



Fapas[®] – Water and Environmental Report DWM010

Drinking Water Microbiology

February-March 2017

PARTICIPANT LABORATORY NUMBER

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SUMMARY

1. The test materials for FAPAS – Drinking Water Microbiology proficiency test DWM010 were dispatched in February 2017. Three test materials were provided for seven examinations in this proficiency test:

Test Material A	DWM0110 Enumeration of total coliforms
Test Material A	DWM0110 Enumeration of <i>Escherichia coli</i>
Test Material B	DWM0210 Colony count after 3 days at 22°C
Test Material B	DWM0210 Colony count after 2 days at 37°C
Test Material C	DWM0310 Enumeration of enterococci
Test Material C	DWM0310 Enumeration of <i>Pseudomonas aeruginosa</i>
Test Material C	DWM0310 Enumeration of <i>Clostridium perfringens</i>

2. An assigned value (x_a) was determined for each test in enumeration and in conjunction with the standard deviation for proficiency (σ_p), was used to calculate a z-score for each result. However, those for *Pseudomonas aeruginosa* are given for information only.
3. Results for this proficiency test are summarised as follows:

examination	assigned value, x_a	units	number of scores, $ z \leq 2$	total number of scores	% $ z \leq 2$
enumeration of total coliforms	7.28	SQRTcfu/100ml	24	27	89
enumeration of <i>Escherichia coli</i>	5.83	SQRTcfu/100ml	26	28	93
colony count after 3 days at 22°C	11.34	SQRTcfu/ml	15	16	94
colony count after 2 days at 37°C	12.73	SQRTcfu/ml	14	17	82
enumeration of enterococci	7.28	SQRTcfu/100ml	16	17	94
enumeration of <i>Pseudomonas aeruginosa</i>	<i>14.06</i>	SQRTcfu/100ml	<i>10</i>	15	<i>67</i>
enumeration of <i>Clostridium perfringens</i>	9.80	SQRTcfu/100ml	9	13	69

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1. INTRODUCTION

1.1. Proficiency Testing

Proficiency testing aims to provide an independent assessment of the competence of participating laboratories. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

Further details of the Fapas[®] – Water and Environmental proficiency tests are available in our protocols [3, 4].

2. TEST MATERIAL

2.1. Preparation

Preparation of the samples for this proficiency test was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [2].

Each test material comprised of a small glass vial containing an inoculum pellet, sealed with a rubber bung and crimp-capped. The organisms present in the inoculum pellets were as follows:

Test Material A	<i>Escherichia coli</i> and <i>Raoultella planticola</i>
Test Material B	<i>Enterococcus faecalis</i> and <i>Staphylococcus epidermidis</i>
Test Material C	<i>Enterococcus faecalis</i> , <i>Clostridium perfringens</i> and <i>Pseudomonas aeruginosa</i>

The test materials were stored at +4°C ±2°C until dispatch.

2.2. Homogeneity

To test for homogeneity, randomly selected test materials were analysed in duplicate by a laboratory meeting the quality requirements of the scheme's accreditation [2].

These data showed sufficient homogeneity and were not included in the subsequent calculation of the assigned values.

2.3. Dispatch

The start date was 14 February 2017. Test materials were sent to 38 participants.

3. RESULTS

The instructions for reporting results were as follows:

- Start the analysis between 14 February and 23 February 2017.
- Report results in cfu/100ml for total coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens* and *Pseudomonas aeruginosa* and in cfu/ml for colony count after 3 days at 22°C and colony count after 2 days at 37°C.

Results were submitted by 37 participants (97%) before the closing date for this test, 08 March 2017.

Each participant was given a laboratory number, assigned in order of receipt of results. The reported results are given in Tables 1-7.

The analytical methods used by each participant are summarised in APPENDIX I.

4. STATISTICAL EVALUATION OF RESULTS

The results submitted by participants were statistically analysed in order to provide an assigned value for each analyte. The assigned values were then used in combination with the standard deviation for proficiency, σ_p , to calculate a z-score [5] for each result. The procedure is detailed in the relevant protocols [3, 4].

Further background on the procedure followed can be found in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [6].

4.1. Calculation of the Assigned Value, x_a

The assigned value, x_a , for each test in enumeration was derived from the consensus of the results submitted by participants. The procedure used to derive this consensus involved a square root transformation of submitted results to obtain a normal distribution.

The following results were excluded from the calculation of the assigned value:

- i) results reported as approximately 100 or 1000 \times greater or smaller than the majority of submitted results (as these were considered to be reporting errors),
- ii) non numerical results i.e. qualitative or semi-quantitative results,
- iii) results where the analysis commenced outside the specified time period.

For the enumeration of total coliforms the distribution of data was skewed. However a bump hunt [7] showed the distribution to be unimodal and therefore, the mode has been set as the assigned value for this proficiency test. The kernel density plot of the distribution of results is presented as an insert to Figure 1.

For the enumeration of *E. coli* this procedure was straightforward and the robust mean was chosen as the assigned value.

For the Colony Count (22°C/3days), Colony Count (37°C/2days), Enterococci and *Clostridium perfringens* enumerations the median was chosen as the assigned value as this was considered the best measure of consensus due to the low number of data points.

For the enumeration of *Pseudomonas aeruginosa*, due to the low number of data points the median was chosen as the assigned value. However the uncertainty of the median was higher than acceptable and therefore the assigned value and z-scores are given for information only.

The assigned values for all examinations are shown in Table 8.

4.2. Standard Deviation for Proficiency, σ_p

A fixed standard deviation has been set at a value that reflects best practice for the analyses in question and the appropriate members of the Fapas[®] Advisory Committee have agreed these values.

The standard deviation for proficiency assessment (σ_p) values are given in Table 8.

4.3. Individual z-Scores

Participants' z-scores were calculated as:

$$z = \frac{(\sqrt{x} - \sqrt{x_a})}{\sigma_p}$$

where x = the participant's reported result,
 x_a = the assigned value
and σ_p = the standard deviation for proficiency.

Participants' z-scores for all analytes are given in Table 1 to Table 7 and shown as histograms in Figure 1 to Figure 7. It is possible for the z-scores published in this report to differ slightly from the z-score that can be calculated using the formula given above. These differences arise from the necessary rounding of the actual assigned values and standard deviations for proficiency prior to their publication in Table 8.

The number and percentage of z-scores in the range $-2 \leq z \leq 2$ are given in Table 9.

Participants' comments are given in Table 10.

5. INTERPRETATION OF SCORES

In normal circumstances, over time, about 95% of z-scores will lie in the range $-2 \leq z \leq 2$. Occasional scores in the range $2 < |z| < 3$ are to be expected, at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

The consideration of a set or sequence of z-scores over time provides more useful information than a single z-score. Examples of suitable methods of comparison are provided in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [6].

6. REFERENCES

- 1 Adobe Certified Document Services
<https://helpx.adobe.com/acrobat/kb/certificate-signatures.html>, accessed 10/03/2017.
- 2 ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing.
- 3 Fapas[®], 2016, Protocol for Proficiency Testing Schemes, Part 1 – Common Principles, Version 5, Issued September 2016.
- 4 Fapas[®], 2016, Protocol for Proficiency Testing Schemes, Part 5 – Fapas[®] – Water and Environmental, Version 5, Issued September 2016.
- 5 AMC Tech Brief No. 74, z-Scores and other scores in chemical proficiency testing – their meanings, and some common misconceptions, *Anal. Methods*, 2016, 8, 5553.
- 6 Thompson, M., Ellison, S.L.R. and Wood, R., 2006, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, **78**, No. 1, 145–196.
- 7 Lowthian, P.J. and Thompson, M., 2002, Bump-hunting for the proficiency tester – searching for multimodality, *Analyst*, **127**, 1359-1364.

Table 1: Results and z-Scores for Enumeration of Total Coliforms

laboratory number	total coliforms		
	assigned value 7.28 SQRTcfu/100ml		
	result cfu/100ml	result SQRTcfu/100ml	z-score
001	52	7.21	0.0
002	40	6.32	-0.6
003	71	8.43	0.8
004	47	6.86	-0.3
005	15	3.87	-2.3
006	38	6.16	-0.7
009	56	7.48	0.1
010	6	2.45	-3.2
011	60	7.75	0.3
012	89	9.43	1.4
015	80	8.94	1.1
016	48	6.93	-0.2
017	51	7.14	-0.1
019	37	6.08	-0.8
021	70	8.37	0.7
022	56.32	7.50	0.1
023	49.0	7.00	-0.2
024	83	9.11	1.2
026	5	2.24	-3.4
027	75	8.66	0.9
028	72	8.49	0.8
029	86	9.27	1.3
030	45	6.71	-0.4
031	51	7.14	-0.1
032	47	6.86	-0.3
033	43	6.56	-0.5
034	# 29.3	5.41	-1.2

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

samples received outside of recommended timeframe for analysis

Table 2: Results and z-Scores for Enumeration of *Escherichia coli*

laboratory number	<i>Escherichia coli</i>		
	assigned value 5.83 SQRTcfu/100ml		
	result cfu/100ml	result SQRTcfu/100ml	z-score
001	36	6.00	0.1
002	15	3.87	-1.3
003	30	5.48	-0.2
004	24	4.90	-0.6
005	2	1.41	-2.9
006	25	5.00	-0.6
008	41	6.40	0.4
009	30	5.48	-0.2
010	14	3.74	-1.4
011	40	6.32	0.3
012	66	8.12	1.5
015	40	6.32	0.3
016	34	5.83	0.0
017	29	5.39	-0.3
018	22	4.69	-0.8
019	33	5.74	-0.1
021	42	6.48	0.4
022	43.9	6.63	0.5
023	38.0	6.16	0.2
024	41	6.40	0.4
026	3	1.73	-2.7
027	29	5.39	-0.3
029	50	7.07	0.8
030	30	5.48	-0.2
031	36	6.00	0.1
032	33	5.74	-0.1
033	43	6.56	0.5
034	# 19.8	4.45	-0.9

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

samples received outside of recommended timeframe for analysis

Table 3: Results and z-Scores for Colony Count after 3 days at 22°C

laboratory number	colony count, 3 days/22°C		
	assigned value 11.34 SQRTcfu/ml		
	result cfu/ml	result SQRTcfu/ml	z-score
004	127	11.27	-0.1
007	125	11.18	-0.1
008	112	10.58	-0.6
010	99	9.95	-1.1
011	140	11.83	0.4
012	134	11.58	0.2
013	94	9.70	-1.3
015	130	11.40	0.1
017	154	12.41	0.9
019	141	11.87	0.4
020	110	10.49	-0.7
024	154	12.41	0.9
025	156.36	12.50	0.9
027	99	9.95	-1.1
033	20	4.47	-5.5
037	153	12.37	0.8

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 4: Results and z-Scores for Colony Count after 2 days at 37°C

laboratory number	colony count, 2 days/37°C		
	assigned value 12.73 SQRTcfu/ml		
	result cfu/ml	result SQRTcfu/ml	z-score
004	178	13.34	0.5
007	135	11.62	-0.9
010	139	11.79	-0.8
011	195	13.96	1.0
012	192	13.86	0.9
013	120	10.95	-1.4
015	140	11.83	-0.7
017	187	13.67	0.8
019	175	13.23	0.4
020	183	13.53	0.6
021	175	13.23	0.4
022	80.80	8.99	-3.0
024	176	13.27	0.4
025	75.00	8.66	-3.3
027	162	12.73	0.0
033	64	8.00	-3.8
037	147	12.12	-0.5

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 5: Results and z-Scores for Enumeration of Enterococci

laboratory number	enterococci		
	assigned value 7.28 SQRTcfu/100ml		
	result cfu/100ml	result SQRTcfu/100ml	z-score
001	48	6.93	-0.3
004	44	6.63	-0.5
005	42	6.48	-0.6
008	59	7.68	0.3
010	17	4.12	-2.5
011	55	7.42	0.1
012	59	7.68	0.3
014	61	7.81	0.4
016	53	7.28	0.0
017	52	7.21	-0.1
019	54	7.35	0.1
022	34.3	5.86	-1.1
024	59	7.68	0.3
026	67	8.19	0.7
027	31	5.57	-1.4
029	44	6.63	-0.5
033	63	7.94	0.5

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 6: Results and z-Scores for Enumeration of *Pseudomonas aeruginosa*

laboratory number	<i>Pseudomonas aeruginosa</i>		
	assigned value <i>14.06</i> SQRTcfu/100ml		
	result cfu/100ml	result SQRTcfu/100ml	z-score
001	200.5	14.16	<i>0.1</i>
004	123	11.09	<i>-2.0</i>
005	20	4.47	<i>-6.4</i>
010	120	10.95	<i>-2.1</i>
011	303	17.41	<i>2.2</i>
012	225	15.00	<i>0.6</i>
014	195	13.96	<i>-0.1</i>
017	173	13.15	<i>-0.6</i>
019	confluent growth		
022	366.0	19.13	<i>3.4</i>
024	160	12.65	<i>-0.9</i>
027	250	15.81	<i>1.2</i>
033	250	15.81	<i>1.2</i>
034	# 38.3	6.19	<i>-5.2</i>
035	140	11.83	<i>-1.5</i>
037	252	15.87	<i>1.2</i>

samples received outside of recommended timeframe for analysis
italics indicate for information only

Table 7: Results and z-Scores for Enumeration of *Clostridium perfringens*

laboratory number	<i>Clostridium perfringens</i>		
	assigned value 9.80 SQRTcfu/100ml		
	result cfu/100ml	result SQRTcfu/100ml	z-score
004	179	13.38	2.4
005	56	7.48	-1.5
011	80	8.94	-0.6
012	111	10.54	0.5
014	116	10.77	0.6
016	91	9.54	-0.2
017	78	8.83	-0.6
019	240	15.49	3.8
024	104	10.20	0.3
026	96	9.80	0.0
027	1	1.00	-5.9
033	86	9.27	-0.3
036	10500.00	102.47	61.8

z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 8: Assigned Values and Standard Deviations for Proficiency

examination	data points, n	assigned value, x_a	units	uncertainty u	standard deviation for proficiency, σ_p
enumeration of total coliforms	24	7.28	SQRTcfu/100ml	0.243	1.50
enumeration of <i>Escherichia coli</i>	25	5.83	SQRTcfu/100ml	0.164	1.50
colony count after 3 days at 22°C	16	11.34	SQRTcfu/ml	0.349	1.25
colony count after 2 days at 37°C	17	12.73	SQRTcfu/ml	0.337	1.25
enumeration of enterococci	17	7.28	SQRTcfu/100ml	0.191	1.25
enumeration of <i>Pseudomonas aeruginosa</i>	14	<i>14.06</i>	SQRTcfu/100ml	<i>0.706</i>	1.50
enumeration of <i>Clostridium perfringens</i>	11	9.80	SQRTcfu/100ml	0.382	1.50

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Table 9: Number and Percentage of z-Scores where $|z| \leq 2$

examination	number of scores where $ z \leq 2$	total number of scores	% $ z \leq 2$
enumeration of total coliforms	24	27	89
enumeration of <i>Escherichia coli</i>	26	28	93
colony count after 3 days at 22°C	15	16	94
colony count after 2 days at 37°C	14	17	82
enumeration of enterococci	16	17	94
enumeration of <i>Pseudomonas aeruginosa</i>	<i>10</i>	15	<i>67</i>
enumeration of <i>Clostridium perfringens</i>	9	13	69

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Table 10: Participants' Comments

participant number	comments (examination)
001	Colilert-18 IDEXX (Test Material A)
001	Enterolert-DW (IDEXX) and Pseudalert (IDEXX) (Test Material C)
007	Yes the method is accredited to EN ISO 6222- Enumeration of culturable micro-organism- Colony count by Inoculation in a nutrient agar culture mediums (Test Material B)
013	PCA (PLATE COUNT AGAR) (Test Material B)
016	Colilert 18 method according to ISO 9308-2:2012 was used (Test Material A)
019	All colonies show yellow halo under membrane (Test Material A)
025	ISO 4833:2003 colony count. pour plate method was used. (Test Material B)
030	Given sample DWM0110 was tested as per IS 15185: 2002 (Test Material A)
033	Preumptive TC= 43cfu/100ml, Presumptive EC=40cfu/100ml. All TC confirmed as EC, therefore 43 confirmed E.coli and 43 confirmed T.coliforms. (Test Material A)
033	very low results. new lenticule package. (Test Material B)

comments are as submitted by participants

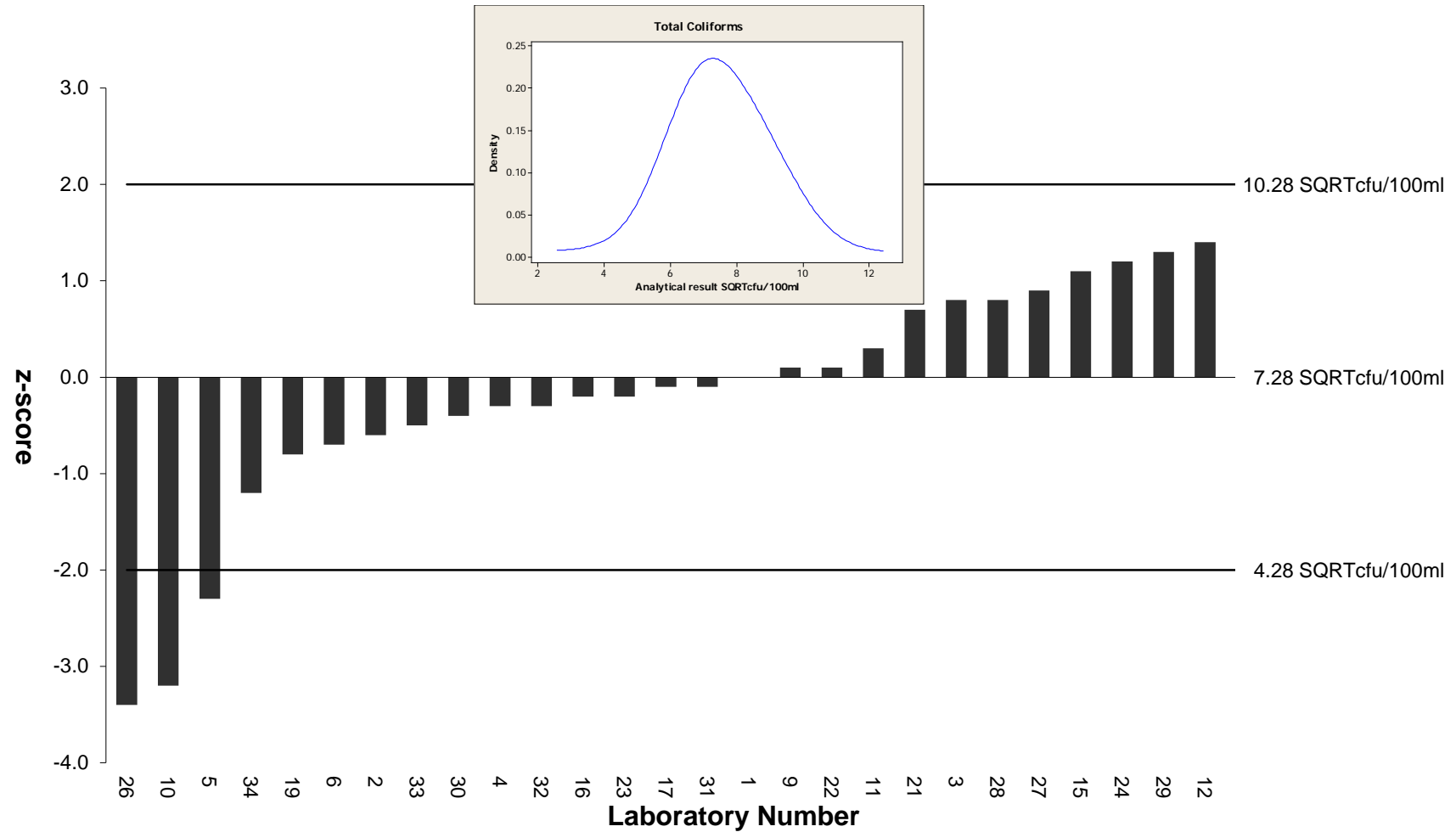


Figure 1: z-Scores for Enumeration of Total Coliforms

The insert presents the kernel density plot

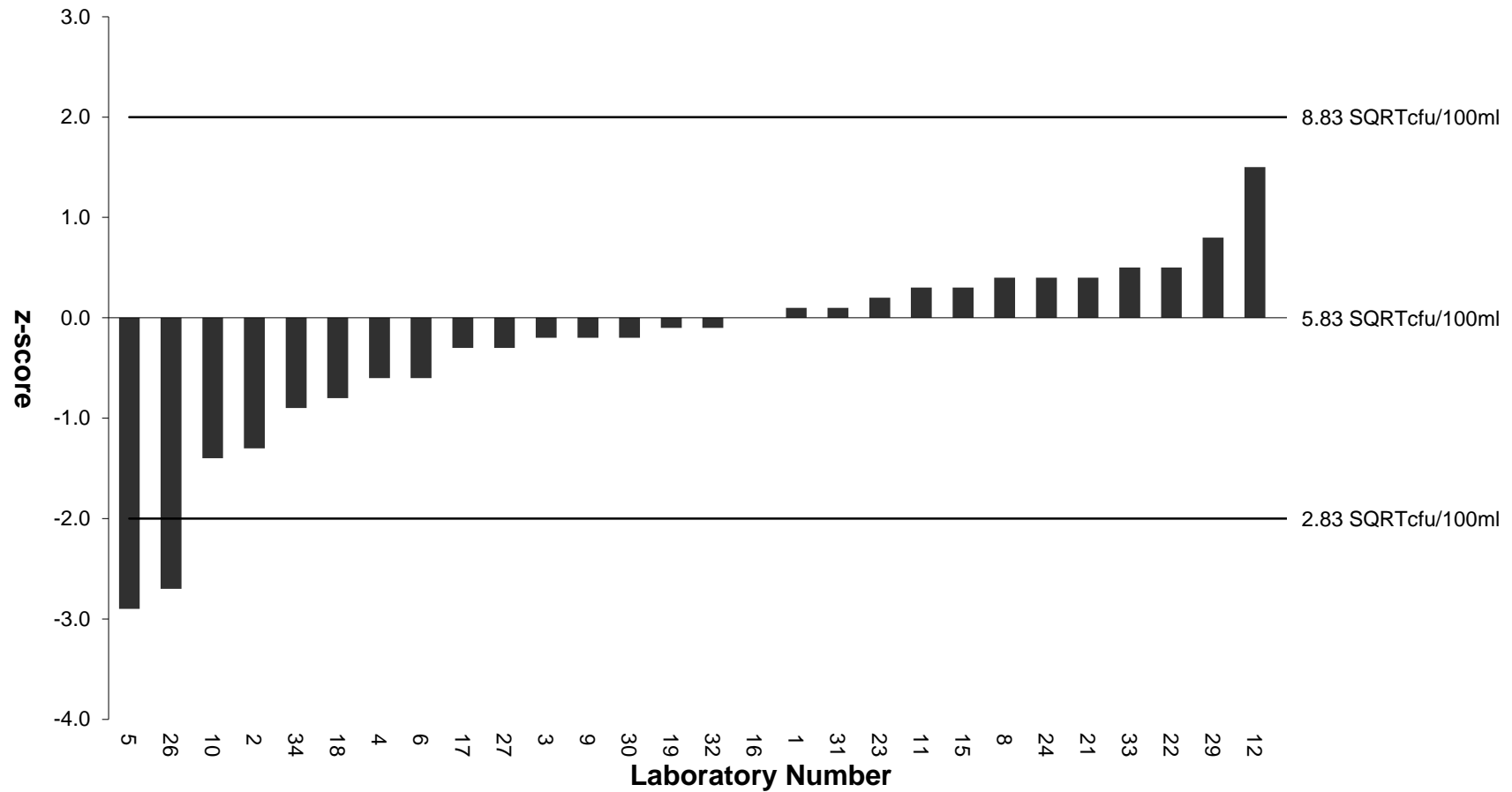


Figure 2: z-Scores for Enumeration of *Escherichia coli*

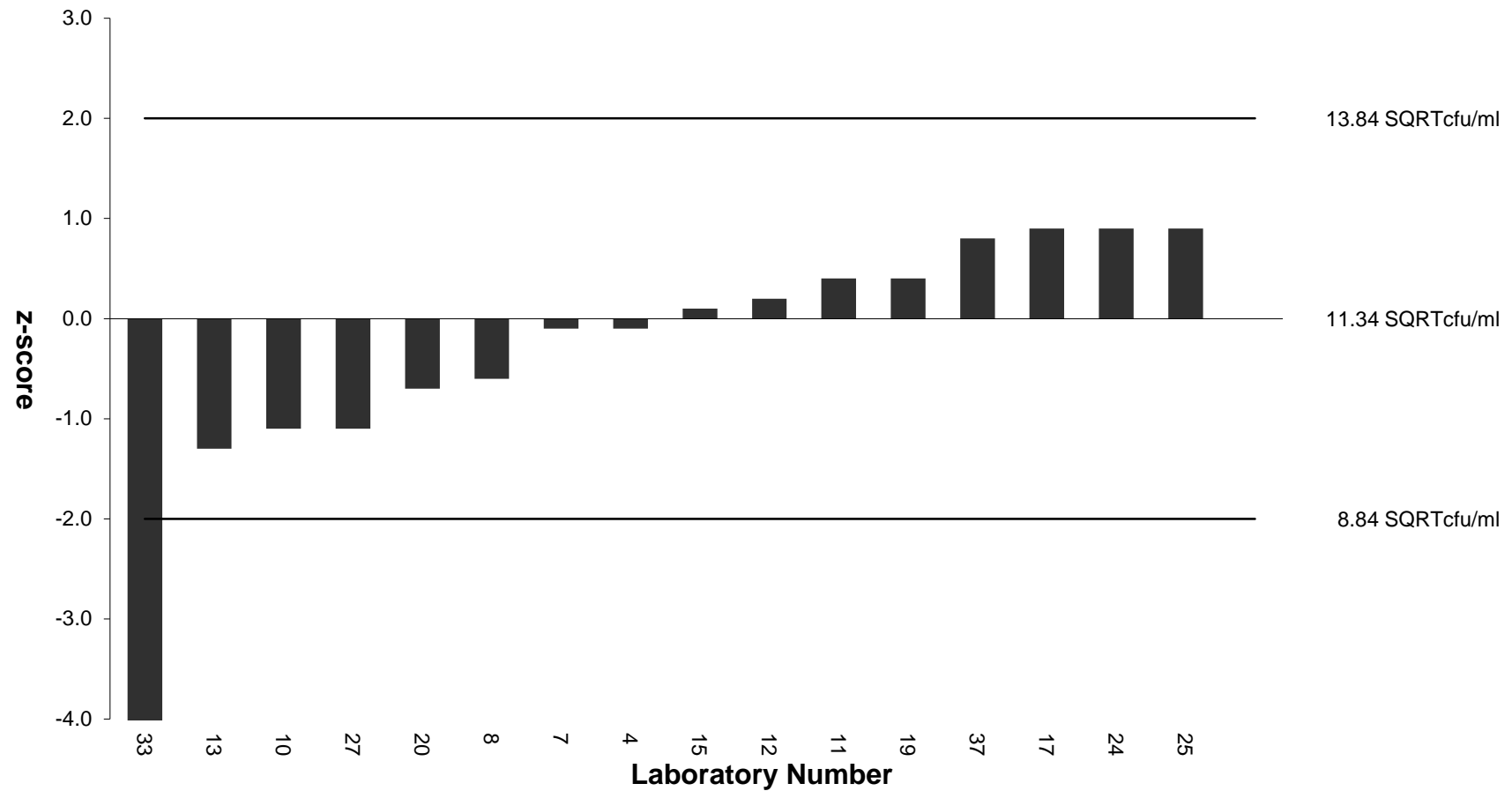


Figure 3: z-Scores for Enumeration for Colony Count after 3 days at 22°C

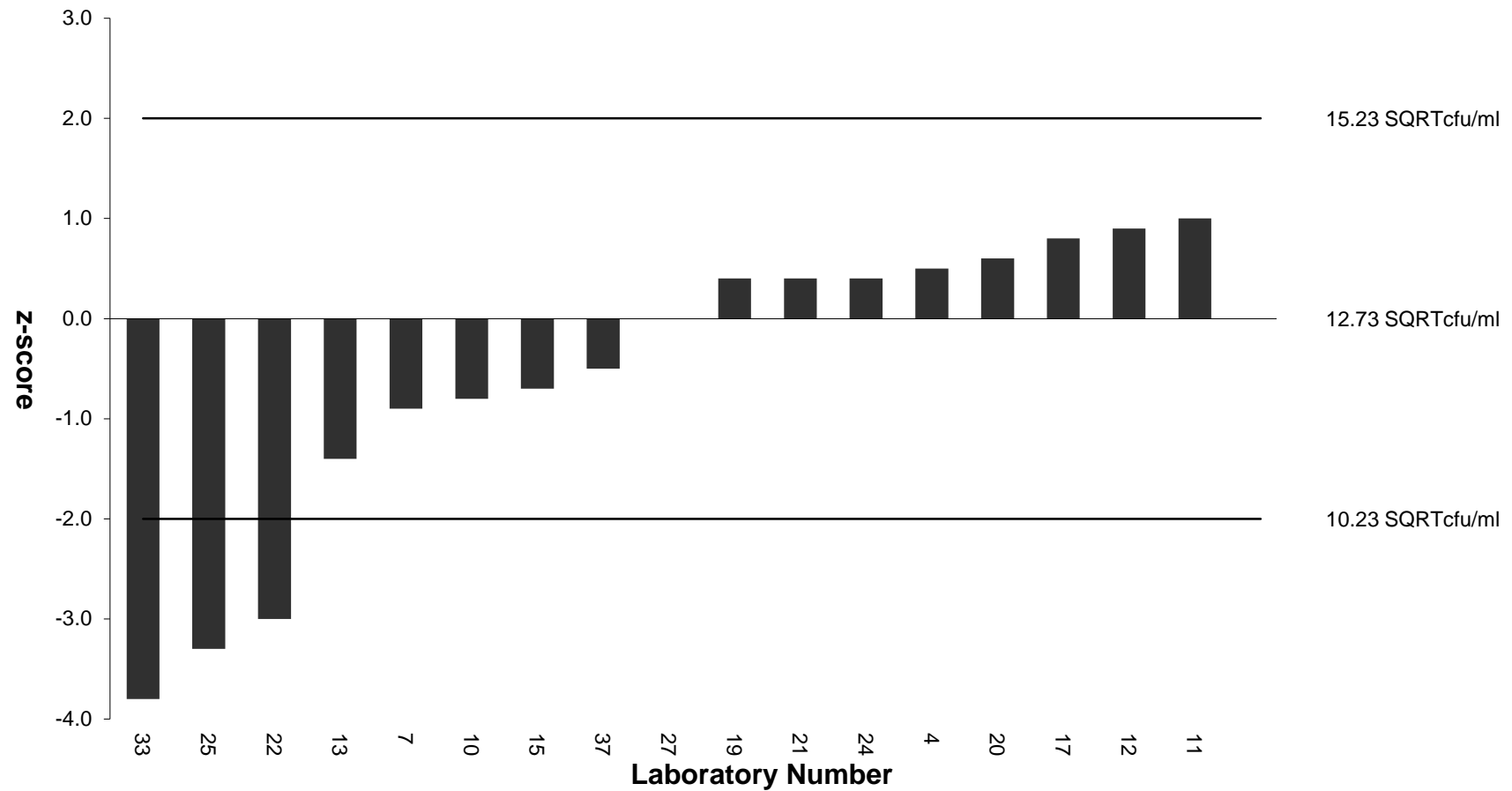


Figure 4: z-Scores for Enumeration for Colony Count after 2 days at 37°C

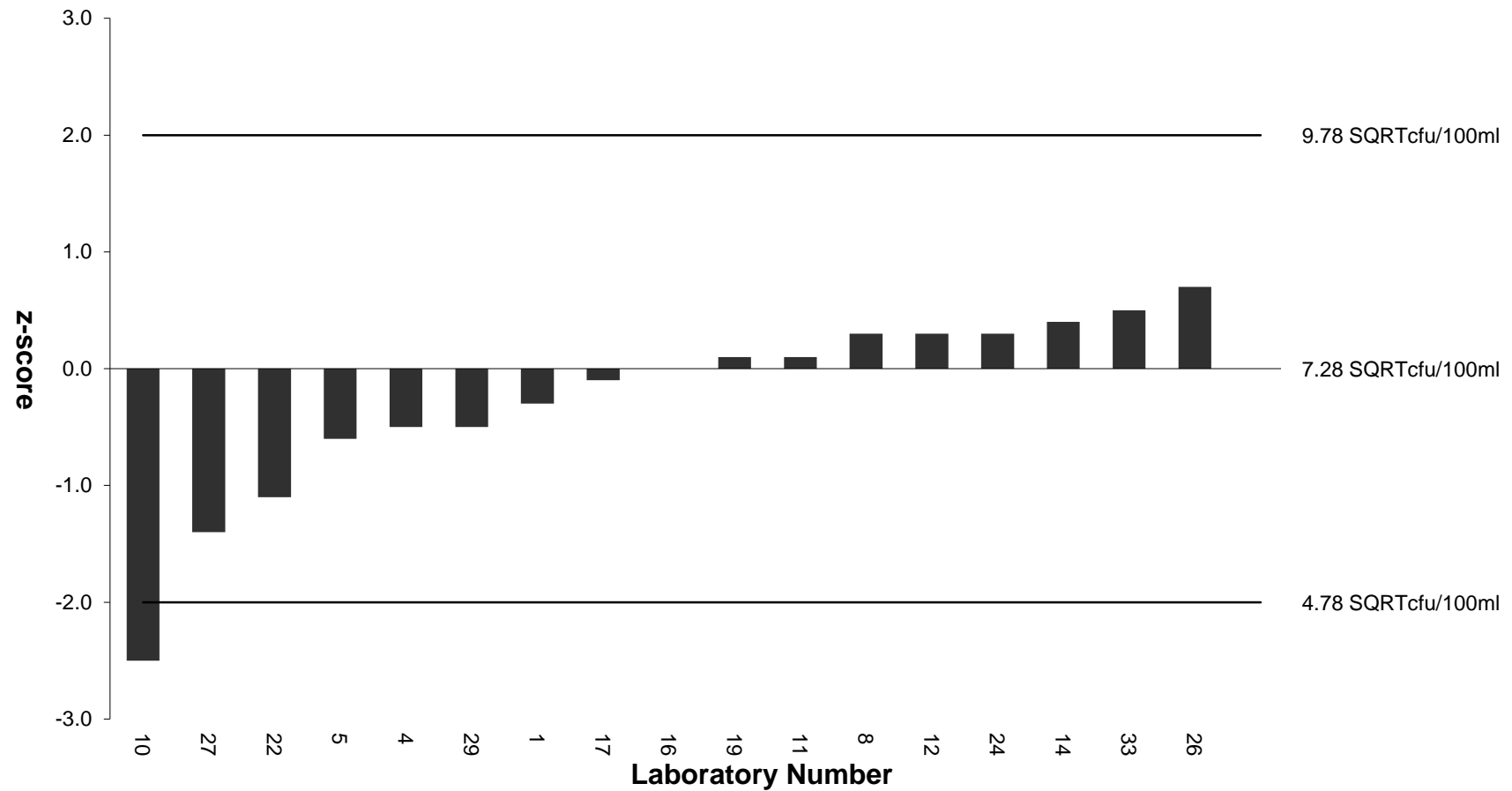


Figure 5: z-Scores for Enumeration of Enterococci

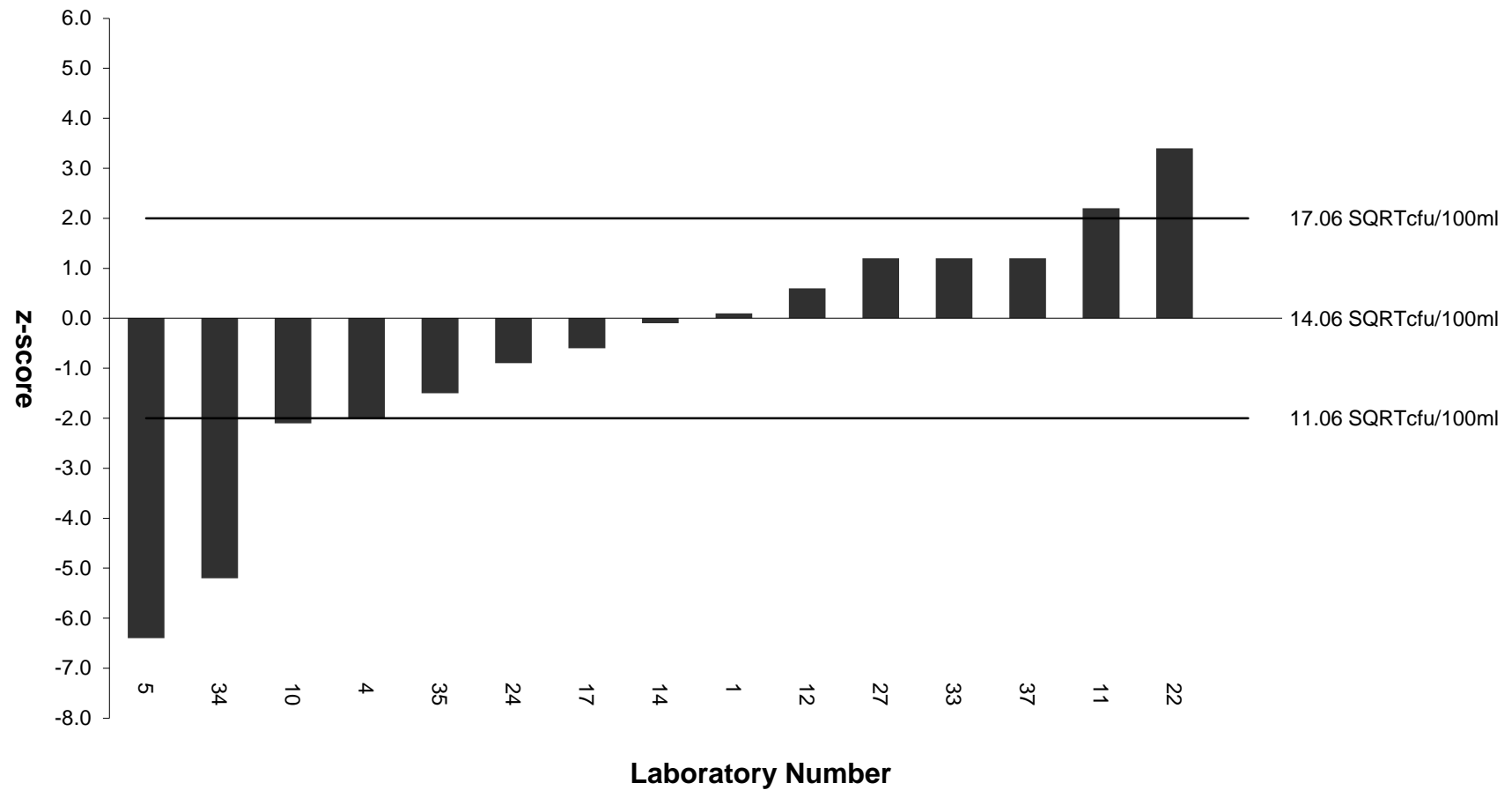


Figure 6: z-Scores for Enumeration of *Pseudomonas aeruginosa*

this histogram is given for information only

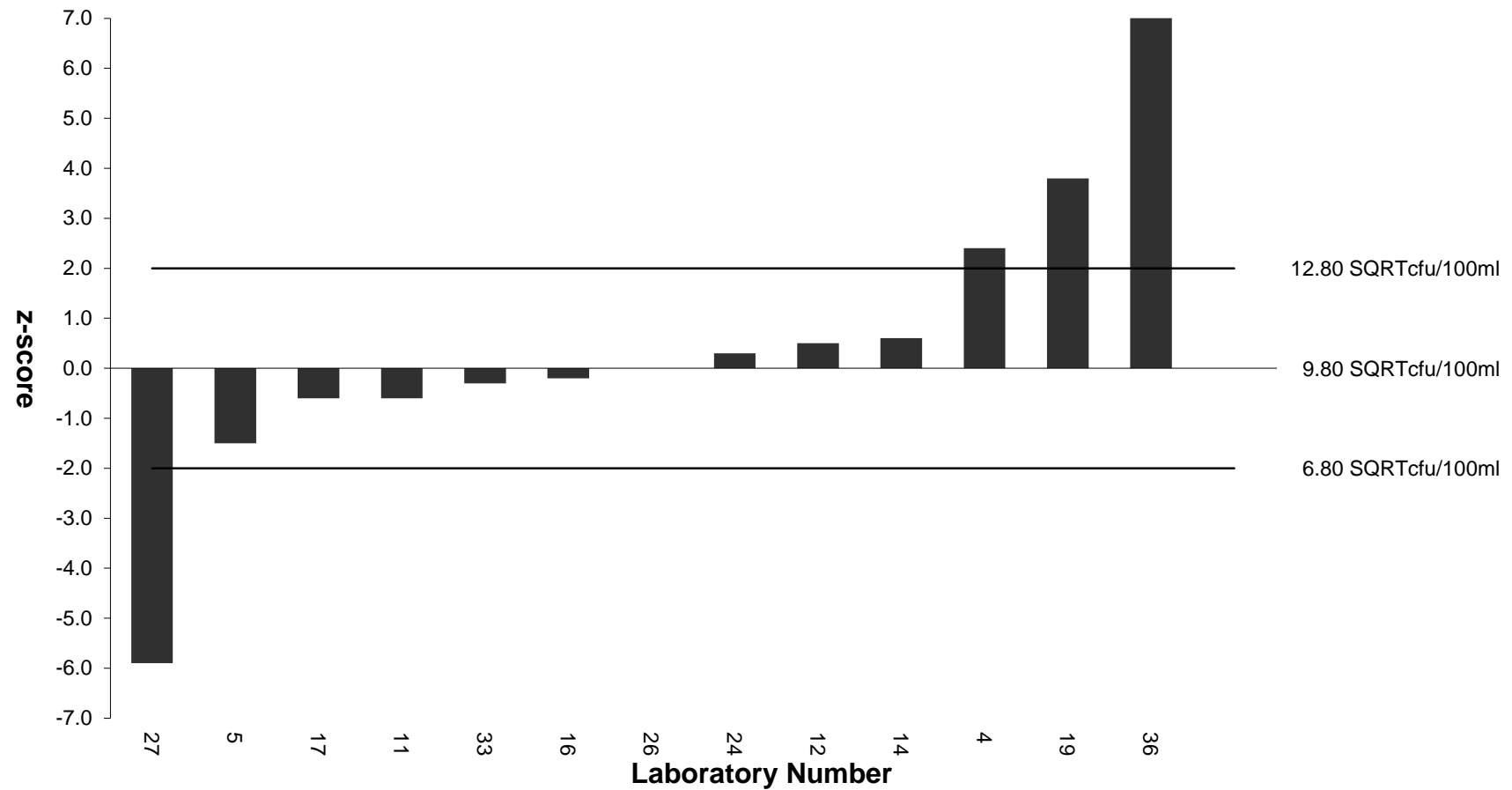


Figure 7: z-Scores for Enumeration of *Clostridium perfringens*

APPENDIX I: Analytical Methods Used by Participants

Methods are tabulated according to the information supplied by participants, but some responses may have been combined or edited for clarity.

Total Coliforms

Accredited Method Used	laboratory number
yes	002 004 011 012 017 019 021 022 023 024 027 028 029 030 031 032 033 034
no	001 005 016

National or International Standard (ISO)	laboratory number
ISO 9308-1:2014 (membrane filtration)	005 021 027 031
ISO 9308-2:2012 (MPN - most probable number)	001 012 016
SCA MoDW (2009) - Part 4	004 024 033
3M petrifilm	028
AOAC Official Method 2005.03	034
IS 15185:2002	030 032
IS:15185-2002	023
MKS EN ISO 9308-1	017
national methodic recomendation 4.2.1018-01	002
NF EN ISO 9308-1: 2000	019
Standard Methods for the examination of water and wastewater 22nd edn	022

If the method is not an ISO Standard, state a reference for the method.	laboratory number
9221	022
APHA9222B	021
IDEXX Colisure	034
ISO	032
ISO:9308-1:2000	023
national methodic recomendation 4.2.1018-01	002
The Microbiology of Drinking Water (2009) part 4	024

Method	laboratory number
membrane filtration	002 004 005 011 017 019 021 023 027 030 031 032 033
Colilert-18	012 016 024 029
multiple tube (MPN - most probable number)	022 034

Media	laboratory number
Colilert-18	001 012 016 024 029
Membrane Lactose Glucoronide Agar (MLGA)	004
Membrane Lauryl Sulphate Broth (MLSB)	033
Chromogenic Coliform Agar (CCA)	005 017 027 031
Lactose TTC Agar	023 030 032
3M petrifilm	028
Endo	002
IDEXX Colilert	034
Lauryal Tryptose Broth	022
m Endo agar	021
TTC Chapman Agar	019

Incubation Time (hours) / Incubation Temperature (°C)	laboratory number
4/30 then 14/37	004 027 031 033
18/35.5	001
18/37	024
20/37	016
22hrs / 37C	012
24/35	021 028
24/35	034
24/36	005
24/37	002 017 032
24/37 then 24/44	030
41 hours / 37°C	023
44 / 37	019
48/35	022

Confirmation of the identity of the colonies grown	laboratory number
yes	002 004 005 011 017 019 021 022 023 028 030 031 032 033
no	001 012 016 024 027 034

Number of colonies used for confirmation	laboratory number
1	022 028
10	004 017 032 033
2	005
45	030
5	011 031
7	021
92	019
all	002
Confirmed result	024

Confirmation Tests	laboratory number
Oxidase	002 004 005 017 019 023 030 032 033
Indole	004 030 031 032 033
Lactose fermentation	002 004 021 033
API	011 022

Confirmation (Selective Media)	laboratory number
Tryptone Nutrient Agar	031
Brilliant Green Bile Broth (BGBB)	011 021 022 030
Lactose Peptone Water	002 004
3M Petrifilm	028
confirmed result	024
lactose peptone water and tryptone water	033
Tryptone Soy Agar	005
Tryptone Water, Nutrient Agar	032

Escherichia coli

Accredited Method Used	laboratory number
yes	002 004 008 011 012 019 021 022 023 024 027 029 030 031 032 033 034
no	001 005 016 018

National or International Standard (ISO)	laboratory number
ISO 9308-1:2014 (membrane filtration)	005 021 027 031
ISO 9308-2:2012 (MPN - most probable number)	001 012 016
SCA MoDW (2009) - Part 4	004 008 024 033
AOAC Official Method 2005.03	034
APAT CNR IRSA 7030 metodo F man 29:2003	018
IS 15185:2002	030 032
IS:15185-2002	023
MKS EN ISO 9308-1	017
national standard 31955-2012	002
NF EN ISO 9308-1: 2000	019
Standard Methods for the examination of water and wastewater 22nd edn	022

If the method is not an ISO Standard, state a reference for the method.	laboratory number
9221 F	022
APAT CNR IRSA 7030 metodo F man 29:2003	018
APHA9221F	021
IDEXX Colisure	034
ISO	032
ISO:9308-1:2000	023
national standard 31955-2012	002
The Microbiology of Drinking Water (2009) part 4	024

Method	laboratory number
membrane filtration	002 004 005 008 011 017 018 019 021 023 027 030 031 032 033
Colilert-18	012 016 024
multiple tube (MPN - most probable number)	022 029 034

Media	laboratory number
Colilert-18	001 012 016 024 029
Membrane Lactose Glucoronide Agar (MLGA)	004 008
Membrane Lauryl Sulphate Broth (MLSB)	033
Chromogenic Coliform Agar (CCA)	005 017 027 031
Lactose TTC Agar	023 030 032
IDEXX Colilert	034
Lauryal Tryptose Broth	022
m Endo agar	021
mFC Agar	002
TBX	018
TTC Chapman Agar	019

Incubation Time (hours) / Incubation Temperature (°C)	laboratory number
4/30 then 14/37	004 027 031
4/30 then 14/44	008 033
18/35.5	001
18/37	024
18-24/44	018
20/37	016
22hrs / 37C	012
24/35	021
24/35	034
24/36	005
24/37	032
24/37 then 24/44	030
24/44	002 017
24/44.5	022
41 hours / 37°C	023
44 / 37	019

Confirmation of the identity of the colonies grown	laboratory number
yes	002 004 011 017 019 021 022 023 030 031 032 033
no	005 008 012 016 018 024 027 034

Number of colonies used for confirmation	laboratory number
1	022
10	004 017 032 033
45	030
5	011 021 031
92	019
all	002
Confirmed result	024
Not required	008

Confirmation Tests	laboratory number
Oxidase	004 017 023 030 032 033
Indole	002 004 017 019 023 030 031 032 033
beta-Galactosidase	021
Lactose fermentation	004 033
API	011 022

Confirmation (Selective Media)	laboratory number
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Tryptone Nutrient Agar	031
Brilliant Green Bile Broth (BGBB)	011 022
Lactose Peptone Water	004
Tryptone Water	023 030
confirmed result	024
EC MUG Broth	021
lactose peptone water and tryptone water	033
Not required	008
Tryptone Water, Nutrient Agar	032

Colony Counts (22°C/3 days)

Accredited Method Used	laboratory number
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yes	004 011 012 017 019 024 027 033
no	008

National or International Standard (ISO)	laboratory number
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ISO 6222:1999	027
SCA MoDW (2012) - Part 7	004 008 012 024 033
MKS EN ISO 6222	017
NF EN ISO 6222:1999	019

If the method is not an ISO Standard, state a reference for the method.	laboratory number
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The Microbiology of Drinking Water (2012)- Part 7- Methods for the enumeration of heterotrophic bacteria.	024
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Method	laboratory number
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Pour plate	004 008 012 017 019 024 027 033
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Media	laboratory number
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Yeast Extract Agar (YEA)	004 008 012 017 019 024 027 033
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Colony Counts (37°C/2 days)

Accredited Method Used	laboratory number
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yes	004 011 012 017 019 021 024 027 033
no	022

National or International Standard (ISO)	laboratory number
ISO 6222:1999	027
SCA MoDW (2012) - Part 7	004 012 024 033
membrane filtration	021
MKS EN ISO 6222	017
NF EN ISO 6222:1999	019
Standard Methods for the examination of water and wastewater 22nd edn	022

If the method is not an ISO Standard, state a reference for the method.	laboratory number
9230A,B	022
APHA9215D	021
The Microbiology of Drinking Water (2012)- Part 7- Methods for the enumeration of heterotrophic bacteria.	024

Method	laboratory number
Spread plate	022
Pour plate	004 012 017 019 024 027 033
membrane filtration	021

Media	laboratory number
Plate Count Agar (PCA)	022
Yeast Extract Agar (YEA)	004 012 017 019 024 027 033
m HPC agar	021

Enterococci

Accredited Method Used	laboratory number
yes	004 008 011 012 014 016 017 019 024 029
no	001 005 022 027 033

National or International Standard (ISO)	laboratory number
ISO 7899-2:2000	005 014 016 027
SCA MoDW (2012) - Part 5	004 008 012 024 033
MKS EN ISO 7899-2	017
NF EN ISO 7899-2	019
Standard Methods for the examination of water and wastewater 22nd edn	022

If the method is not an ISO Standard, state a reference for the method.	laboratory number
9230	022
The Microbiology of Drinking Water (2012)- Part 5-Methods for the isolation and enumeration of Enterococci by membrane filtration.	024

Method	laboratory number
membrane filtration	004 005 008 012 014 016 017 019 024 027 033
multiple tube (MPN - most probable number) Enterolert	022 001 029

Media	laboratory number
Slanetz and Bartley Agar	004 005 008 012 014 016 017 019 024 027 033
Azide dextrose	022

Incubation Time (hours) / Incubation Temperature (°C)	laboratory number
44/37	008 016
48/37	004 005 012 014 017 027 033
4/37 then 40/44	024
24/44	001
48/35	022
48/37 then 2/44	019

Confirmation of the identity of the colonies grown	laboratory number
yes	004 005 008 011 012 014 016 017 019 022 024
no	001 027 029 033

Number of colonies used for confirmation	laboratory number
10	004 012 017 024
100%	022
135 in 250 ml	019
26	008
42	005
5	011
61	014
All	016

Confirmation Tests	laboratory number
Aesculin hydrolysis by sub-culture	012 024
Aesculin hydrolysis by membrane transfer	008 014 016
Catalase reaction	004
Bile tolerance	004
Bile aesculin	017
Bile Esculin Azide Agar	005
pfizer selective enterococcus agar, Brain heart infusion broth	022
the membrane is transferred onto Bile Aesculin Azide agar and incubated at 44 degrees for 2 hrs.	019

Pseudomonas aeruginosa

Accredited Method Used	laboratory number
yes	004 011 012 017 019 022 027 033 034
no	001 005 014 024

National or International Standard (ISO)	laboratory number
ISO 16266:2006	005 014 027
SCA MoDW (2010) - Part 8	004 012 033
MKS EN ISO 16266	017
Standard Methods for the examination of water and wastewater 22nd edn	022
The Microbiology of Drinking Water (2002)- part 8-Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration.	024

If the method is not an ISO Standard, state a reference for the method.	laboratory number
9213F	022
IDEXX Pseudalert Test Kit	034
NF EN ISO 16266:2008	019
The Microbiology of Drinking Water (2002)- part 8-Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration.	024

Method	laboratory number
membrane filtration	004 005 012 014 017 019 024 027 033
multiple tube (MPN - most probable number)	022
Pseudalert	001 034

Media	laboratory number
Pseudomonas Agar with CFC supplement	004
Pseudomonas Agar with CN supplement	012 014 017 019 024 027 033
Asparagine broth	022
Cetrimide	005

Incubation Time (hours) / Incubation Temperature (°C)	laboratory number
48/37	004 005 012 014 017 024 027 033
24/35	034
44 / 36	019
48/36	022

Confirmation of the identity of the colonies grown	laboratory number
yes	004 005 011 012 014 017 019 022 024 033
no	027 034

Number of colonies used for confirmation	laboratory number
10	004 012 017 024 033
100%	022
12	014
5	005 011
confluent growth but all fluoresce in 250ml	019

Tests used to confirm identity	laboratory number
Oxidase	004 012 017 033
Casein hydrolysis	012 024 033
API 20 NE	011
Acetamide Broth	005
Acetamide, API-VITEK	022
fluoresce under UV;ability to produce ammonia from acetamide	014

Clostridium perfringens

Accredited Method Used	laboratory number
yes	011 012 014 016 017 019 024 036
no	004 005 027 033

National or International Standard (ISO)	laboratory number
ISO 6461-2:1986 (membrane filtration)	016
ISO 14189:2013 (membrane filtration)	014
SCA MoDW (2010) - Part 6	004 012 024 033 036
MKS EN ISO 26461-2	017
NF EN ISO 26461-2	019

If the method is not an ISO Standard, state a reference for the method.	laboratory number
98/83/EC	005
The Microbiology of Drinking Water (2010). Methods for the examination of waters and associated materials. Part 6 Methods for the isolation and enumeration of sulphite reducing clostridia and <i>Clostridium perfringens</i> by membrane filtration.	024

Method	laboratory number
anaerobic jar	004 012 014 033
membrane filtration	005 016 017 019 024 027 036

Media	laboratory number
Tryptose Sulphite Cycloserine Agar (TSC) without egg	012 014 017 024 027 033 036
mCP	016
Membrane Clostridium Perfringens (m-CP) Agar	005
Tryptose Sulphite without cycloserine	019

Incubation Time (hours) / Incubation Temperature (°C)	laboratory number
21/37	027
21/44	004 005 012 014 016 017 024 033 036
21/48	019

Confirmation of the identity of the colonies grown	laboratory number
yes	004 005 011 012 014 016 017 019 024
no	027 033 036

Number of colonies used for confirmation	laboratory number
10	004 012 017 024
120 in 50ml	019
18	014
5	011
56	005
All	016

Tests used to confirm identity	laboratory number
Nitrate/nitrite reduction	017
Lactose fermentation	004 017
API 20A	011
acid phosphatase reaction	012 014 016 024
ammonium hydroxide vapor	005

Method used for the acid phosphatase	laboratory number
by dropping reagent on the colonies	012 017
by smearing colony on pre-soaked filter paper	014 024
Exposure of colonies to ammonium hydroxide	016
Exposure to ammonium hydroxide vapor	005

APPENDIX II: Fapas[®] SecureWeb, Protocol and Contact Details

1. Fapas[®] SECUREWEB

Access to the secure area of our website is only available to participants in our proficiency tests. Please contact us if you require a UserID and Password. Fapas[®] SecureWeb allows participants to:

- Obtain their laboratory numbers for the proficiency tests in which they have participated.
- View the results they submitted in past and current proficiency tests.
- Submit their results and methods for current tests.
- Review future tests they have ordered.
- Order proficiency tests, reference materials and quality control materials.
- Freely download copies of reports, in Acrobat PDF format, of proficiency tests in which they have participated.

2. PROTOCOL

The Protocols [3, 4] set out how Fapas[®] – Water and Environmental is organised. Copies can be downloaded from our website.

3. CONTACT DETAILS

This report was prepared and authorised on behalf of Fapas[®] by Emma Hutton (Round Coordinator). Participants with any comments or concerns about this proficiency test should contact:

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