



European Union Reference Laboratory (EU-RL) and Health Protection Agency (HPA) EQA Shellfish Scheme

Escherichia coli and *Salmonella* spp. EQA

EU-RL ring trial reference number: RT 44

Sample distributions: SF038, SF039 and SF040

Contents	Page number
Samples	2
Results	2
Distribution results SF038 to SF040	3
Performance assessment	9
Summary	10
References	10
Charts	11
Appendices	16

Article 32 of Regulation (EC) 882/2004 sets out the organisational responsibilities for EU Reference Laboratories (EU-RL) with respect to comparative (proficiency) testing (PT).

This PT scheme is intended to provide comparative testing samples for laboratories undertaking examination of live bivalve molluscs from production areas in accordance with Regulation (EC) No. 854/2004 and products placed on the market in accordance with Regulation (EC) No. 2073/2005.

The scheme is organised in collaboration with the Health Protection Agency (HPA) (<http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/ExternalQualityAssessmentProficiencyTesting/EQAOrPTForFoodWaterAndEnvironmentalMicrobiology/ShellfishScheme/>).

The EU stipulated reference method for enumeration of *E. coli* in live bivalve molluscs in ISO TS 16649-3, Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (Anon 2005). The EU stipulated reference method for detection of *Salmonella* spp. in live bivalve molluscs is ISO 6579, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Anon 2002). These methods, or an alternative method validated according to ISO 16140 must be used for official control testing of live bivalve molluscs for compliance with EU Regulations (Anon 2003).

Performance assessments are carried out on participants results. Performance assessments are valuable tools to help laboratories identify any ongoing problems with their procedures or analyses. Scores are given for results returned for each distribution and cumulative scores are calculated for 2 and 3 distributions.

If you are experiencing problems please contact the EU-RL (contact details below), or alternately refer to the troubleshooting guide included as Appendix I of this report. Further advice on microbiological testing of bivalve molluscan shellfish can be obtained via the EU-RL website www.eurlcefas.org

Distribution period:	March – November 2011
Report date:	5th March 2011
Report compiled by:	Louise Stockley
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Samples

Sample preparation

Samples distributed consisted of LENTICULE™ discs containing fully characterised bacterial isolates. The proportions and types of organisms were designed to mirror those found in freshly harvested bivalve molluscs. Samples were packaged according to IATA regulations and distributed with preparation instructions and report forms.

Participation

Twenty laboratories participated in the EQA scheme comprising 16 Member State NRLs, 1 EFTA NRL and 2 laboratories in accession countries. All participants were requested to examine the samples using Official Control methods i.e. those methods routinely used for official control analysis of live bivalve molluscs. NRLs Belgium, Bulgaria, The Czech Republic, Finland, Greece, Iceland, Latvia, Lithuania and Slovakia did not participate in the mandatory 2 distributions per year.

Results

Reference results

For each distribution 10 reference samples were examined by the organising laboratory. Reference analyses were performed using ISO TS 16649-3 for the enumeration of *Escherichia coli* and ISO 6579 for the detection of *Salmonella* spp..

Participants' analysis and scoring system

Results reported by NRLs for *E. coli* and *Salmonella* spp. examinations are shown in Tables 2, 4, 6, 8, 10 and 11. Reported *E. coli* MPN values were compared to the median MPN from all participants' results, reference results were omitted from this calculation. The result charts were compiled using \log_{10} transformed MPN values (Figures 1 - 5). Upper and lower acceptability limits were calculated as the participants' median ± 3 standard deviations (SD) and ± 5 SD. SD calculations were based on the expected inherent variability of the five tube 3 dilution MPN method which is $0.26 \log_{10}$. Performance assessment was according to the algorithm in Appendix II.

Distribution SF038: Sample SF0084

Contents – *Escherichia coli* (10^2), *Enterobacter agglomerans* and *Klebsiella oxytoca* ($10^3 - 10^4$).

Table 1 Participants and reference results median, median ± 3 and ± 5 SD - SF0084

	Median MPN/100g	Median -3SD MPN/100g	Median -5SD MPN/100g	Median +3SD MPN/100g	Median +5SD MPN/100g
Reference results	1.1×10^2	1.8×10^1	5.5×10^0	6.6×10^2	2.2×10^3
Participants results	1.1×10^2	1.8×10^1	5.5×10^0	6.6×10^2	2.2×10^3

Reference results

E. coli MPN – $<20 - 6.5 \times 10^2$ per 100g. *Salmonella* spp. – Not detected in 25g.

Participants results - SF0084 (Figure 1)

Table 2 Results reported by participants and scores allocated - SF0084

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	110	170	12	Not detected	2
391	330	330	12	Not detected	2
403	130	170	12	Not detected	2
413	140	140	12	Not detected	2
493	DNP	DNP	-	DNP	-
583	80	80	12	Not detected	2
597	78	20	12	Not detected	2
601	40	70	12	Not detected	2
649	110	110	12	Not detected	2
651	170	490	12	Not detected	2
653	110	50	12	Not detected	2
658	110	70	12	Not detected	2
701	170	80	12	Not detected	2
703	50	130	12	Not detected	2
715	40	20	12	Not detected	2
718	20	50	12	Not detected	2
719	230	80	12	Not detected	2
720	330	230	12	Not detected	2
744	130	110	12	Not detected	2
1498	230	130	12	Not detected	2
1527	100	100	12	-	-
1578	20	40	12	Not detected	2

DNP – Did not participate in this distribution,

Discussion – SF0084

Participation – Eighteen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. All laboratories returned results and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratory 493 did not take part in this distribution.

***E. coli* MPN** – All laboratories reported replicate results within the expected range and received a maximum score of 12.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp. correctly reported *Salmonella* spp. as not detected and received a maximum score of 2.

Distribution SF038: Sample SF0085

Contents – *Escherichia coli* (10^3), *Salmonella* London 3,10:l,v;1,6 (10 – 20) and *Proteus mirabilis* (10^2).

Table 3 Participants and reference results median, median ± 3 and ± 5 SD - SF0085

	Median MPN/100g	Median -3SD MPN/100g	Median -5SD MPN/100g	Median +3SD MPN/100g	Median +5SD MPN/100g
Reference results	7.9×10^2	1.3×10^2	4.0×10^1	4.8×10^3	1.6×10^4
Participants results	1.1×10^3	1.8×10^2	5.5×10^1	6.6×10^3	2.2×10^4

Reference results

E. coli MPN – 1.4×10^2 – 4.6×10^3 per 100g. *Salmonella* spp. – Detected in 25g.

Participants results - SF0085 (Figure 2)

Table 4 Results reported by participants and scores allocated - SF0085

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	700	2400	12	Detected	2
391	790	1300	12	Detected	2
403	1300	1100	12	Detected	2
413	1700	1700	12	Detected	2
493	DNP	DNP	-	DNP	-
583	1100	1700	12	Detected	2
597	490	460	12	Detected	2
601	790	490	12	Detected	2
649	1700	1300	12	Detected	2
651	790	1300	12	Detected	2
653	790	2400	12	Detected	2
658	500	750	12	Detected	2
701	790	790	12	Detected	2
703	790	330	12	Detected	2
715	310	750	12	Detected	2
718	460	1700	12	Detected	2
719	1300	1400	12	Detected	2
720	1300	2400	12	Detected	2
744	790	1300	12	Detected	2
1498	1700	1300	12	Detected	2
1527	520	520	12	NE	-
1578	1700	1300	12	Detected	2

DNP – Did not participate in this distribution, NE – Not examined

Discussion – SF0085

Participation - Eighteen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. All laboratories returned results and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratory 493 did not take part in this distribution.

***E. coli* MPN** – All laboratories reported replicate results within the expected range and received a maximum score of 12.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp. correctly reported *Salmonella* spp. as detected and received a maximum score of 2.

Distribution SF039: Sample SF0086

Contents – *Escherichia coli* (10^4), *Candida tropicalis* (10^4), *Clostridium perfringens* (10^2) and *Pseudomonas aeruginosa* (10^3).

Table 5 Participants and reference results median, median ± 3 and ± 5 SD - SF0086

	Median MPN/100g	Median -3SD MPN/100g	Median -5SD MPN/100g	Median +3SD MPN/100g	Median +5SD MPN/100g
Reference results	9.2×10^3	1.5×10^3	4.6×10^2	5.5×10^4	1.8×10^5
Participants results	9.9×10^3	1.6×10^3	4.9×10^2	6.0×10^4	2.0×10^5

Reference results

E. coli MPN – 1.5×10^3 – 5.5×10^4 per 100g. *Salmonella* spp. - Not detected in 25g.

Participants results - SF0086 (Figure 3)

Table 6 Results reported by participants and scores allocated - SF0086

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	3500	5400	12	Not Detected	2
391	16000	16000	12	Not Detected	2
403	9200	9200	12	Not Detected	2
413	17000	17000	12	Not Detected	2
493	DNP	DNP	-	DNP	-
583	22000	17000	12	Not Detected	2
597	2400	9200	12	Not Detected	2
601	7900	7900	12	Not Detected	2
649	22000	17000	12	Not Detected	2
651	7000	5400	12	Not Detected	2
653	7900	14000	12	Not Detected	2
658	DNP	DNP	-	DNP	-
701	5400	16000	12	Not Detected	2
703	9200	16000	12	Not Detected	2
715	5400	9100	12	NE	-
718	11000	11000	12	Not Detected	2
719	4900	3300	12	Not Detected	2
720	9200	16000	12	Not Detected	2
744	11000	3500	12	Not Detected	2
1498	17000	16000	12	Not Detected	2
1527	9160	10600	12	NE	-
1578	54000	92000	9	Not Detected	2

DNP – Did not participate in this distribution, NE – Not examined.

Discussion – SF0086

Participation - Seventeen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. All laboratories returned results and were included in the assessment. Laboratories 715 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 493 and 658 did not take part in this distribution.

***E. coli* MPN** – Nineteen laboratories reported replicate results within the expected range and received a maximum score of 12. Laboratory 1578 reported one replicate MPN result between ± 3 and ± 5 SD of the participants' median.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp. correctly reported *Salmonella* spp. as not detected and received a maximum score of 2.

Distribution SF039: Sample SF0087

Contents *Escherichia coli* (10^2), *Salmonella* London 3,10:l,v;1,6 (10^3) and *Proteus mirabilis* (10^2).

Table 7 Participants and reference results median, median ± 3 and ± 5 SD - SF0087

	Median MPN/100g	Median -3SD MPN/100g	Median -5SD MPN/100g	Median +3SD MPN/100g	Median +5SD MPN/100g
Reference results	1.1×10^3	1.8×10^2	5.5×10^1	6.6×10^3	2.2×10^4
Participants results	7.4×10^2	1.2×10^2	3.7×10^1	4.5×10^3	1.5×10^4

Reference results

E. coli MPN – 1.3×10^2 – 4.7×10^3 per 100g. *Salmonella* spp. - Detected in 25g.

Participants results - SF0087

Table 8 Results reported by participants and scores allocated - SF0087

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	1700	700	12	Detected	2
391	790	790	12	Detected	2
403	2400	1300	12	Detected	2
413	490	490	12	Detected	2
493	DNP	DNP	-	DNP	-
583	330	490	12	Detected	2
597	1300	490	12	Detected	2
601	330	490	12	Detected	2
649	1700	2400	12	Detected	2
651	1100	1300	12	Detected	2
653	1300	1700	12	Detected	2
658	DNP	DNP	-	DNP	-
701	460	80	9	Detected	2
703	230	230	12	Detected	2
715	700	310	12	Detected	2
718	940	330	12	Detected	2
719	700	1100	12	Detected	2
720	1300	1700	12	Detected	2
744	700	1300	12	Detected	2
1498	490	790	12	Detected	2
1527	380	260	12	NE	-
1578	1100	2200	12	Detected	2

DNP – Did not register for this distribution, NE – Not examined

Discussion – SF0087

Participation – Seventeen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. All laboratories returned results and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratory 493 and 658 did not take part in this distribution.

***E. coli* MPN** – Nineteen laboratories reported replicate results within the expected range and received a maximum score of 12. Laboratory 701 reported one replicate MPN result between ± 3 and ± 5 SD of the participants' median.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp correctly reported *Salmonella* spp. as detected and received a maximum score of 2.

Distribution SF040: Sample SF0088

Contents – *Escherichia coli* (10^3), *Salmonella* Liverpool (serotype 1,3,19:d,e,n,z₁₅) (10^4), *Hafnia* spp. (10^3) and *Pseudomonas putida* (10^3).

Reference results

***E. coli* MPN** – 9.0×10^2 – 3.2×10^4 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 9 Participants and reference results median, median ± 3 and ± 5 SD - SF0088

	Median MPN/100g	Median -3SD MPN/100g	Median -5SD MPN/100g	Median +3SD MPN/100g	Median +5SD MPN/100g
Reference results	7.9×10^3	1.3×10^3	4.0×10^2	4.8×10^4	1.6×10^5
Participants results	5.4×10^3	9.0×10^2	2.7×10^2	3.3×10^4	1.1×10^5

Participants results - SF0088 (Figure 4)

Table 10 Results reported by participants and scores allocated - SF0088

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	5400	9200	12	Detected	2
391	5400	9200	12	Detected	2
403	2300	7900	12	Detected	2
413	DNP	DNP	-	DNP	-
493	11000	11000	12	Detected	2
583	9200	>18000	7	Detected	2
597	3500	5400	12	Detected	2
601	DNP	DNP	-	DNP	-
649	1100	2400	12	Detected	2
651	4600	3500	12	Detected	2
653	7900	4900	12	Detected	2
658	DNP	DNP	-	DNP	-
701	5400	3500	12	Detected	2
703	1700	9200	12	Detected	2
715	NR	NR	-	NR	-
718	4900	3300	12	Detected	2
719	13000	11000	12	Detected	2
720	9200	5400	12	Detected	2
744	16000	5400	12	Detected	2
1498	3500	9200	12	Detected	2
1527	6020	6080	12	NE	-
1578	DNP	DNP	-	DNP	-

DNP – Did not register, NR – No results returned, NE – Not examined.

Discussion – SF0088

Participation – Fifteen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. Seventeen laboratories returned results and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratory 715 did not return results for this distribution. Laboratory 413, 601, 658 and 1578 did not take part in this distribution.

***E. coli* MPN** – Sixteen laboratories reported replicate results within the expected range and received a maximum score of 12. Laboratory 583 reported one replicate MPN result as a high censored result.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp. correctly reported *Salmonella* spp. as detected and received a maximum score of 2.

Distribution SF040: Sample SF0089
Contents – *Bacillus pumilus* (10^3), *Hafnia alvei* (10^2) and *Serratia liquefaciens* (10^2).

Reference results
***E. coli* MPN** – <20 - 0 per 100g. ***Salmonella* spp.** – Not detected in 25g.

Participants results - SF0089 (Figure 5)
Table 11 Results reported by participants and scores allocated - SF0089

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	<20	<20	12	Not detected	2
391	<20	<20	12	Not detected	2
403	<20	<20	12	Not detected	2
413	DNP	DNP	-	DNP	-
493	<20	<20	12	Not detected	2
583	<20	<20	12	Not detected	2
597	<18	<18	12	Not detected	2
601	DNP	DNP	-	DNP	-
649	<20	<20	12	Not detected	2
651	<20	<20	12	Not detected	2
653	<20	<20	12	Not detected	2
658	DNP	DNP	-	DNP	-
701	<20	<20	12	Not detected	2
703	<20	<20	12	Not detected	2
715	NR	NR	-	NR	-
718	<20	<20	12	Not detected	2
719	<20	<20	12	Not detected	2
720	<20	<20	12	Not detected	2
744	<20	<20	12	Not detected	2
1498	<20	<20	12	Not detected	2
1527	<20	<20	12	NE	-
1578	DNP	DNP	-	DNP	-

DNP – Did not register, NR – No results returned, NE – Not examined.

Discussion – SF0089
Participation - Fifteen NRLs, 1 EFTA and 2 accession country laboratories received material for examination. Seventeen laboratories returned results and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratory 715 did not return results for this distribution. Laboratory 413, 601, 658 and 1578 did not take part in this distribution.

***E. coli* MPN** – All laboratories reported replicate results within the expected range and received a maximum score of 12.

***Salmonella* spp.** – All laboratories that reported results for *Salmonella* spp. correctly reported *Salmonella* spp. as not detected and received a maximum score of 2.

Participation

At the 10th workshop of microbiological NRLs for bivalve shellfish it was noted (Resolution 12) that the performance of NRLs in PT for *E. coli* and *Salmonella* spp. was excellent. The EURL agreed that NRLs may participate in a minimum of 2 distributions each year if showing satisfactory performance. Table 12 shows the participation of NRLs for 2011 with 64% (18 NRLs) participating in 2 or more distributions and 7 NRLs not participating in 1 distribution. The EURL recommends that NRLs not registered to the EURL/HPA scheme should join this scheme.

Table 12 : Number of EURL/HPA EQA distributions analysed by NRLs in 2011

Country	Number of EQA distributions			
	None	1	2	3
Austria			✓	
Belgium/ Luxembourg	✓			
Bulgaria	✓			
Croatia				✓
Czech Republic	✓			
Denmark				✓
Finland	✓			
France				✓
Germany				✓
Greece		✓		
Hungary			✓	
Iceland	✓			
Ireland				✓
Italy			✓	
Italy				✓
Latvia	✓			
Lithuania		✓		
Netherlands				✓
Norway			✓	
Poland				✓
Portugal				✓
Romania				✓
Slovakia	✓			
Slovenia				✓
Spain				✓
Sweden				✓
UK				✓

Performance Assessment

A cumulative performance assessment was undertaken on participant's results over two and three distributions for both *E. coli* MPNs and *Salmonella* spp. from March 2011 to November 2011 and are summarised in Tables 13 and 14 respectively.

For participants analysing the mandatory 2 distributions a year, a performance assessment was based on the rules for participation in 3 distributions, where a cumulative score of >70% identifies good performance. Performance assessment for participation in 2 distributions is under review and a percentage for good performance will be calculated for future reports.

Participant's who achieved <40% of the maximum possible score in a single distribution and <70% over two or three distributions for *E. coli* enumeration and/or *Salmonella* spp. detection should in the first instance refer to the troubleshooting guide included as Appendix I.

***E. coli* MPN assessment**

Twenty laboratories completed 2 or 3 distributions in 2011 and were therefore subject to a full performance assessment. All laboratories achieved a cumulative total of >70% for the two or three distributions analysed.

Table 13 Summary of performance of participants over three distributions, SF038 to SF040 - *E. coli*

Lab no.	Distribution SF038		Distribution SF039		Distribution SF040		All distributions		
	SF0084	SF0085	SF0086	SF0087	SF0088	SF0089	Cumulative score	Max score	%
121	12	12	12	12	12	12	72	72	100
391	12	12	12	12	12	12	72	72	100
403	12	12	12	12	12	12	72	72	100
413	12	12	12	12	-	-	48	48	100
493 ^a	-	-	-	-	12	12	-	-	-
583	12	12	12	12	7	12	67	72	93
597	12	12	12	12	12	12	72	72	100
601	12	12	12	12	-	-	48	48	100
649	12	12	12	12	12	12	72	72	100
651	12	12	12	12	12	12	72	72	100
653	12	12	12	12	12	12	72	72	100
658 ^a	12	12	-	-	-	-	-	-	-
701	12	12	12	9	12	12	69	72	96
703	12	12	12	12	12	12	72	72	100
715	12	12	12	12	-	-	48	48	100
718	12	12	12	12	12	12	72	72	100
719	12	12	12	12	12	12	72	72	100
720	12	12	12	12	12	12	72	72	100
744	12	12	12	12	12	12	72	72	100
1498	12	12	12	12	12	12	72	72	100
1527	12	12	12	12	12	12	72	72	100
1578	12	12	9	12	-	-	45	48	94

^a full performance assessment was not carried as laboratories completed less than two distributions during 2011.

Salmonella spp. Assessment

Eighteen laboratories completed either 2 or 3 distributions in 2011 and were therefore subject to a full performance assessment. All laboratories achieved a cumulative total of >70% for the two or three distributions analysed.

Table 14 Summary of performance of participants over three distributions, SF038 to SF040 Salmonella spp.

Lab no.	Distribution SF038		Distribution SF039		Distribution SF040		All distributions		
	SF0084	SF0085	SF0086	SF0087	SF0088	SF0089	Cumulative score	Max score	%
121	2	2	2	2	2	2	12	12	100
391	2	2	2	2	2	2	12	12	100
403	2	2	2	2	2	2	12	12	100
413	2	2	2	2	-	-	8	8	100
493 ^a	-	-	-	-	2	2	-	-	-
583	2	2	2	2	2	2	12	12	100
597	2	2	2	2	2	2	12	12	100
601	2	2	2	2	-	-	8	8	100
649	2	2	2	2	2	2	12	12	100
651	2	2	2	2	2	2	12	12	100
653	2	2	2	2	2	2	12	12	100
658 ^a	2	2	-	-	-	-	-	-	-
701	2	2	2	2	2	2	12	12	100
703	2	2	2	2	2	2	12	12	100
715 ^a	2	2	-	2	-	-	-	-	-
718	2	2	2	2	2	2	12	12	100
719	2	2	2	2	2	2	12	12	100
720	2	2	2	2	2	2	12	12	100
744	2	2	2	2	2	2	12	12	100
1498	2	2	2	2	2	2	12	12	100
1527 ^a	-	-	-	-	-	-	-	-	-
1578	2	2	2	2	-	-	8	8	100

^a full performance assessment was not carried as laboratories completed less than two distributions during 2011.

Summary

In 2011 Seventeen laboratories completed two or three distributions between March 2011 and November 2011 and therefore were subject to a full performance assessment. For *E. coli* examination and *Salmonella* spp. detection all laboratories achieved a score of >70% over two or three distributions.

References

Anon 2005. ISO TS 16649-3:2005. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide. Geneva, Switzerland

Anon. 2002. ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. Geneva, Switzerland.

Anon 2003. ISO 16140 Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods, Geneva, Switzerland.

Figure 1. Distribution SF038: Sample SF0084

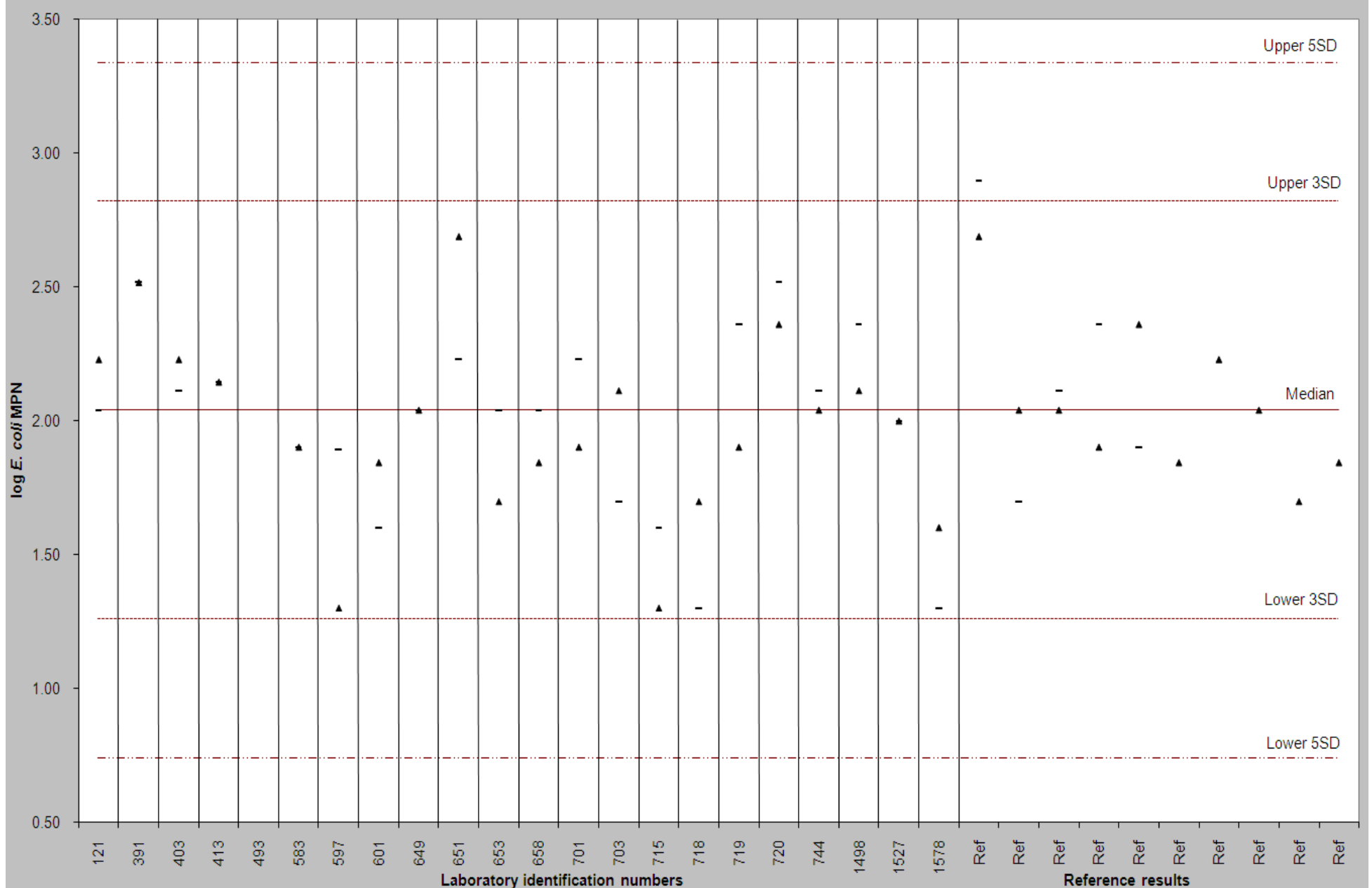


Figure 2. Distribution SF038: Sample SF0085

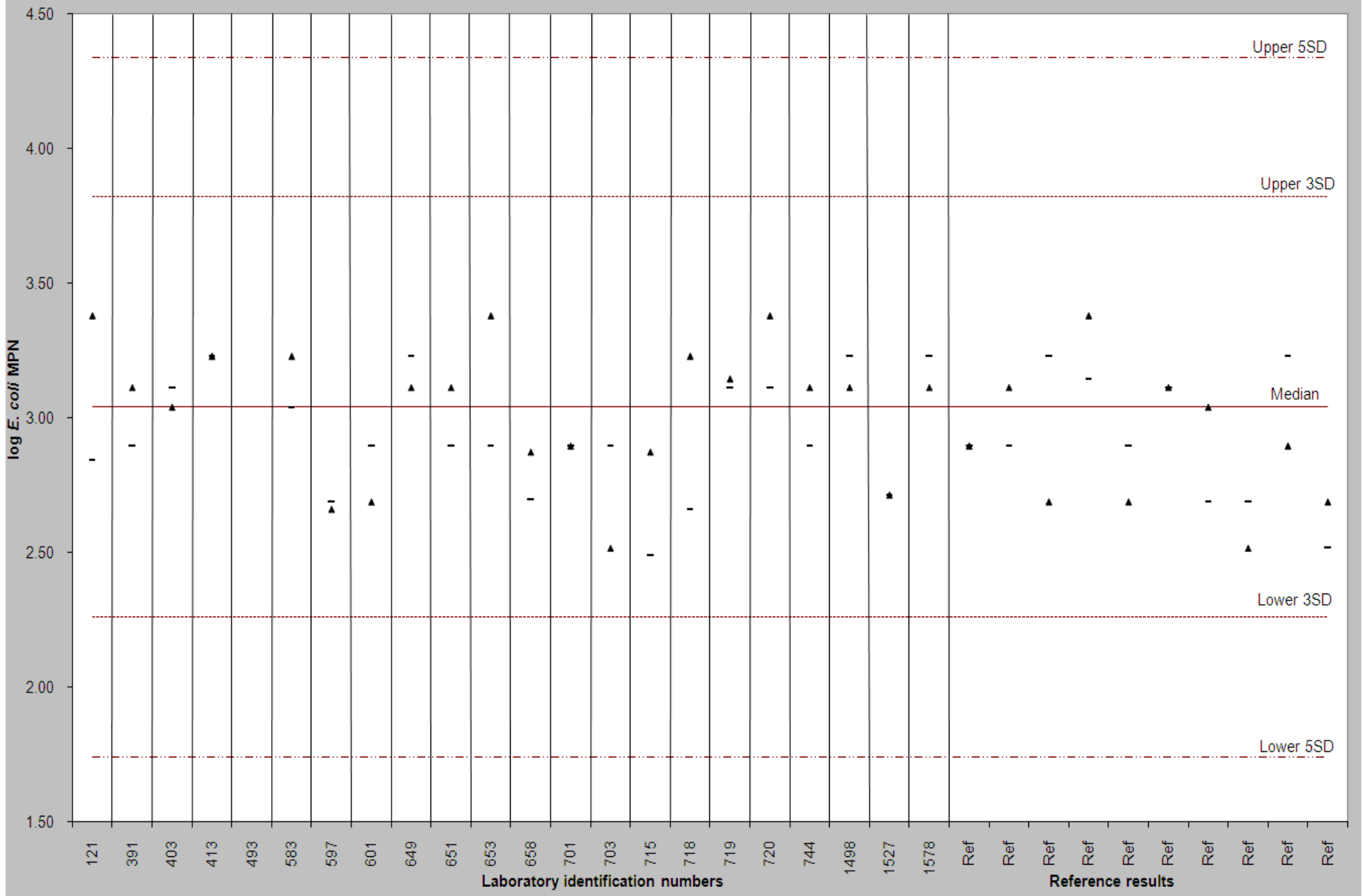


Figure 3. Distribution SF039: Sample SF0086

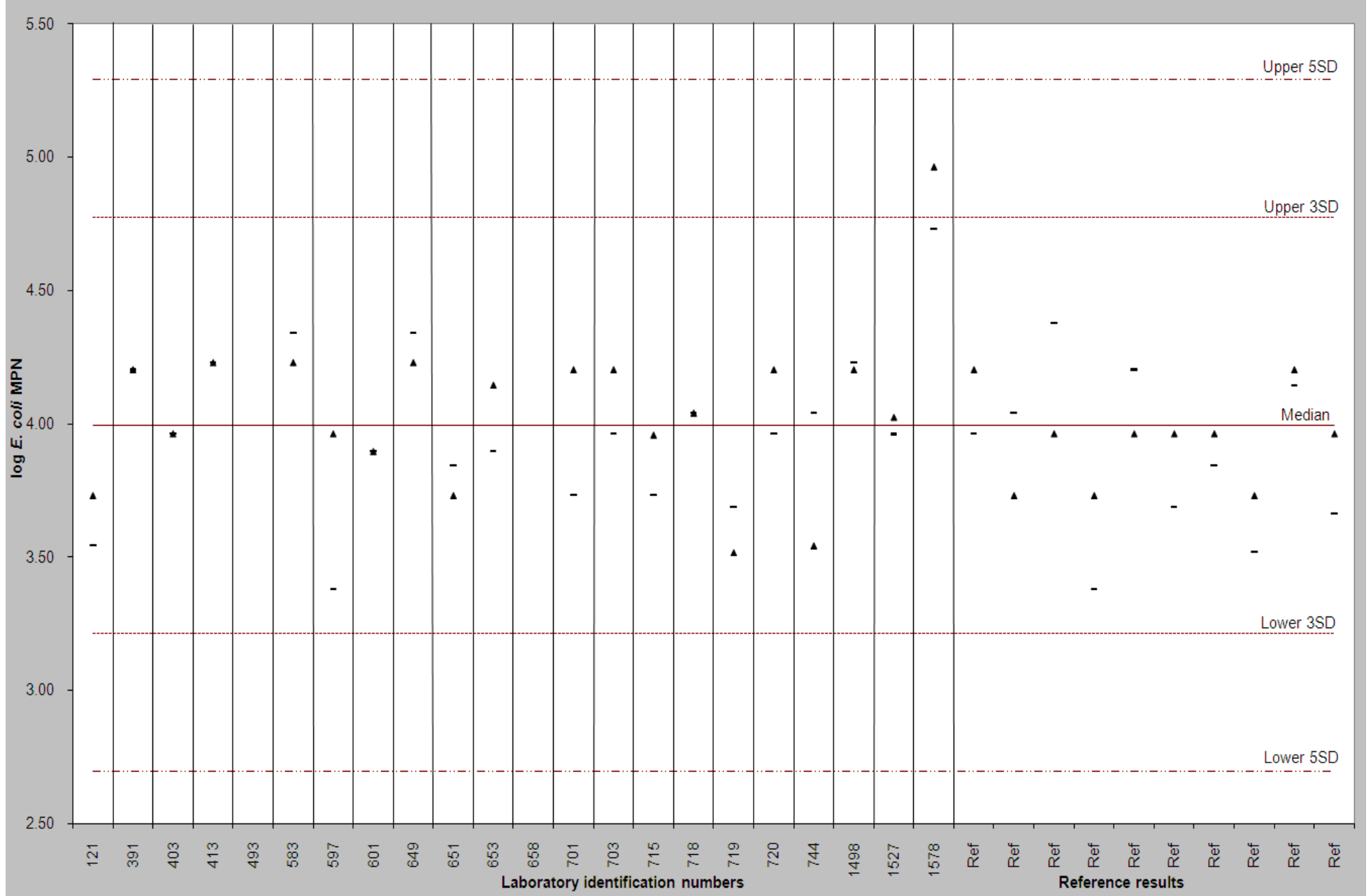


Figure 4. Distribution SF039: Sample SF0087

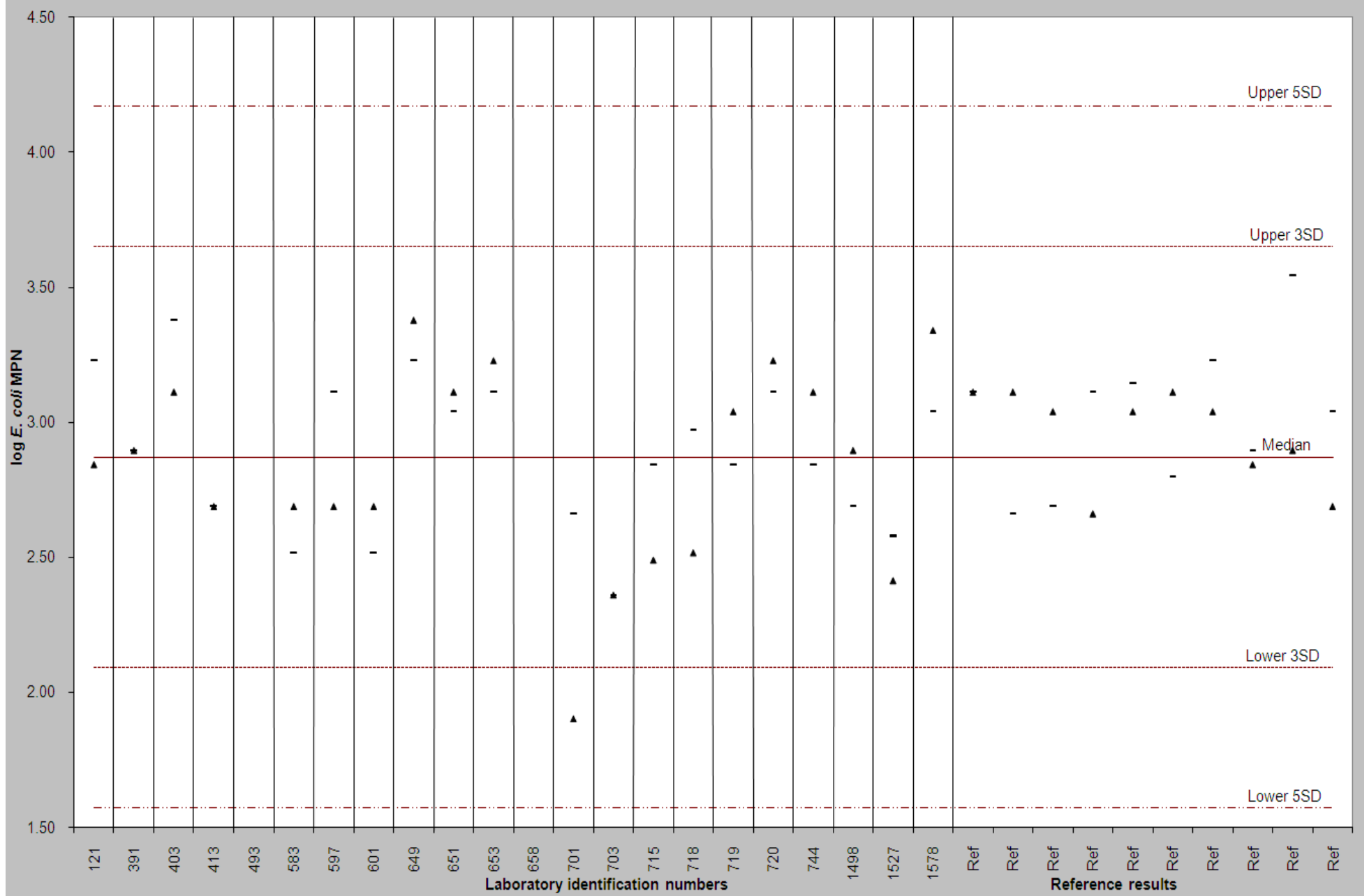
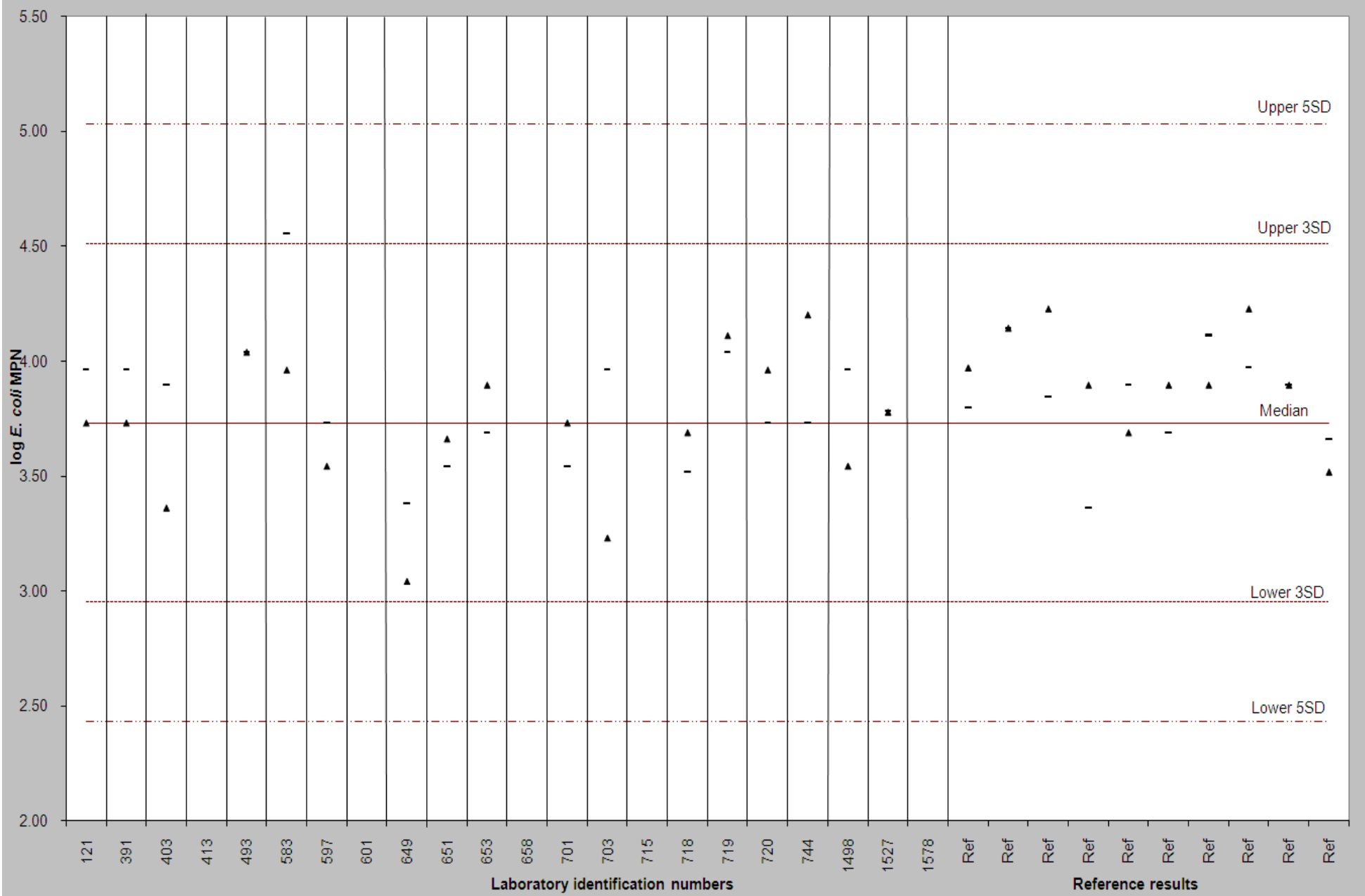


Figure 5. Distribution SF040: Sample SF0088



Appendix I:**Troubleshooting advice**

1. Methods – Ensure that the method used is appropriate for the examination of the sample.
 - a. Ensure that any dilutions have been calculated correctly.
 - b. Ensure that MPN tables (if used) are interpreted correctly.
2. Culture Medium - Check the quality control data for media to ensure that they are within specifications and performing adequately.
3. Equipment - Check that the equipment used for the procedures (incubators, refrigerators, measuring instruments) are calibrated and performing adequately.
4. Staff Training - Check that the staff performing the tests are fully trained and familiar with all the procedural steps.
5. Clerical Procedures - Check that the sample labeling, laboratory numbering and clerical procedures are adequate have you procedures for ensuring that test results are reported accurately and on time.
6. Accreditation- Check that quality procedures are documented and adhered to at all times.
7. Internal quality controls (IQC) – Ensure that adequate controls are in place and that and documentation for dealing with IQC failures is appropriate.

Further advice can be obtained from the EU-RL on request.

Appendix II:
***E. coli* MPN scores allocated to participants returning 2 replicate results**

Result	Returning of results	Score allocated		Total score
		Replicate 1	Replicate 2	
One replicate MPN result reported is outside the expected range and falls between the median $\pm 3SD$ and median $\pm 5SD$ value	2	5	2	9
Both replicates MPN results are outside the expected range and fall between the median $\pm 3SD$ and median $\pm 5SD$ value	2	2	2	6
One replicate MPN result reported is outside the median $\pm 5SD$ value	2	5	0	7
Both replicates MPN results are outside the expected range. The first falls between the median $\pm 3SD$ and median $\pm 5SD$ value and the second falls outside the median $\pm 5SD$ value.	2	2	0	4
Both replicates MPN results reported is outside the median $\pm 5SD$ value	2	0	0	2

***E. coli* MPN scores allocated to participants returning 1 single replicate results**

Result	Returning of results	Score allocated	Total score
Single replicate MPN result reported is within the expected range	2	5	7
Single replicate MPN result reported only and falls between the median $\pm 3SD$ and median $\pm 5SD$ value	2	2	4
Single replicate MPN result reported is outside the median $\pm 5SD$ value	2	0	2

***E. coli* score deductions**

Result	Score deducted
Tube combination inconsistent with MPN reported, ISO 7218 or 5 x 3 MPN tables provided by the EURL.	2
Sample not examined or results returned late - no explanation received	2
High censored result (e.g. MPN = >18000 per 100g)	No score allocated

***Salmonella* spp scoring**

Result	Score allocated
Fully correct results	2
Misleading result, e.g. failure to isolate <i>Salmonella</i>	0

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