

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

According to the regulation (EC 2073/2005 Article 5), food business operators may use other methods than listed in Annex 1 if it can be demonstrated 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 in Annex I, and if a proprietary method, certified by a third party in accordance with ISO 16140 or another international recognised protocol (like NordVal protocol).

By “..procedures provide at least equivalent guarantees” we understand methods that give equivalent results, i.e. methods that provide results that do not differ significant statistically. This means that alternative methods to those referred to in Annex 1 do not necessarily have to apply the same principle, however, need to provide equivalent results.

The table below lists the microorganisms and the referred reference methods given in the EC 2073/2005 Annex 1 along with alternative NMKL and NordVal methods that provide at least equivalent guarantees / results.

Microorganism	Reference method	Alternative NMKL method Expected to give equivalent results	Alternative NordVal methods (Validated acc. to NordVal/ISO 16140)
<i>Listeria Monocytogenes</i> - detection thereof	EN/ISO 11290-1 Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> Part 1: Detection method <u>Validation status:</u> Collaboratively validated	NMKL 136 <i>Listeria monocytogenes</i> . Detection and enumeration in foods and feeding stuffs <u>Validation status:</u> Collaboratively validated <u>Comment:</u> The method applies similar principle as EN/ISO 11290-1.	NordVal 022 RAPID' <i>L.mono</i> Detection + enumeration (Ref. method: ISO 11290-1) NordVal 025 LightCycler <i>L.mono</i> Detection Kit (Ref. method: ISO 11290-1) NordVal 037 IQ-Check <i>Listeria monocytogenes</i> II (Ref. method: ISO 11290-1)

Microorganism	Reference method	Alternative NMKL method Expected to give equivalent results	Alternative NordVal methods (Validated acc. to NordVal/ISO 16140)
<i>Listeria Monocytogenes</i> - enumeration thereof	EN/ISO 11290-2 Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> Part 2: Enumeration method <u>Validation status:</u> Not collaboratively validated	NMKL 136 <i>Listeria monocytogenes</i>. Detection and enumeration in foods and feeding stuffs <u>Validation status:</u> Collaboratively validated <u>Comment:</u> The method applies similar principle as EN/ISO 11290-2.	NordVal 022 RAPID' <i>L.mono</i> Detection + enumeration (Ref. method: ISO 11290-2)
<i>Salmonella</i>	EN/ISO 6579 Horizontal method for the detection of <i>Salmonella spp.</i> <u>Validation status:</u> Partly validated	NMKL 71 <i>Salmonella</i>. Detection in foods. <u>Validation status:</u> Collaboratively validated <u>Comments:</u> ISO 6579 includes two selective enrichment procedures (RVS and MKTTn), NMKL 71 includes the RVS procedure. The principles of the methods are similar for the RVS part. The methods have also been compared in studies.	NordVal 001 Transia Plate <i>Salmonella</i> Gold (Ref. method: ISO 6579) NordVal 010 Bioline <i>Salmonella</i> ELISA Test Optima / RayAI <i>Salmonella</i> Optima (Ref. method: ISO 6579) NordVal 023 LightCycler <i>Salmonella</i> Detection Kit (Ref. method: ISO 6579) NordVal 030 BAX <i>Salmonella</i> PCR (BAX Classic and BAX Q7) BAX System with Automated Detection PCR Assay for Screening <i>Salmonella</i> (Ref. method: ISO 6579) NordVal 031 <i>Salmonella</i> detection method by real-time PCR (Ref. method: NMKL 71, NMKL 187) NordVal 032 RAPID' <i>Salmonella</i> - 24 hours test method (Ref. method: ISO 6579) NordVal 038

Microorganism	Reference method	Alternative NMKL method Expected to give equivalent results	Alternative NordVal methods (Validated acc. to NordVal/ISO 16140)
			iQ-Check TM <i>Salmonella</i> II kit (Ref. method: ISO 6579) NordVal 041 <i>Salmonella</i> detection method: DNA extraction by boiling followed by real-time PCR (Ref. method: NMKL 187)
<i>E.coli</i>	ISO 16649 Horizontal method for the enumeration of beta-glucuronidase-positive <i>Escherichia coli</i> Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide <u>Validation status:</u> Not collaboratively validated	NMKL 125 Thermotolerant coliform bacteria and <i>Escherichia coli</i>. Enumeration in food and feed. <u>Validation status:</u> Collaboratively validated NMKL 96 Bacterial Examinations in Fresh and Frozen seafood. <u>Validation status:</u> Collaboratively validated <u>Comments:</u> The method applies a different principle than ISO 16649. However, the two methods are considered to provide equivalent results. 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.	NordVal 014 3M Petrifilm <i>E.coli</i>/Coliform Count Plate (Ref. method: ISO 16649-2, ISO 4832, AOAC 991.14) NordVal 020 RAPID' <i>E.coli</i> 2 AGAR (Ref. method: ISO 16649-2, ISO 4832) NordVal 036 Compact Dry EC Method for the Enumeration of <i>E.coli</i> and Coliforms (Ref. method: ISO 16649-2, ISO 4832) NordVal 027 3M Petrifilm Select <i>E.coli</i> Count Plate (Ref. method: ISO 16649-2)
Aerobic microbes – surface	ISO 4833 Horizontal method for the	On surfaces:	On surfaces:

Microorganism	Reference method	Alternative NMKL method Expected to give equivalent results	Alternative NordVal methods (Validated acc. to NordVal/ISO 16140)
	enumeration of microorganisms. Colony-count technique at 30°C . <u>Validation status:</u> Collaboratively validated on milk The ISO method is a colony count method tested on milk, and does not describe enumeration on surfaces, which is the scope in the regulation. Recommended method should be methods similar to NMKL 5	NMKL 5 Aerobic microorganisms and presumptive Enterobacteriaceae. Enumeration on surfaces and utensils. <u>Validation status:</u> Collaboratively validated	NordVal 018 Hygicult TPC (Ref. method: NMKL 5)
Aerobic microbes - in foods	ISO 4833 Horizontal method for the enumeration of microorganisms. Colonycount technique at 30 degrees C. <u>Validation status:</u> Collaboratively validated on milk	NMKL 86 Aerobic microorganisms. Determination in foods at 30°C, 20°C or 6,5°C. <u>Validation status:</u> Not collaboratively validated <u>Based on:</u> The method applies similar principle as ISO 4833.	NordVal 012 3M Petrifilm, AerobicCount Plate (Ref. method: ISO 4833) NordVal 033 Compact Dry TC Method for the Enumeration of Total Viable Organisms (Ref. method: ISO 4833)
<i>Enterobacteriaceae</i> – surface	ISO 21528-2 Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method <u>Validation status:</u> Not validated collaboratively This ISO method is a colony count method		

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	and does <u>not</u> describe enumeration on surface, which is the scope in the regulation. Recommended method should be methods similar to NMKL 5, NordVal 018		
<i>Enterobacteriaceae</i> - in foods	ISO 21528-2 Horizontal methods for the detection and enumeration of <i>Enterobacteriaceae</i> - Part 2: Colony-count method <u>Validation status:</u> Not validated collaboratively	NMKL 144 <i>Enterobacteriaceae</i>. Determination in foods and feeds. <u>Validation status:</u> Collaboratively validated <u>Comments:</u> The method applies similar principle as ISO 21528-2, except for 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.	NordVal 029 3M Petrifilm <i>Enterobacteriaceae</i> Count Plate (Ref. method: ISO 21528-2) NordVal 034 Compact Dry ETB Method for the Enumeration of <i>Enterobacteriaceae</i> (Ref. method: ISO 21528-2)
<i>Stafylococcus aureus</i>	ISO 6888-1 + 2 Horizontal method for the enumeration of coagulase-positive staphylococci (<i>Stafylococcus aureus</i> and other species) Part 1: Technique using Baird-Parker agar medium Part 2: Technique using rabbit plasma fibrinogen agar medium	NMKL 66 Coagulase positive staphylo cocci. Enumeration in foods. <u>Validation status:</u> Collaboratively validated <u>Comments:</u> The method applies similar principle as ISO 6888. 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	NordVal 019 3M Petrifilm Express Count System (Ref. method: ISO 6888-1)

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	<u>Validation status:</u> Validated collaboratively	method states that the pour-plate method is used with BP-RPF agar thereby lowering the detection limit.	
<i>E.coli</i> O 157	EN/ISO 16654 Horizontal method for the detection of <i>Escherichia coli</i> O157 <u>Validation status:</u> Not validated collaboratively	NMKL 164 <i>Escherichia coli</i> O157. Detection in food and feeding stuffs. <u>Validation status:</u> Collaboratively validated <u>Comments:</u> The method applies similar principle as ISO 16654. There are only minor non-technical differences.	NordVal 026 LightCycler food proof <i>E. coli</i> O 157 Detection Kit in combination with ShortPrep foodproof II Kit (Ref. method: ISO 16654)