



Nordic Committee on Food Analysis
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Memorandum of Equivalence

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Comparison of NMKL and ISO methods for methods given in the Annex of the EU Commission Regulation (EC 2073/2005) on microbiological criteria

Are the methods equivalent?

Nordic experts have reviewed NMKL and ISO methods in order to find whether or not the methods may be regarded as equivalent. The methods considered are methods of interest laid down in the Annex of the Commission Regulation (EC) No 2073/2005 on microbiological criteria.

The reasons for the comparisons are:

- The reference methods in the Annex of the commission regulation 2073/2005 refer to ISO standards only. In order to use NMKL methods, the methods should be proven equivalent to the reference methods.
- Most of the ISO standards are not collaboratively tested, and hence if a NMKL and ISO method are considered equivalent, ISO might adopt the study results of the particular NMKL method. As it is important to have collaboratively validated methods as reference methods, the EU commission has given CEN mandate to collaboratively validate the specific ISO methods. The commission has requested NMKL to contribute, as NMKL may have equivalent validated methods and as NMKL's referees have experience in arranging collaborative method performance studies.

The Annex enclosed gives a very brief summary of the comparisons between ISO methods referred to in EC 2073/2005 and the respective NMKL methods. The experts' complete comparisons of the methods are available as pdf-files on NMKL's homepage: www.nmkl.org.

MICRO-ORGANISM	METHODS		EQUAL YES/NO	CONCLUSION
<p><i>Listeria Monocytogenes</i></p>	<p>EN/ISO 11290-1 + 2 Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> Part 1: Detection method Part 2: Enumeration method (part 1 is collaboratively validated, but not part 2)</p>	<p>NMKL 136 <i>Listeria monocytogenes</i>. Detection and enumeration in foods and feeding stuffs (collaboratively validated)</p>	<p>YES</p>	<p><u>Detection of <i>L.monocytogenes</i></u>: Both methods 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 the enrichment steps are plated out on a <i>L. monocytogenes</i> specific isolation medium. The NMKL method gives the options to use ALOA or LMBA or Chromogenic Listeria Agar medium basically alike ALOA and on another solid selective isolation medium, which is optional. In the ISO method LMBA is not described. [The NMKL collaborative study showed that there is no statistically difference whether you use ALOA, LMBA, OCLA or LCA.] Both methods confirm <i>L. monocytogenes</i> colonies by use the same morphological and biochemical tests.</p> <p><u>Enumeration of <i>L.monocytogenes</i></u>: The initial suspension is incubated for 1 h 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 <i>L. monocytogenes</i> specific isolation medium, ALOA or LMBA or Chromogenic Listeria Agar medium basically alike ALOA, (in duplicates – considered to be removed by both NMKL and ISO). In the ISO method only ALOA is described. [The NMKL collaborative study showed no difference between the media tested]. After incubation presumptive <i>L. monocytogenes</i> colonies are counted and confirmed using appropriate morphological and biochemical tests.</p>
<p><i>Salmonella</i></p>	<p>EN/ISO 6579 Horizontal method for the detection of <i>Salmonella spp.</i> (prev. version collaboratively validated)</p>	<p>NMKL 71 <i>Salmonella</i>. Detection in foods. (collaboratively validated)</p>	<p>YES</p>	<p>ISO method includes two selective enrichment procedures (RVS and MKTTn), while NMKL method includes one procedure (RVS). The methods are equal to the respective part of the ISO method.</p>
<p><i>E.coli</i></p>	<p>ISO 16649 Horizontal method for the enumeration of beta-glucuronidase-positive <i>Escherichia coli</i></p>	<p>NMKL 125 Thermotolerant coliform bacteria and <i>Escherichia coli</i>. Enumeration in food</p>	<p>NO</p>	<p>The ISO and NMKL methods apply two different principles for the detection of <i>E.coli</i>. The NMKL method use 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</p>

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	Part 1: Colony-count technique at 44°C .. Part 2: Colony-count technique at 44°C.. Part 3: Most probable number technique ... (not collaboratively validated)	and feed. (collaboratively validated) NMKL 96 Bacterial Examination in Fresh and Frozen seafood. (collaboratively validated)		the indication of presence of <i>E. coli</i> is the cleavage of the chromogenic ingredient giving rise to a particular colour in <i>E. coli</i> . The ISO method is less time consuming as there is no need for lengthy confirmation steps. The two methods apply different principles and media and cannot be considered as equal . The validation that has been performed for the NMKL method cannot be used for the ISO method. However, they may probably be used interchangeably as they cover more or less the same . None of the methods will cover <i>E. coli</i> that does not grow at lower temperatures (such as <i>E. coli</i> O157), but both methods will work fine for the enumeration of <i>E. coli</i> in hygiene purposes.
Aerobic microbes – surfaces	ISO 4833 Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C . (collaboratively validated on raw and pasteurized milk)	NMKL 5 Aerobic micro-organisms and presumptive Enterobacteriaceae. Enumeration on surfaces and utensils. (collaboratively validated)	NO	The NMKL and ISO methods are not comparable . The NMKL method uses swab or contact plate method. The ISO method is a colony count method tested on raw and pasteurized milk, and does not describe enumeration on surfaces as the scope in the regulation.
<i>Enterobacteriaceae</i> – surface	ISO 21528-2 Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method (not collaboratively validated)	NMKL 5 Aerobic micro-organisms and presumptive Enterobacteriaceae. Enumeration on surfaces and utensils. (collaboratively validated)	NO	The NMKL and ISO methods are not comparable . The NMKL method uses swab or contact plate method. The ISO method is a colony count method and does not describe enumeration on surfaces as the scope in the regulation.
Aerobic microbes	ISO 4833 Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C.	NMKL 86 Aerobic microorganisms. Determination in foods at 30°C, 20°C or 6,5°C.	YES	These methods are equivalent . NMKL could consider for adoption the collaborative studies referred to in the ISO 4833.

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	(collaboratively validated on raw and pasteurized milk)	(not collaboratively validated)		
<i>Enterobacteriaceae</i>	ISO 21528-2 Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method (not collaboratively validated)	NMKL 144 <i>Enterobacteriaceae</i> . Determination in foods and feeds. (collaboratively validated)	YES	The methods may be considered as equal . There are however two minor differences: 1) The ISO method prescribes two plates of VRBG-agar per dilution and NMKL one plate. 2) The ISO method prescribes confirmation by tests for fermentation of glucose and presence of oxidase while NMKL is using oxidase test only.
<i>Stafylococcus aureus</i>	ISO 6888-1 + 2 Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) Part 1: Technique using Baird-Parker agar medium Part 2: Technique using rabbit plasma fibrinogen agar medium (collaboratively validated)	NMKL 66 <i>Staphylococcus aureus</i> . Enumeration in foods. (collaboratively validated)	YES	The methods may be considered as equal . The NMKL method includes the possibility of including Blood agar, but does not recommend using this as a single medium, but more like an extra plate. The ISO method states that the pour-plate method is used with BP-RPF agar thereby lowering the detection limit.
<i>E.coli O 157</i>	EN/ISO 16654 Horizontal method for the detection of Escherichia coli O157 (not collaboratively validated)	NMKL 164 Escherichia coli O157. Detection in food and feeding stuffs. (collaboratively validated)	YES	The two methods are very similar. Both methods relies 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 <i>E. coli</i> O157. However there are also minor differences in the methods. The two methods may be considered as equivalent .
<i>Campylo-</i>	EN/ISO 10272-1	NMKL 119 (rev)	YES	Both methods have same enrichment procedure. In the NMKL method pre-

MICRO-ORGANISM	METHODS		EQUAL YES/NO	CONCLUSION
<i>bacter</i>	Horizontal method for detection and enumeration of <i>Campylobacter</i> spp. -- Part 1: Detection method (not collaboratively validated)	Thermotolerant <i>Campylobacter</i> . Detection and enumeration of in food (collaboratively validated)		incubation is optional. The collaborative study showed that for the samples included, there was no statistical significant difference in the results with or without the pre incubation. In the NMKL method the enrichment is carried out on mCCDA or AHB (one media). In the ISO method the enrichment should be carried out on 2 culture media (mCCDA + one optional). The enrichment conditions are the same. Both methods use motility and cell morphology plus oxidase and catalase tests for confirmation of <i>Campylobacter</i> . The ISO method has however some more confirmation steps. As the revised NMKL method no 119 is considered to be equivalent to ISO 10272-1, the collaborative study conducted on <i>Campylobacter</i> is valid also for ISO 10272-1.
<i>Salmonella</i> in faeces	EN/ISO 6579/Amd.1 MSRV method <i>Salmonella</i> in faeces. (under collaborative study)	NMKL XX <i>Salmonella</i> . Detection in foods, faeces and materials from primary animal production using MSRV. (not collaboratively validated)	YES	None of the methods are published yet. The methods are equivalent for faeces ; however, the NMKL method will have a broader scope.
<i>Yersinia enterocolitica</i>	EN/ISO 10273 Horizontal method for the detection of presumptive pathogenic <i>Yersinia enterocolitica</i> . (not collaboratively validated)	NMKL 117 (rev) <i>Yersinia enterocolitica</i> . Detection in foods. (collaboratively validated but the method is not published as the results were not satisfactory)	YES	The NMKL method is an adoption of the EN/ISO method. A method performance study arranged by NMKL shows that there is a need for further study and possible method improvement.