CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

100601

The AOAC Research Institute hereby certifies the method known as:

foodproof® *E. coli* O157 Detection Kits (5'Nuclease and Hybridization Probes)
with **food**proof® ShortPrep II Kit

manufactured by

BIOTECON Diagnostics GmbH
Hermannswerder Haus 17
14473 Potsdam, Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (March 3, 2022 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

March 3, 2022
Date

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SUBMITTING COMPANY
BIOTECON Diagnostics GmbH
Hermannswerder Haus 17
14473 Potsdam, Germany

METHOD NAME
foodproof® *E. coli* O157 Detection Kits (5'Nuclease and Hybridization Probes)
with **food**proof® ShortPrep II Kit

CATALOG NUMBERS
KIT 2300 42; KIT 2301 71

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APPLICABILITY OF METHOD
Target organism – *E. coli* O157

Matrixes – (25 g) - Camembert cheese, egg salad, raw milk, mayonnaise, hamburger meat, bockwurst sausage, coalfish, fresh salmon, apple juice, raw vegetables

Performance claims - This advanced PCR-method has been designed to reduce the time taken to achieve results from PCR reactions and to enable the user to monitor the amplification of the PCR product simultaneously, in real-time.

REFERENCE METHODS
http://www.cfsan.fda.gov/~ebam/bam-4a.html, Hitchins A.D., Feng P., Watkins W.D., Rippey S.R. and Chandler S.A. 1998, Bacteriological Analytical Manual BAM/AOAC 8th Edition. AOAC International. Gaithersburg, pp 4.01-4.29 Escherichia coli and coliform bacteria. (8)

EN ISO 16654:2001: Horizontal method for the detection of *E. coli* O157, Beuth publishing, Berlin, 1-14, 2003 (10)

ORIGINAL CERTIFICATION DATE
November 01, 2006

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2022.

METHOD MODIFICATION RECORD
1. **February 2011 Level 2**
2. **2014 Level 2**
3. **February 2018 Level 1**
4. **December 2020 Level 1**
5. **March 2022 Level 1**

SUMMARY OF MODIFICATION
1. **Change in probes.**
2. **Change in Taq.**
3. **Editorial changes.**
4. **Editorial/reformatting of inserts.**
5. **Rebranding to include Hygiena, editing, and formatting changes to inserts and labeling.**

Under this AOAC® *Performance Tested*℠ License Number, 100601 this method is distributed by:
None

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None

PRINCIPLE OF THE METHOD (1)
The method describes the detection of *E. coli* O157 in food.
1. The ShortPrep **food**proof II Kit is used for the isolation of *E. coli* O157 DNA from the enriched food sample. Cells are pre-lysed due to mixing with lysis reagent and mechanical disruption. DNA is extracted by incubation with the ShortPrep **food**proof II Kit Resuspension buffer.
2. Using the sequence-specific primers in the LightCycler **food**proof *E. coli* O157 Detection Kit in a polymerase chain reaction (PCR), the LightCycler Instrument and its associated reagents amplify and simultaneously detect fragments of a gene located in the O antigen gene cluster of *E. coli* of the serotype O157.
The co-amplification of an internal control (IC) prevents misinterpretation of false-negative results due to inhibition of the amplification.
The LightCycler Instrument detects both amplification products in real time through fluorescence generated by sequence-specific pairs of Hybridization Probes. For each amplicon, one Hybridization Probe is labeled at the 5'-end with a LightCycler Red fluorophore (LightCycler Red 640 for the detection of the *E. coli* O157 amplicon, LightCycler Red 705 for the detection of the internal control). To avoid extension, the 3'-end is modified by phosphorylation. The other Hybridization Probe is labeled at the 3'-end with Fluorescein.
During the annealing phase of each PCR cycle, these probes hybridize to an internal sequence of the amplicon. Only while hybridized in close proximity to each other, fluorescein, the donor fluorophore, which is excited by the light source of the LightCycler Instrument, transfers part of the excitation energy to the LightCycler Red, the acceptor fluorophore (fluorescence resonance energy transfer (FRET)).
The LightCycler Instrument measures the emitted fluorescence of the LightCycler Red fluorophores.
Three food types, camembert, raw vegetables salad and raw milk showed a high bacterial background . The competitive flora quickly metabolized the nutrients in normal enrichment media and also in selective media. *E. coli* O157 also grew, but much slower than without the bacterial background. This was reflected in the high cp-values of the PCR-method and the difficulties of the FDA BAM-method to detect *E. coli* O157.

DISCUSSION OF THE VALIDATION STUDY (1)

Highest detection sensitivity is important for food associated pathogens like *E. coli* O157 because smallest contaminations can cause the most fatal consequences for all involved persons and, from another point of view, also for the involved food company.

For the detection of those pathogens real-time PCR is a reliable method. It offers a very high degree of accuracy because amplification of organism-specific DNA segments allows the specific detection of very low cell copy numbers.

This could be confirmed by comparing the BIOTECON Diagnostics/Roche LightCycler **food**proof *E. coli* O157 Detection Kit (including ShortPrep **food**proof II Kit sample preparation) with the official BAM/FDA and ISO reference methods.

The LightCycler **food**proof *E. coli* O157 Detection Kit in combination with ShortPrep **food**proof II Kit successfully detected low and high numbers of *E. coli* O157 in a variety of foodstuffs. There were some deviations in results between PCR and the microbiological reference methods.

In nine of ten foodstuffs the uninoculated samples were tested negative with both methods, PCR and reference method. In one case, raw milk, the PCR gave a positive result, whereas the FDA BAM method gave a negative result. This sample was rechecked with both, microbiology and PCR, with the same outcome. Raw milk is often naturally contaminated with *E. coli* O157, therefore it was not unlikely to detect this parameter in an uninoculated sample. Following the AOAC RI regulations, this result has to be declared false positive because it could not be confirmed by the reference method.

Regarding the inoculated samples, all tested food groups showed fractional positive results at least for the low inoculation level.

Both methods produced identical results in six foodstuffs (60 %): mayonnaise, egg salad, frozen salmon, apple juice, minced meat and large frankfurters.

In four foodstuffs the alternative PCR-method gave more positive results at both inoculation levels than the reference method. These foodstuffs were: camembert, coalfish, raw vegetables salad and raw milk.

Also, coalfish showed a high background flora, but clearly lower cp-values than the three above mentioned foodstuffs. This could be due to the fact that coalfish harbored more gram positive microorganisms whose growth were inhibited through the selective enrichment step. This allowed the target organisms to grow better. However, the agar plates showed many different colony morphologies, where typical and untypical sorbitol fermenting bacteria and non-sorbitol fermenting bacteria were represented in the same ratio.

Altogether, it was observed that the bacterial background had a bigger effect on the microbiological reference method than on the PCR method. Regarding the PCR, only the cp-values were influenced, whereas a distinctive competitive flora influenced the results of analysis of the reference method.

Following the results of a study from M. A. Grant (5), high levels of microorganisms which are harmless, but which are present in large numbers may conceal small numbers of *E. coli* O157. TCSMAC plates streaked after the standard enrichment of the FDA BAM, Chapter 4A (2002) method, are commonly covered with sorbitol-positive colonies to the extent that it is difficult to see whether small numbers of target sorbitol-negative colonies are present. Hitchins et al. (6) reported difficulties due to high levels of contaminating coliforms which may mask the presence of E. coli O157:H7 strains when grown on SMAC agar. A general laboratory practice is that 1 different colony (of the same size) can be separated among 100 colonies (150 colonies is the maximum level) on a 9 cm standard Petri dish. In other words, 1 % of well-grown colonies can be separated depending on their color and/or shape.

Table 15.8: Tested *E. coli* O157 serotypes of the inclusivity study (1)

Serovars	Number of strains	Positive results	Origin
E. coli O157:H7	38	38	animal faeces, human, food
E. coli O157:H-	19	19	faeces, food
E. coli O157:H16	2	2	food
E. coli O157 (H not known)	1	1	animal faeces, human, food

Table 15.9: Exclusivity results of tested non O157-*E. coli* strains (1)

Strain # (BCD* collection)	Serovar	Group	Result	Origin
5643	O2:H5	EHEC	negative	not specified
12534	O3:H-	EHEC	negative	cow faeces
14194	O4:H-	EHEC	negative	not specified
7853	O5:H-	EHEC	negative	calf
7885	O6:H10		negative	bowel for sausage preparation
14205	O7:H16	EHEC	negative	not specified
14195	O8:H-	EHEC	negative	ground beef
14197	O9:10	EHEC	negative	not specified
14213	O10:H4	EHEC	negative	not specified
12551	O12:H-	EHEC	negative	cow faeces
8244	O15:H-	ETEC	negative	not specified
12505	O17:H-	EHEC	negative	raw milk
8245	O20	ETEC	negative	not specified
7879	O22:H8	EHEC	negative	ground beef
7841	O23:H-	EHEC	negative	not specified
8331	O24		negative	not specified
8227	O25:H42	ETEC	negative	not specified
14191	O26:H11	EHEC	negative	cow faeces
8256	O28	EIEC	negative	not specified
14236	O32:H8	EHEC	negative	not specified
8327	O37		negative	not specified

12532	O39:H40	EHEC	negative	cow faeces
8346	O44		negative	not specified
12514	O46:H-	EHEC	negative	ground beef
14238	O48:H-	EHEC	negative	not specified
8318	O55:H-	EHEC	negative	not specified
7836	O57:H-	EHEC	negative	not specified
12517	O62:H8	EHEC	negative	ground beef
12521	O65:H-	EHEC	negative	minced meat with onions
5646	O69:H-	EHEC	negative	not specified
14239	O74:H39	EHEC	negative	cow faeces
7884	O75:H-		negative	not specified
14237	O77:H18	EHEC	negative	not specified
5581	O78:H11	ETEC	negative	not specified
14229	O82:H8	EHEC	negative	cow faeces
14222	O84:H-	EHEC	negative	not specified
7567	O86	EPEC	negative	not specified
14231	O91:H10	EHEC	negative	not specified
5647	O101:H9	EHEC	negative	not specified
14210	O103:H42	EHEC	negative	cow faeces
12515	O104:H12	EHEC	negative	ground beef
12541	O105:H18	EHEC	negative	cow faeces
14217	O107:H11	EHEC	negative	not specified
8341	O110:H17		negative	not specified
7876	O111:H-	EHEC	negative	human
8255	O112	EIEC	negative	not specified
14204	O113:H-	EHEC	negative	not specified
8592	O114	EPEC	negative	not specified
14230	O116:H21	EHEC	negative	cow faeces
12547	O118:H-	EHEC	negative	cow faeces
7572	O119	EPEC	negative	not specified
14192	O121:H10	EHEC	negative	not specified
8264	O124	EIEC	negative	not specified
7573	O125	EPEC	negative	not specified
14228	O126:H20	EHEC	negative	not specified
7576	O127	EPEC	negative	not specified
7839	O128:H2	EHEC	negative	not specified
7860	O129:H-	EHEC	negative	cow faeces
12533	O136:H19	EHEC	negative	cow faeces
12502	O138:H8	EHEC	negative	attested milk
7578	O142	EPEC	negative	not specified
8253	O143:H-	EIEC	negative	not specified
8252	O144:H-	EIEC	negative	not specified
7881	O145:H-	EHEC	negative	not specified
14203	O146:H21	EHEC	negative	not specified
8233	O148	ETEC	negative	not specified
8246	O152:H-	EIEC	negative	not specified
14208	O153:H25	EHEC	negative	not specified
12548	O155:H-	EHEC	negative	cow faeces
14215	O156:H-	EHEC	negative	ground beef
7583	O158	EPEC	negative	not specified
8261	O164	EIEC	negative	not specified
8259	O167:H5	EIEC	negative	not specified

*BCD = BIOTECON Diagnostics

Table 15.10: Exclusivity results of tested non-*E. coli* strains (1)

Strain #	Organism	Result	Origin
BCD 8832	*Bacillus cereus*	negative	garden soil
BCD 3134	*Burkholderia cepacia*	negative	onion
DSM4688	*Campylobacter jejuni*	negative	bovine faeces
BCD 5792	*Citrobacter freundii*	negative	contaminated cutting fluids
BCD 14571	*Citrobacter rodentium*	negative	hamster
DSM 30053	*Enterobacter aerogenes*	negative	sputum
DSM 30054	*Enterobacter cloacae*	negative	spinal fluid
DSM 4581	*Enterobacter intermedius*	negative	not specified
BCD 7888	*Escherichia blattae*	negative	hindgut of cockroach
NCTC 12128	*Escherichia fergusonii*	negative	faeces of 1-year-old boy
BCD 8467	*Escherichia hermanii*	negative	toe of 17-year old female
BCD 5611	*Escherichia vulneris*	negative	human wound
BCD 8930	*Hafnia alvei*	negative	soil under the nickel-accumulating tree *Sebertia acuminata*
DSM 2026	*Klebsiella pneumoniae*	negative	sewage
BCD 5609	*Klebsiella terrigena*	negative	drinking water
SLCC 7054	*Listeria monocytogenes*	negative	rabbit
DSM 30164	*Morganella morganii*	negative	stool of a summer diarrhoea case
BCD 8600	*Pantoea agglomerans*	negative	knee laceration
BCD 8929	*Pantoea dispersa*	negative	not specified
BCD 4701	*Proteus mirabilis*	negative	not specified
DSM 4539	*Providencia stuartii*	negative	faeces of infant
DSM 50071	*Pseudomonas aeruginosa*	negative	animal room water bottle
BCD 2439	*Pseudomonas fluorescens*	negative	raw milk for cheese production
BCD 8721	*Rahnella aquatilis*	negative	chicken
BCD 6203	*Salmonella* Aqua (O-gr. N)	negative	not specified
BCD 5242	*Salmonella bongori* (O-gr. Y)	negative	not specified
BCD 2288	*Salmonella* Choleraesuis (O-gr. C1)	negative	human stool
BCD 2431	*Salmonella enterica* subsp. *salamae* (O-gr. 58)	negative	not specified
BCD 5200	*Salmonella enterica* subsp. *arizonae* (O-gr. Z)	negative	not specified
BCD 2425	*Salmonella enterica* subsp. *diarizonae* (O-gr. X)	negative	not specified
BCD 5237	*Salmonella enterica* subsp. *houtenae* (O-gr. V)	negative	not specified
BCD 5697	*Salmonella enterica* subsp. *indica* (O-gr. S)	negative	not specified
BCD 2263	*Salmonella* Enteritidis (O-gr. D1)	negative	not specified
BCD 6204	*Salmonella* Morningside (O-gr. N)	negative	not specified
BCD 2220	*Salmonella* Urbana (O-gr. N)	negative	not specified
DSM 4487	*Serratia liquefaciens*	negative	raw milk
BCD 7551	*Shigella boydii* (O7)	negative	not specified
NCTC 4837	*Shigella dysenteriae* (O1)	negative	not specified
BCD 7562	*Shigella flexneri* (O3b)	negative	not specified
BCD 4301	*Shigella sonnei*	negative	not specified
DSM 20231	*Staphylococcus aureus*	negative	human pleural fluid
DSM 2171	*Vibrio alginolyticus*	negative	spoiled horse mackerel, causing food poisoning
DSM 6904	*Vibrio harveyi*	negative	seawater
DSM 10027	*Vibrio parahaemolyticus*	negative	shirasu food poisened victim
DSM 30189	*Vibrio proteolyticus*	negative	intestine of *Limnoria tripunctata*
DSM 8992	*Yersinia pseudotuberculosis*	negative	turkey
BCD 6046	*Yokenella regensburgei*	negative	human wrist wound

(DSM = German Collection of Microorganisms, SLCC = Seeligers *Listeria* Culture Collection, NCTC = National Collection of Type Cultures)

Table 15.6: Results of the In-house repeatability study (1)

Food	No. of Samples	Inoculation Level (MPN) cells per 25 gram	No. of positive tested samples			Chi Square Values
			PCR presumpt.	PCR confirm.	Cultural method	
Mayonnaise[1]	20	0.9	7	7	7	0
	20	9.5	20	20	20	0
	5	-	0		0	0
Camembert[1]	20	1.5	3	2	1 (2)[3]	0
	20	7.0	20	15	14 (15)	0
	5	-	0		0	0
Coal fish[1]	20	1.7	6	5	5 (5)	0
	20	24.0	20	20	20	0
	5	-	0		0	0
Egg salad[1]	20	1.5	13	13	13	0
	20	11.5	20	20	20	0
	5	-	0		0	0
Raw vegetables salad[1]	20	2.0	17	9	7 (9)	0.5
	20	11.8	19	10	10 (10)	0
	5	-	0		0	0
Frozen salmon[1]	20	1.5	15	15	14 (15)	0
	20	28.0	20	20	20	0
	5	-	0		0	0
Apple juice[1]	20	< 0.8	11	11	10 (11)	0
	20	5.5	20	20	20	0
	5	-	0		0	0
Raw milk[1]	20	< 0.8	5	4	3(4)	0
	20	3.8	10	6	5 (6)	0
	5	-	1		0	0
Minced meat[2]	20	2.1	13	13	11 (13)	0.5
	20	8.0	20	20	19 (20)	0
	5	-	0		0	0
Large Frankfurters[2]	20	1.6	14	14	14	0
	20	6.5	20	20	20	0
	5	-	0		0	0

1: food samples tested according to FDA BAM, Chapter 4A (2002)
2: food samples tested according to EN ISO 16654:2001
3: Numbers in brackets: results from repetitions, see text below

DISCUSSION OF MODIFICATION DATA APPROVED FEBRUARY 2011 (11)

For this method extension a repeatability study/method comparison with three different food matrixes was accomplished. Moreover, the in- and exclusivity of the real-time PCR system have been examined with a wide spectrum of different isolates. Therefore, the **food**proof *E. coli* O157 Detection Kits (5'Nuclease and Hybridization Probes) with **food**proof ShortPrep II Kit (formerly BIOTECON Diagnostics **food**proof *E. coli* O157 Detection Kit in combination with the **food**proof ShortPrep II Kit were tested on two different real-time PCR instruments, the LightCycler 480 System from Roche Diagnostics and the Mx3005P from Agilent/Stratagene. The repeatability study and the inclusivity and exclusivity studies gave the expected results. No deviations occurred all results were within the expected range.

Inclusivity (11)

Strain-No. (internal)	Serotype	Origin/source
4735	O157:H-	unknown
4738	O157:H7	human feces
4946	O157:H7	human
4948	O157	unknown
5579	O157:H7	unknown
5580	O157:H7	unknown
5854	O157:H7	unknown
5855	O157:H-	unknown
7840	O157:H-	unknown
7842	O157:H-	unknown

7844	O157:H-	unknown
7848	O157:H-	unknown
7851	O157:H-	unknown
7852	O157:H-	unknown
7854	O157:H7	unknown
7855	O157:H7	unknown
7875	O157:H-	unknown
8275	O157:H7	unknown
8325	O157:H7	unknown
12503	O157:H-	milk
12507	O157:H-	sausages
12518	O157:H7	minced meat (cattle)
12538	O157:H7	bovine feces
14173	O157:H7	bovine feces
14174 (ATCC 43895)	O157:H7	Hamburger
14175	O157:H7	raw milk
14176	O157:H-	human feces
14177	O157:H16	ground beef
14178	O157:H-	milk
14190	O157:H7	human feces
14200	O157:H7	minced meat (cattle)
14211	O157:H7	minced meat (cattle)
14226	O157:H7	bovine feces
14227	O157:H7	bovine feces
14240	O157:H7	bovine feces
14241	O157:H7	bovine feces
14242	O157:H7	human feces
14243	O157:H7	human feces
14244	O157:H7	human feces
14245	O157:H7	human feces
14246	O157:H7	human feces
14247	O157:H7	human feces
14248	O157:H7	human feces
14249	O157:H7	human feces
Strain-No. (internal)	**Serotype**	**Origin/source**
14250	O157:H7	human feces
14251	O157:H7	human feces
14252	O157:H7	human feces
14253	O157:H7	human feces
14254	O157:H7	human feces
14255	O157:H7	Salami
14256	O157:H7	ground beef
14257	O157:H7	ground beef
14258 (NCTC 12079)	O157:H7	human feces
14259	O157:H-	human feces
14260	O157:H-	sausages
14261	O157:H-	ground beef
14262	O157:H16	ground beef
14263	O157:H-	raw milk
14264	O157:H-	intestine, sheep
14265 (NCTC 12080)	O157:H-	human feces

The **food**proof *E. coli* O157 Detection Kit detected various isolates of *E. coli* of serotype O157 with both real-time PCR instruments. No false negative results occurred.

Exclusivity (11)

No.	Organism	Strain-Nr. (internal)	Strain-Nr. (external)	Strain Origin/source
1	*Citrobacter koseri*	4958	DSM 4595	Throat
2	*Cronobacter sakazakii*	4955	DSM 4485	Child's throat
3	*Enterobacter cloacae* subsp. *cloacae*	15136	DSM 30054	Spinal Fluid
4	*E. coli* O7:H- (STEC)	12509	LM 1126	Meat (lamb)
5	*E. coli* O8:H- (STEC)	12512	LM 1364	Minced meat (cattle)
6	*E. coli* O8:H27 (STEC)	12504	LM 841	beef
7	*E. coli* O17:H- (STEC)	12505	LM 872	Raw milk
8	*E. coli* O22:H- (STEC)	12506	LM 1046	Minced meat (cattle)
9	*E. coli* O22:H8 (STEC)	14206	1608	Minced meat (cattle)
10	*E. coli* O23:H15 (STEC)	12511	LM 1328	Raw milk cheese
11	*E. coli* O26:H- (STEC)	5856	H 2459/96/1	Unknown
12	*E. coli* O26:H11 (STEC)	5853	H 73/96/1	Unknown
13	*E. coli* O26:H11 (STEC)	5858	H 2955/96/1	Unknown
No.	**Organism**	**Strain-Nr. (internal)**	**Strain-Nr. (external)**	**Strain Origin/source**
14	*E. coli* O46:H- (STEC)	12514	LM 1394	Minced meat (cattle)
15	*E. coli* O48:H21 (STEC)	5852	H 509/95	Unknown
16	*E. coli* O55:H- (STEC)	5851	H 774/89	Unknown
17	*E. coli* O84:H21 (STEC)	12508	LM 1119	Meat (lamb)
18	*E. coli* O101:H9 (STEC)	5647	EH VUB 60	Unknown
19	*E. coli* O103:H2 (STEC)	5648	7828/95	Unknown
20	*E. coli* O103:H2 (STEC)	5857	H 2947/96/1	Unknown
21	*E. coli* O104:H12 (STEC)	12515	LM 1398	Minced meat (cattle)
22	*E. coli* O111:H2 (STEC)	5849	H 946/87/1	Unknown
23	*E. coli* O138:H8 (STEC)	12502	LM 680	milk
24	*E. coli* Ont:H- (STEC)	12510	LM 1247	Meat (lamb)
25	*E. coli* Orauh:H23 (STEC)	12513	LM 1389	Minced meat (cattle)
26	*Escherichia vulneris*	5611	DSM 4564	Human wound
27	*Hafnia alvei*	8930	DSM 30163	Human clinical isolate
28	*Klebsiella pneumoniae* subsp. *pneumoniae*	4957	DSM 30102	Water
29	*Salmonella enterica* subsp. *enterica* (Enteritidis)	14151	2627/00	Human stool
30	*Shigella flexneri*	2144	DSM 4782	Unknown

The **food**proof *E. coli* O157 Detection Kit was specific for *E. coli* of serotype O157 on both real- time PCR instruments. No false positive results occurred.

Table 3: Results of the repeatability study with 3 food matrixes tested by PCR and microbiologically according to the FDA-BAM or USDA/FSIS methods (11)

Food	No. of Samples	Inoculation Level (Determination via MPN) cells per 25 gram	Inoculation Level cells per 1 gram	PCR	Cultural Con-firmation	FDA-BAM or USDA/FSIS
Egg salad**	20	1.1	0.04	5	5	6
	20	11.5	0.46	16	16	15
	5	-	-	0	0	0
Bockwurst*	20	0.2	0.007	6	6	6
	20	5.8	0.23	10	10	10
	5	-	-	0	0	0
Apple juice**	20	0.4	0.015	7	7	7
	20	5.8	0.23	14	14	14
	5	-	-	0	0	0

* Food matrix tested according to the USDA/FSIS method

** Food matrixes tested according to the FDA-BAM method

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