ISO 161654:2001 Validation Study

Update and further steps

M/381 Mandate

- Launched in 2006
- Re-launched in 2007
- Projects approved in 2008
- Funds released in 2011
- Study carried out in 2012
- Report in 2013
- TWO YEARS EARLIER THAN THE DEADLINE!!!

Nr. 164
2. udg.
2005

NORDISK METODIKKOMITÉ FOR NÆRINGSMIDLER

NORDIC COMMITTEE ON FOOD ANALYSIS

No 164
2. Ed.
2005

Escherichia coli O157. Påvisning i levnedsmidler og foder.

Denne NMKL-metode er kollaborativt valideret for levnedsmidler i en metode-afprøvning.

1. FORMÅL OG ANVENDELSESOMRÅDE

Denne metode omhandler kvalitativ bestemmelse af *E. coli* O157 i levnedsmidler og foder.

2. DEFINITIONER

E. coli er en fakultativt anaerob Gram-negativ stav tilhørende familien *Enterobacteriaceae*. *E. coli* er katalase-positiv, oxidase-negativ, indol-positiv, reducerer nitrat og danner luft udfra glukose.

Hos den typiske patogene *E. coli* O157 kan flagel antigenet H7 være tilstede eller mangle (H-). Bakterien er sorbitol-negativ indenfor 24 timer efter udstrygning samt glucoronidase-negativ. Sorbitolpositive varianter er beskrevet. Bakterien er enterohemolysin-positiv på vaskede fåreblodsceller. Patogene stammer indeholder et stort plasmid som bærer gener, der er associeret med virulens (EHEC plasmid). Patogene varianter er i besiddelse af shigatoksingenerne *stx*1 og/eller *stx*2 (også benævnt *vt*1 og *vt*2) og gener (*eae*), der koder for adhæsion til tarmepitheliet.

3. REFERENCER

3.1 NMKL Nr. 91, 4. udg. 2002: Prøveudtagning og forbehandling af levnedsmidler og foderstoffer til kvantitativ mikrobiologisk undersøgelse.

3.2 NMKL Nr. 5, 2 udg. 1994: Handledning i kvalitetssäkring for mikrobiologiska laboratorier.

3.3 NMKL Nr. 19, 1998: Harmonisering af mikrobiologiske metoder.

Escherichia coli O157. Detection in food and feeding stuffs.

This NMKL method is validated for food in a collaborative study.

1. SCOPE AND FIELD OF APPLICATION

This procedure describes the qualitative determination of *E. coli* O157 in food and feeding stuffs.

2. DEFINITIONS

E. coli is a facultative anaerobic Gram-negative rod belonging to the family *Enterobacteriaceae*. *E. coli* is catalase-positive, oxidase-negative, indole-positive, reduces nitrate and produces gas from glucose.

The typical pathogenic *E. coli* O157 strains either express the flagella antigen H7 or lack flagella antigen (H-), are sorbitol-negative within 24 hours after plating, and are glucoronidase negative. Sorbitol-positive strains have been described. The bacteria are enterohemolysin-positive on washed sheep erythrocytes. Pathogenic strains contain a large plasmid harboring genes associated with virulence (EHEC plasmid). Pathogenic strains harbor the shigatoxin genes *stx*1 and/or *stx*2 (also named *vt*1 and *vt*2) and carry genes (*eae*) coding for adhesion to the intestinal epithelium.

3. REFERENCES

3.1 NMKL No. 91, 4 ed. 2002: Sampling and pre-treatment of foods and animal feedstuffs, for quantitative microbiological examination.

3.2 NMKL No. 5, 2 ed. 1994: Quality Assurance Guidelines for microbiological laboratories.

3.3 NMKL No. 19, 1998: Harmonization of microbiological methods (available in Finnish and

Design of the study

- In 2006 CEN requested to WG6 to assess the equivalence between the ISO 16654 and the NMKL method No 164, 2. Ed. 2005
- NMKL method No 164 validated in a collaborative study in 2002
- Project for validation in mandate M/381 included a reduced study as agreed by CEN in 2007 in Cairo (only one epidemiologically relevant matrix)

Mandate M/381 study

	Samples E.coli O157
number of labs	**15**
stam	**E.coli O157 NCCB 100282**
method for homogenity testing	**ISO 16654**
media	**CT-SMAC**
incubation	**24h 37°C**
matrix	**milk**
volume	**10 ml**
labelling	**sample 1 to 24 coded ad random**

blanco	120	sample code	3	4	5	8	12	13	14	24
low level (10-50 cfu/ml)	120	sample code	1	9	15	16	17	19	20	23
high level (100-500 cfu/ml)	120	sample code	2	6	7	10	11	18	21	22

Homogenity testing	**10x**	low level (sample codes)	1	9	15	16	17	19	20	23	1	9
	10x	high level (sample codes)	2	6	7	10	11	18	21	22	2	6
blanco testing	**10x**	blanco (sample codes)	3	4	5	8	12	13	14	24	3	4

Samples stable for one year @-20°C!

Mandate M/381 Results

		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15
expected	*Sample*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*	*Result*
L	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	12	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
0	13	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
L	15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	24	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0

Mandate M/381 Next steps

- Meeting of the PLs of the Mandate next week in Brussels
- Meeting with the participant labs early next year
- Design the report based on the study
- Combine report of the study with that from the NMKL validation
- Present to the commission within 2013 year ???