

Comparison of two methods for the detection of *Escherichia coli* serogroup O157 in foods and feeding stuffs: ISO 16654:2001: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157 and Nordic Committee on Food Analysis: NMKL No 164, 2. Ed. 2005: *Escherichia coli* O157. Detection in food and feeding stuffs.

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Date: 28 November 2006.

At the ISO/TC34 SC9 meeting in Prague in June 2006 it was decided in resolution N° 297 under “NMKL – Cooperation agreement between NMKL and ISO/TC34 SC9” that “Jeppe Boel has to check whether the NMKL method is equivalent to the ISO method”. The following document has been written in response to this request.

ISO and NMKL have both published culture based reference methods for the detection of *Escherichia coli* O157 in food and feeding stuffs. The two methods are very similar and both methods relies on the same principles: 1: Enrichment in modified tryptic soy broth supplemented with novobiocin (mTSBn), 2: separation and concentration by means of immunomagnetic particles coated with antibodies to *E. coli* O157 (immuno-magnetic separation – IMS), 3: Isolation by subculture onto two selective indicative isolation media (cefixime potassium supplemented Sorbitol MacConkey (SMAC) agar as mandatory isolation media and the other isolation media is free of choice), and 4: Confirmation of the *E. coli* O157. However there are also minor differences in the methods. In Table 1 a list of important similarities and differences between the two methods are given.

The NMKL method has been evaluated in a collaborative study (Normark, C (2005): Collaborative study of method for detection of *Escherichia coli* O157 in food - NMKL No. 164, 1999. SLV rapport 2/2005 (National Food Administration, Uppsala, Sweden)). The ISO method has not been collaboratively evaluated. If the two methods are considered equivalent, this would mean, that the collaborative validation of the NMKL method also could be considered as documentation of the ISO method.

Table 1 Comparison of NMKL and ISO methods for the detection of Eschericia coli O157 in food and feed.

Step	ISO	NMKL	Comments
Scope	Detection in food and feed	Detection in food and feed	No differences
Test size, Sample preparation	x g or x ml to 9 x ml of enrichment broth. Homogenization (Stomacher)	25 grams of food to 225 ml of enrichment broth. Homogenization (Stomacher)	Except for specification of the protein source, no difference in the prescribed mTSBn
Enrichment broth	Enrichment broth Modified Tryptone Soya Broth: Enzymatic digest of casein 17.0 g Enzymatic digest, soya 3.0 g Dipotassium hydrogen phosphate (K ₂ HPO ₄) 4.0 g Sodium chloride 5.0 g D(+) Glucose 2.5 g Bilesalt no. 3 1.5 g Distilled or equal water 1000 ml Novobiocin 20 mg/l. pH 7.4±0.2	Enrichment broth: Modified Tryptone Soya Broth: Pancreatic digest, casein 17.0 g Papain digest, soybean 3.0 g Dipotassium hydrogen phosphate (K ₂ HPO ₄) 4.0 g Sodium chloride 5.0 g Glucose 2.5 g Bilesalt no. 3 1.5 g Distilled or equal water 1000 ml Novobiocin 20 mg/l. pH 7.4±0.2	
Enrichment	Incubate 6 hours at 41.5°C and further 12-18 hours (total 18-24 hours).	Incubate the samples at 41.5±0.5°C for 6-8 hours and/or 18-24 hours for 6-8 h and/or 18-24 hours.	Variation in the specification of the “short” incubation period
Immunomagnetic separation	IMS should be carried out after 6 hours and again, if necessary, after 12 hours to 18 hours of incubation Method relies on non-specified (commercially) available antibody-coated beads.	IMS should be carried out after 6-8 h and again after 12h to 18 hours of incubation Method relies on non-specified (commercially) available antibody-coated beads.	Both methods refer to non-specified antibody-coated beads. This could be regarded as potentially problematic because of differences in the performance of different “bead systems” and the quality of the applied antibodies. In the Nordic countries most laboratories use beads from Dynal (see www.invitrogene.com)

			Comment on the ISO method: when is it “necessary” to use IMS after both 6 and 18-24 hours of incubation.																																		
Plating out onto selective agar	<p>The affinity purified IMS bead-bacteria complex (2x50µl) is seeded on to mandatory media (CT-SMAC) and a second isolation medium free of choice).</p> <p>CT-SMAC – composition:</p> <table border="0"> <tr><td>Enzymatic digest of casein</td><td>17.0 g</td></tr> <tr><td>Enzymatic digest of animal tissues</td><td>3.0 g</td></tr> <tr><td>Bile salts No.3</td><td>1.5 g</td></tr> <tr><td>Sodium chloride</td><td>5.0 g</td></tr> <tr><td>Neutral red</td><td>0.03 g</td></tr> <tr><td>Agar</td><td>9-18 g</td></tr> <tr><td>Sorbitol</td><td>10.0 g</td></tr> <tr><td>Crystal violet</td><td>0.001 g</td></tr> <tr><td>Distilled or equal water</td><td>1000 ml</td></tr> </table> <p>pH 7,1 ±0.5</p> <p>Cefixime concentration of 0.05 mg/l potassium tellurite concentration of 2.5 mg/l.</p>	Enzymatic digest of casein	17.0 g	Enzymatic digest of animal tissues	3.0 g	Bile salts No.3	1.5 g	Sodium chloride	5.0 g	Neutral red	0.03 g	Agar	9-18 g	Sorbitol	10.0 g	Crystal violet	0.001 g	Distilled or equal water	1000 ml	<p>The affinity purified IMS bead-bacteria complex (2x50µl) is seeded on to mandatory media (CT-SMAC) and a second isolation medium free of choice).</p> <p>CT-SMAC – composition:</p> <table border="0"> <tr><td>Peptone</td><td>20.0 g</td></tr> <tr><td>Agar</td><td>15.0 g</td></tr> <tr><td>Sorbitol</td><td>10.0 g</td></tr> <tr><td>Bile salts No.3</td><td>1.5 g</td></tr> <tr><td>Sodium chloride</td><td>5.0 g</td></tr> <tr><td>Neutral red</td><td>0.03 g</td></tr> <tr><td>Crystal violet</td><td>0.001 g</td></tr> <tr><td>Distilled or equal water</td><td>1000 ml</td></tr> </table> <p>pH: no specification (mistake?)</p> <p>Cefixime concentration of 0.05 mg/l potassium tellurite concentration of 2.5 mg/l.</p>	Peptone	20.0 g	Agar	15.0 g	Sorbitol	10.0 g	Bile salts No.3	1.5 g	Sodium chloride	5.0 g	Neutral red	0.03 g	Crystal violet	0.001 g	Distilled or equal water	1000 ml	<p>The composition of the CT-SMAC agar is very similar in the two methods. As the case with mTSBn, a difference in the peptone sources are observed.</p> <p>Potassium tellurite sensitive strains have been reported by Germany (reference? - Anecdotal evidence). Neither the NMKL or ISO method addresses this by recommending that the second isolation media should be free of tellurite. Not much is know about the possible presence of cefixime sensitive strains.</p> <p>The use of a cottonswab to streak out the plates could be controversial. An anecdotal argument for using the cottonswab is that it helps “breaking down” aggregates of <i>E. coli</i> O157, thereby facilitating the distribution of the target organism on the agar plates. But it is also clear that a cotton swab absorbs some of the liquid (and bacteria), and thus reduces the seeded volume.</p>
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Plating technique:	The bacteria-bead complex is streaked out with a sterile loop	The bacteria-bead complex is streaked out with a cotton swab on half of the plate and on the remaining half with a sterile loop.																																			

Incubation of selective indicative isolation plates	CT-SMAC: 37°C for 18-24 hours, other isolation media at its recommended temperature and specified time.	37°C for 18-24 hours for both isolation media	
Reading of isolation media	Description of the morphology of the typical sorbitol negative <i>E. coli</i> O157 on CT-SMAC. Other media inspect typical colonies.	Description of the morphology of the typical sorbitol negative <i>E. coli</i> O157 on CT-SMAC. Other media inspect typical colonies.	Essentially the same formulation with regard to morphology on CT-SMAC
Confirmation	Up to five typical colonies from each of the isolation media are seeded on to non-selective agar. The colonies are tested for indole formation in tryptone/tryptophan medium. Indole forming isolates are investigated serologically by slide agglutination. Further characterization: Send strain to reference laboratory	Test typical colonies (no number given). Seed onto non-selective agar (e.g. blood agar). Colonies are tested for indole formation in tryptone tryptophane media. Indole forming isolates are investigated serologically by slide agglutination. It is stated, that it is also possible to use slide agglutination tests on colonies that grows on the isolation media. Further characterization: Send strain to reference laboratory	Essentially the same procedure
QA, test strain	Control strain: Non VT producing <i>E. coli</i> O157 Recommends that spiked samples are used to obtain routine in reading the selective indicative agar plate.	Control strain: Non VT producing <i>E. coli</i> O157, ATCC 43888. Recommends that spiked samples are used to obtain routine in reading the selective indicative agar plate.	Essentially the same procedure.