Proficiency Testing

Food Microbiology

January 2017

Jonas Ilbäck







Edition Version 1 (2017-04-26)

Editor in chief Hans Lindmark, head of Biology department, National Food Agency

Responsible for the scheme Jonas Ilbäck, microbiologist, Biology department, National Food Agency

PT January 2017 is registered as no. 2016/03619 at the National Food Agency.

Proficiency Testing Microbiology – Food

January 2017



Accred. no. 1457 Proficiency testing ISO/IEC 17043

Quantitative analyses

- Aerobic microorganisms, 30 °C
- Enterobacteriaceae
- Thermotolerant Campylobacter
- Listeria monocytogenes

Qualitative analyses

- Thermotolerant *Campylobacter*
- Listeria monocytogenes
- Salmonella
- Escherichia coli O157
- Pathogenic Vibrio spp.
- Yersinia enterocolitica

National Food Agency, Biology department, Box 622, SE-751 26 Uppsala, Sweden

Abbreviations

Media

ALOA	Agar Listeria according to Ottaviani and Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BPW	Buffered Peptone Water
BA	Blood Agar
BS	Bromthymol blue Saccharose agar
CIN	Cefsulodin Irgasan Novobiocin agar
CT-SMAC	Cefixime-tellurite-sorbitol-MacConkey-agar
ITC	Irgasan Ticarcillin potassium Chlorate broth
mCCDA	Modified Charcoal Cephoperazone Deoxycholate Agar
MKTTn	Muller-Kauffmann tetrathionate/novobiocin broth
MPCA	Milk Plate Count Agar
MRB	Modified Rappaport Broth
MSRV	Modified Semi-solid Rappaport-Vassiliadis enrichment media
mTSB	Modified Tryptone Soya Broth
PCA	Plate Count Agar
PSB	Peptone Sorbitol Bile salts broth
RVS	Rappaport-Vassiliadis Soy peptone broth
SP	Salt Polymyxin broth
SSDC	Salmonella/Shigella Sodium Deoxycholate Calcium chloride agar
TCBS	Thiosulphate Citrate Bile salts Sucrose agar
TSA	Tryptic Soya Agar
TSBY	Tryptone Soya Broth with Yeast extract
XLD	Xylose Lysine Deoxycholate agar
VRBG	Violet Red Bile Glucose agar

Organisations

AFNOR	French National Standardization Association
AOAC	AOAC INTERNATIONAL
ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
SLV/NFA	Livsmedelsverket/National Food Agency, Sweden

Contents

General information on results evaluation	
Results of the PT round January 2017 5	
- General outcome 5	
- Aerobic microorganisms, 30 °C 6	
- Enterobacteriaceae	
- Thermotolerant Campylobacter	
- Listeria monocytogenes	
- Salmonella	
- Escherichia coli O157 14	
- Pathogenic Vibrio spp	
- Yersinia enterocolitica16	
Outcome of the results of individual laboratory – assessment	
- Box plot	
Test material and quality control	
- Test material	
- Quality control of the mixtures	
References	

Annex 1: Results obtained by the participants

Annex 2: z-scores of all participants

General information on results evaluation

Statistical evaluation of the results

Highly deviating values that did not belong to a strictly normal distribution were identified as statistical outliers (Grubbs' test modified by Kelly (1)). In some cases, subjective adjustments were made to set limits, based on knowledge of the mixture's contents. Outliers and false results were not included in the calculations of means and standard deviations. Results reported as "> value" were excluded from the evaluation. Results reported as "< value" were interpreted as being zero (negative result). All reported results are presented in Annex 1.

According to EN ISO/IEC 17043, for which the proficiency testing programme is accredited, it is mandatory for the participating laboratories to report method information for all their analyses. Method information is sometimes difficult to interpret, since many laboratories report a medium that that is not included in the standard method that they refer to. Results from laboratories that report contradictory data on methods/media have either been excluded from the method analysis, or been added to the group of "Others", together with results from methods and media that are only used by 1-2 laboratories.

Mean values and standard deviations are normally provided for the different analyses. When the total number of reported results for an analysis is fewer than 20, the median is provided instead of the mean value. For method groups with fewer than 5 results, only the number of false results and outliers are provided.

Uncertainty of measurement for the assigned values

The uncertainty of measurement for an assigned value is calculated as the standard deviation divided by the square root of the number of correct results ("standard error"). The assigned value of evaluated parameters is the mean value of the participants results.

Table and figure legends

Tables

- N number of laboratories that performed the analysis
- n number of laboratories with satisfactory result
- m mean value in \log_{10} cfu/ml (false results and outliers excluded)
- s standard deviation
- F number of false positive or false negative results
- < number of low outliers
- > number of high outliers
- global results for the analysis
 - values discussed in the text

Figures

Histograms of the analytical results for each mixture are presented. The mean value of the analysis results is indicated in each histogram.

- values within the interval of acceptance (Annex 1)
- outliers
- \Box false negative results
- * values outside of the x-axis scale

Results of the PT round January 2017

General outcome

Samples were sent to 153 laboratories, 33 in Sweden, 102 in other European countries, and 18 outside Europe. Of the 147 laboratories that reported results, 64 (44 %) provided at least one result that received an annotation. In the previous round with similar analyses (January 2016), the proportion was 41 %.

Individual results for each analysis in the PT round are listed in Annex 1 and are also available on the website after logging in: <u>www2.slv.se/absint</u>.

Table 1 *Microorganisms in each mixture and % of deviating results (N: number of reported results, F%: false positive or false negative, X%: outliers).*

		Mix	cture A			Mi	xture B Mixture C 0% 3% 0% 12% 12% 12%						
% participants 0 annotation 1 annotation 2 annotation >2 annotation	s with n n ns ons	26%	6 0%	69%	6	19	6 0%	889	6	12%	% 0%	859	%
Organisms		Aeromonas hydro Campylobacter co Escherichia coli o Listeria monocyto	ophila oli 0157 ogenes	a Bacillus cereus C. Micrococcus sp. Salmonella Enteritidis Presinia enterocolitica 2s Vibrio cholerae Vibrio enterocolitica F% X% Target N F% X%						Campylobacter Proteus mirabil Salmonella Ente Vibrio parahaer	<i>jejuni</i> is eritidis nolyticu	15	
Analysis		Target	N	F%	X%	Target	Ν	F%	Х%	Target	Ν	F%	Х%
Aerob. micro 30 °C	org.	A. hydrophila	129	1	1	<i>Micrococcus</i> sp.	126	0	10	P. mirabilis	127	0	4
Enterobacteri	aceae	E. coli O157 (A. hydrophila)	105	30	0	<i>Y.enterocolitica</i> <i>S.</i> Enteritidis	104	0	4	P. mirabilis	abilis 104 2		9
Thermotol.	Quant.	C coli	11	27	0	_	11	0	0	C jajuni	11	0	0
Camp.	Qual.	0.000	24	4	-		23	4	-	C. jejuni	23	0	-
L. mono-	Quant.	L. mono-	61	2	13		62	0	0		62	2	0
cytogenes	Qual.	cytogenes	95	1	-	-	93	0	-	-	93	0	-
Salmonella		-	117	3	-	S. Enteritidis	117	1	-	S. Enteritidis	117	6	-
E. coli O157		E. coli O157	25	0	-	-	24	0	-	-	24 0		-
Path. Vibrio s	spp.	-	20	10	-	V. cholerae	19	11	-	V. para- haemolyticus	19	11	-
Y. enterocolit	ica	-	13	0	-	Y.enterocolitica	erocolitica 12 0 12 0				0	-	

(*microorganism*): false positive before confirmation

Aerobic microorganisms, 30 °C

Mixture A

A strain of *Aeromonas hydrophila* was present in the highest concentration, and thus most colonies were from this species. The analyses were without problem for the majority of the 129 laboratories, but the results had a rather wide distribution, with a relatively large number of results lower than the main peak. One false negative result was also reported. Statistically, only one low outlier was identified among the low results. Participants should however be aware that the same mixture has been used also in a previous PT round (January 2016). In that earlier PT round, the results were distributed around a distinct peak, and results lower than log_{10} 3.5 were considered outside the accepted interval.

Mixture B

A strain of *Micrococcus* sp. was present in the highest concentration, and thus most colonies were from this species. As a whole, the results were distributed around a distinct peak, but 10 low and 2 high outliers were reported.

Mixture C

A strain of *Proteus mirabilis* was present in the highest concentration, and thus most colonies were from this species. The analysis was without problem for the laboratories, and the results were distributed around a distinct peak. Three low and 2 high outliers were reported.

General remarks

The results were for the most part without problem for the laboratories, with a relatively small number of outliers for mixture B, and occasional outliers for mixtures A and C. The wider range of results reported for mixture A does not have an obvious explanation. Results lower than the main peak are mainly associated with the use of PCA, and the methods NMKL 86 (2006 and 2013) and ISO 4833 (2003 and 2013). These methods are highly similar, and all stipulate incubation for 72 h at 30 °C. In contrast, the incubation time and temperature for $3M^{TM}$ PetrifilmTM Aerobic Count (Petrifilm AC) varies depending on the method, and is for example 48 h at 35 °C according to AOAC® 990.12 and 72 h at 30 °C according to AFNOR 3M 01/1-09/89. Despite this, the results from Petrifilm AC are more clustered compared to those from PCA. Possibly the surface spreading technique used with this substrate is more gentle to the bacteria compared to the pour plate method used in NMKL 86:2013 and ISO 4833-1:2013. A milder treatment of the bacteria could also help explain the higher results for Petrifilm AC, compared to other media, in mixture A.

Media	N	N Mixture A							Μ	ixture	B			Mixture C					
Media	IN	n	m	s	F	<	>	n	m	s	F	<	>	n	m	S	F	<	>
All results	129	127	4.22	0.40	1	1	0	114	4.67	0.12	0	10	2	122	4.23	0.14	0	3	2
PCA	81	80	4.15	0.36	0	1	0	77	4.66	0.12	0	2	1	76	4.20	0.13	0	3	0
Petrifilm AC	20	20	4.53	0.31	0	0	0	14	4.64	0.13	0	4	0	20	4.31	0.10	0	0	0
TSA	10	10	4.36	0.23	0	0	0	9	4.70	0.14	0	0	1	9	4.31	0.21	0	0	1
MPCA	9	8	4.09	0.51	1	0	0	9	4.70	0.09	0	0	0	8	4.29	0.15	0	0	1
Other	9	9	4.20	0.58	0	0	0	5	4.67	0.13	0	4	0	9	4.24	0.10	0	0	0

Results from analysis of aerobic microorganisms at 30 °C.



Enterobacteriaceae

Mixture A

No target organism for the analysis of Enterobacteriaceae was present in the recommended dilutions. Laboratories that analysed the undiluted sample may however have detected *E. coli* O157, which is positive for the analysis of Enterobacteriaceae, and was present at a low concentration ($\log_{10} 0.75$ cells/ml in the undiluted sample). Results corresponding to the concentration of *E. coli* O157 were therefore judged as correct. However, 31 of the 105 reporting laboratories had clear false positive results with concentrations considerably higher than that of *E. coli* O157. Most of the false results corresponded to the concentration of *Aeromonas hydrophila*. The included strain of *A*.

hydrophila grows on violet red bile glucose agar (VRBG) with colonies that can be interpreted as belonging to Enterobacteriaceae. They should however be distinguished from Enterobacteriaceae in subsequent confirmation, since colonies of *A. hydrophila*, in contrast to Enterobacteriaceae, are oxidase positive. Identification of *A. hydrophila* as Enterobacteriaceae may be a consequence of not confirming or with having problems in the confirmation.

Mixture B

Strains of *Yersinia enterocolitica* and *Salmonella* Enteritidis were target organisms for the analysis, which in general was without problem for the laboratories. The results were distributed well, and 4 high outliers were reported.

Mixture C

A strain of *Proteus mirabilis* was target organism for the analysis. The analysis was without problem for the majority of laboratories, however low outliers were reported by 9 laboratories. Two laboratories reported false negative results.

General remarks

The analyses of mixtures B and C were as a whole without problem for the laboratories. As in previous proficiency testing rounds, the majority of the laboratories reported the use of either NMKL 144:2005 or ISO 21528-2:2004. Consequently, most laboratories (77 %) reported VRBG as medium. Among the remaining laboratories 20 % used 3MTM PetrifilmTM Enterobacteriaceae (Petrifilm EB) and 3 % used other media. Regardless of method and media, equivalent results were reported for both mixture B and C.

For mixture C, 9 low outliers were reported. All but one of these results were reported by laboratories that used VRBG and followed NMKL 144:2005 or ISO 21528-2:2004. Enterobacterieaceae are oxidase negative and Gram-negative bacteria. They ferment glucose, with the production of acid by-products. On VRBG they form pink/red colonies, with or without a bile precipitation zone. None of the 9 outliers in mixture C were reported by users of Petrifilm EB, and it is possible that the colour indicator in Petrifilm EB assists in detecting acid by-products of glucose fermentation. In NMKL 144:2005, presumptive colonies from VRBG are confirmed by a negative oxidase test. In ISO 21528-2:2004, presumptive colonies are confirmed both with an oxidase test and with a glucose fermentation test. Low outliers were however reported both by laboratories that performed confirmation tests and those that did not. So far, no plausible explanation for the low outliers and false negative results has been identified. At the National Food Agency, the colonies growing on VRBG were without doubt identified as belonging to Enterobacteriaceae.

Metod	N		Ν	Aixture	Α				Miz	xture B	6			Mixture C					
Metou	14	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	105	74	-	-	31	0	0	100	2.09	0.28	0	0	4	93	4.05	0.32	2	9	0
VRBG	81	64	-	-	17	0	0	77	2.09	0.29	0	0	3	70	4.00	0.33	2	8	0
Petrifilm EB	20	8	-	-	12	0	0	19	2.10	0.23	0	0	1	20	4.16	0.25	0	0	0
Other	4	2	-	-	2	0	0	4	-	-	0	0	0	3	-	-	0	1	0

Results of Enterobacteriaceae analysis



Thermotolerant Campylobacter

Mixture A

A strain of *Campylobacter coli* was target organism. Of the 11 laboratories that performed the quantitative analysis, 3 reported false negative results. In contrast, only 1 of the 24 laboratories in the qualitative analysis reported a false negative result.

Mixture B

No target organism for this analysis was present in mixture B. All laboratories reported correct negative results for the quantitative analysis, whereas 1 false positive result was reported for the qualitative analysis.

Mixture C

A strain of *Campylobacter jejuni* was target organism. The analysis was without problem for the laboratories, and all reported results in both the quantitative and qualitative analysis were without remark.

General remarks

Eleven laboratories performed the quantitative analyses, which makes it difficult to evaluate the results statistically. Of the participants, 6 followed NMKL 119:2007 and 5 followed ISO/TS 10272-2:2006. False results were reported by users of both methods. All laboratories except one reported the use of modified charcoal cephoperazone deoxycholate agar (mCCDA).

The results in the quantitative analysis had a relatively large distribution, something that has been observed in several earlier PT rounds. *Campylobacter* spp., are sensitive to

mechanical stress and to dehydration. Differences in the results might therefore be a consequence of a harsh surface spreading. At the National Food Agency, *Campylobacter* spp., are carefully spread onto the plates and the final drying of the bacterial suspension is done by leaving the lids of the plates slightly open.

Similar to the quantitative analysis, NMKL 119:2007 and ISO 10272-1:2006 were the most widely used methods for the qualitative analysis, and the majority of laboratories used mCCDA. Five laboratories used other methods (*e.g.* PCR-based methods and VIDAS), or other methods in combination with the NMKL and ISO methods. Somewhat more laboratories reported results for the qualitative analysis, but the results are still difficult to evaluate statistically.

The number of laboratories that performed a confirmation test was high; 92 % of the 24 laboratories in the qualitative analysis reported some type of confirmation. *Campylobacter* spp. are Gram-negative, oxidase positive and catalase positive bacteria. They can also be confirmed by their appearance; spiral-shaped rods that display a characteristic darting/rotating movement. Further, *C. jejuni, C. coli* and *C. lari* can be separated by differences in their hydrolysis of hippurate and indoxyl acetate and their sensitivity/resistance to nalidixic acid and cephalothin.

Method	N		Miz	xtur	e A				Μ	ixtu	re B				Miz	tur	e C		
Method	11	n	\mathbf{Med}^*	s	F	<	>	n	m	s	F	<	>	n	\mathbf{Med}^*	s	F	<	٧
All results	11	8	0.93	-	3	0	0	11	-	-	0	-	-	11	2.10	-	0	0	0
NMKL 119	6	4	0.75	-	2	0	0	6	-	-	0	-	-	6	2.09	-	0	0	0
ISO 10272-2	5	4	1.32	-	1	0	0	5	-	-	0	-	-	5	2.10	-	0	0	0

Results of thermotolerant Campylobacter quantitative analysis

* Med = median

Mathad	N	N	lixture	A	N	lixture	B	Ν	lixture	С
Method	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	24	23	Pos	1	23	Neg	1	23	Pos	0
NMKL 119	13	13	Pos	0	13	Neg	0	13	Pos	0
ISO 10272-1	6	5	Pos	1	5	Neg	0	5	Pos	0
Other	5	5	Pos	0	4	Neg	1	5	Pos	0

Results of thermotolerant Campylobacter qualitative analysis





Listeria monocytogenes

Mixture A

A strain of *Listeria monocytogenes* was target organism for the analysis. The quantitative analysis was without problem for the majority of the 62 laboratories; however 7 laboratories reported results that were all clearly lower than the main peak. Such low values for *L. monocytogenes* have been seen in previous PT rounds (January 2016), where they have statistically been determined as outliers. In the current PT round, the 7 values below $\log_{10} 2.0$ constituted a minor peak, clearly separated from the main peak. In these situations, the statistical test used is insensitive to discern these values as outliers. Considered individually, they were however easily determined as outliers. Taken together, values below $\log_{10} 2.0$ were therefore considered to be statistically unlikely, and were regarded as low outliers. The low outliers could not be attributed to the use of a specific method or media. In addition to the low outliers, one high outlier and one false negative result were reported. In the qualitative analysis, one of 95 laboratories reported a false negative result.

Mixture B

No target organism for this analysis was present in mixture B. The analyses were without problem for the laboratories, and all reported results in both the quantitative and qualitative analysis were without remarks.

Mixture C

No target organism for this analysis was present in mixture C. All laboratories that performed the qualitative analysis reported correct negative results. For the quantitative analysis, one laboratory reported a false positive result. The false positive result was possibly incorrectly reported, as it would have been unreasonably high even if *Listeria* sp. had been present in the mixture.

General remarks

With the exception of the low outliers in mixture A, the analyses were without problem for the laboratories. Regardless of method and media, equivalent results were reported.

ISO 11290-1 and ISO 11290-2 were the most used methods for the qualitative and quantitative analyses, respectively. The qualitative method (ISO 11290-1) is based on primary enrichment in half Fraser broth, followed by secondary enrichment in Fraser

broth. Aliquots from both half Fraser and Fraser are plated onto selective agar for Listeria according to Ottaviani and Agosti (ALOA) and also onto a second selective medium chosen by the individual laboratory. Typical colonies of L. monocytogenes are green-blue on ALOA due to β -glucosidase activity, and surrounded by an opaque halo due to hydrolysis of inositol in the media. The halo is sometimes weak or may not be present at all. Confirmation of Listeria spp. is by a positive catalase test and a positive Gram staining result. Confirmation of L. monocytogenes is by β -haemolysis on blood agar (BA), carbohydrate utilization (fermentation of rhamnose but not xylose) and increased and decreased β -haemolysis in the presence of *Staphylococcus aureus* and Rhodococcus equi respectively (CAMP test). In the quantitative method (ISO 11290-2), an initial suspension of the sample is made in buffered peptone water (BPW) or in half Fraser broth, and aliquots from this are transferred to ALOA. Confirmation is carried out essentially as in the qualitative method. The qualitative and quantitative methods utilized in NMKL 136 - the second most used method for the quantitative analysis in this PT round – are similar to the ISO methods. At the National Food Agency, colonies of L. monocytogenes present in mixture A were on ALOA blue-green and surrounded by a distinct opaque zone. In subsequent confirmation, the strain displayed β haemolysis on BA, and fermented rhamnose but not xylose.

New versions of both ISO 11290-1 (detection method) and ISO 11290-2 (enumeration method) are scheduled for publication in 2017. In the revised methods, identification of *Listeria* spp. is included as a mandatory step. The incubation time in Fraser broth will also be shortened from 48 h to 24 h, and confirmation with catalase test and CAMP test will be optional. Gram staining will be optional if the isolation agar allows distinction between pathogenic and non-pathogenic *Listeria* spp.

Method	N		M	ixture A	4			ľ	Μ	lixtu	ire E	3			Μ	ixtu	ire (2	
Method	IN	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	62	52	2.72	0,11	1	7	1	62	-	-	0	-	-	61	-	-	1	-	-
ISO 11290-2	25	22	2.73	0,13	0	2	1	25	-	-	0	-	-	24	-	-	1	-	-
NMKL 136	17	14	2.74	0,10	0	3	0	17	-	-	0	-	-	17	-	-	0	-	-
RAPID' L.mono	14	13	2.69	0,10	0	1	0	14	-	-	0	-	-	14	-	-	0	-	-
Other	6	3	-	-	1	1	0	6	-	-	0	-	-	6	-	-	0	-	-

Results of L. monocytogenes quantitative analysis

Mathad	N	N	lixture	A	N	Aixture	B	N	Aixture	С
Wiethod	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	95	95	Pos	1	93	Neg	0	93	Neg	0
ISO 11290-1	28	28	Pos	0	27	Neg	0	27	Neg	0
RAPID' L.mono	18	18	Pos	0	18	Neg	0	18	Neg	0
NMKL 136	16	16	Pos	0	16	Neg	0	16	Neg	0
VIDAS®	15	15	Pos	0	15	Neg	0	15	Neg	0
PCR	7	7	Pos	0	7	Neg	0	7	Neg	0
Other	11	10	Pos	1	10	Neg	0	10	Neg	0

Results of L. monocytogenes qualitative analysis



Salmonella

Mixture A

No target organism for this analysis was present in mixture A. Of the 117 laboratories that performed the analysis, 3 reported false positive results.

Mixture B

A strain of *Salmonella* Enteritidis was target organism for the analysis. One laboratory reported a false negative result.

Mixture C

The same strain of *Salmonella* Enteritidis as in mixture B was target organism for the analysis in mixture C. Seven laboratories reported false negative results.

General remarks

The analyses were generally without problem, and no differences in results could be attributed to the use of a specific method or medium.

The same strain of *S*. Enteritidis was present in both mixture B and C, and in a similar concentration ($\log_{10} 2.06$ and 2.02 for mixtures B and C, respectively). Despite this, whereas only 1 laboratory reported a false negative result for mixture B, 7 laboratories reported false negative results for mixture C. No obvious explanation to this discrepancy could be found. All 7 laboratories also reported the use of a confirmation test. At the National Food Agency, the strain of *S*. Enteritidis was easily identified and displayed typical colony morphology on XLD and BrillianceTM in both mixtures.

NMKL 71:1999 was the most used method, followed by ISO 6579:2002, VIDAS, and various PCR-based methods. Nineteen laboratories used rare methods (used by 3 laboratories or less), that in the table below are included in the group "Other method", together with laboratories that stated the use of more than one method, and laboratories that used older versions of the NMKL and ISO methods.

NMKL 71:1999 and ISO 6579:2002 are very similar. Both are based on preenrichment in buffered peptone water (BPW), followed by selective enrichment in Rappaport-Vassiliadis soy peptone broth (RVS) and subsequent plating onto selective xylose lysine deoxycholate agar (XLD) and a second selective agar medium chosen by the individual laboratory. ISO 6579:2002 differs from NMKL 71:1999 in that it also includes selective enrichment in Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn). Confirmation is by biochemical (e.g. mannitol, urea) and serological (*Salmonella* poly O- and poly H-antiserum agglutination) verifications. On XLD, typical *Salmonella* colonies are transparent red and have a black center. As a complementary media to XLD, most laboratories in this PT round used a chromogenic media containing the substrates magenta-caprylate and X-glucoside, that detect caprylate esterase and β -glucosidase activity, respectively. On these media (*e.g.* BrillianceTM and COMPASS) colonies of *Salmonella* typically have a magenta/purple colour (caprylate esterase + and β -glucosidase –) that distinguish them from other microorganisms that may grow on the media.

A new ISO method for *Salmonella* is now available; ISO 6579-1:2017. Major changes compared to ISO 6579:2002 include the use of modified semi-solid Rappaport-Vassiliadis enrichment media (MSRV). This is suitable for detection of motile *Salmonella*, and is allowed for all sample types. For confirmation, colonies can be tested directly from the selective media if they are well separated on the plate. In the confirmation step, detection of β -galactosidase and indole test are optional, and a positive result for both O- and H-antigen agglutination is a requirement for a strain to be considered as *Salmonella*.

NMKL 187:2007 was used by 6 laboratories. This method is intended for the detection of motile *Salmonella*, and therefore differs from NMKL 71:1999 in that the selective enrichment broth (RVS) is substituted by MSRV. The method was recently revised, and the new NMKL 187:2016 includes clarifications on the complementary selective agar medium and the MSRV composition, as well as additional paragraphs for faecal samples and materials from primary animal production.

Mathad	N	N	lixture	A	N	lixture	В	Ν	lixture	С
Wiethoa	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	117	114	Neg	3	116	Pos	1	110	Pos	7
NMKL 71:1999	41	40	Neg	1	41	Pos	0	37	Pos	4
ISO 6579:2002	21	21	Neg	0	21	Pos	0	21	Pos	0
VIDAS	15	14	Neg	1	15	Pos	0	15	Pos	0
PCR method	15	15	Neg	0	15	Pos	0	15	Pos	0
NMKL 187:2007	6	6	Neg	0	6	Pos	0	6	Pos	0
Other method	19	18	Neg	1	18	Pos	1	16	Pos	3

Results of Salmonella qualitative analysis

Escherichia coli O157

Mixture A

A strain of *E. coli* O157 was target organism for the analysis in mixture A. All 25 laboratories that performed the analysis correctly reported positive results.

Mixture B

No target organism for this analysis was present in mixture B. All laboratories that performed the analysis correctly reported negative results.

Mixture C

No target organism for this analysis was present in mixture C. All laboratories that performed the analysis correctly reported negative results.

General remarks

Regardless of method and media, all laboratories performing the analysis reported correct results. The majority of laboratories used methods based on isolation of *E. coli* O157 on cefixime tellurite sorbitol MacConkey agar (CT-SMAC). For pre-enrichment, modified tryptone soya broth (mTSB) was the most common medium. The strain of *E. coli* O157 present in mixture A is sorbitol negative, and formed transparent colonies with a dark center on CT-SMAC at the National Food Agency. It should be noted that one laboratory reported the use of ISO 7251:2005, which is a Most Probable Number Method (MPN) for the detection of presumptive *E. coli*, and not suited for the identification of *E. coli* O157.

Mathad	N	N	lixture	A	N	Aixture	B	N	Aixture	С
Wiethod	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	25	25	Pos	0	24	Neg	0	24	Neg	0
ISO 16654:2001	7	7	Pos	0	7	Neg	0	7	Neg	0
NMKL 164:2005	3	3	Pos	0	3	Neg	0	3	Neg	0
EB-SM-5036	4	4	Pos	0	4	Neg	0	4	Neg	0
VIDAS	3	3	Pos	0	3	Neg	0	3	Neg	0
PCR method	3	3	Pos	0	3	Neg	0	3	Neg	0
Other	5	5	Pos	0	4	Neg	0	4	Neg	0

Results of E. coli O157 qualitative analysis

Pathogenic Vibrio spp.

Mixture A

No target organism for this analysis was present in mixture A. Of the 20 laboratories that performed the analysis, 2 reported a false positive result.

Mixture B

A strain of *Vibrio cholerae* was target organism for the analysis. Two false negative results were reported.

Mixture C

A strain of *Vibrio parahaemolyticus* was target organism for the analysis. Two false negative results were reported.

General remarks

Only 20 laboratories performed the analysis, and most used similar methods and media. It is therefore difficult to evaluate differences between methods and media.

The majority of laboratories followed either NMKL 156:1997 or ISO/TS 21872-1:2007. ISO/TS 21872-1:2007 is based on enrichment in alcaline peptone water with 2 % NaCl (APW 2 %), followed by plating onto selective thiosulphate citrate bile salts sucrose (TCBS) agar plates. The procedure in NMKL 156:1997 is similar, but in addition to APW 2% also includes enrichment in salt polymyxin (SP) broth.

At the National Food Agency, *V. cholerae* in mixture B formed typical yellow colonies on TCBS, regardless of whether enrichment was carried out in APW 2% or in SP. Likewise, the strain of *V. parahaemolyticus* in mixture C formed typical blue-green

colonies on TCBS. Upon confirmation, both strains were oxidase positive and sensitive to vibriostaticum O129.

At the National Food Agency, *P. mirabilis* formed atypical small light green colonies on TCBS in the analysis of mixture C. These were oxidase negative, and could thus be distinguished from *V. parahaemolyticus* present on the same plate.

Mathad	N	N	lixture	A	N	lixture	B	N	lixture	С
Ivietnoa	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	20	18	Neg	2	17	Pos	2	17	Pos	2
NMKL 156:1997	11	10	Neg	1	9	Pos	2	9	Pos	2
ISO/TS 21872-1:2007	7	7	Neg	0	6	Pos	0	6	Pos	0
Other	2	1	Neg	1	2	Pos	0	2	Pos	0

Results of pathogenic Vibrio spp. qualitative analysis

Yersinia enterocolitica

Mixture A

No target organism for this analysis was present in mixture A. All 13 laboratories that performed the analysis correctly reported negative results.

Mixture B

A strain of *Yersinia enterocolitica* was target organism for the analysis. All laboratories that performed the analysis correctly reported positive results.

Mixture C

No target organism for this analysis was present in mixture C. All laboratories that performed the analysis correctly reported negative results.

General remarks

The analyses were without problem for the laboratories, and no false results were reported. Most laboratories followed NMKL 117:1996 or ISO 10273:2003. The method in ISO 10273:2003 is based on parallel enrichment in semi-selective peptone sorbitol bile salts broth (PSB) and irgasan ticarcillin potassium chlorate broth (ITC), followed by isolation on cefsulodin irgasan novobiocin agar (CIN) and *Salmonella/Shigella* sodium deoxycholate calcium chloride agar (SSDC) respectively. NMKL 117:1996 differs somewhat, and is based on pre- and cold-enrichment in PSB, as well as selective enrichment in modified Rappaport broth (MRB). Following enrichment, colonies are isolated on CIN, but SSDC may also be used. Presumptive colonies are subcultured on bromthymol blue saccharose agar (BS) and saccharose positive colonies (yellow) are selected for confirmation.

Colonies of *Y. enterocolitica* have a typical appearance on CIN, with a red "bull's eye" center and an outer transparent zone. At the National Food Agency, the strain of *Y. enterocolitica* in mixture B formed typical colonies on both CIN and on BS. The colonies were oxidase negative upon confirmation.

Two laboratories used in-house methods that were based on PCR. One laboratory followed NMKL 163:2013, which is based on growth in semi-selective PSB or in non-selective tryptone soya broth with yeast extract (TSBY), followed by DNA extraction

and real-time PCR detection of the chromosomal virulence-associated *ail* gene in *Y*. *enterocolitica*. Subculturing from the enrichment media onto CIN plates is optional. The method is suitable when high contamination levels are suspected, and use of NMKL 117:1996 or ISO 10273:2003 is recommended for samples with low suspected levels of *Y. enterocolitica*.

A revised version of ISO 10273 is scheduled for publication during early 2017. In the revised method, characteristic *Y. enterocolitica* colonies isolated on CIN can be confirmed either with the traditional biochemical confirmation steps, or with real-time PCR detection of the *ail* gene as in NMKL 163:2013.

Mathad	N	N	lixture	A	N	lixture	B	N	lixture	С
Method	IN	n	+/-	F	n	+/-	F	n	+/-	F
All results	13	13	Neg	0	12	Pos	0	12	Neg	0
ISO 10273:2003	6	6	Neg	0	5	Pos	0	5	Neg	0
NMKL 117:1996	3	3	Neg	0	3	Pos	0	3	Neg	0
NMKL 163:2013	1	1	Neg	0	1	Pos	0	1	Neg	0
Other	3	3	Neg	0	3	Pos	0	3	Neg	0

Results of Y. enterocolitica qualitative analysis

Outcome of the results of individual laboratory - assessment

The reported results of all participating laboratories are listed in Annex 1, together with the minimum and maximum accepted values for each analysis. Results that received a remark (false results and outliers) are highlighted in yellow, with bold font.

When laboratories appear to have mistakenly analysed the wrong mixture, the corresponding results are written in italics.

Z-scores (see below) for individual analyses are shown in Annex 2 and can be used as a tool by laboratories when following up on the results.

The laboratories are not grouped or ranked based on their results. The performance of a laboratory as a whole can only be evaluated from the number of false results and outliers that are listed in Annex 1 and below the box plots.

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (2). Samples for follow-up can be ordered, free of charge via our website: <u>www.livsmedelsverket.se/en/PT-extra.</u>

Z-scores, box plots and deviating results

In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated from all laboratory results for each analysis.

The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates the results of that individual laboratory, with false results excluded, are close to the general mean values calculated for all laboratory results. The range of z-scores is indicated by the size of the box and, for most laboratories, by lines and/or circles above and beneath the box. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

Box plots and numbers of deviating results for each laboratory

- Z-scores are calculated according to the formula: z = (x-m)/s, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories.
- Outliers are included in the figures after being calculated to z-scores in the same way as for other results.
- False results do not generate any z-scores, and are not included in "No. of results".
- Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism generate a *z*-score of 0.
- The laboratory median value is illustrated by a horizontal red line in the box.
- The box includes 50 % of a laboratory's results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box.
- A circle is for technical reasons shown in the plot when a value deviates to certain degree^{*} from the other values. This does not by itself indicate that the value is an outlier.
- *z*-scores >+4 and <-4 are positioned at +4 and -4, respectively, in the plot.
- The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.
- * < [lowest value in the box $-1.5 \times$ (highest value in the box- lowest value in the box)] <u>or</u> > [highest value in the box + 1.5 × (highest value in the box - lowest value in the box)].

















Test material and quality control

Test material

Each laboratory received three manufactured freeze-dried microbial mixtures, designated A-C. The test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (3). Before analysing the samples, the contents of each vial had to be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

Nr:1	N/:	St	train
Mixture	Microorganism	SLV no. ²	Reference ³
A	Aeromonas hydrophila	SLV-454	CCUG 30208
	Campylobacter coli	SLV-271	CCUG 45147
	Escherichia coli O157	SLV-479	SMI 81186
	Listeria monocytogenes	SLV-444	CCUG 69007
В	Bacillus cereus	SLV-516	CCUG 44740
	Micrococcus sp	SLV-055	CCUG 35073
	Salmonella Enteritidis	SLV-436	-
	Vibrio cholerae	SLV-530	CCUG 45388
	Yersinia enterocolitica	SLV-408	CCUG 45643
С	Campylobacter jejuni	SLV-540	Chicken, 2003
	Proteus mirabilis	SLV-374	CCUG 43605
	Salmonella Enteritidis	SLV-436	-
	Vibrio parahaemolyticus	SLV-529	CCUG 38981

Table 2. Microorganisms present in mixtures A-C.

 ¹ The links between the mixtures and the randomised sample numbers are shown in Annex 1.
 ² Internal strain identification no. at the National Food Agency
 ³ Origin or culture collection (CCUG: Culture Collection University of Gothenburg, Sweden ; ATCC: American Type Culture Collection, SMI: Public Health Agency of Sweden)

Quality control of the mixtures

It is essential to have aliquots of homogeneous mixture and equal volume in all vials in order to allow comparison of all freeze-dried samples from one mixture. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the mixtures or on 5 vials if an "old" mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a mixture is approved if, for each analysis, the values obtained for the test of reproducibility (T) and the test "Index of dispersion" between vials (I₂) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and I₂, see references 4 and 5 respectively.)

		\mathbf{A}^{1}			B ²			C ²	
Analysis and method	m	Т	I_2	m	Т	I_2	m	Т	I_2
Aerobic microorganisms 30 °C NMKL method no. 86	4.501	1.28	0.46	4.701	1.34	1.01	4.491	1.61	1.56
Enterobacteriaceae NMKL method no. 144	-	-	-	2.477	1.36	0.71	4.384	1.10	0.55
Thermotolerant campylobacter, quant. NMKL method no. 119	1.456	2.89	0.58	-	-	-	2.948	1.30	1.53
Thermotolerant campylobacter, qual. NMKL method no. 119	Pos	-	-	Neg	-	-	Pos	-	-
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136	2.810	1.10	0.14	-	-	-	-	-	-
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136	Pos	-	-	Neg	-	-	Neg	-	-
Salmonella NMKL method no. 71	Neg	-	-	2.062*	1.24	0.34	2.023*	3.50	0.70
Escherichia coli O157 NMKL method no. 164	0.752	1.00	0.00^{**}	Neg	-	-	Neg	-	-
Pathogenic Vibrio spp. NMKL method no. 156	Neg	-	-	3.098*	1.72	9.17	2.485*	2.85	1.86
Yersinia enterocolitica NMKL method no. 117	Neg	-	-	2.402*	1.39	1.63	Neg	-	-

Table 3. Concentration mean (m), T and I_2 values from the quality control of the mixtures; m is expressed in log_{10} cfu (colony forming units) per ml of sample.

- No target organism and therefore no value

n = 5 vials analysed in duplicate

 2 n = 10 vials analysed in duplicate

* Value based on results from analysis of parallel mixture

** Low value due to a small number of colonies on the plates

References

- 1. Kelly, K. 1990. Outlier detection in collaborative studies. J. Assoc. Off. Anal. Chem. 73:58-64.
- 2. Anonymous, 2015. Protocol, Microbiology. Drinking Water & Food, The National Food Agency, Sweden.
- 3. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. J. Appl. Bacteriol. 74:143-148.
- 4. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.
- Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockefeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A.; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.

Annex 1 Results of the participating laboratories - January 2017

All results are in log₁₀ cfu per ml sample. Results reported as "< value" have been regarded as zero. Results reported as "> value" are exluded from the calculations. A dash indicates the analysis was not performed. Outliers and false results are highlighted and summarized for each analysis at the end of the table

Lab no.	Vial	Aerobic micro- organisms 30 °C C A B C 2 4.22 4.59 4.15 4 0.00 4.60 4.24		Enter	robacteri	iaceae	The Car	ermotole npyloba	erant Incter	Listeria	топосу	togenes	The Car	ermotolei npylobad	rant cter	mo	Listeria nocytoge	enes	s	almonel	la	Escher	richia co (VT-neg)	li 0157	۴	Pathogen Vibrio sp	ic p.	Yersini	ia entero	colitica	Lab no.	
	ABC	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	1
1254	312	4.22	4.59	4.15	<1	1.95	3.97	-	-	-	2.98	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	125
1594	132	4.22	4.69	4.24	<1	2.33	4.27	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	1594
1970	312	4.46	4.68	4	<1	2	1.95	0.9	<1	2.18	2.86	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Neg	Pos	-	-	-	1970
2035	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	203
2050	132	4.16	4.85	4.54	<1	1.9	4.06	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	2050
2058	132	4.18	4.68	3.89	-	-	-	-	-	-	2.65	<1	<1	-	-		Pos	Neg	Neg	-			-	-	-	-			-	-	-	2058
2072	312	4.43	4.66	4.26	<1	2.15	4.23	<1	<1	1.92	2.72	<1	<1	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	2072
2151	213	4.4	3.2	4.2	-	-	-	0	0	1.38	1.78	0	0	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	215
2221	321	3.6	4.61	4.22	<1	2.33	4.25	-	-	-	2.7	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	2221
2324	213	4.28	4.69	4.24	3.75	1.85	3.96	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	neg	neg	-	-	-	-	-	-	2324
2380	213	4.71	4.80	4.41	- 24	-	-	-	-	-	-	-	-	-	-	-	Pos	neg	neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	238
2402	132	4.09	-	4.20	3.4	2.09	3.34	-	-	-	2.54	- 1	1	-	-	-	- Doc	- Nog	- Nog	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	2402
2037	231	3.40	5.05	4.11		2.00	1.0	-	-	-	2.04	<1	<1	-	-	-	FUS	neg	neg	Neg	Pos	Nog		-	-	Neg	- Pos	Pos	-	-	-	203
2704	2 1 3	4 48	4.63	4.07	-1	2 1 1	4 08				27	-1	-1		-		Pos	Nea	Nea	Neg	Pos	Pos			-	ivey	-	-		-	-	270/
2745	$\frac{2}{3}$ 1 2	4 13	4.00	4.2	~1	2.11	4.00	_	_	_	2.51	0	0	_	_	_	Pos	Neg	Neg	Neg	Pos	Pos	_	_	_	_	_	_	_	_	_	274
2764	213	4 89	4 97	4 65	4.3	2 74	4 58	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Nea	Nea	-	-	-	-	-	-	2764
2842	132	-	-	-		-	-	1.6	<1	1.9	-	-	-	Pos	Nea	Pos	Pos	Nea	Nea	-	-	-	Pos	Nea	Neg	Nea	Pos	Pos	Nea	Pos	Nea	2842
2915	231	4.45	4.62	4.11	-	-	-	-	-	-	>2	<1	<1	-	-	-	Pos	Nea	Nea	Nea	Pos	Pos	-	-	-	-	-	-	-	-	-	291
2920	213	4.32	4.69	4.24	0	2.05	4.21	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	2920
2944	123	4.41	4.85	4.26	<1	1.85	3.23	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	2944
3159	132	4.71	4.7	4.35	<1	1.9	4.15	-	-	-	2.77	<2	<2	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	3159
3305	213	4.4	4.8	4.32	<1	2.28	4.54	-	-	-	2.7	0	0	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	330
3457	123	-	-	-	<1	2.33	3.32	-	-	-	2.6	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Neg	-	-	-	3457
3533	132	3.48	4.61	3.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	3533
3587	213	4.29	4.63	4.28	<1	1.93	4.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3587
3595	231	4.42	4.7	4.25	<1	2.3	3.88	-	-	-	2.8	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	3595
3626	312	4.4	4.6	4.2	<1	1.9	4.2	0.9	<1	2.2	2.8	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	3626
3868	231	4.32	4.7	4.28	<1	1.6	4.08	0.6	0	2.48	2.81	0	9	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	3868
3878	312	3.36	2.91	4.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Neg	-	-	-	-	-	-	-	-	-	3878
3925	213	4.28	4.77	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	392
4064	312	2.34	4.59	2.12	<1	1.43	3.94	-	-	-	-	-	-	-	-	-	- Dee	- Nor	- Neg	-	-	-	-	-	-	-	-	-	-	-	-	4064
4100	3 2 1 2 1 2	3.07	4.00	4.Z	< 2 02	1.00	4.11	-	-	-	2.11	<1	<1	-	-	-	POS	neg	neg	Neg	Pos	Pos	- Doc	- Nog	- Nog	-	-	-	-	-	-	4100
41/1	213	4.04	4.07	4.23	3.03	2.30	4.23		-	-	-	-	-	-	-	-	- Pos	- Nog	- Nog	Neg	Pos	Pos	POS	neg	ineg	-	-	-	-	-	-	417
4240	312 312	4.10	4.40	4.10	-1	1 98	3 01					-	-		-		Pos	Neg	Neg	Neg	Pos	Pos			-		-	-		-	-	4240
4339	321		-	50		-	-	_	_	_	3.85	-1	-1	_	_	_	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Nea	Nea	_	_	_	Neg	Pos	Nea	4330
4352	231	4 76	4.38	4.38	4.23	2.08	4 56	-	-	-	2.84	<1	<1	Pos	Nea	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Nea	Pos	Pos	-	-	-	4352
4400	213	4.61	4.86	-	3.63	2.26	3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4400
4560	213	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Nea	Nea	Nea	Pos	Pos	-	-	-	-	-	-	-	-	-	4560
4562	132	4.43	4.41	4.43	<1	2.48	4.2	1.34	<1	2.32	2.76	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Neg	Pos	Neg	4562
4633	321	4.19	4.66	4.21	<1	1.94	4.03	-	-	-	<1	<1	<1	-	-	-	Neg	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	4633
4635	231	4.61	4.6	4.15	<1	4.18	3.15	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	4635
4664	321	4.45	4.81	4.32	0	2.15	4.08	-	-	-	1.68	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Neg	Pos	-	-	-	4664
4683	231	3.74	4.5	4.11	2	3.59	3.85	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	4683
4817	321	3.64	4.59	4.01	-	-	-	-	-	-	2.64	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Neg	Pos	Neg	4817
4840	132	3.11	3.63	3.58	<1	1.77	3.15	-	-	-	2.78	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	4840
4879	213	-	-	-	-		-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	4879
4889	321	4.08	4.65	4.26	0	2.08	4.18	-	-	-	2.57	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	4889
4944	321	3.11	4.72	4.32	<1	2	4.19	-	-	-	2.8	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	4944
4980	231	4.41	4.74	4.33	2.96	1.92	4.34	-	-	-	2.71	<1	<1	- D	- N	- Dc-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	- D	-	4980
5018	213	4.2	4.65	4.13	<1	2.18	2.68	-	-	-	2.65	<1	<1	POS	iveg	POS	POS	iveg	iveg	iveg	POS	POS	1 -	-	-	- 1	-	-	Neg	POS Doc	Neg	5018
5100	213	- 3 31	-	30	-		-		-	-		-	-		-	-		-	-	Nec	- Pos	- Pos		-	-		-	-	Neg	Pos	iveg	5100
5100	212	3.31 4.64	4.43	3.9 4 38			-	1 1	-	-	1 1	-	-	1]	-	-	Pos	- Nec	- Nea	Neg	Pos	Pos	1]	-	-	1]	-	-			-	5129
m	213	4.04	4.668	4 23/	_	2 086	4 044	0 088	0	2 067	2 717	0	0		- nea	-	nos	nea	neg	neg	009	nos		nea	- nea	nea	-	-	nea	-	- nea	m
s		0.397	0.123	0.138	_	0.283	0.316	0.300	0	0.288	0 113	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
<u> </u>		5.001	5	200	1	0.200	0.070	00	v	0.200		v	ÿ																			

Lab no	Vial	Aer orga	obic micr nisms 30	ro-) °C	Enter	robacteri	aceae	The Can	rmotol npylob	erant acter	Listeria	топосу	rtogenes	The Ca	ermotolei mpylobad	rant cter	mo	Listeria	enes	s	almonel	la	Escher	richia col (VT-neg)	li 0157	P V	athogeni ibrio sp	ic p.	Yersin	ia entero	colitica	Lab no.
	ABC	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	A	В	С	Α	В	С	Α	В	С	1
5200	312	3.09	4.8	4.19	<1	2.08	4.09	-	-	-	2.65	<1	<1	-	-	-	Pos	Neg	Neg	Pos	Pos	Pos	-	-	-	-	-	-	-	-	-	5200
5204	213	4.6	-	4.4	4.2	2.3	4.2	<1	<1	2	2.7	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5204
5329	132	3.82	4.65	4.13	<1	1.84	3.93	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	5329
5333	132	-	-	-	-	-		-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5333
5342	213	3.85	4.52	4.22	<1	1.9	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5342
5352	132	4.64	4.74	4.27	<1	1.97	4.20	-	-	-	2.78	<0	<0	- Doc	- Nog	- Poo	Pos	neg	neg	neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5352
5523	213	4 11		-	3 75	1			-		-	-		Pos	ivey	F05	Pos	-		Nea		-	Pos	-	-	Nea	-	-	Neg	-		5523
5545	312	-	-	-	-		-	-	-	-	-	-	-	-	-	-	Pos	Nea	Nea	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5545
5553	132	4.31	4.83	4.28	<1	2	4.18	-	-	-	1.74	<1	<1	Pos	Nea	Pos	Pos	Nea	Nea	Nea	Pos	Pos	Pos	Nea	Nea	-	-	-	-	-	-	5553
5615	213	4.48	4.57	4.28	<1	2.11	3.85	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5615
5632	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5632
5701	132	4.2	4.7	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5701
5801	123	4.09	4.46	4.03	1.3	2.3	4.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5801
5808	123	4.33	4.75	4.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5808
5883	312	4.35	4.51	4.16	<1	2.07	4.03	-	-	-	2.69	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	5883
5950	132	4.45	4.6	4.19	3.79	2.6	4.18	0.3	<1	2	2.76	<1	<1	Pos	neg	Pos	Pos	neg	neg	neg	Pos	Pos	Pos	neg	neg	neg	POS	Pos	neg	Pos	iveg	5950
6100	132	4 34	- 4 81	- 4 46	_	1	-		-	-	-	-	-	-	-	-	1	-	-	Neg	- Pos	- Pos	Pos	- Nea	- Nea	-	-	-	-	-	-	6100
6175	213	4.04	4 75	4.04	<1	17	3 78	_	-	_	_	_	_	_	_	_	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	_	_	_	_	_	-	6175
6180	321	4.4	4.45	4.15	<1	1.95	1.74	-	-	-	2.78	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6180
6224	231	4.86	4.79	4.5	4.14	2.55	4.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6224
6232	132	3.45	4.71	3.26	2.76	1.85	0.48	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6232
6253	312	<1	4.8	4.26	3.64	2.17	4.18	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6253
6343	132	4.23	4.78	4.26	2.4	2	4.22	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6343
6352	213	4.19	4.37	3.97	<1	1.74	3.66	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6352
6368	321	4.45	4.64	4.2	<1	2.51	4.32	-	-	-	2.79	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	6368
6456	321	4.30	4.04	4.34	-	2 1 1	4 17				-	-		-		-	Pos	- Nea	- Nea	Neg	Pos	Pos	1	-	-	-	-	-	-			6456
6594	132	4.3	4.72	4.23	1.6	1.78	3.8	_	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Nea	Nea	-	-	-	-	-	-	6594
6647	321	3.99	4.88	4.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6647
6658	123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6658
6686	231	4.41	4.67	4.32	4.21	4.22	4.2	-	-	-	2.61	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	6686
6720	123	-	-	-	-	-	-	-	-	-	2.3	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	6720
6762	213	4.74	3.42	4.36	4.24	2.41	4.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6762
6071	312	-	-	-	- 4 25	-	-	-	-	-	-	-	-	-	-	-	Pos	neg	neg	-	-	-	-	-	-	-	-	-	-	-	-	6071
7182	1 2 3 2 1 3	4.59	4.1	4.10	4.25	2.0	3.90 4.27		-		-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-		-	7182
7191	321	3.7	4.8	4.04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Nea	-	-	-	Pos	Pos	Nea	-	-	-	7191
7232	312	4.28	4.73	4.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7232
7242	231	4.3	4.1	4.45	0	3.77	2.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7242
7248	132	4.56	4.66	4.23	<1	1.9	3.28	-	-	-	2.84	<1	<1	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7248
7253	312	-	-	-	-	-	-	-	-	-	2.71	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7253
7282	312	4.16	4.65	4.2	<1	2.18	4.22	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7282
7302	132	-	-	-	-	-	-	-	-	-	-	-	-	Pos	POS	Pos	-	-	-	Neg	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	7302
7334	1 2 3 2 1 3	4.1	4.40	4.5	<1	2.19	4.12	-			-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	- Nea	- Nea	-	-	-	-		-	7334
7627	$\frac{2}{3}21$	4.2	5.33	4.17		1	-	-	-		_	-	-	_	-	-		-	-	Neg	Pos	Neg	Pos	Neg	Neg	_	-	-	_	-	-	7627
7688	321	4.32	4.61	4.2	<1	2.04	4.23	-	-	-	2.78	<1	<1	Pos	Nea	Pos	Pos	Nea	Nea	Neg	Pos	Pos	Pos	Nea	Neg	-	-	-	Nea	Pos	Nea	7688
7728	213	4.15	4.72	4.19	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7728
7750	213	4.26	4.48	4.15	3.49	2.04	1.95	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	7750
7825	132	4.77	4.81	4.36	3.52	2.88	4.21	-	-	-	2.77	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7825
7876	123	4	4.7	4.3	<1	2.1	3.7	-	-	-	2.83	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7876
7882	123	4.4	4.49	4.36	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7882
7930	312	4.44	4.69	4.25	<1	2.24	4	-	-	-	2.8	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	7930
7940	312	4.39	4.03 1 77	4.79	-1	2.26	- 4 28	1	-	-	2 76	-	-	1	-	-	Pos	- Nea	- Nea	Nea	- Pos	- Pos	1]	-	-		-	-	1	-	-	7940
7968	231	4.37	4,73	4.22	<1	1.78	4.11		-	-	2.85	<1	ں 1	Pos	Nea	Pos	Pos	Neg	Nea	Neg	Pos	Pos	1 -	-	-	- I	-	-	_	-	-	7968
8042	213	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8042
8066	312	-	-	-	-	-	-	-	-	-	2.64	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8066
m		4.224	4.668	4.234	-	2.086	4.044	0.988	0	2.067	2.717	0	0	pos	neg	pos	pos	neg	neg	neg	pos	pos	pos	neg	neg	neg	pos	pos	neg	pos	neg	m
S		0.397	0.123	0.138	-	0.283	0.316	0.420	0	0.288	0.113	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S

Lab no	Vial	Aer orga	robic mic anisms 30	ro- D°C	Enter	robacteri	iaceae	The <i>Can</i>	rmotolei 1pylobad	rant c <i>ter</i>	Listeria	monocy	togenes	The Ca	ermotolei mpylobad	ant ter	то	Listeria locytoge	enes	s	almonel	la	Eschei	richia col (VT-neg)	i 0157	P V	athogen /ibrio sp	nic op.	Yersini	a entero	colitica	Lab no.
	АВС	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	1
8068	132	4.04	4.66	4.3	0	1.85	0	-	-	-	2.76	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8068
8165	231	-	-	-	<1	1.84	4.08	0.96	<1	2.25	-	-	-	-	-	-	Pos	-	-	-	Pos	Pos	Pos	Neg	Neg	-	-	-	Neg	Pos	Neg	8165
8252	132	4.77	4.61	4.38	-1	1.95	4.32	-	-	-	1.86	-1	-1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8252
8313	312	4.1	4.55	4 28	<1	2.32	4 15	_	-	-	2.69	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-		-	-	8313
8333	231	4.23	4.72	4.51	3.36	2	<1	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	8333
8397	321	4.49	3.3	4.45	3.78	2.2	4.15	-	-	-	1.84	<1	<1	-	-	-	Pos	Neg	Neg	-	-	-	-	-	-	-	-	-	-	-	-	8397
8435	213	4.29	4.84	4.25	<1	1.7	3.96	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8435
8529	213	4.49	4.74	4.26	<1	2.17	4.3	-	-	-	2.83	<0	<0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos		-	-	-	-	-	-	-	-	8529
8568	321	4.46	4.67	4.28	4.13	2.32	4	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	-	-	-	-	-	-	8568
8620	213	4.59	4.79	4.32	0	1.95	1.78	-	-	-	1.3	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	- Nog	- Boo	- Poo	-	-	-	8626
8657	321	4.44	4.01	4.30	-	-	4.11	-	-	-	2.12	-	-	-	-	-	-	-	-	neg	-	-	-	-	-	-	FUS -	-	-	-	-	8657
8734	321	4.65	4.18	4.23	4.11	2,18	4.13	-	-	-	-	-		-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8734
8742	321	4.18	4.73	4.26	3.75	2	4.08	-	-	-	2.77	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8742
8756	213	4.55	4.2	4.21	<1	2.48	4.26	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8756
8766	213	4.5	5.5	4.4	<1	1.9	4	-	-	-	2.6	0	0	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	Neg	Pos	Neg	8766
8862	213	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	8862
8918	312	4.48	4.54	4.33	<1	2.15	4.04	-	-	-	2.59	<0	<0	- Doc	- Nog	- Poo	Pos	Neg	Neg	- Nog	- Boo	- Doc	- Boo	- Nog	- Nog	- Nog	- Boo	- Poo	- Nog	- Boo	- Nog	8918
9002	$\frac{123}{312}$	4.34	4.74	4.25	0	1 99	4.27	-	-	-	1.83	0	0	F05	neg	-	Pos	Neg	Neg	Neg	Pos	Pos	F05	ivey	iveg	-	-	-	iveg	-	-	9002
9034	123	4.4	4.6	4.3	<1	2	4	1.3	0	2.1	-	-	-	Pos	Nea	Pos	Pos	Nea	Nea	Neg	Pos	Pos	-	-	-	-	-	-	Nea	Pos	Nea	9034
9051	321	3.85	4.79	4.2	0	1.95	4.23	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	9051
9078	321	2.85	4.83	4.6	3.01	2	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078
9086	123	4.27	4.64	4.14	-		-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	-	-	-	-	-	-	-	-	-	9086
9217	132	4.5	3.2	4.2	3.3	2.2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9217
9429	321	4.23	4.65	4.15	<1	1.88	3.9	-	-	-	-	-	-	- Boo	- Nog	- Poo	Pos	Neg	Neg	Neg	Pos	Pos	- Boo	- Nog	- Nog	Bos	- Boo	- Poo	-	-	-	9429
9441	1231	3.52	4.0	4.2	<1	2	4 87	_	-	-	2.03	<1	~1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-		-	-		-	-	9441
9453	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9453
9512	321	3.3	4.47	4.15	2.78	1.87	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9512
9555	321	4.14	4.68	4.05	<1	2.05	3.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9555
9662	231	4.4	4.67	4.23	4	2.95	4.15	-	-	-	2.69	<1	<1	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	-		-	-	-	-	9662
9716	132	3.87	4.87	4.25	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	Neg	Pos	Pos	-	-	-	Neg	Pos	Pos	-	-	-	9716
9/4/	$3 \angle 1$ 2 1 3	3.71	4.52	3.83	4 04	- 1 78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9/4/
9903	$\frac{2}{3}12$	4.45	4.70	4.11	<1	1.70	3.9	_	-	-	_	-	-	-	-	-	Pos	Nea	Nea	Neg	Pos	Nea	-	-	-	-	-	-		-	-	9903
9950	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950
																																-
N		129	126	127	105	104	104	11	11	11	61	62	62	24	23	23	95	93	93	117	117	117	25	24	24	20	19	19	13	12	12	N
Min		0	2.91	2.12	0	1.4	0	1 60	0	1.38	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Min
Med		4.89	5.50	4.79	4.3	4.22	4.87	1.60	0	2.48	3.85	0	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Med
m		4.224	4.668	4.234	0.066	2.015	4.044	0.988	0	2.067	2.717	0	0	DOS	nea	DOS	DOS	nea	nea	nea	DOS	DOS	DOS	nea	nea	nea	DOS	DOS	nea	DOS	nea	m
s		0.397	0.123	0.138	0.285	0.283	0.316	0.420	Ő	0.288	0.113	Ő	Ő	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s
F+		0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	3	0	0	0	0	0	2	0	0	0	0	0	F+
F-		1	0	0	0	0	2	3	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	2	2	0	0	0	F-
			2	3	31	4	9	0	0	0	1	0	0		-	-		-	-		-	-		-	-	1	-			-	-	\leq
< OK		2.85	4.37	3.83	0	1.40	3.11	0.30	õ	1.38	2.30	õ	õ	_	_	_	_	_	-	_	-	-	-	-	-	-	-	_	-	-	-	< 0K
> 0K		4.89	5.05	4.65	1.60	3.04	4.87	1.60	Ő	2.48	2.98	Ő	Õ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	> 0K

N = number of analyses performed Min = lowest reported result

Max = highest reported result m = Median = median value s = s

m = mean value s = standard deviation

F+ = false positive < = low outlier F- = false negative > = high outlier

< OK = lowest accepted value
 >OK = highest accepted value

High outliers should be regarded as false positive results

Annex 2 Z-scores of all participants - January 2017

Z-scores are calculated according to the formula: z = (x-m)/s, where x = result of the individual laboratory, m = mean of the results of all participating laboratories, s = standard deviation of the results from all participating laboratories. Correct negative results in quantitative analyses and correct results in qualitative analyses have obtained a z-score of zero. False results did not generate a z-score. Z-scores from outliers are not real z-scores, but are a practical means to express the results from the outliers. Very low and high z-scores are here limited to -4 and +4 respectively.

	2	<	z	≤3	8,		Z	>3
--	---	---	---	----	----	--	---	----

Lab no.	Vial	Ae org	robic mic anisms 3	cro- 0 °C	Enter	obacteria	aceae	The <i>Car</i>	ermotoler npylobad	ant ster	Listeria	топосу	togenes	Ther Cam	motole pyloba	rant cter	L mono	.isteria ocytogo	enes	Sa	lmone	lla	Esch 015	erichia 7 (VT-ı	<i>coli</i> neg)	Pa Vit	thoge brio s	nic op.	Y ente	'ersinia Procoli	a tica	Lab no.
	ABC	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	
1254	312	-0.011	-0.629	-0.610	-0.232	-0.482	-0.236				2.336	0.000	0.000				0	0	0	0	0	0										1254
1594	1 3 2	-0.011	0.183	0.041	-0.232	0.862	0.714													0	0	0	0	0	0							1594
1970	312	0.594	0.101	-1.694	-0.232	-0.305	-4.000	-0.209	0.000	0.394	1.269	0.000	0.000	0	0	0	0	0	0	0	0	0				0		0				1970
2035	132	0.460	1 404	2 200	0 000	0.650	0.040													0	0	0										2035
2050	1 2 2	-0.102	0.401	2.209	-0.232	-0.659	0.049				0.507	0.000	0.000				0	0	0	0	0	0										2050
2038	3 1 2	0.112	-0.061	0 185	-0 232	0 226	0 587		0 000	-0 508	0.025	0.000	0.000		0	0	0	0	0	0	0	0				0	0	0				2038
2151	2 1 3	0.443	-4.000	-0 249	0.202	0.220	0.007		0.000	-2.381	-4.000	0.000	0.000	0	Ő	õ	õ	õ	õ	Ő	0	0				Ŭ	Ŭ	Ŭ				2151
2221	3 2 1	-1.573	-0.467	-0.104	-0.232	0.862	0.650		0.000		-0.153	0.000	0.000	Ũ	Ũ	Ũ	Õ	Õ	õ	Ő	Õ	Õ										2221
2324	2 1 3	0.141	0.183	0.041	4.000	-0.836	-0.267										-			0	0	0	0	0	0							2324
2386	2 1 3	1.224	1.563	1.270													0	0	0	0	0	0										2386
2402	1 3 2	1.174		0.185	4.000	-0.659	-2.229													0	0	0										2402
2637	3 1 2	-1.951	-1.279	-0.899	3.278	-0.022	-4.000				-1.575	0.000	0.000				0	0	0	0	0	0										2637
2670	231	-2.052	3.105	-1.188													_		_	0	0					0	0	0				2670
2704	2 1 3	0.645	-0.305	-0.249	-0.232	0.084	0.113				-0.153	0.000	0.000				0	0	0	0	0	0										2704
2745	312	-0.238	0.832	-1.694	-0.232	1.358	-0.077				-1.842	0.000	0.000				0	0	0	0	0	0	0	0	0							2745
2/04	2 1 3	1.070	2.430	3.004	4.000	2.313	1.095	1 /56	0.000	-0 578				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2/04
2042	231	0 569	-0 386	-0 800				1.450	0.000	-0.570		0 000	0 000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2042
2920	2 1 3	0.303	0.000	0.000	-0 232	-0 128	0 524					0.000	0.000				0	0	0	0	0	0										2920
2944	1 2 3	0.468	1.481	0.185	-0.232	-0.836	-2.577										0	0	0	Ő	Õ	Õ				0	0	0				2944
3159	1 3 2	1.224	0.264	0.836	-0.232	-0.659	0.334				0.469	0.000	0.000				0	0	0	0	0	0				-		-				3159
3305	2 1 3	0.443	1.076	0.619	-0.232	0.686	1.568				-0.153	0.000	0.000	0	0	0	0	0	0		0	0	0	0	0							3305
3457	123				-0.232	0.862	-2.292				-1.042	0.000	0.000				0	0	0	0	0	0				0	0					3457
3533	132	-1.876	-0.467	-1.984																0	0	0				0	0	0				3533
3587	2 1 3	0.166	-0.305	0.330	-0.232	-0.553	0.113										_	_	_	_	_	_										3587
3595	231	0.493	0.264	0.113	-0.232	0.756	-0.520	0.000	0 000	0.400	0.736	0.000	0.000	~	~	~	0	0	0	0	0	0										3595
3626	312	0.443	-0.548	-0.249	-0.232	-0.659	0.492	-0.209	0.000	0.463	0.736	0.000	0.000	0	0	0	0	0	0	0	0	0										3020
3000	231	0.241	-4 000	-0.500	-0.232	-1.720	0.115	-0.922	0.000	1.434	0.625	0.000		0	0	0	0	0	0	0	0	0										3000
3925	213	0 141	0.832	-0.971																0	0	0										3925
4064	3 1 2	-4.000	-0.629	-4.000	-0.232	-2.321	-0.331													Ŭ	0	Ŭ										4064
4100	321	-1.397	-0.061	-0.249	-0.232	-0.836	0.207				0.469	0.000	0.000				0	0	0	0	0	0										4100
4171	213	0.796	0.020	-0.032	4.000	0.969	0.587													0	0	0	0	0	0							4171
4246	312	-0.162	-1.685	-0.538	-0.232	-0.305	0.081										0	0	0	0	0	0										4246
4288	312	0.292	0.426	0.908	-0.232	-0.376	-0.425										0	0	0	0	0	0										4288
4339	321										4.000	0.000	0.000		_	_	0	0	0	0	0	0	0	0	0	_	_	_	0	0	0	4339
4352	231	1.350	-2.334	1.053	4.000	-0.022	1.631				1.092	0.000	0.000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				4352
4400	213	0.972	1.563		4.000	0.615	-2.356										0	0	0	_	0	0										4400
4560	213	0.522	2 050	1 1 2 4	0.222	1 202	0 505	0.042	0.000	0 007	0 /11	0.000	0.000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4560
4002	321	-0.022	-2.030	-0 176	-0.232	-0.517	-0.046	0.043	0.000	0.007	0.411	0.000	0.000	0	U	U	U	0	0	0	0	0	U	0	U	0	U	U	0	U	U	4502
4635	231	0.000	-0.548	-0.610	-0.232	4.000	-2.830					0.000	0.000				0	0	0	0	0	0										4635
4664	3 2 1	0.569	1.157	0.619	-0.232	0.226	0.113				-4.000	0.000	0.000				õ	õ	ŏ	ŏ	ŏ	ŏ				0		0				4664
4683	231	-1.220	-1.360	-0.899	4.000	4.000	-0.615										0	0	0	0	0	0				-		-				4683

Lab no.	Vial	Ae orga	obic micr misms 30	o- ℃	Enter	obacteria	iceae	The Car	rmotole npyloba	rant cter	Listeria	топосу	togenes	Theri Cam	motole pyloba	rant cter	L mono	isteria ocytoge	enes	Sal	monella		Esche 0157	erichia 7 (VT-r	<i>coli</i> neg)	Pa <i>Vil</i>	thogei brio sp	nic op.	۲ ente	′ersini erocoli	a itica	Lab no.
	АВС	Α	В	C A B C A				В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С		
4817 4840 4879	321 132 213	-1.472 -2.808	-0.629 ·	1.622	-0.232	-1.119	-2.830				-0.686 0.558	0.000 0.000	0.000 0.000	0	0	0	0 0	0 0	0 0	0 0	0 0	0	0	0	0	0	0	0	0	0	0	4817 4840 4879
4889 4944 4980 5018	3 2 1 3 2 1 2 3 1 2 1 3	-0.366 -2.808 0.468 -0.061	-0.118 0.426 0.588 -0.142	0.149 0.619 0.691 -0.755	-0.232 -0.232 4.000 -0.232	-0.025 -0.305 -0.588 0.332	0.416 0.461 0.935 -4.000				-1.326 0.736 -0.064 -0.597	0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000	0	0	0	0 0 0 0	0 0 0 0	0 0 0	0 0 0 0	0 0 0 0	0 0 0 0				0	0	0	0	0	0	4889 4944 4980 5018
5028 5100 5128 5200 5204	2 1 3 1 3 2 2 1 3 3 1 2 2 1 3	-2.304 1.048 -2.864 0.947	-1.928 - -0.223 1.100 -	- 2.417 1.053 -0.335 1.197	-0.232 4.000	-0.025 0.756	0.128 0.492		0.000	-0.231	-0.571 -0.153	0.000 0.000	0.000 0.000	0	0	0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0 0	0 0 0 0							0	0	0	5028 5100 5128 5200 5204
5329 5333 5342	132 132 213	-1.019	-0.142	0.755	-0.232	-0.871	-0.362										0	0	0	0	0	0										5329 5333 5342
5352 5447	132	1.048	0.588	0.258	-0.232	-0.411	0.682				0.558	0.000	0.000	0	0	0	0	0	0	0	0	0										5352 5447
5523 5545 5553	3 1 2 3 1 2 1 3 2	-0.288 0.216	1.319	0.330	4.000 -0.232	-0.305	0.429				-4.000	0.000	0.000	0	0	0	0 0 0	0 0	0 0	0 0 0	0 0	0 0	0 0	0	0	0			0			5523 5545 5553
5615 5632 5701 5801	2 1 3 1 3 2 1 3 2 1 2 3	0.645 -0.061	-0.792 0.264	0.330	-0.232	0.084	-0.615										0 0	0 0	0 0	0 0	0 0	0 0										5615 5632 5701 5801
5808 5883 5950	1 2 3 3 1 2 1 3 2	0.267 0.317 0.569	0.670 -1.279 -0.548	0.330 -0.538 -0.321	-0.232	-0.057 1.818	-0.046 0.429	-1.636	0.000	-0.231	-0.242 0.380	0.000 0.000	0.000 0.000	0	0	0	0 0	0 0	0 0	0 0 0	0 0 0	0 0 0	0	0	0	0	0	0	0	0	0	5808 5883 5950
5993 6109 6175 6180	1 2 3 1 3 2 2 1 3 3 2 1	0.292 -0.364 0.438	1.157 0.670 -1.790	1.631 •1.405 •0.639	-0.232 -0.232	-1.366 -0.468	-0.837 -4.000				0.540	0.000	0.000				0 0	0 0	0 0	0 0 0	0 0 0	0 0 0	0	0	0							5993 6109 6175 6180
6224 6232 6253	231 132 312	1.602 -1.959	0.994 0.321 1.076	1.920 • 4.000 0.185	4.000 4.000 4.000	1.641 -0.853 0.297	1.663 -4.000 0.429										0	0	0	0 0	0 0	0 0										6224 6232 6253
6352 6368 6443	2 1 3 3 2 1 3 1 2	-0.086 0.562 0.342	-0.199 -0.223	-1.911 -0.220 0.764	-0.232 -0.232	-1.225 1.481	-1.216 0.878				0.603	0.000	0.000				0	0	0	0 0 0	0 0 0	0 0 0 0				0	0	0				6352 6368 6443
6456 6594 6647	321 132 321	-0.011 0.191 -0.590	-0.629 0.426 1.725	0.474 -0.032 -0.610	-0.232 4.000	0.098 -1.083	0.397 -0.774										0	0	0	0 0	0 0	0 0	0	0	0							6456 6594 6647
6686 6720 6762	1 2 3 2 3 1 1 2 3 2 1 3	0.468 1.300	0.020 -4.000	0.619 0.908	4.000 4.000	4.000 1.145	0.492 0.587				-0.953 -3.708	0.000 0.000	0.000 0.000				0 0	0 0	0 0	0 0	0 0	0										6686 6720 6762
6870 6971 7182	3 1 2 1 2 3 2 1 3	0.922	-4.000 -0.873	-0.538 0.619	4.000 4.000	1.818 -0.199	-0.267 0.714										0	0	0		0						0					6870 6971 7182
7191 7232 7242 7248	321 312 231 132	-1.321 0.141 0.191 0.846	1.076 0.507 -4.000 -0.061	-1.405 0.980 1.559 -0.032	-0.232	4.000	-4.000 -2.419				1 092	0.000	0 000	0	0	0	0	0	0	0	0	0					υ					7191 7232 7242 7248
7253	3 1 2 3 1 2	-0.162	-0.142	-0.249	-0.232	0.332	0.556				-0.064	0.000	0.000	5	5	5	0	0 0	0	0	0 0	0 0										7253

Lab no.	Vial	Aer orga	obic mic Inisms 30	ro- D °C	Enter	obacteria	aceae	The Car	rmotole npyloba	rant cter	Listeria	топосу	togenes	Ther Cam	motole <i>pyloba</i>	rant cter	L mono	.isteria ocytog	enes	Sa	lmonell	a	Esche 0157	erichia 7 (VT-r	<i>coli</i> neg)	Pa Vil	thoge brio s	nic op.	۱ ente	'ersini erocoli	a tica	Lab no.
	АВС	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	
7302 7330 7334 7627 7688	1 3 2 1 2 3 2 1 3 3 2 1 3 2 1	-0.313 -0.061 -0.086 0.241	-1.685 -0.386 4.000 -0.467	0.474 -0.465 4.000 -0.249	-0.232	0.367 -0.163	0.239 0.587				0.558	0.000	0.000	0	0	0	0	0	0	0 0 0 0	0 0 0 0	0 0 0	0 0 0 0	0 0 0 0	0 0 0 0				0	0	0	7302 7330 7334 7627 7688
7728 7750 7825 7876 7882 7930	2 1 3 2 1 3 1 3 2 1 2 3 1 2 3 3 1 2	-0.187 0.090 1.368 -0.565 0.443 0.544	0.426 -1.522 1.173 0.264 -1.441 0.183	-0.321 -0.610 0.901 0.474 0.908 0.113	4.000 4.000 -0.232	-0.163 2.815 0.049 0.544	-4.000 0.527 -1.090 -0.141				0.425 1.003 0.736	0.000 0.000 0.000	0.000 0.000 0.000	0	0	0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0	0	0							7728 7750 7825 7876 7882 7930
7940 7962 7968 8042 8066	3 1 2 3 1 2 2 3 1 2 1 3 3 1 2	0.418 0.267 0.367	-0.305 0.840 0.507	4.000 0.417 -0.104	-0.232 -0.232	0.597 -1.083	0.739 0.207				0.345 1.180 -0.686	0.000 0.000 0.000	0.000 0.000 0.000	0	0	0	0 0 0	0 0 0	0 0 0	0 0 0 0	0 0 0 0	0 0 0 0										7940 7962 7968 8042 8066
8068 8165 8252 8260 8313 8333	1 3 2 2 3 1 1 3 2 1 3 2 3 1 2 2 3 1	-0.464 1.376 -0.313 0.317 0.015	-0.061 -0.467 -1.116 -0.223 0.426	0.474 1.053 -2.128 0.330 1 992	-0.232 -0.232 3.278 -0.232 -0.232 4 000	-0.836 -0.871 -0.482 -2.427 0.827 -0.305	0.113 0.872 -4.000 0.334	-0.066	0.000	0.636	0.380 -4.000 -0.242	0.000 0.000 0.000	0.000 0.000 0.000				0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0 0	0 0 0 0	0 0 0 0 0	0	0	0				0	0	0	8068 8165 8252 8260 8313 8333
8397 8435 8529 8568 8626	3 2 1 2 1 3 2 1 3 3 2 1 3 2 1 2 1 3	0.670 0.156 0.670 0.594 0.922	-4.000 1.392 0.588 0.020 0.994	1.559 0.098 0.185 0.330 0.619	4.000 -0.232 -0.232 4.000 -0.232	0.403 -1.370 0.297 0.827 -0.482	0.334 -0.270 0.809 -0.141 -4.000				-4.000 1.003 -4.000	0.000 0.000 0.000	0.000 0.000 0.000				0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0	0	0							8397 8435 8529 8568 8626
8628 8657 8734 8742 8756	1 2 3 3 2 1 3 2 1 3 2 1 2 1 3 2 1 3	0.533 1.073 -0.112 0.821	-0.444 -3.958 0.507 -3.795	1.054 -0.032 0.185 -0.176	-0.232 4.000 4.000 -0.232	-0.305 0.332 -0.305 1.393	0.201 0.271 0.113 0.682				-0.011 0.469	0.000	0.000				0	0	0	0 0 0 0	0 0 0	0				0	0	0	0	0	0	8628 8657 8734 8742 8756
8862 8918 8955 9002	2 1 3 2 1 3 3 1 2 1 2 3 3 1 2	0.645 0.292 0.972	-1.035 0.588 -0.629	0.691 0.113 0.619	-0.232 -0.232 -0.232	0.226 -0.305 -0.340	-0.014 0.714 0.745				-1.042 -1.131 0.736 -4.000	0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000	0	0	0	0 0 0	0 0 0	0 0 0	0	0	0	0	0	0	0	0	0	0	0	0	8862 8918 8955 9002
9034 9051 9078 9086 9217	1 2 3 3 2 1 3 2 1 1 2 3 1 3 2	0.443 -0.943 -3.464 0.103 0.695	-0.548 0.994 1.319 -0.191 -4.000	0.474 -0.249 2.643 -0.704 -0.249	-0.232 -0.232 4.000 4.000	-0.305 -0.482 -0.305 0.403	-0.141 0.587 -1.090 -0.141	0.743	0.000	0.116				0	U	U	0	0	0	0	0	0							0	U	U	9034 9051 9078 9086 9217
9429 9436 9441 9453 9512	3 2 1 2 3 1 1 2 3 3 2 1 3 2 1	0.015 0.015 -1.775 -2.329	-0.142 -0.548 -0.548 -1.604	-0.610 -0.249 -0.393 -0.610	-0.232 -0.232 -0.232 4.000	-0.729 3.374 -0.305 -0.765	-0.457 -2.957 2.612 0.176				-0.597 -1.842	0.000 0.000	0.000 0.000	0	0	0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0	0	0		0	0				9429 9436 9441 9453 9512
9555 9662 9716 9747 9890	3 2 1 2 3 1 1 3 2 3 2 1 2 1 3	-0.212 0.443 -0.893 -1.296 0.569	0.101 0.020 1.644 -1.198 0.751	-1.333 -0.032 0.113 -2.923 -0.899	-0.232 4.000 4.000	-0.128 3.056 -1.083	-0.932 0.334 0.492				-0.242	0.000	0.000				0 0	0 0	0 0	0 0	0 0	0 0				0	0	0				9555 9662 9716 9747 9890
9903 9950	3 1 2 3 2 1	-0.061	-1.360	-0.249	-0.232	-0.659	-0.457										0	0	0	0	0											9903 9950

Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most laboratories carry out some form of internal quality assurance, but their analytical work also has to be evaluated by an independent party. Such external quality control of laboratory competence is commonly required by accreditation bodies and can be done by taking part in proficiency testing (PT).

In a proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The organiser evaluates the results and compiles them in a report.

The National Food Agency's PT program offers

- > External and independent evaluation of laboratories analytical competence.
- > Improved knowledge of analytical methods with respect to various types of organisms.
- ➢ Expert support.
- > Tool for inspections regarding accreditation.
- ➢ Free extra material for follow-up analyses.

For more information visit our website: www2.slv.se/absint

The National Food Agency's reference material

As a complement to the proficiency testing, but without specific accreditation, the National Food Agency also manufactures a number of reference materials (RM) for internal quality control of food and drinking water microbiological analyses, including pathogens.

More information is available on our website: www.livsmedelsverket.se/en/RM-micro