are given in Table 9.

Table 9: Results of E. coli with ISO 16649-2:2001 and NMKL 125, 2005

Method	**ISO 16649-2:2001**			**NMKL 125:2005**		
No of labs	16			40		
Sample solution	A	B	C	A	B	C
No of outliers	0	3	2	3	3	3
Mean (log cfu/g)	0	3.60	3.09	0	3.72	3.17
Standard deviation (log cfu/g)		0.15	0.16		0.12	0.12

The results are overlapping, and the precision is satisfactory. The results with the NMKL method fall within the confidence interval of the results of the ISO method, which illustrates that the methods provide equivalent results for these samples. This is also shown in the figure below.

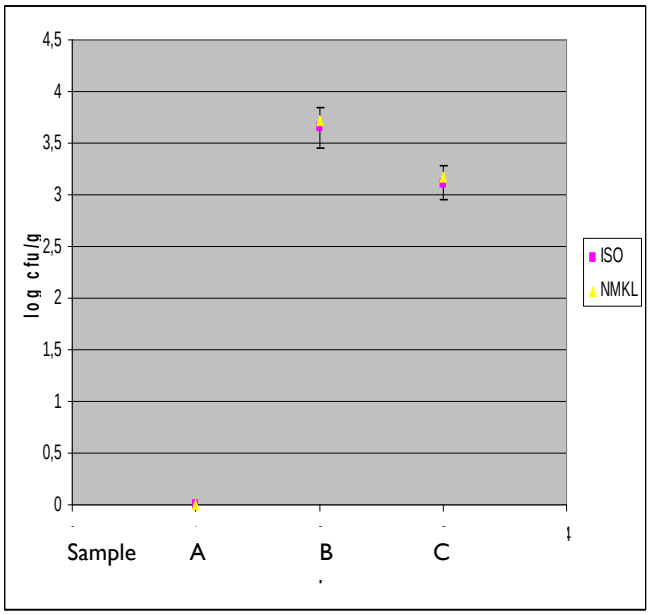


Figure 7: Results (in log cfu/ml) of E. coli with ISO 16649-2:2001 and NMKL 125, 2005 for sample solutions A, B and C

The ISO method and the NMKL method apply two different principles for the detection of *E. coli*. The NMKL method uses bile salts, the fermentation of lactose, and high temperature (44 °C) as the selective principle. The ISO method uses the detection of the enzyme β-glucuronidase on a solid agar containing a chromogenic ingredient. The selective principle is bile salts and high temperature (44 °C), and the indication of presence of *E. coli* is the cleavage of the chromogenic ingredient, giving rise to a particular colour in *E. coli*.

The two methods apply different principles and media, and cannot be considered as equal. However, they may cover more or less the same analysis area. None of the methods will cover *E. coli* that does not grow at lower temperatures (such as *E. coli* O157), but both methods will be suitable for the enumeration of *E. coli* for hygiene purposes.

The methods provide equivalent results for the samples in this PT scheme.

E. coli O157 – Comparison of results using the ISO 16654: 2001 and NMKL 164, 2005

Ten laboratories applied ISO 16654, and nine laboratories applied NMKL 164, for the detection of *E. coli* O157 in the PT scheme arranged in January 2012 (Table 1, page 2). The results are given in Table 10 and Figure 8.

Table 10: Results for E. coli O157 with ISO 16654 and NMKL 164

Method	**ISO 16654**			**NMKL 164**		
No of labs	10			9		
Sample solution	A	B	C	A	B	C
No of positives	1	9	7	1	8	9
No of negatives	9	1	3	8	1	0
POD*	0.10	0.90	0.70	0.11	0.89	1
Lower limit**	0.02	0.60	0.40	0.02	0.57	0.70
Upper limit**	0.40	0.98	0.89	0.44	0.98	1

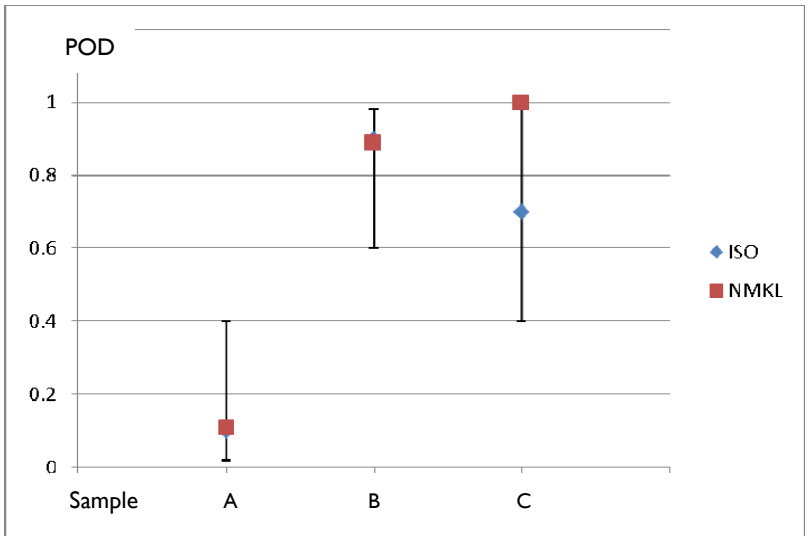
*POD = probability of detection = no of positives/ no of laboratories
** Upper and lower limit of 95% confidence interval according to E.B Wilson

The two methods are very similar. Both methods rely on the same principles:

1: Enrichment in modified tryptic soy broth with novobiocin (mTSBn)
2: Immunomagnetic separation
3: Subculture onto two selective media
4: Confirmation of the *E. coli* O157

There are only minor differences between the methods.

Figure 8: POD for E. coli O157



The probability of detecting *E. coli* O157, including the confidence level for ISO 16654, is illustrated in the figure above. The probability of detecting *E. coli* O157 in sample A is low (10%), which is good as sample A does not contain *E. coli* O157. Thus, positive results of sample A are false positives.

As the results of the NMKL method fall within the confidence level for the probability of detecting *E. coli* O157 with the ISO method, the methods provide equivalent result for the samples analysed.

Applicable analytical methods according to Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs

According to regulation EC 2073/2005 Article 5, food business operators may use other methods than those listed in Annex I of the regulation, provided it can be demonstrated to the satisfaction of the competent authority that these procedures provide at least equivalent guarantees. The table below lists the microorganisms and the referred reference methods given in EC 2073/2005 Annex I, along with alternative NMKL and NordVal methods that provide at least equivalent guarantees.

Microorganisms	Reference method	Alternative methods providing equivalent guarantees	
Listeria monocytogenes **- detection**	**EN/ISO 11290-1** Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1: Detection method	**NMKL 136** *Listeria monocytogenes.* Detection in foods and feeds, and enumeration in foods	**NordVal 022** RAPID' *L.mono* Detection + enumeration **NordVal 025** LightCycler *L.mono* Detection Kit **NordVal 037** IQ-Check *Listeria monocytogenes* II
Listeria monocytogenes **- enumeration**	**EN/ISO 11290-2** Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 2: Enumeration method	**NMKL 136** *Listeria monocytogenes.* Detection in foods and feeds, and enumeration in foods	**NordVal 022** RAPID' *L.mono* Detection + enumeration
Salmonella	**EN/ISO 6579** Horizontal method for the detection of *Salmonella spp.*	**NMKL 71** *Salmonella.* Detection in foods **NordVal 001** Transia Plate Salmonella Gold **NordVal 010** Bioline *Salmonella* ELISA Test Optima / RayAl *Salmonella* Optima **NordVal 023** Foodproof® *Salmonella* Detection Kit **NordVal 030** BAX *Salmonella* PCR	**NordVal 031** *Salmonella* detection method by real-time PCR **NordVal 032** RAPID' *Salmonella* - 24 hours test method **NordVal 038** iQ-Check TM *Salmonella* II kit **NordVal 041** *Salmonella* detection method: DNA extraction by boiling followed by real -time PCR **NordVal 028** Bioline *Salmonella* ELISA Test Optima, RayAl *Salmonella* Optima
Aerobic microbes in foods	**ISO 4833** Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 °C.	**NMKL 86** Aerobic microorganisms. Enumeration in foods at 30 °C, 20 °C or 6.5 °C.	**NordVal 012** 3M Petrifilm, AerobicCount Plate **NordVal 033** Compact Dry TC Method for the Enumeration of Total Viable Organisms

Micro-organisms	Reference method	Alternative methods providing equivalent guarantees	
Aerobic mikrobes – surface	**ISO 4833** Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 °C . The ISO method does not describe enumeration on surfaces, which is the scope described in the regulation.		**NMKL5** Aerobic microorganisms and presumptive *Enterobacteriaceae*. Enumeration on **surfaces** and utensils. **NordVal 018** Hygicult TPC
E. coli	**ISO 16649** Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli Part 1, 2 and 3	**NMKL 125** Thermotolerant coliform bacteria and *Escherichia coli*. Enumeration in foods and feeds. **NMKL 96** Bacterial Examinations in Fresh and Frozen seafood.	**NordVal 014** 3M Petrifilm *E. coli*/Coliform Count Plate **NordVal 020** RAPID' *E. coli* 2 AGAR **NordVal 036** Compact Dry EC Method for the Enumeration of *E. coli* and Coliforms **NordVal 027** 3M Petrifilm Select *E. coli* Count Plate
***Enterobacteriaceae* – surface**	**ISO 21528-2** Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method The ISO method does not describe enumeration on surfaces, which is the scope described in the regulation.		**NMKL 5** Aerobic microorganisms and presumptive *Enterobacteriaceae*. Enumeration on **surfaces** and utensils. **NordVal 018** Hygicult TPC
***Enterobacteriaceae* in foods**	**ISO 21528-2** Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method	**NMKL 144** *Enterobacteriaceae*. Enumeration in foods and feeds.	**NordVal 029** 3M Petrifilm *Enterobacteriaceae* Count Plate **NordVal 034** Compact Dry ETB Method for the Enumeration of *Enterobacteriaceae*
Stafylococcus aureus	**ISO 6888-1 + 2** Horizontal methods for the enumeration of coagulase-positive *staphylococci*	**NMKL 66** *Stafylococcus aureus*. Enumeration in foods.	**NordVal 019** 3M Petrifilm Express Count System **NordVal 043** Compact Dry X-SA Method
***E. coli* O157**	**EN/ISO 16654** Horizontal method for the detection of *Escherichia coli* O157	**NMKL 164** *Escherichia coli* O157. Detection in foods and feeds.	**NordVal 026** LightCycler food proof *E. coli* O 157 Detection Kit in combination with ShortPrep foodproof II Kit

Renewed NordVal Certificates

TRANSIA® PLATE *Listeria* spp.
NordVal Certificate No. 002

TRANSIA® PLATE *Listeria* spp. is an ELISA kit, based on a two step sandwich-type reaction, where specific proprietary antibodies ensure detection of *Listeria* spp. (*L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri, L. welshimeri* og *L. grayi*) in foods and feeds.

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 for 22-26 h at 30 °C ± 1 °C, followed by
- TRANSIA® PLATE *Listeria* test after heating of 1 to 2 mL of the enrichment broth Fraser 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 is compared with EN/ISO 11290-1:2004: Horizontal method for the detection and enumeration of *Listeria monoctyogenes* – Part 1: Detection method.

In 2007, Institut Pasteur de Lille, France, carried out an extensive method comparison and a collaborative method validation study with 14 participating laboratories. The studies showed that there was no statistical difference in the results between TRANSIA® PLATE *Listeria* spp. and the reference method for the detection of *Listeria* spp. in foods and feeds.

Obtained results:
Relative accuracy: 98-99%
Relative specificity: 99-100%
Relative sensitivity: 97-100%
Agreement between the methods: very good - Kappa > 0.80
Limit of detection: 1-10 cfu per 25 g or 25 ml sample for all matrices

Photo from www.biocontrolsystems.com

BioControl is the manufacturer and supplier of TRANSIA® PLATE *Listeria* spp.

See **www.nmkl.org** for information on:

- NMKL publications (methods, procedures, protocols)
- NordVal Certificates
- Workshops, seminars
- Nordic reference laboratories, NRL and EU-RL and their task
- Comparisons between ISO and NMKL methods according to EU 2073/2005
- Link to providers of PT– schemes
- The working programme of NMKL

Bioline *Salmonella* ELISA Test SELECTA/ RayAl *Salmonella* SELECTA
NordVal Certificate No. 028

Bioline *Salmonella* ELISA Test SELECTA / RayAl *Salmonella* SELECTA is an immuno-enzymatic test which uses 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 for 6-10 h at 37 °C ± 1 °C, and then
- incubation in SELECTA broth for 18-24 h at 41.5°C ± 0.5 °C, followed by an
- immuno-enzymatic test after heating of an aliquot of SELECTA broth.

Confirmation of positive samples is not necessary, unless required by legislation. The method is tested on foods and animal feeds.

Bioline *Salmonella* ELISA Test SELECTA / RayAl *Salmonella* SELECTA has been compared with ISO 6579:2002 in extensive validation studies in 2004 and 2008. According to the comparison and collaborative studies no statistical differences were found between the Bioline *Salmonella* ELISA Test SELECTA/ RayAl *Salmonella* SELECTA and the reference method for the detection of *Salmonella* in foods and animal feeds. The alternative method meets the acceptance criteria given in the validation protocol of NordVal.

Obtained results:
Relative accuracy: 98-99%
Relative specificity: 97-100%
Relative sensitivity: 98-99%
Agreement between the methods: very good - Kappa > 0.80
Limit of detection> 1-10 cfu per 25 g or 25 ml sample for all matrices.

Diatek AG, Switzerland (www.diatek.ch) (previously Bioline Aps, Denmark) is the manufacturer and supplier of Bioline *Salmonella* ELISA Test SELECTA. RayAl Ltd, UK, (www.rayal.com) is the supplier of the same product named RayAl *Salmonella* SELECTA.

- **Rapid'*Salmonella* method, short protocol**
- **Rapid'*Salmonella* method, double enrichment protocol (24h)**
- **Rapid'*Salmonella* LATEX confirmation**

NordVal Certificate No. 032

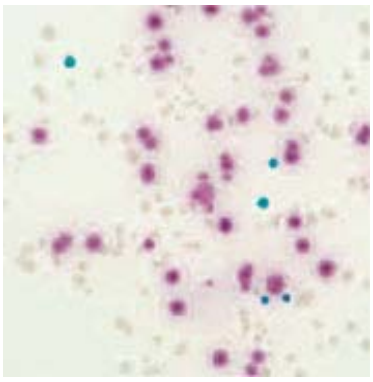
RAPID'*Salmonella* is a chromogenic agar medium, the principle of which relies on demonstration of two enzymatic activities. The RAPID'*Salmonella* test methods approved by NordVal are:

RAPID'*Salmonella* mehod - Short protocol:

- selective enrichment in Buffered Peptone Water and RAPID'*Salmonella* supplement at 41.5 °C ± 1 °C for 18h ± 2h
- plating out on RAPID'*Salmonella*
- selective isolation by incubation at 37 °C ± 1 °C for 24 h ± 2 h

RAPID'*Salmonella* method – Double enrichment protocol:

- pre-enrichment in Buffered Peptone Water at 37 °C ± 1 °C for 18 h ± 2 h
- selective enrichment in RVS at 41,5°C ± 1 °C for 24 h ± 2 h
- plating out on RAPID'*Salmonella*
- selective isolation by incubation at 37 °C ± 1 °C for 24 h ± 2 h



Salmonella spp. appear as typical magenta colonies.

Confirmation is carried out on Rapid'*Salmonella* Latex confirmation test.

Photo from www.bio-rad.com

The methods have been tested and found applicable for the analyses of foods and feeds.

The comparison study of the double enrichment protocol was carried out in 2005, and the validation study of the short protocol and the *Salmonella* LATEX confirmation test were carried out in 2009. In 2011, the sample preparation of the short protocol was modified by addition of a red colour agent. The protocols are compared against the reference method EN/ISO 6579:2002: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.

RAPID'*Salmonella* – Double enrichment protocol

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

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

The relative sensitivity is satisfactory for all products. The relative specificity is slightly low (80%), yielding 20% false positive, and hence confirmation is necessary.

RAPID'*Salmonella* – Short protocol

A total of 324 samples of meat products, vegetables, seafood products, dairy products, ovo products, and animal feed products were analysed both with the RAPID'*Salmonella* method and the reference method. The following results were obtained:

	After screening	After confirmation
Relative accuracy	91%	91%
Relative specificity	92%	96%
Relative sensitivity	90%	99%

In the screening, the relative sensitivity is low as the sensitivity of the reference method is poorer than the alternative method.

The detection level was found to be 1-10 cfu in a sample of 25 g or 25 ml for all matrices.

The results of the collaborative study, where 15 laboratories analysed samples of pasteurised milk artificially contaminated with strains of *Salmonella typhimurium,* yielded satisfactory results.

Bio-Rad Laboratories, France (www.bio-rad.com) is the manufacturer and supplier of RAPID'*Salmonella*.

3M™Petrifilm™ Select *E. coli* Count Plate
NordVal certificate No. 027

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

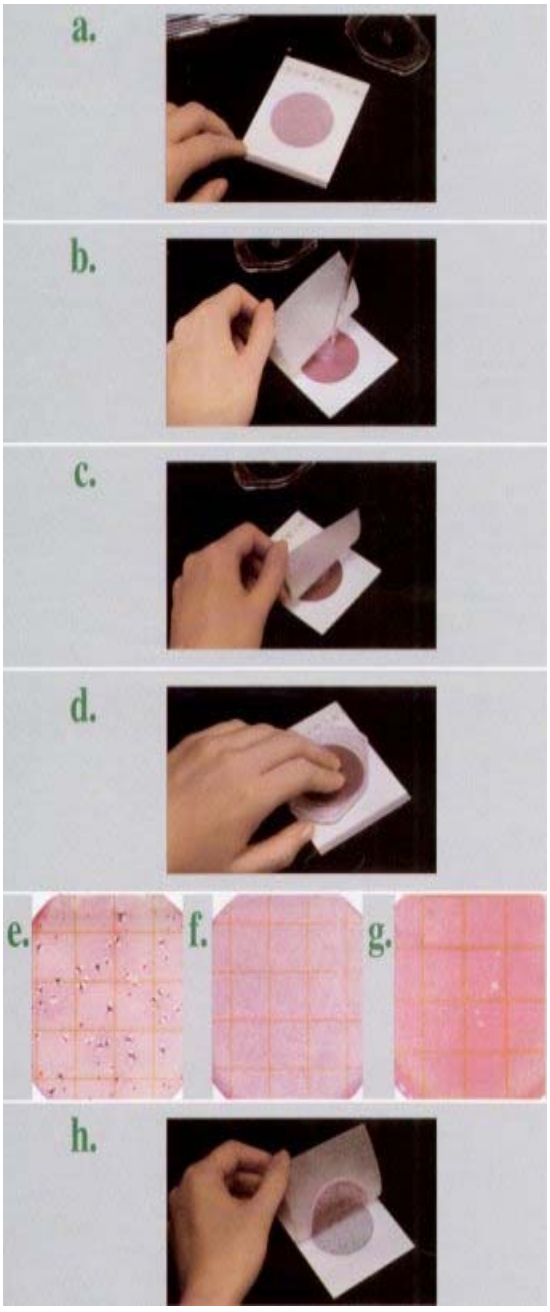
- Inoculation of 3M Petrifilm count plate with 1ml of sample
- Incubation for 24 h ± 2 h at 42 °C ± 1 °C
- Interpretation: After 24 h ± 2 h at 42 °C ± 1 °C, *E. coli* colonies appear as dark green to light blue-green colonies, regardless of gas production. Colonies other than *E. coli* are difficult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

The method has been tested on foods. The method is not applicable for the enumeration of b-glucuronidase negative *E. coli*, such as *E. coli* O157:H7.

3M™Petrifilm™ Select *E. coli* Count Plate was tested at the expert laboratory ADRIA Développement, Quimper, France, where it was compared against the reference method ISO 16649 Part-2, 2001: Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*—Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

Validation studies were carried out in 2001, 2003 and 2005 on meat products, poultry, dairy products, seafood products, vegetables and egg products.

According to the studies, no statistical differences were found between the 3M™Petrifilm™ Select *E. coli* Count Plate and the reference method ISO 16649 Part-2, 2001.



Photos from https://www.msu.edu/course/fsc/441/3mc&ec.html

NordVal certificates can be downloaded from the list of the methods approved by NordVal at www.nmkl.org

3M™Petrifilm™ *Enterobacteriaceae* Count Plate NordVal certificate No. 029

The Petrifilm Entrobacteriaceae (EB) Count Plate is a sample-ready 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.

Three steps:

- Inoculation of 3M Petrifilm count plate with 1ml of sample
- Incubation for 24 h ± 2 h 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 with or without yellow zones.

Photo from www.3m.com

The method is tested on foods. 3M™Petrifilm™ *Entrobacteriaceae* Count Plate was tested at the expert laboratory ADRIA Développement, Quimper, France, where it was compared against the reference method: ISO 21528:2004: Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae.* Part 2: Colony-count method.

Validation studies were carried out in 1997 and 2005 on meat products, poultry, dairy products, seafood products, vegetables and egg products. According to the studies, no statistical differences were found between the 3M™Petrifilm™ *Entrobacteriaceae* Count Plate and the reference method ISO 21528:2004 Part-2.

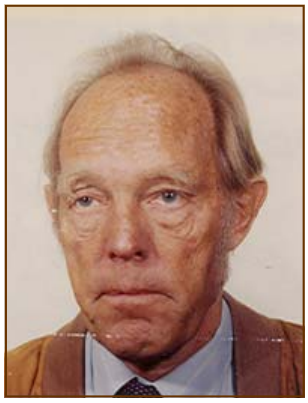
Workshops on Recovery in Chemical Analytical Measurement

In October / November 2012, NMKL will arrange workshops on Recovery, including apparent recovery, calibration recovery, recovery factors, measurement uncertainty related to recovery, method validation and method criteria.

Lars Jorhem, Sweden will be the lecturer of the workshops, which will be held at the
- Technical University of Denmark, Copenhagen,
- Norwegian Veterinary Institute, Oslo,
- National Food Agency, Uppsala, Sweden,
- Matis, Iceland and
- Evira, Finland.

Information about the workshops will be made available on the NMKL homepage.

In Memory of Professor Niels Skovgaard



Professor Niels Skovgaard passed away on 16 February 2012, at the age of 87. Niels Skovgaard had a remarkable career over a period of 60 years. Everybody engaged in food safety knew him or at least had heard of him. For many years, I have travelled to numerous meetings with him, and appreciated his sharp remarks and deep insight in the interaction of microbes and our food and animals.

After graduation as a veterinarian from the Royal Veterinary and Agricultural University (KVL) in Copenhagen in 1951, and a time as veterinary practitioner, he very soon joined the veterinary public health service in Denmark, dealing with meat inspection, food control and administration.

His teaching career started in 1965 as a lecturer within food microbiology and food hygiene at KVL, where he in 1973 was appointed professor. A position he held until his official retirement at the age of 70 in 1994.

During his time as professor and also after his retirement, Niels Skovgaard contributed actively with his profound knowledge as advisor and teacher for several international organizations such as: the World Health Organization (WHO), the Food and Agricultural Organization (FAO), the EU, the World Association of Veterinary Food Hygienists (WAVFH), the International Organization for Standardization (ISO), the International Union of Microbiological Societies (IUMS) and International Commission of Food Microbiology and Hygiene (ICFMH). In addition, he was frequently used as a scientific consultant by the Danish Government and the Danish food industry.

Niels Skovgaard devoted much of his career to Nordic cooperation within the field of food microbiology and the development of analytical methods. Over a period of 40 years he participated in the Danish national committee of NMKL as a highly respected and dedicated member. He even attended a meeting in January 2012. Skovgaard served several periods as secretary, as well as president, for the Nordic Committee on Food Analysis (NMKL).

In the Nordic microbiology group, he will be remembered for bringing forward proposals for new work items, such as emerging microbes of global interest. In this respect, he acted as a true lecturer with deep insight in the subject. We will all miss him as a source of profound knowledge, and – not to forget – his ability to convey his messages with his special type of humour keeping his audience alert!

Niels Skovgaard was co-founder of the International Journal of Food Microbiology published by ICFMH, and he was appointed president of this organization from 1989 to 1996, followed by an appointment as Honorary President.

Niels Skovgaard has published more than 300 scientific articles in Danish, Nordic and International magazines, and he has contributed to the publication of several instructive text books for students. Especially during his retirement, he spent considerable time reviewing scientific books and acting as referee of articles on food microbiology and hygiene. Niels Skovgaard was an honoury member of several international organisations and universities, and in Denmark, Niels Skovgaard was honoured with the title Knight of 1st Class of the Royal Order of Dannebrog.

But his true legacy is neither his books nor his many publications; it is the many students that have taken over and are continuing his remarkable work in many different organizations, journals, panels, and committees.

Sven Qvist, Denmark

From AOAC EUROPE - NMKL/NordVal International Symposium on

Rapid Methods - chemical, microbiological and sensory analysis of foods

held on 7 – 8 May 2012 in Copenhagen, Denmark

About 160 persons from 22 countries participated at the symposium, which was a arranged in cooperation with AOAC Europe. In addition to Europeans, there were participants from China, Brazil and USA. The feedback from participants and exhibitors was in general very positive.

Great thanks are extended to the moderators, to the 40 lecturers, the 29 poster contributors and the exhibitors from 3M, AB Sciex, Agilent Technologies, BIOTECON Diagnostics, Thermo Scientific, Bruker, Statens Serum Institut, Food Diagnostics, IonSense, r-biopharm, Neogen Europe Ltd and Romer Labs.

Silvia Sponza, University of Veterinary Medicine, Vienna, got the AOAC Europe Poster Award for the poster: "Fast GC-FID method for the determination of iridoids in *Plantago* species."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Analysis
with DART
ANSR


Food authenticity


Food Testi
Agricultu


Food Testing &
Agriculture

Available NMKL Procedures

No 1, 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	Yleiset ohjeet aistinvaraisten laboratorioiden laadunvarmistukseen (avail. Danish/Finnish)
No 7, 1998	Checking of UV/VIS spectrophotometers
No 8, 4. Ed. 2008	Measurement of uncertainty in quantitative microbiological examination of foods
No 9, 2. Ed. 2007	Evaluation of method bias using certified reference materials.
No 10, 2001	Control of microbiological media
No 11, 2.Ed. 2010	Procedure for sensory analysis of drinking water
No 12, 2002	Guide on sampling for analysis of foods
No 13, 2003	Volumetric control
No 14, 2004	SENSVAL: Guidelines for internal control in sensory analysis laboratories
No 15, 2004	Temperature control in microbiological laboratories
No 16, 2005	Sensory quality control
No 17, 2006	Guidelines for requirement specifications for food analyses
No 18, 2006	The use of reference materials, reference strains and control charts in a food microbiological laboratory
No 19, 2007	Guideline for sensorial analysis of food containers/packages
No 20, 2007	Evaluation of results from qualitative methods
No 21, 2008	Guide for sensory analysis of fish and shellfish
No 22, 2008	Considerations regarding evaluation of immunochemical test kits for food analysis
No 23, 2008	Guide on quality assurance in microbiological laboratories (replacing NMKL Report No. 5)
No 24, 2010	Guidelines for quality assurance for food chemical laboratories
No 25, 2012	Recovery information in analytical measurement