SADCMET Water PT 2018
Evaluation Workshop

Dr. K. Luden

Topics

- About me
- Proficiency Testing - General remarks
- Statistical evaluation
  - Assigned value - Algorithm A
  - standard deviation (in PT) - Limitation?!
  - z-score
- Evaluation of 10th PT

NLGA

- **N**iedersächsisches **L**andes**g**esundheits**a**mt
- Governmental Institute of Public Health of Lower Saxony
  - Consultancy / advice to Ministry of Health and public health services
  - department of environmental health: laboratory within public health services for drinking water, bathing water, pool and spas, cooling towers
  - PT for drinking water since 1995

NLGA PT

Drinking Water PT	Methods	Rounds/ year	Samples/ Round
E. coli / Coliform Bacteria	ISO 9308-1 (2001), ISO 9308-2, ISO 9308-1 (2014)	4	600
Colony Counts 22°C/36°C	ISO 6222 , TrinkwV 2001 Anl. 5 I d bb	4	600
Enterococci	ISO 7899-2, Chromocult®, Enterolert®-DW	4	600
Clostridium perfringens	TrinkwV 2001 Anl. 5, ISO 14189	2	500
Pseudomonas aeruginosa	ISO 16266, Pseudalert®	2	500
Legionella	ISO 11731-2, ISO 11731:1998, ISO 11731:2017	2/2	500
Other PTS	**Methods**	**Rounds/ year**	
E. coli (bathing water)	ISO 9308-3	1	300
Enterococci (bathing water)	ISO 7899-1, ISO 7899-2	1	300
Bacteriophages	ISO 10705-2	1	30

Samples

Participants: 800 from Germany, 40 from Austria/Switzerland

other countries: UK, Lithuania, Hungary, Luxembourg

Proficiency Testing

- different types for performance evaluation of
  - laboratory
  - method
  - material
    (e.g. reference material)

Proficiency Testing - Providers View

- Define the purpose of the PT
- Choose parameters and matrix (type of sample) taking into account methods used for analysis
- Choose level of contamination
- Prepare samples
- Collect reported data
- Evaluate
- Write a report (and distribute)
- provide further necessary and interesting information

Assigned value

ISO/IEC Guide 43-1:1997

Method	Determined by
known value	***specific test items***
certified reference material	definite methods
reference material	***analysis, measurement or comparison of the test item alongside a reference material or standard, traceable to a national or international standard***
Consensus values from expert laboratories	demonstrable competence in the determination of the measurand, using validated methods known to be highly precise and accurate, and comparable to methods in general use (e.g. Reference Laboratories)
Consensus values from participant laboratories	***using statistics... (no details given) with consideration of the effects of extreme values***

Assigned value

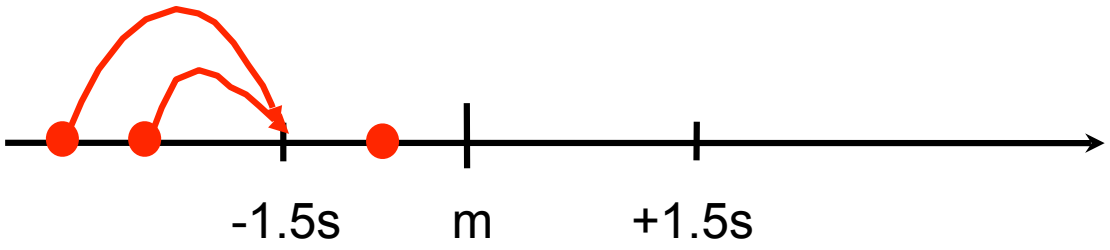
ISO 13528:2009

Statistical methods for use in proficiency testing by interlaboratory comparisons

- details for consensus value from participants results:
- advantage: easy to realise, cheap and particularly useful with operationally defined measurement methods
- Disadvantage: biased if the results are biased
- There might be no consensus
- e.g. Algorithm A

Algorithm A – ISO 13528

- robust estimates of the mean and standard deviation of the data to which it is applied
- starts with median and MAD $s^*=1.483 \times \text{median } |x_i - x^*|$
- limit data at $x^* + 1.5s^*$ and $x^* - 1.5x^*$
- extreme values trimmed to $1.5x^*$
- Calculation of Arithmetic mean and SD repeated (*iterative calculation possible*)



Algorithm A

				iteration		
		x_i	$\lvert x_i - med(x_i)\rvert$			
				1	2	3
S=1,5 x s*				2,26254	2,27852	2,29952
x* - S				2,63644	2,52919	2,49037
x* + S	CFU	sqr		7,16152	7,08624	7,08941
1	2	1,41	3,48	2,63644	2,52919	2,49037
2	2	1,41	3,48	2,63644	2,52919	2,49037
3	3	1,73	3,17	2,63644	2,52919	2,49037
4	3	1,73	3,1669287	2,63644	2,52919	2,49037
5	3	1,73	3,1669287	2,63644	2,52919	2,49037
6	4	2,00	2,8989795	2,63644	2,52919	2,49037
7	5	2,24	2,6629115	2,63644	2,52919	2,49037
8	7	2,65	2,2532282	2,64575	2,64575	2,64575
9	7	2,65	2,2532282	2,64575	2,64575	2,64575
53	37	6,08	1,183783	6,08276	6,08276	6,08276
54	42	6,48	1,5817612	6,48074	6,48074	6,48074
55	43	6,56	1,658459	6,55744	6,55744	6,55744
56	62	7,87	2,9750284	7,16152	7,08624	7,08941
57	75	8,66	3,7612746	7,16152	7,08624	7,08941
58	76	8,72	3,8188184	7,16152	7,08624	7,08941
59	94	9,70	4,7963802	7,16152	7,08624	7,08941
Median x*		4,89898	1,01710	4,80772	4,78989	4,78550
s*		1,50836		1,51902	1,53302	1,54236

z-score

The *z*-score for a proficiency test result x_i is calculated as:

$$z_i = (x_i - x_{pt}) / \sigma_{pt}$$

Where

x_{pt} is the assigned value, and

σ_{pt} is the standard deviation for proficiency assessment

$$\text{z-score} = \frac{\text{test result} - \text{assigned value}}{\text{standard deviation } pt}$$

PT 2018

- material used: freeze dried material from Swedish National Food Administration (NFA)
- distribution of bacteria in the material usually approximates normal distribution after square root transformation
- ***obvious outliers removed***
- square root transformed results are used in the evaluation of this PT
- Algorithm A applied

- acceptable standard deviation limited for evaluation
- z-scores are calculated of the transformed results

Evaluation procedure

- square root transformation of the results: x to $\sqrt{x}$
- calculation of mean x^* and standard deviation s^* using Algorithm A (robust method)
- the acceptable maximal standard deviation for the PT based on the experience was decided to be 15% or 20 % depending on the parameter
  therefore the target value x_{pt} and standard deviation σ_{pt} of the PT were assigned as:

$$x^* = x_{pt}$$

$$s^* = \sigma_{pt} \quad \text{if} \quad s^* < 0.15x_{pt}$$

$$0.15x_{pt} = \sigma_{pt} \quad \text{if} \quad s^* \geq 0.15x_{pt}$$

Algorithm A

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PT 2018

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- Algorithm A applied
- acceptable standard deviation limited for evaluation
- z-scores are calculated of the transformed results

Evaluation procedure

- range:

  lower warning signal $x_{pt} - 2\sigma_{pt}$

  upper warning signal $x_{pt} + 2\sigma_{pt}$

  lower action signal $x_{pt} - 3\sigma_{pt}$

  upper action signal $x_{pt} + 3\sigma_{pt}$

- retransformation (square all values)
- rounding to whole numbers (results are counts and there are no parts of bacteria)

Example: Coliform Bacteria

x_{pt} = 5.420 calculated by using **Algorithm A of** participants results

σ = 1.515 **28 %** of x_{pt}

σ_{pt} = 0.813 **15 %** of x_{pt}

calculation of range

lower warning limit (LWL) $x_{pt} - 2\sigma_{pt} = 5.420 - (2*0.813) = 3.794$

upper warning limit (UWL) $x_{pt} + 2\sigma_{pt} = 5.420 + (2*0.813) = 7.046$

retransformation **rounding**

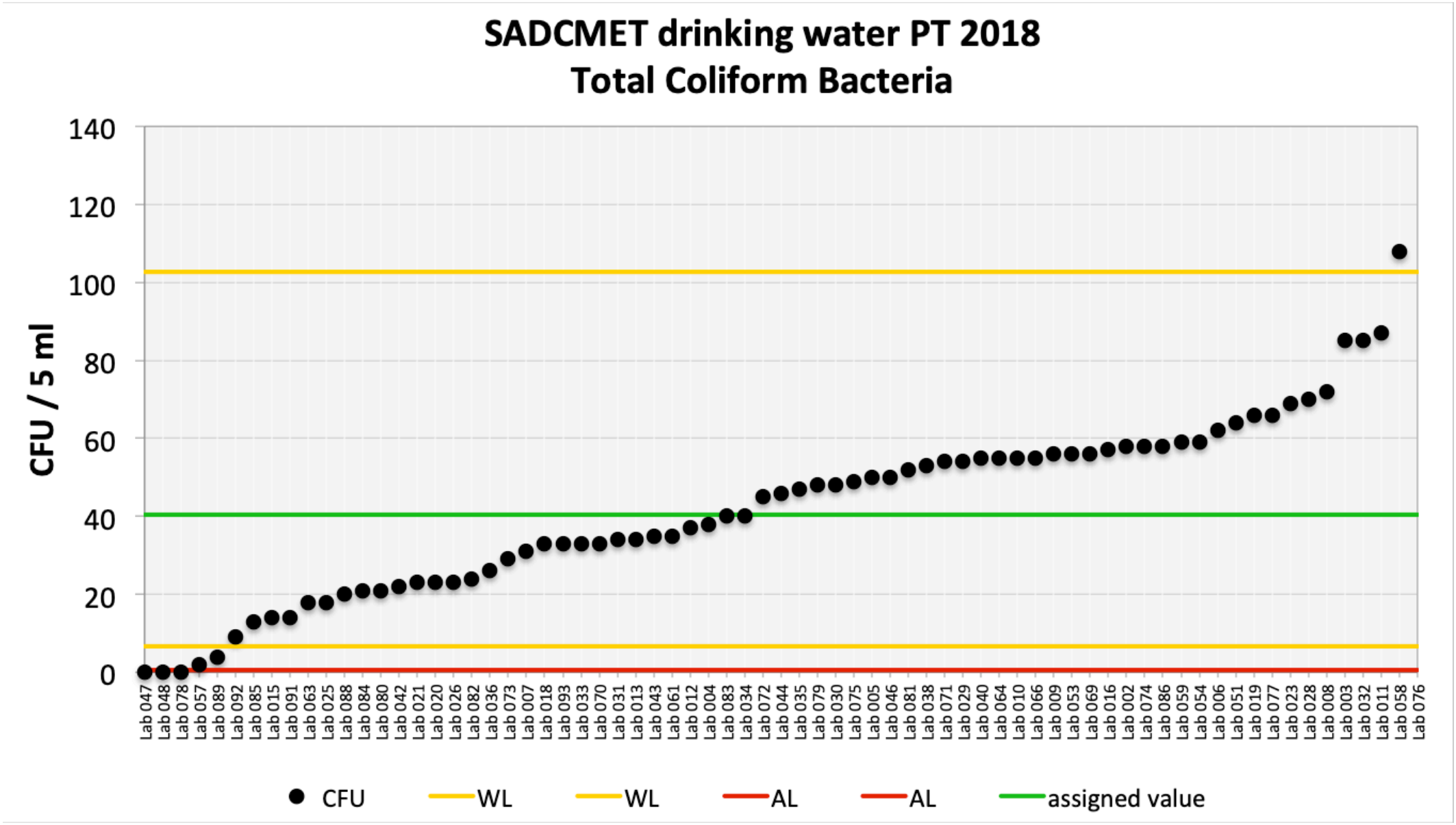
assigned value (CFU/10 ml): $x_{pt}^2 = (5.420)^2 = 29.38 \Rightarrow$ **29**

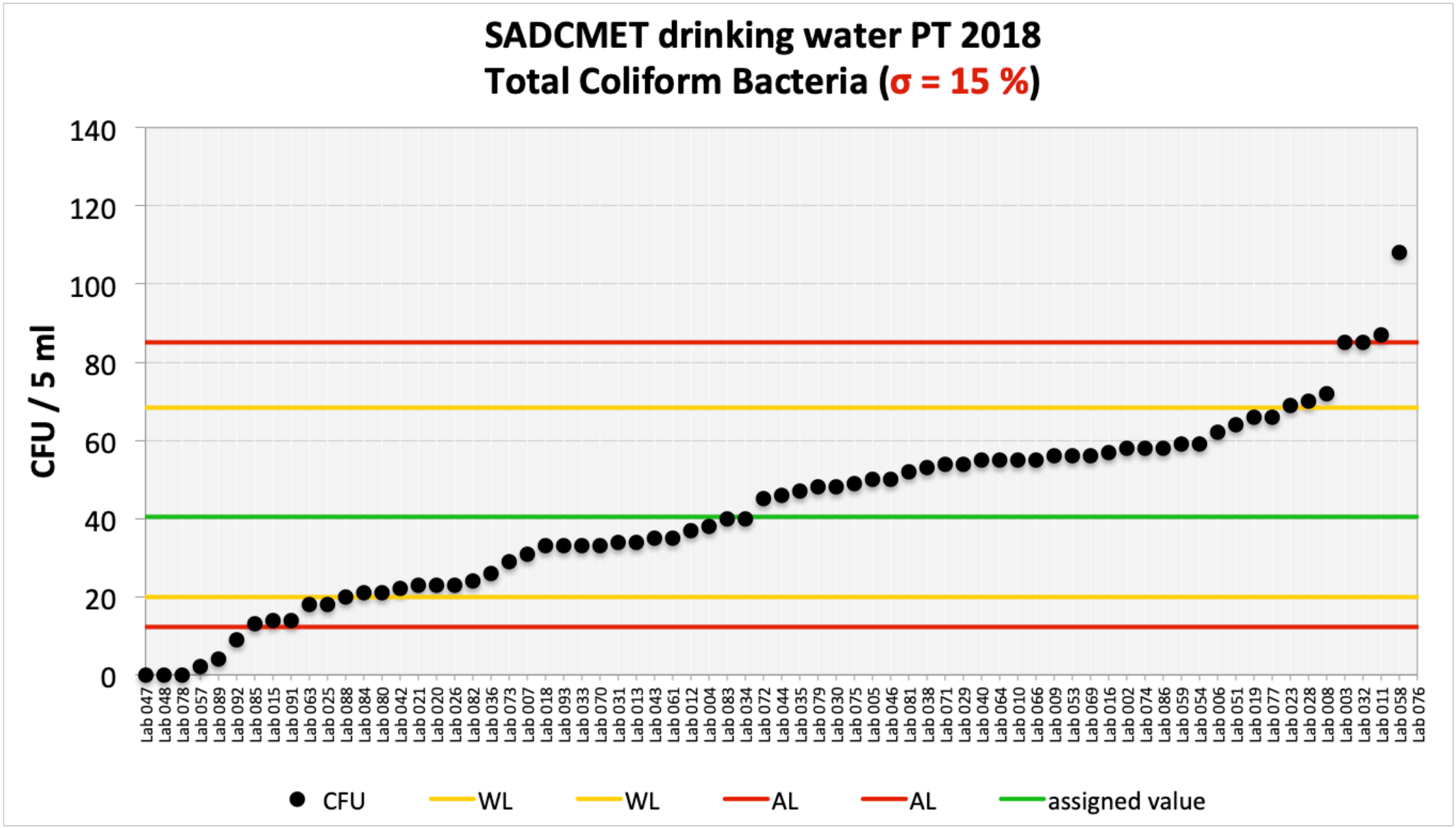
lower warning limit (LWL): $(x_{pt} - 2\sigma_{pt})^2 = (3.794)^2 = 14.40 \Rightarrow$ **14**

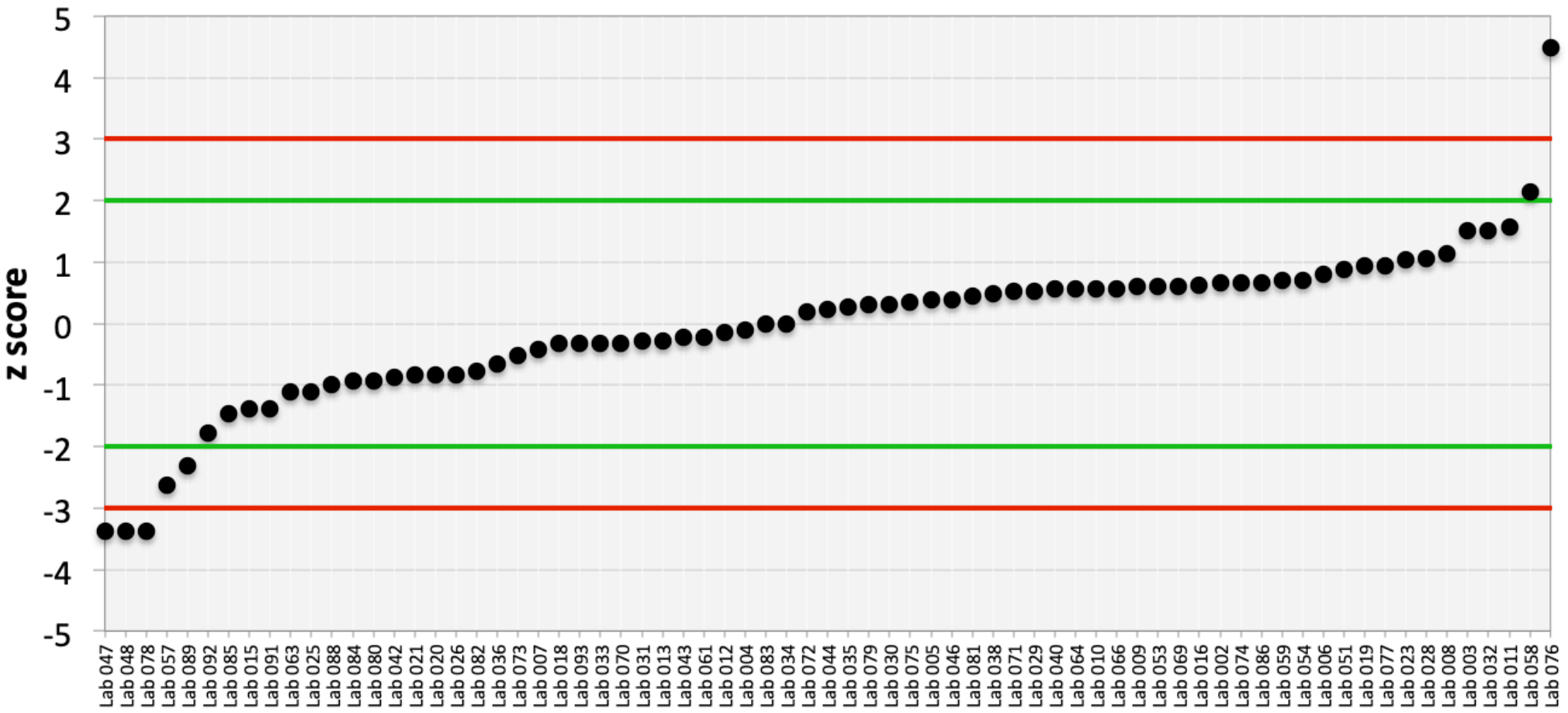
upper warning limit (UWL): $(x_{pt} + 2\sigma_{pt})^2 = (7.046)^2 = 49.65 \Rightarrow$ **50**

Summary

Parameter	Vol.	AlgA	SD AlgA	n	assigned value CFU	RSD %	RSD (in sqr)	σ	comment
Total Coliform Bacteria	5 ml	6,358	1,887	71	40	30	0,15	0,954	All values included
Coliform bacteria at 44°C	5 ml	5,088	2,503	54	26	49	0,15	0,763	All values included
E. coli	5 ml	4,535	1,880	63	21	41	0,15	0,680	1100 excluded
Intestinal Enterococci	5 ml	8,767	1,271	46	77	14			All values included
P. aeruginosa	5 ml	4,294	1,416	44	18	33	0,20	0,859	All values included
C. perfringens	5 ml	3,928	2,657	33	15	68	0,20	0,786	All values included
Standard Plate Count 36°C	1 ml	5,478	1,288	62	30	24	0,15	0,822	All values included
Standard Plate Count 22°C	1 ml	5,603	1,266	48	31	23	0,15	0,840	All values included


SADCMET drinking water PT 2018
Total Coliform Bacteria
CFU / 5 ml
140
120
100
80
60
40
20
0
Lab 047
Lab 048
Lab 078
Lab 057
Lab 089
Lab 092
Lab 085
Lab 015
Lab 091
Lab 063
Lab 025
Lab 088
Lab 084
Lab 080
Lab 042
Lab 021
Lab 020
Lab 026
Lab 082
Lab 036
Lab 073
Lab 007
Lab 018
Lab 093
Lab 033
Lab 070
Lab 031
Lab 013
Lab 043
Lab 061
Lab 012
Lab 004
Lab 083
Lab 034
Lab 072
Lab 044
Lab 035
Lab 079
Lab 030
Lab 075
Lab 005
Lab 046
Lab 081
Lab 038
Lab 071
Lab 029
Lab 040
Lab 064
Lab 010
Lab 066
Lab 009
Lab 053
Lab 069
Lab 016
Lab 002
Lab 074
Lab 086
Lab 059
Lab 054
Lab 006
Lab 051
Lab 019
Lab 077
Lab 023
Lab 028
Lab 008
Lab 003
Lab 032
Lab 011
Lab 058
Lab 076
CFU
WL
WL
AL
AL
assigned value


SADCMET drinking water PT 2018
Total Coliform Bacteria (σ = 15 %)
CFU / 5 ml
140
120
100
80
60
40
20
0
Lab 047 Lab 048 Lab 078 Lab 057 Lab 089 Lab 092 Lab 085 Lab 015 Lab 091 Lab 063 Lab 025 Lab 088 Lab 084 Lab 080 Lab 042 Lab 021 Lab 020 Lab 026 Lab 082 Lab 036 Lab 073 Lab 007 Lab 018 Lab 093 Lab 033 Lab 070 Lab 031 Lab 013 Lab 043 Lab 061 Lab 012 Lab 004 Lab 083 Lab 034 Lab 072 Lab 044 Lab 035 Lab 079 Lab 030 Lab 075 Lab 005 Lab 046 Lab 081 Lab 038 Lab 071 Lab 029 Lab 040 Lab 064 Lab 010 Lab 066 Lab 009 Lab 053 Lab 069 Lab 016 Lab 002 Lab 074 Lab 086 Lab 059 Lab 054 Lab 006 Lab 051 Lab 019 Lab 077 Lab 023 Lab 028 Lab 008 Lab 003 Lab 032 Lab 011 Lab 058 Lab 076
CFU
WL
WL
AL
AL
assigned value


SADCMET drinking water PT 2018
Total Coliform Bacteria
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 047
Lab 048
Lab 078
Lab 057
Lab 089
Lab 092
Lab 085
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Lab 091
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Lab 043
Lab 061
Lab 012
Lab 004
Lab 083
Lab 034
Lab 072
Lab 044
Lab 035
Lab 079
Lab 030
Lab 075
Lab 005
Lab 046
Lab 081
Lab 038
Lab 071
Lab 029
Lab 040
Lab 064
Lab 010
Lab 066
Lab 009
Lab 053
Lab 069
Lab 016
Lab 002
Lab 074
Lab 086
Lab 059
Lab 054
Lab 006
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Lab 003
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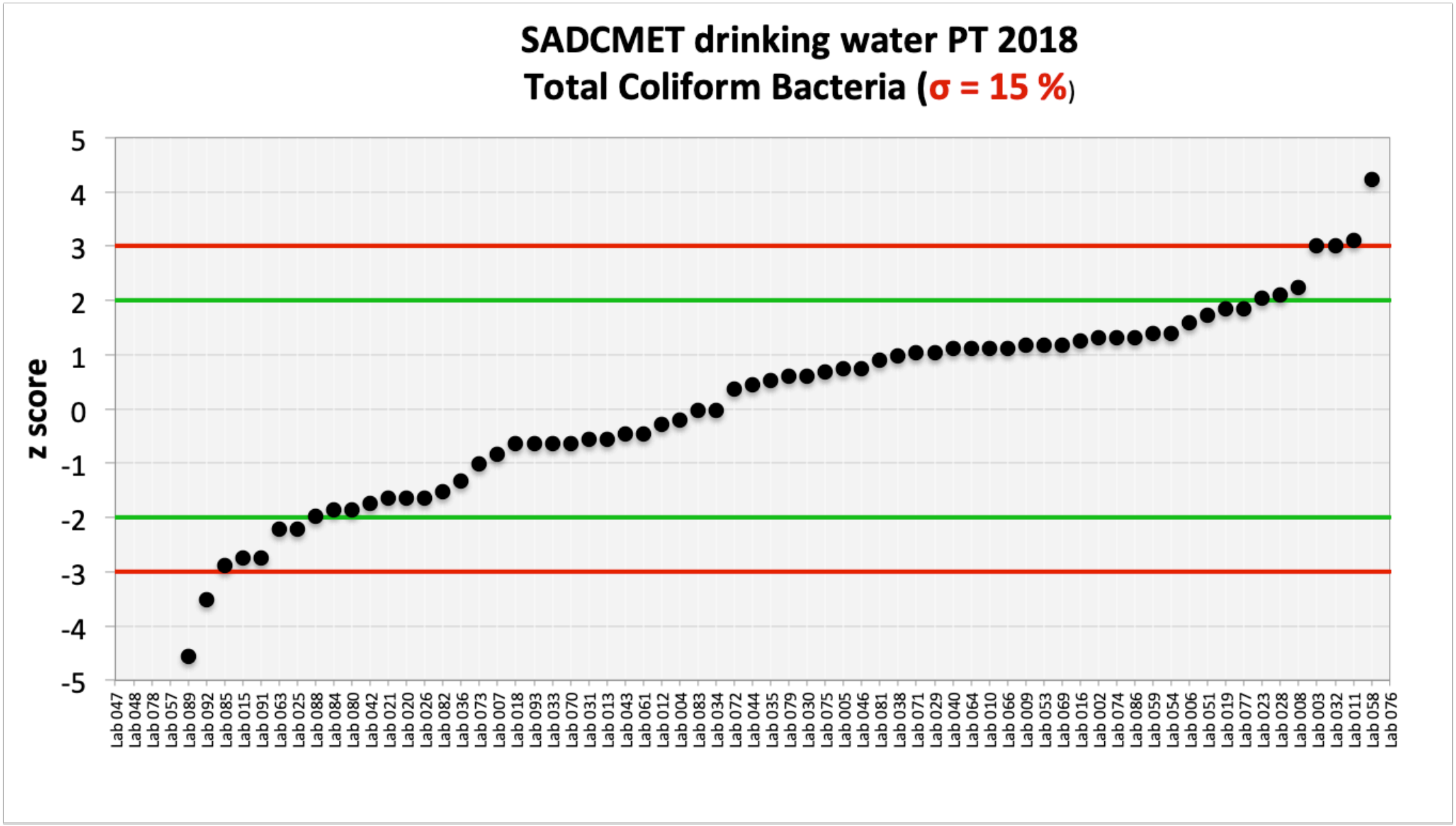
Lab 011
Lab 058
Lab 076


SADCMET drinking water PT 2018
Total Coliform Bacteria (σ = 15 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 047
Lab 048
Lab 078
Lab 057
Lab 089
Lab 092
Lab 085
Lab 015
Lab 091
Lab 063
Lab 025
Lab 088
Lab 084
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Lab 026
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Lab 036
Lab 073
Lab 007
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Lab 093
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Lab 012
Lab 004
Lab 083
Lab 034
Lab 072
Lab 044
Lab 035
Lab 079
Lab 030
Lab 075
Lab 005
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Lab 054
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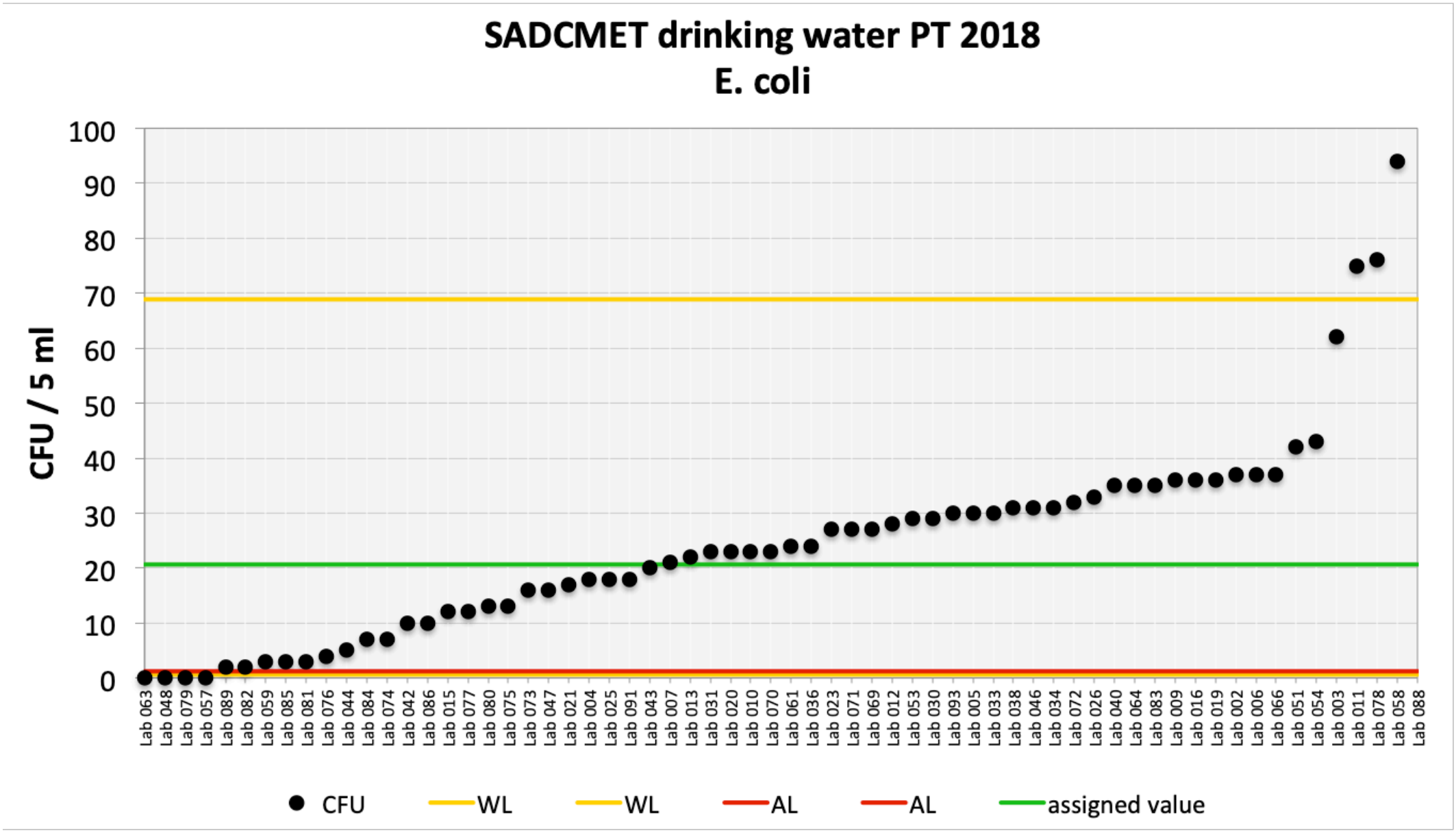
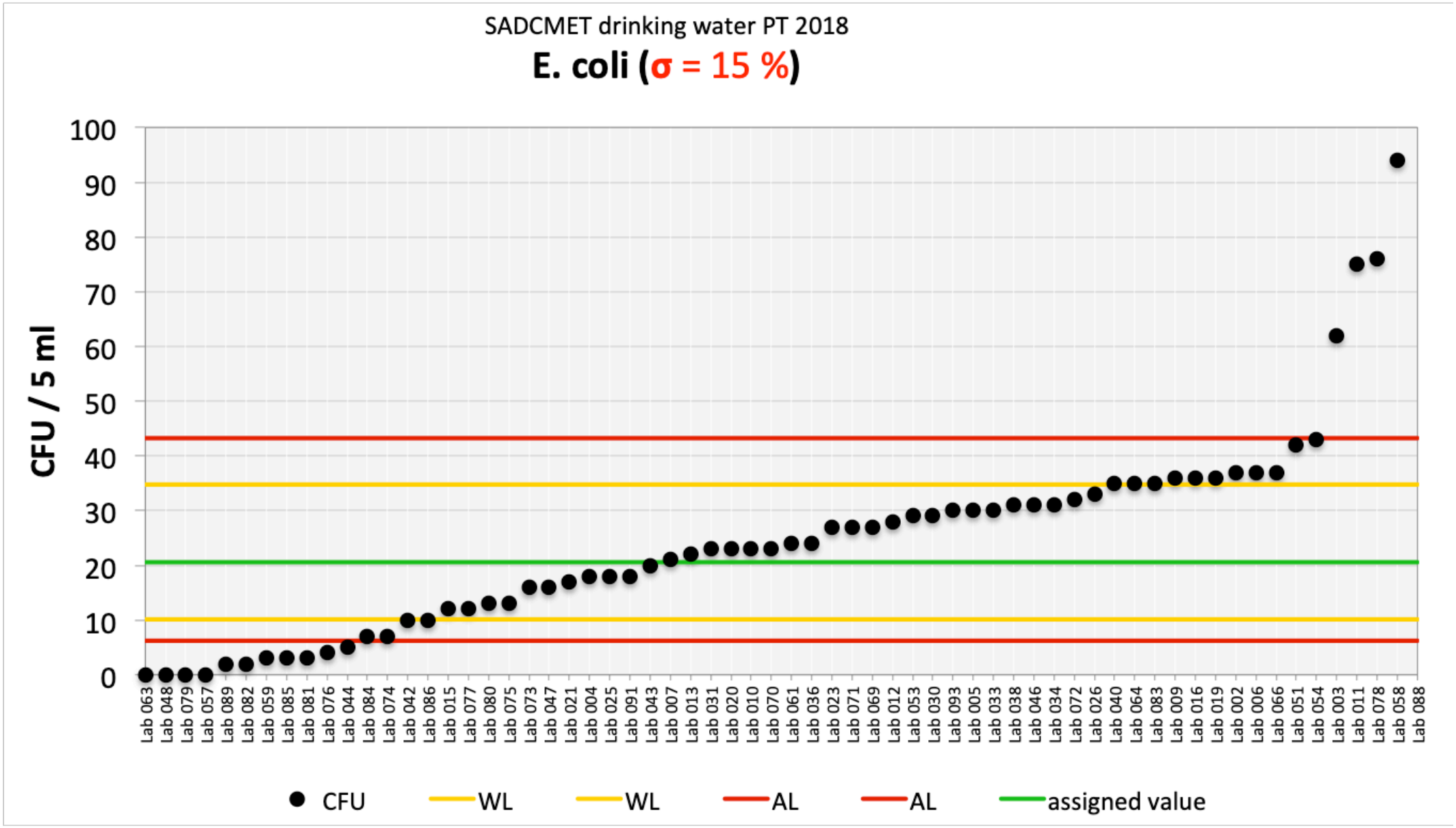
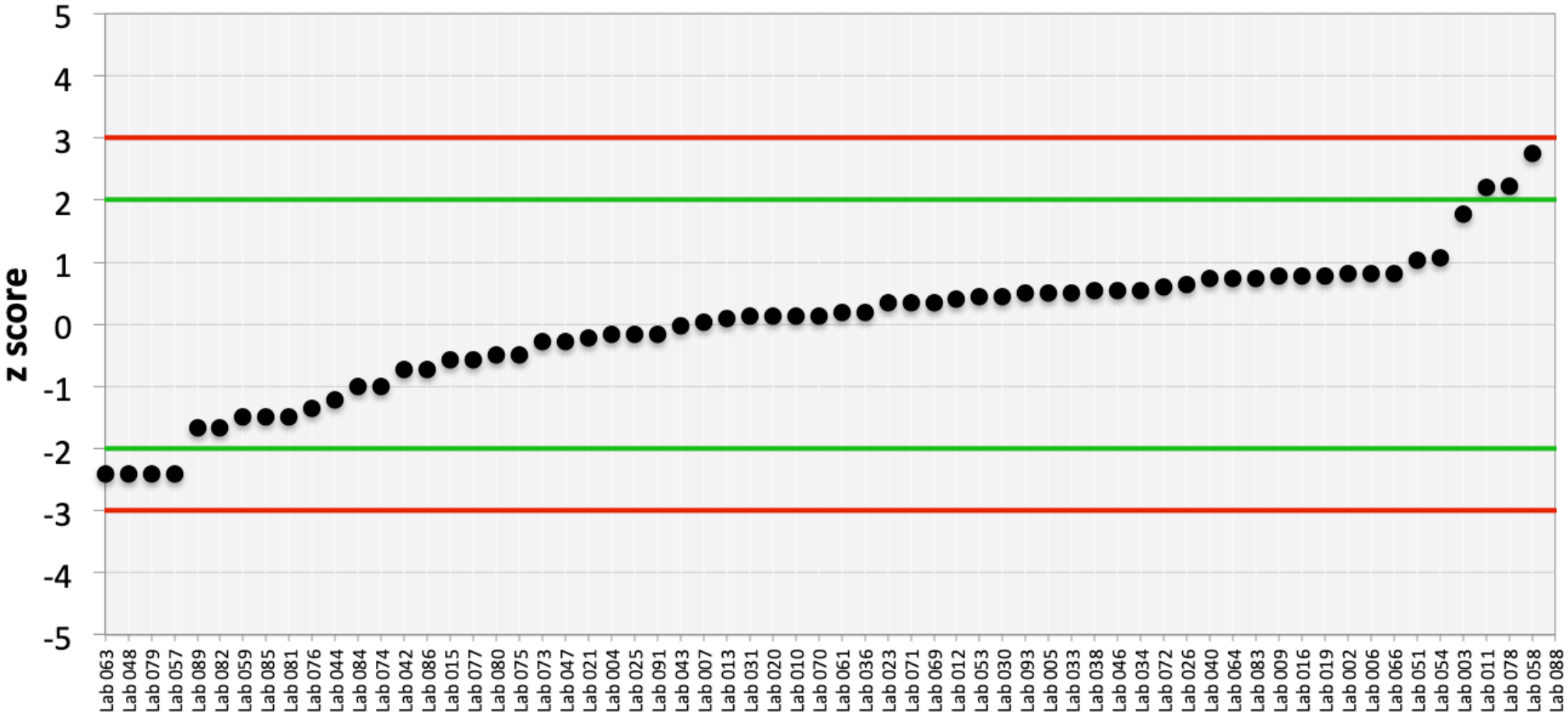
Lab 003
Lab 032
Lab 011
Lab 058
Lab 076


SADCMET drinking water PT 2018
E. coli
CFU / 5 ml
100
90
80
70
60
50
40
30
20
10
0
Lab 063
Lab 048
Lab 079
Lab 057
Lab 089
Lab 082
Lab 059
Lab 085
Lab 081
Lab 076
Lab 044
Lab 084
Lab 074
Lab 042
Lab 086
Lab 015
Lab 077
Lab 080
Lab 075
Lab 073
Lab 047
Lab 021
Lab 004
Lab 025
Lab 091
Lab 043
Lab 007
Lab 013
Lab 031
Lab 020
Lab 010
Lab 070
Lab 061
Lab 036
Lab 023
Lab 071
Lab 069
Lab 012
Lab 053
Lab 030
Lab 093
Lab 005
Lab 033
Lab 038
Lab 046
Lab 034
Lab 072
Lab 026
Lab 040
Lab 064
Lab 083
Lab 009
Lab 016
Lab 019
Lab 002
Lab 006
Lab 066
Lab 051
Lab 054
Lab 003
Lab 011
Lab 078
Lab 058
Lab 088
CFU
WL
WL
AL
AL
assigned value


SADCMET drinking water PT 2018
E. coli (σ = 15 %)
CFU / 5 ml
100
90
80
70
60
50
40
30
20
10
0
Lab 063 Lab 048 Lab 079 Lab 057 Lab 089 Lab 082 Lab 059 Lab 085 Lab 081 Lab 076 Lab 044 Lab 084 Lab 074 Lab 042 Lab 086 Lab 015 Lab 077 Lab 080 Lab 075 Lab 073 Lab 047 Lab 021 Lab 004 Lab 025 Lab 091 Lab 043 Lab 007 Lab 013 Lab 031 Lab 020 Lab 010 Lab 070 Lab 061 Lab 036 Lab 023 Lab 071 Lab 069 Lab 012 Lab 053 Lab 030 Lab 093 Lab 005 Lab 033 Lab 038 Lab 046 Lab 034 Lab 072 Lab 026 Lab 040 Lab 064 Lab 083 Lab 009 Lab 016 Lab 019 Lab 002 Lab 006 Lab 066 Lab 051 Lab 054 Lab 003 Lab 011 Lab 078 Lab 058 Lab 088
CFU
WL
WL
AL
AL
assigned value


SADCMET drinking water PT 2018
E. coli
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 063
Lab 048
Lab 079
Lab 057
Lab 089
Lab 082
Lab 059
Lab 085
Lab 081
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Lab 074
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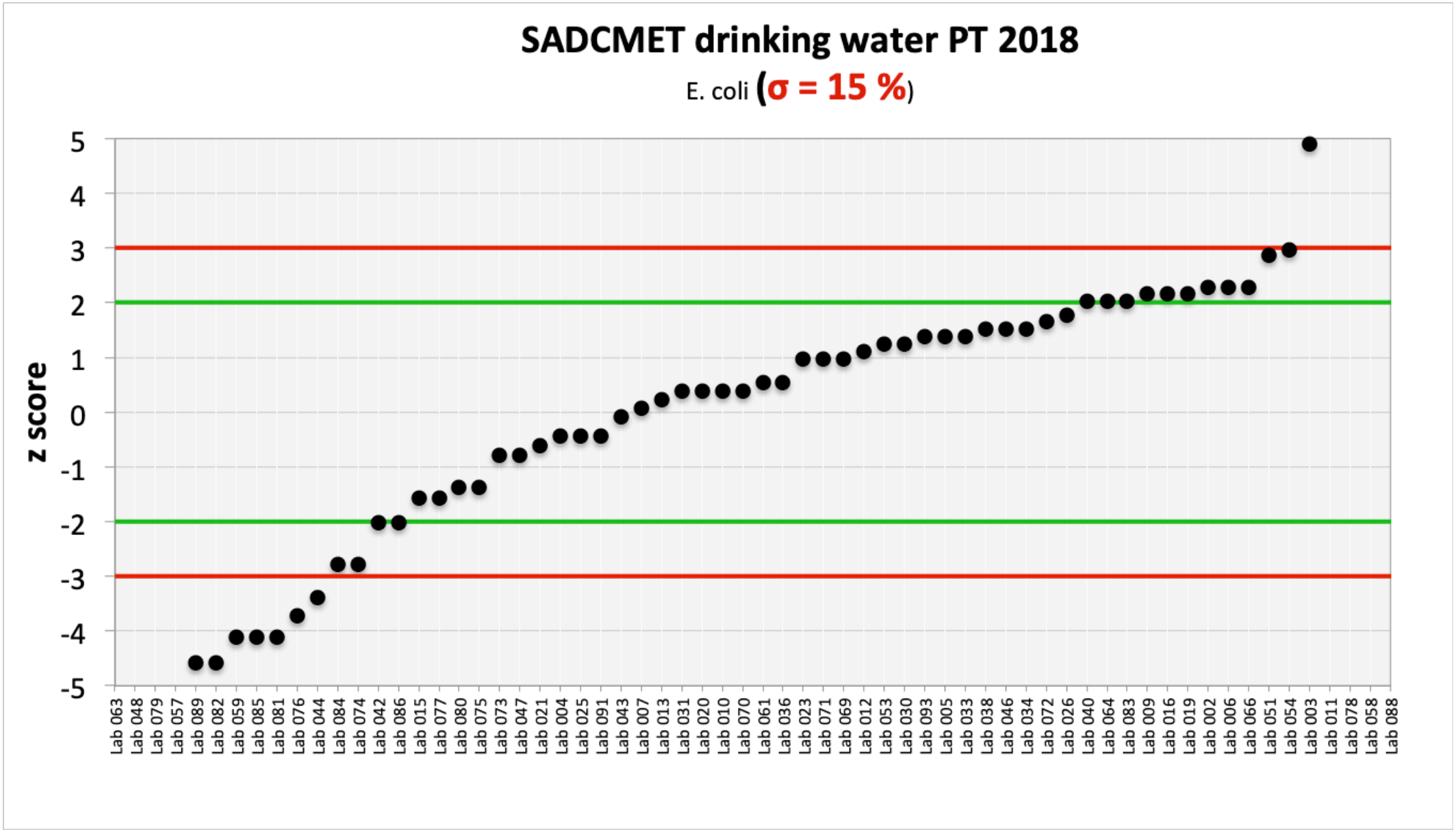
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z score
5
4
3
2
1
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-2
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-5
Lab 063
Lab 048
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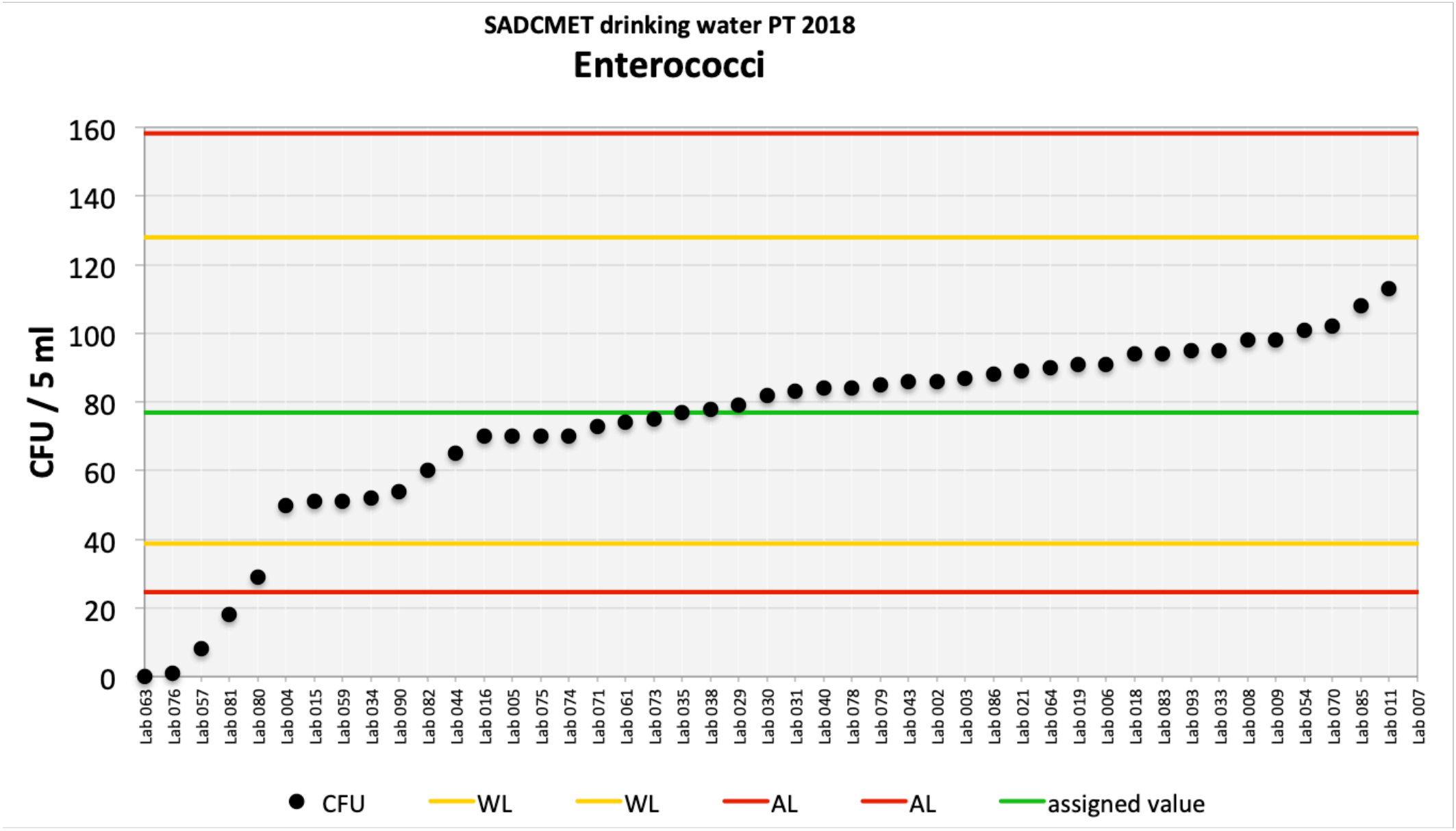
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Enterococci
CFU / 5 ml
160
140
120
100
80
60
40
20
0
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
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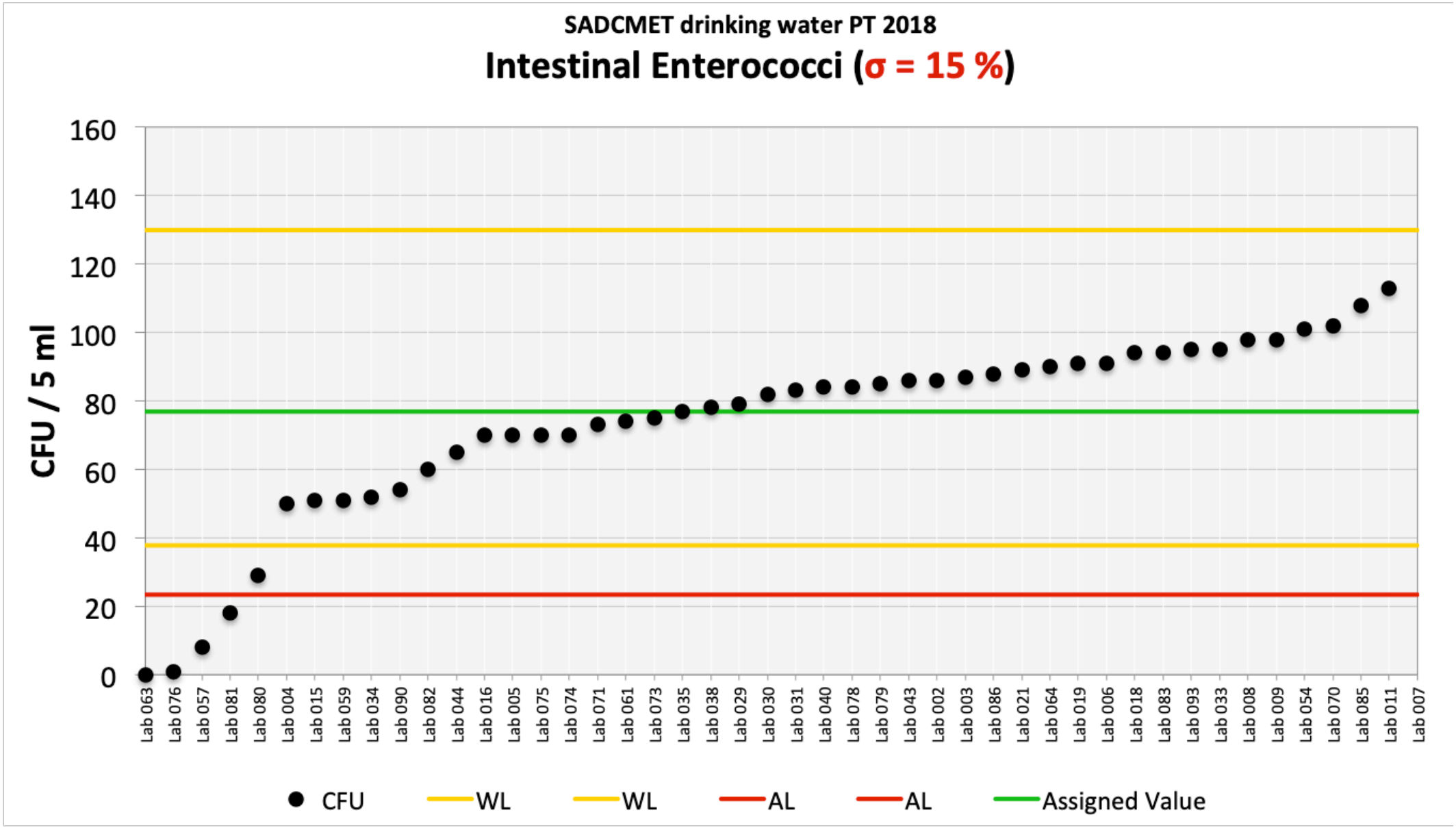
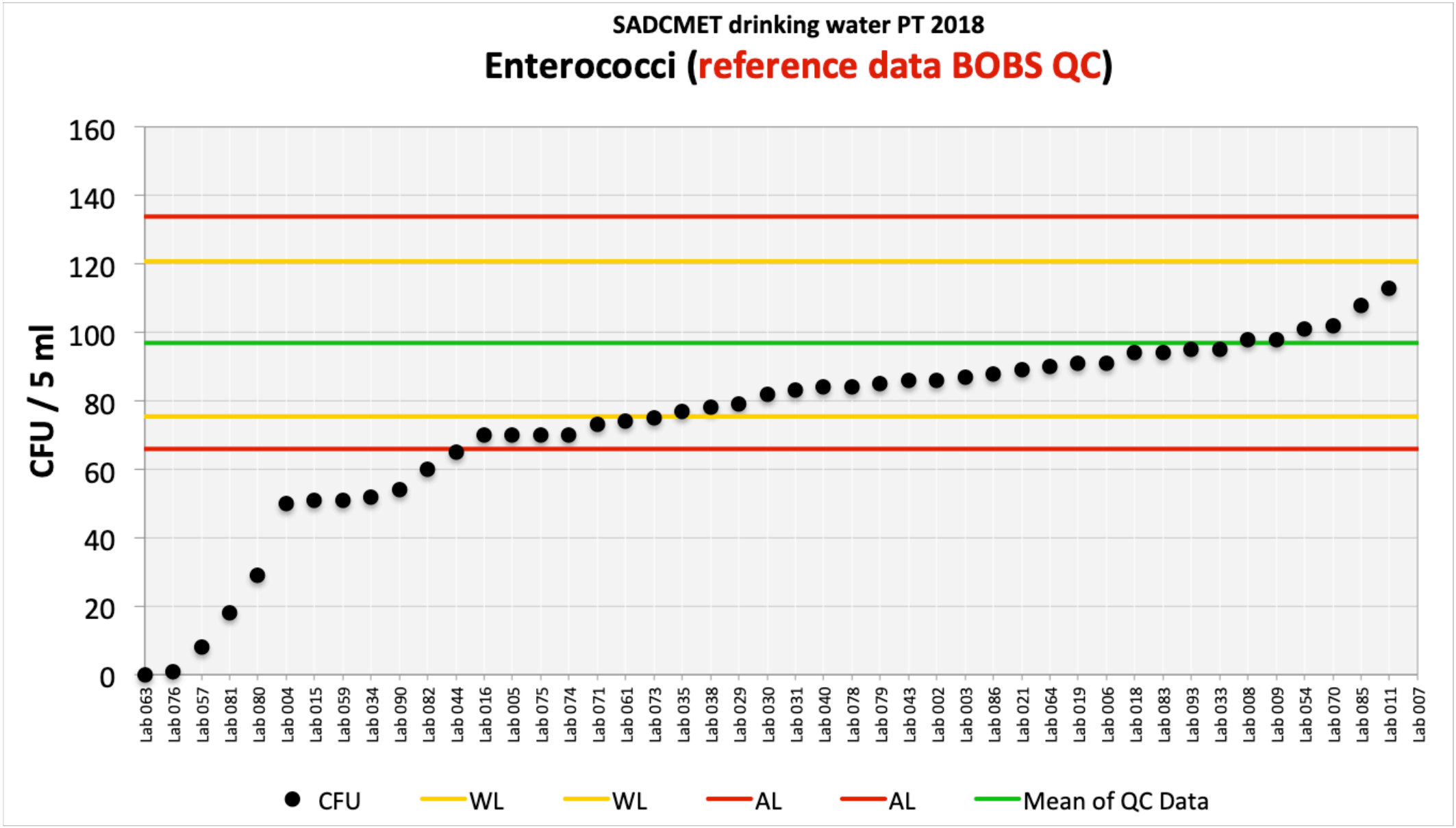
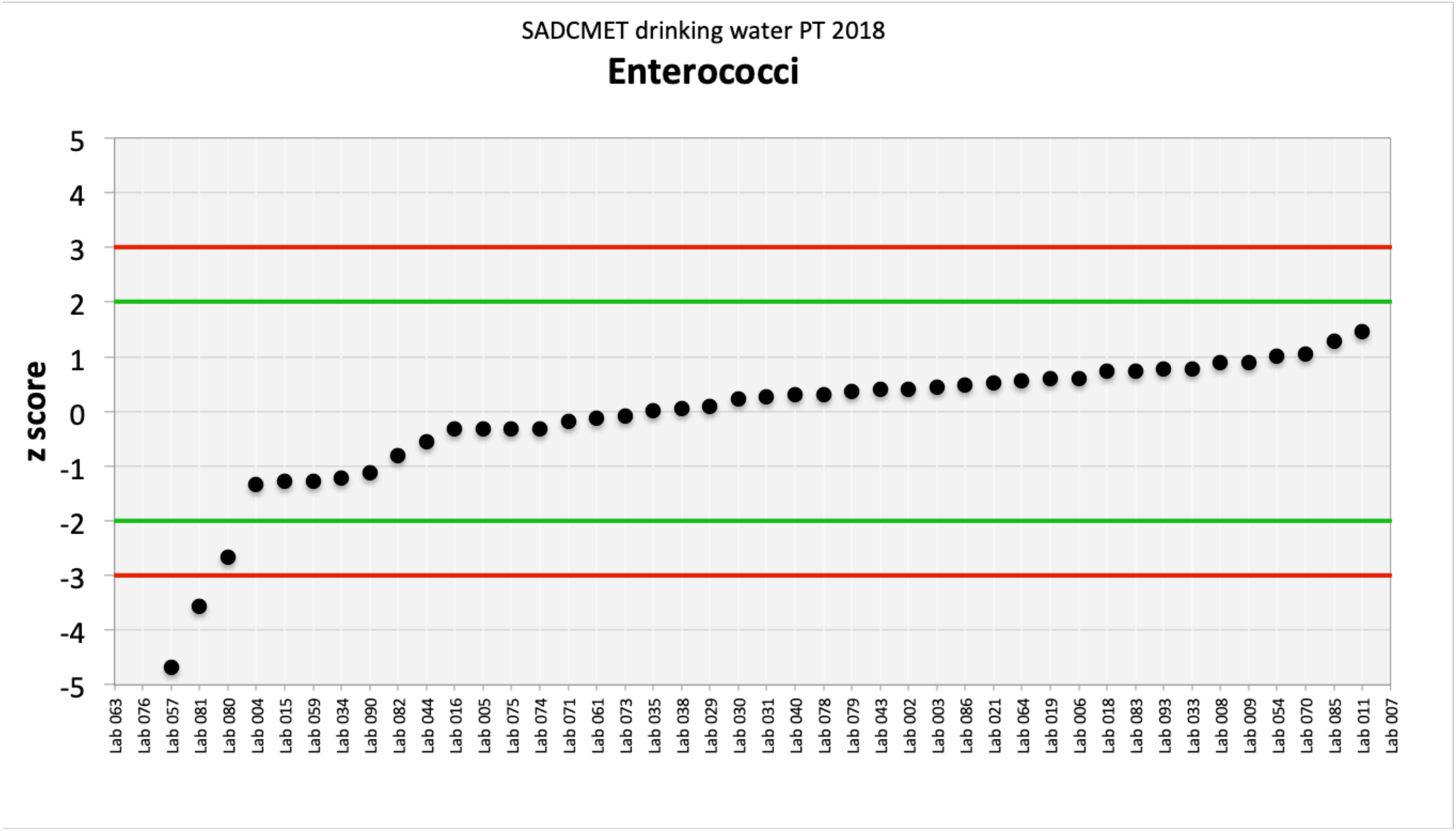
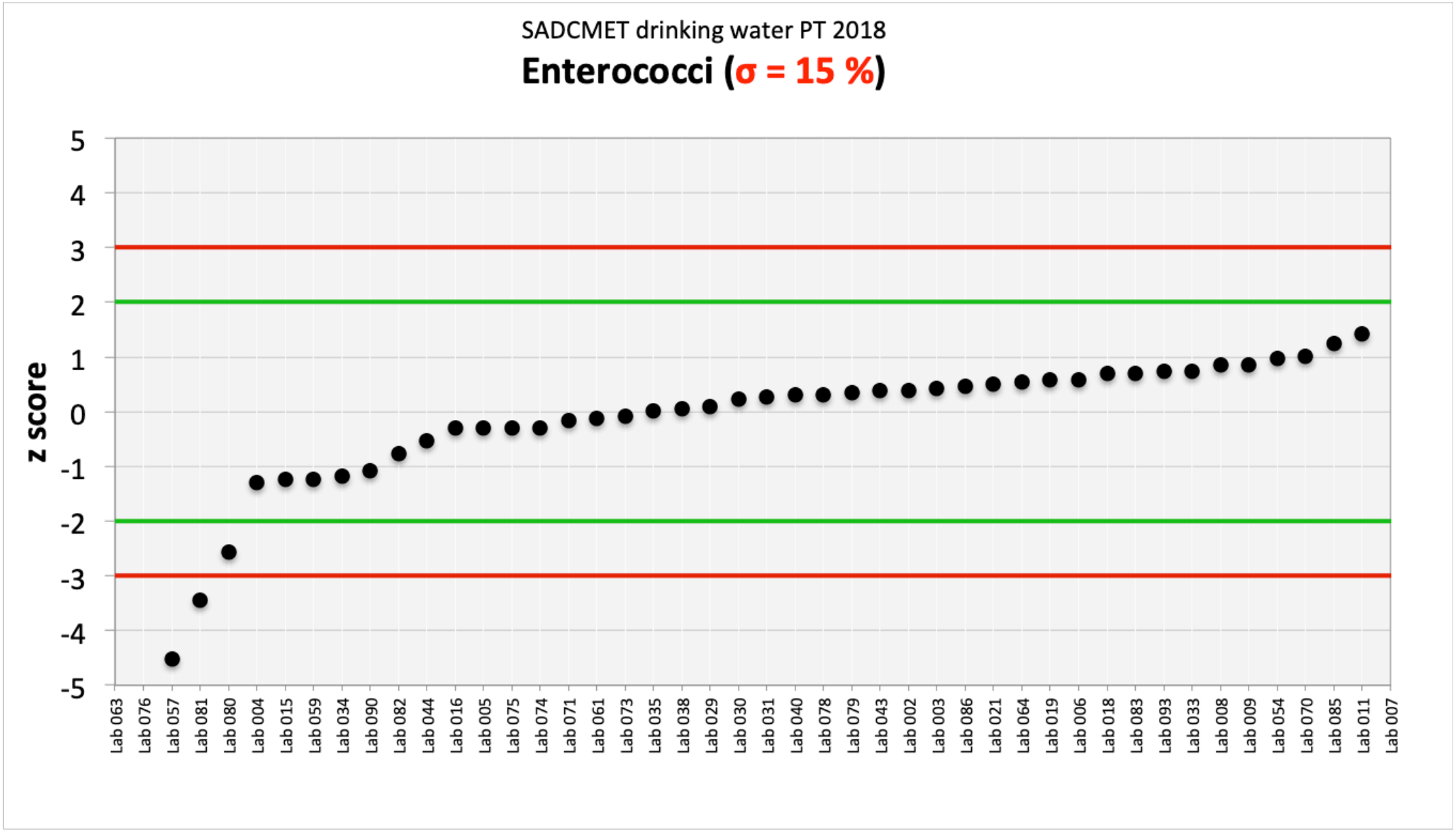
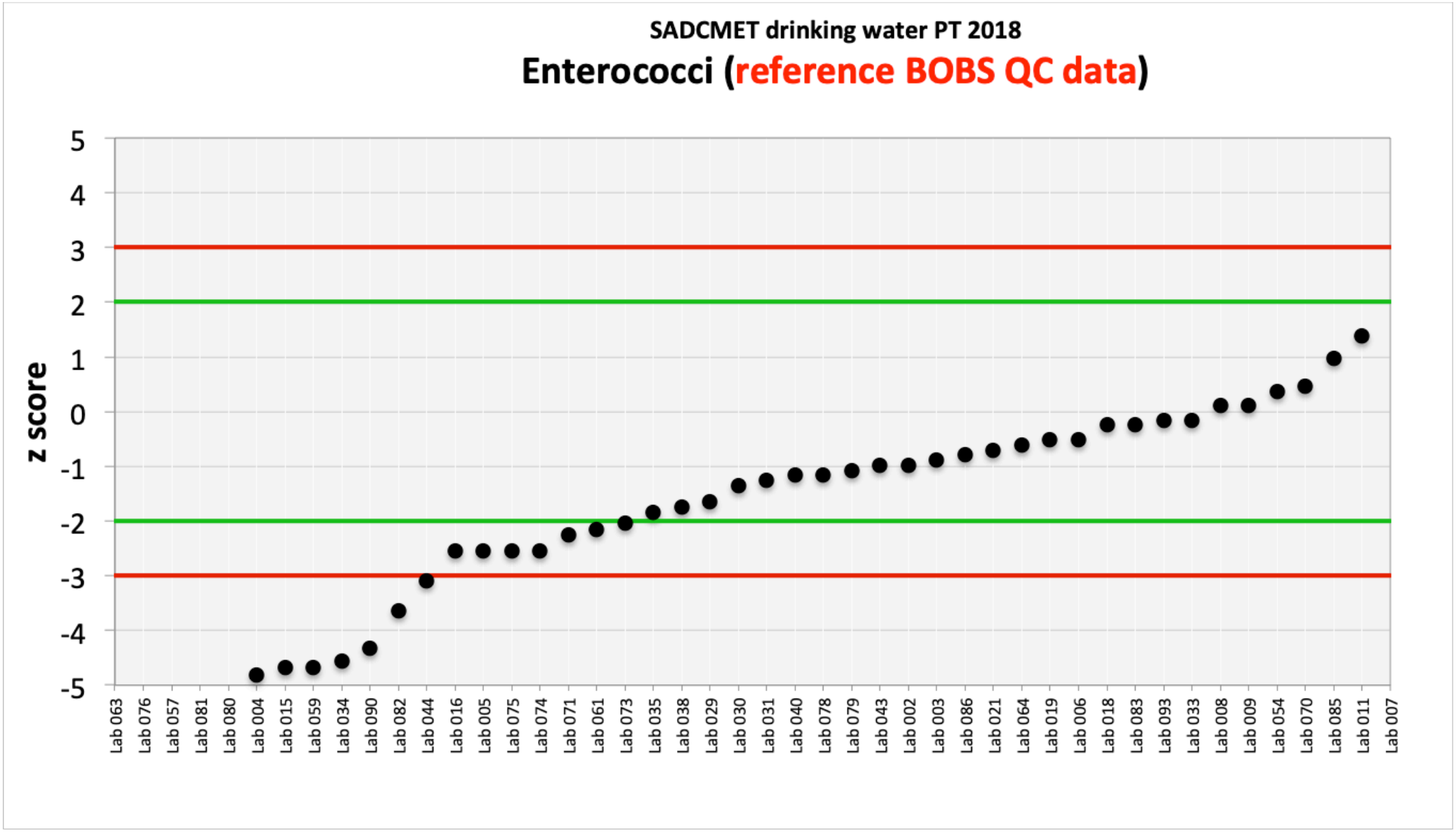
Lab 011
Lab 007
CFU
WL
WL
AL
AL
assigned value

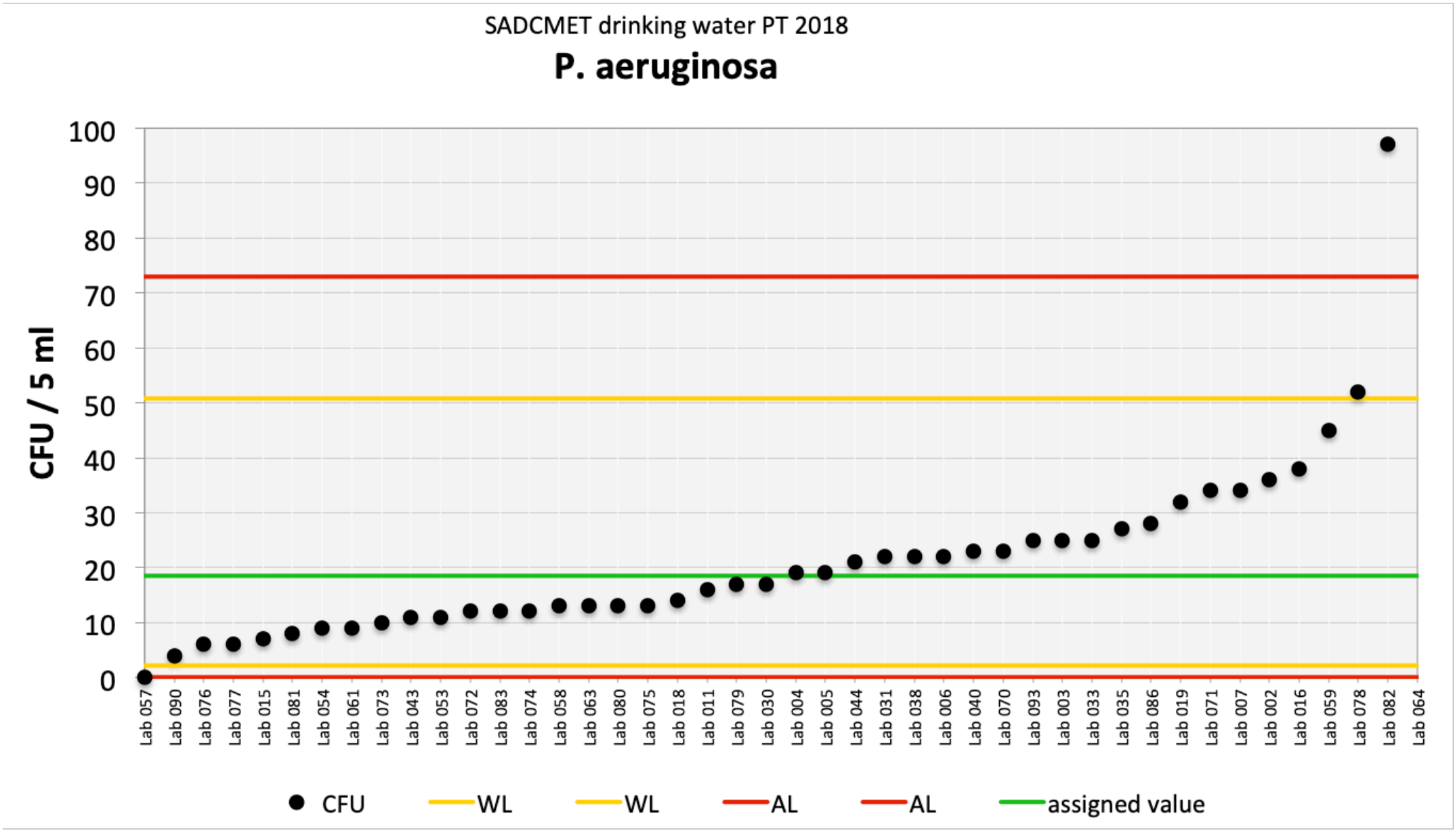

SADCMET drinking water PT 2018
Intestinal Enterococci (σ = 15 %)
CFU / 5 ml
160
140
120
100
80
60
40
20
0
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
Lab 074
Lab 071
Lab 061
Lab 073
Lab 035
Lab 038
Lab 029
Lab 030
Lab 031
Lab 040
Lab 078
Lab 079
Lab 043
Lab 002
Lab 003
Lab 086
Lab 021
Lab 064
Lab 019
Lab 006
Lab 018
Lab 083
Lab 093
Lab 033
Lab 008
Lab 009
Lab 054
Lab 070
Lab 085
Lab 011
Lab 007
CFU
WL
WL
AL
AL
Assigned Value

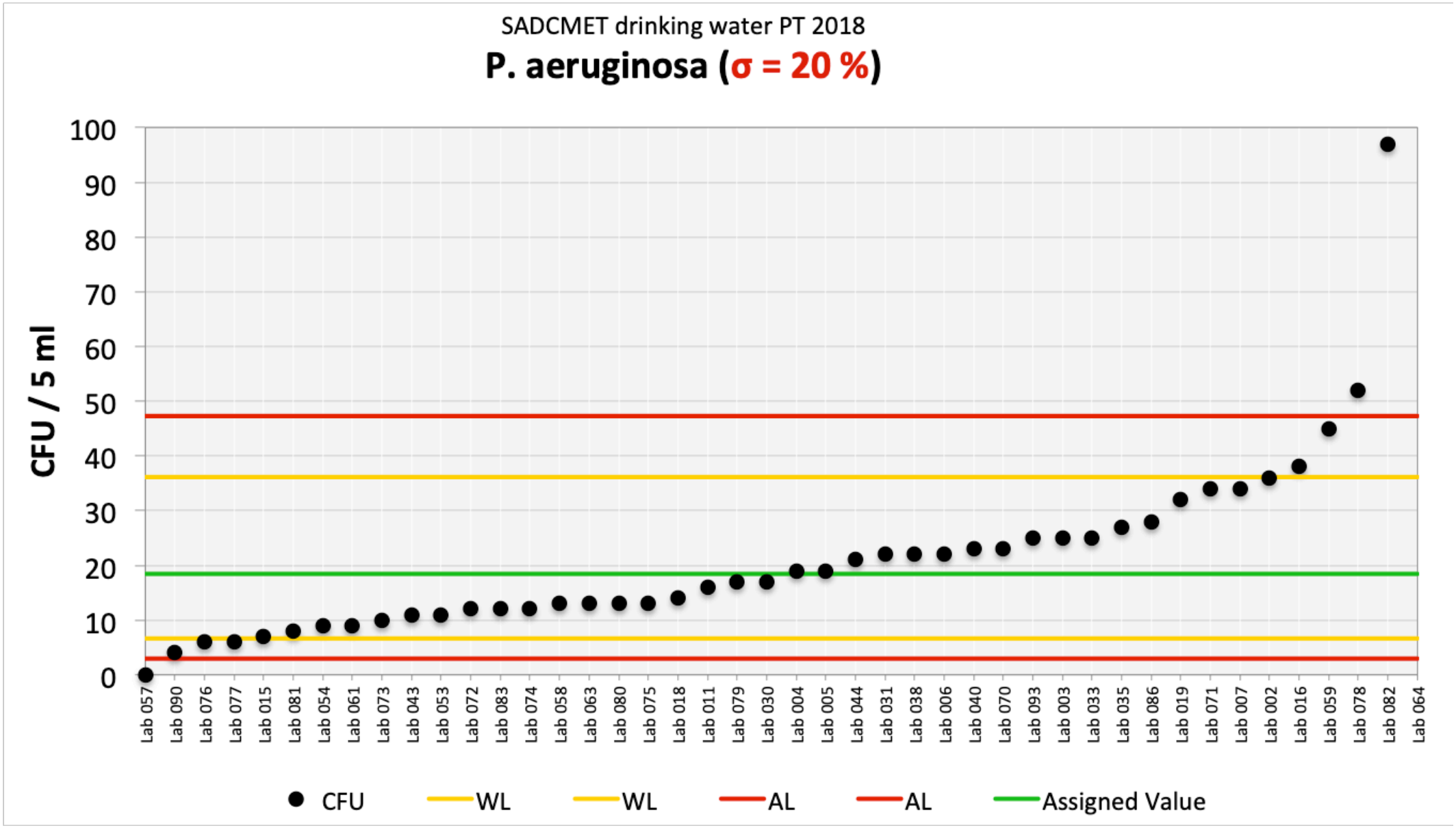

SADCMET drinking water PT 2018
Enterococci (reference data BOBS QC)
CFU / 5 ml
160
140
120
100
80
60
40
20
0
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
Lab 074
Lab 071
Lab 061
Lab 073
Lab 035
Lab 038
Lab 029
Lab 030
Lab 031
Lab 040
Lab 078
Lab 079
Lab 043
Lab 002
Lab 003
Lab 086
Lab 021
Lab 064
Lab 019
Lab 006
Lab 018
Lab 083
Lab 093
Lab 033
Lab 008
Lab 009
Lab 054
Lab 070
Lab 085
Lab 011
Lab 007
CFU
WL
WL
AL
AL
Mean of QC Data

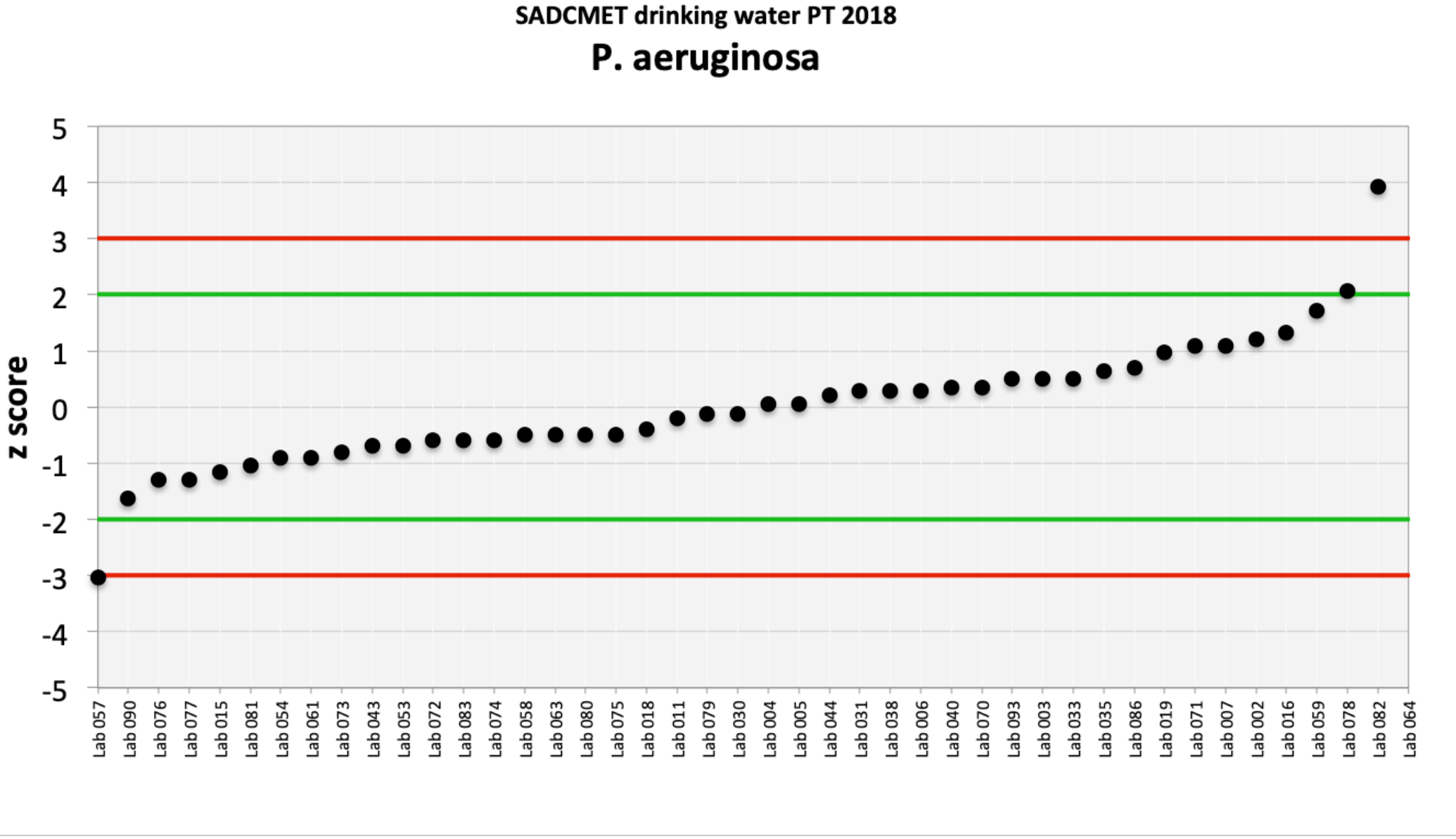

SADCMET drinking water PT 2018
Enterococci
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
Lab 074
Lab 071
Lab 061
Lab 073
Lab 035
Lab 038
Lab 029
Lab 030
Lab 031
Lab 040
Lab 078
Lab 079
Lab 043
Lab 002
Lab 003
Lab 086
Lab 021
Lab 064
Lab 019
Lab 006
Lab 018
Lab 083
Lab 093
Lab 033
Lab 008
Lab 009
Lab 054
Lab 070
Lab 085
Lab 011
Lab 007

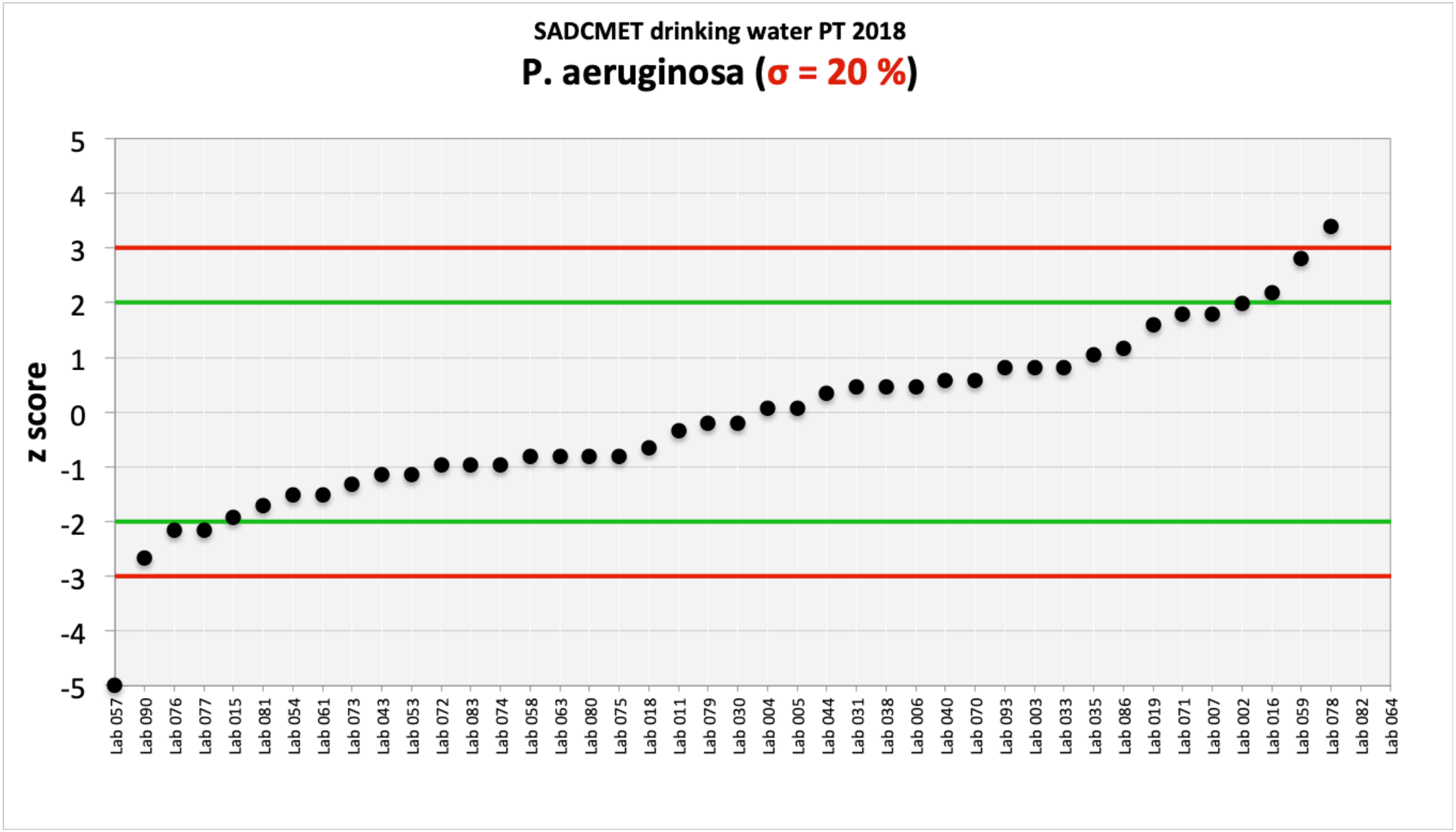

SADCMET drinking water PT 2018
Enterococci (σ = 15 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
Lab 074
Lab 071
Lab 061
Lab 073
Lab 035
Lab 038
Lab 029
Lab 030
Lab 031
Lab 040
Lab 078
Lab 079
Lab 043
Lab 002
Lab 003
Lab 086
Lab 021
Lab 064
Lab 019
Lab 006
Lab 018
Lab 083
Lab 093
Lab 033
Lab 008
Lab 009
Lab 054
Lab 070
Lab 085
Lab 011
Lab 007

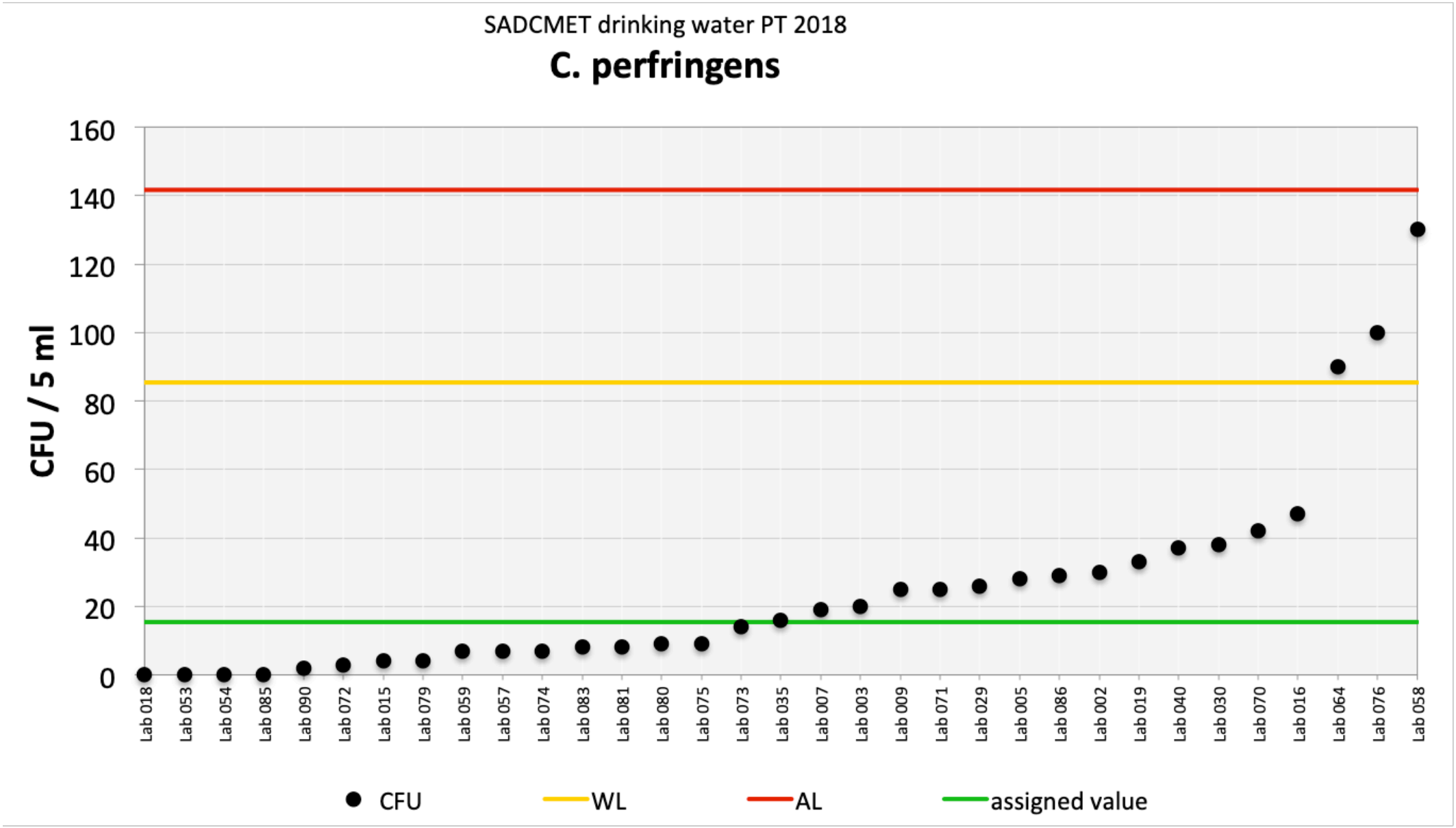

SADCMET drinking water PT 2018
Enterococci (reference BOBS QC data)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 063
Lab 076
Lab 057
Lab 081
Lab 080
Lab 004
Lab 015
Lab 059
Lab 034
Lab 090
Lab 082
Lab 044
Lab 016
Lab 005
Lab 075
Lab 074
Lab 071
Lab 061
Lab 073
Lab 035
Lab 038
Lab 029
Lab 030
Lab 031
Lab 040
Lab 078
Lab 079
Lab 043
Lab 002
Lab 003
Lab 086
Lab 021
Lab 064
Lab 019
Lab 006
Lab 018
Lab 083
Lab 093
Lab 033
Lab 008
Lab 009
Lab 054
Lab 070
Lab 085
Lab 011
Lab 007

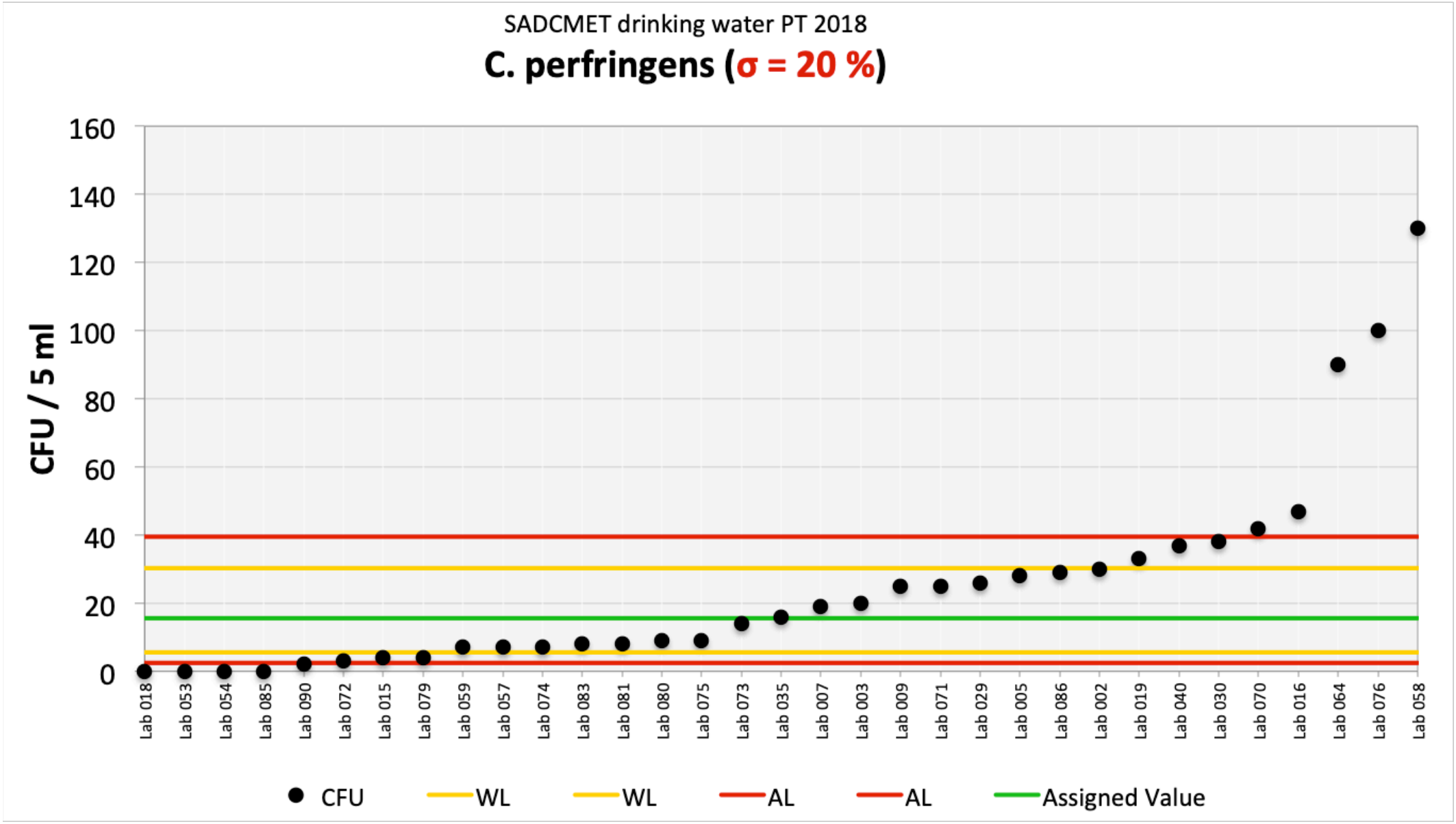

SADCMET drinking water PT 2018
P. aeruginosa
CFU / 5 ml
100
90
80
70
60
50
40
30
20
10
0
Lab 057
Lab 090
Lab 076
Lab 077
Lab 015
Lab 081
Lab 054
Lab 061
Lab 073
Lab 043
Lab 053
Lab 072
Lab 083
Lab 074
Lab 058
Lab 063
Lab 080
Lab 075
Lab 018
Lab 011
Lab 079
Lab 030
Lab 004
Lab 005
Lab 044
Lab 031
Lab 038
Lab 006
Lab 040
Lab 070
Lab 093
Lab 003
Lab 033
Lab 035
Lab 086
Lab 019
Lab 071
Lab 007
Lab 002
Lab 016
Lab 059
Lab 078
Lab 082
Lab 064
CFU
WL
WL
AL
AL
assigned value

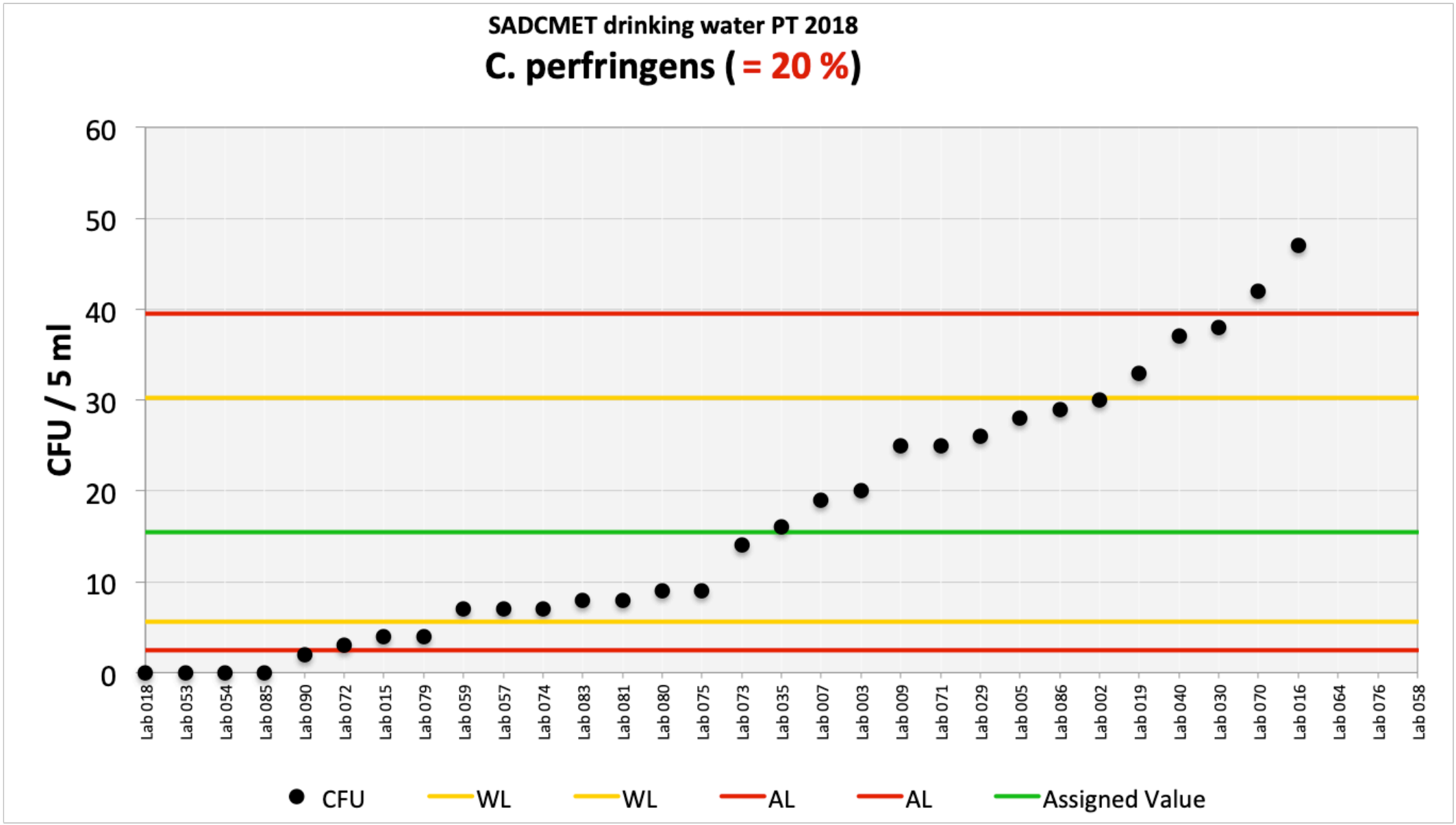

SADCMET drinking water PT 2018
P. aeruginosa (σ = 20 %)
CFU / 5 ml
100
90
80
70
60
50
40
30
20
10
0
Lab 057
Lab 090
Lab 076
Lab 077
Lab 015
Lab 081
Lab 054
Lab 061
Lab 073
Lab 043
Lab 053
Lab 072
Lab 083
Lab 074
Lab 058
Lab 063
Lab 080
Lab 075
Lab 018
Lab 011
Lab 079
Lab 030
Lab 004
Lab 005
Lab 044
Lab 031
Lab 038
Lab 006
Lab 040
Lab 070
Lab 093
Lab 003
Lab 033
Lab 035
Lab 086
Lab 019
Lab 071
Lab 007
Lab 002
Lab 016
Lab 059
Lab 078
Lab 082
Lab 064
CFU
WL
WL
AL
AL
Assigned Value

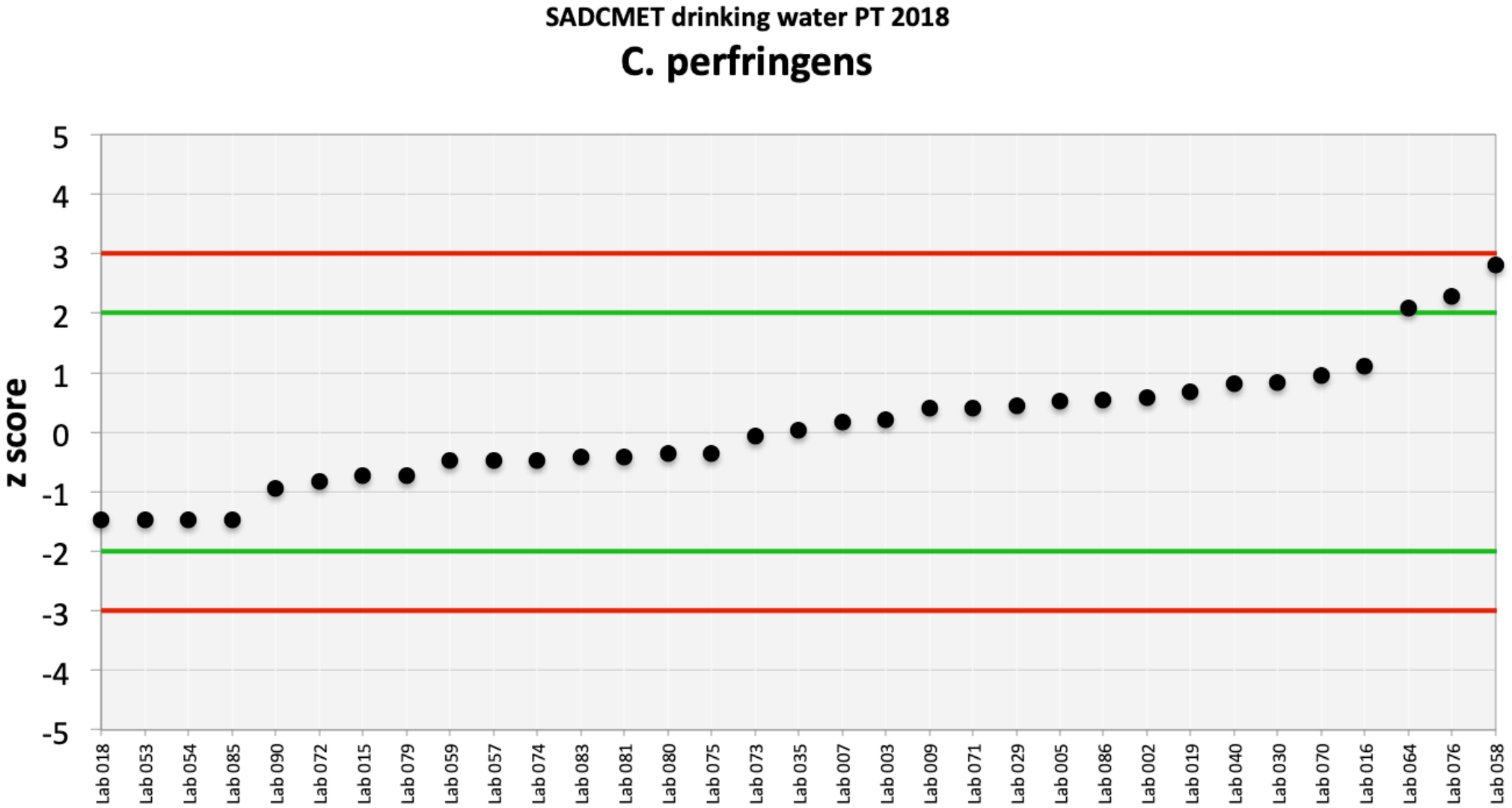

SADCMET drinking water PT 2018
P. aeruginosa
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 057
Lab 090
Lab 076
Lab 077
Lab 015
Lab 081
Lab 054
Lab 061
Lab 073
Lab 043
Lab 053
Lab 072
Lab 083
Lab 074
Lab 058
Lab 063
Lab 080
Lab 075
Lab 018
Lab 011
Lab 079
Lab 030
Lab 004
Lab 005
Lab 044
Lab 031
Lab 038
Lab 006
Lab 040
Lab 070
Lab 093
Lab 003
Lab 033
Lab 035
Lab 086
Lab 019
Lab 071
Lab 007
Lab 002
Lab 016
Lab 059
Lab 078
Lab 082
Lab 064

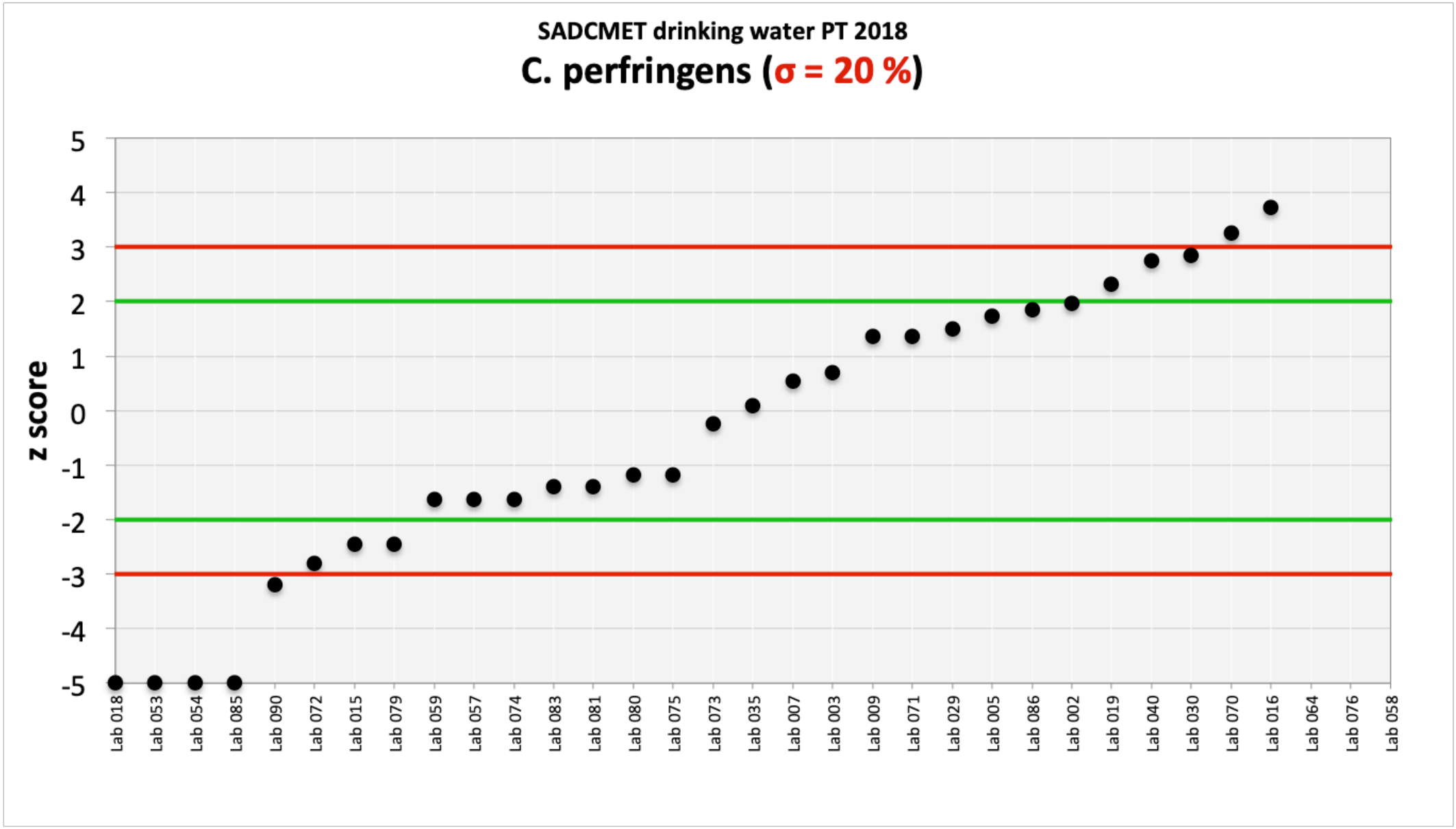

SADCMET drinking water PT 2018
P. aeruginosa (σ = 20 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 057
Lab 090
Lab 076
Lab 077
Lab 015
Lab 081
Lab 054
Lab 061
Lab 073
Lab 043
Lab 053
Lab 072
Lab 083
Lab 074
Lab 058
Lab 063
Lab 080
Lab 075
Lab 018
Lab 011
Lab 079
Lab 030
Lab 004
Lab 005
Lab 044
Lab 031
Lab 038
Lab 006
Lab 040
Lab 070
Lab 093
Lab 003
Lab 033
Lab 035
Lab 086
Lab 019
Lab 071
Lab 007
Lab 002
Lab 016
Lab 059
Lab 078
Lab 082
Lab 064

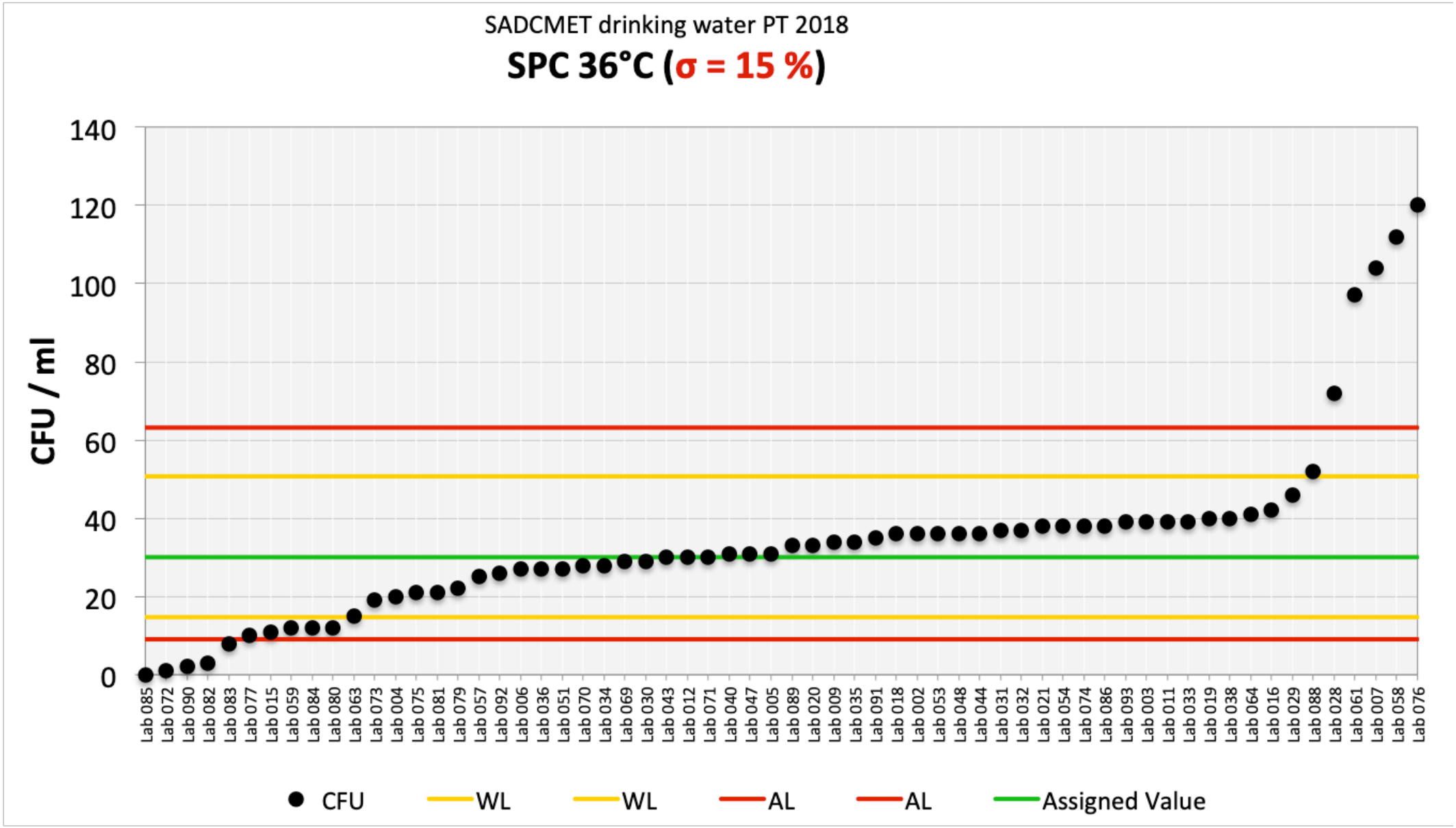

SADCMET drinking water PT 2018
C. perfringens
CFU / 5 ml
160
140
120
100
80
60
40
20
0
Lab 018
Lab 053
Lab 054
Lab 085
Lab 090
Lab 072
Lab 015
Lab 079
Lab 059
Lab 057
Lab 074
Lab 083
Lab 081
Lab 080
Lab 075
Lab 073
Lab 035
Lab 007
Lab 003
Lab 009
Lab 071
Lab 029
Lab 005
Lab 086
Lab 002
Lab 019
Lab 040
Lab 030
Lab 070
Lab 016
Lab 064
Lab 076
Lab 058
CFU
WL
AL
assigned value

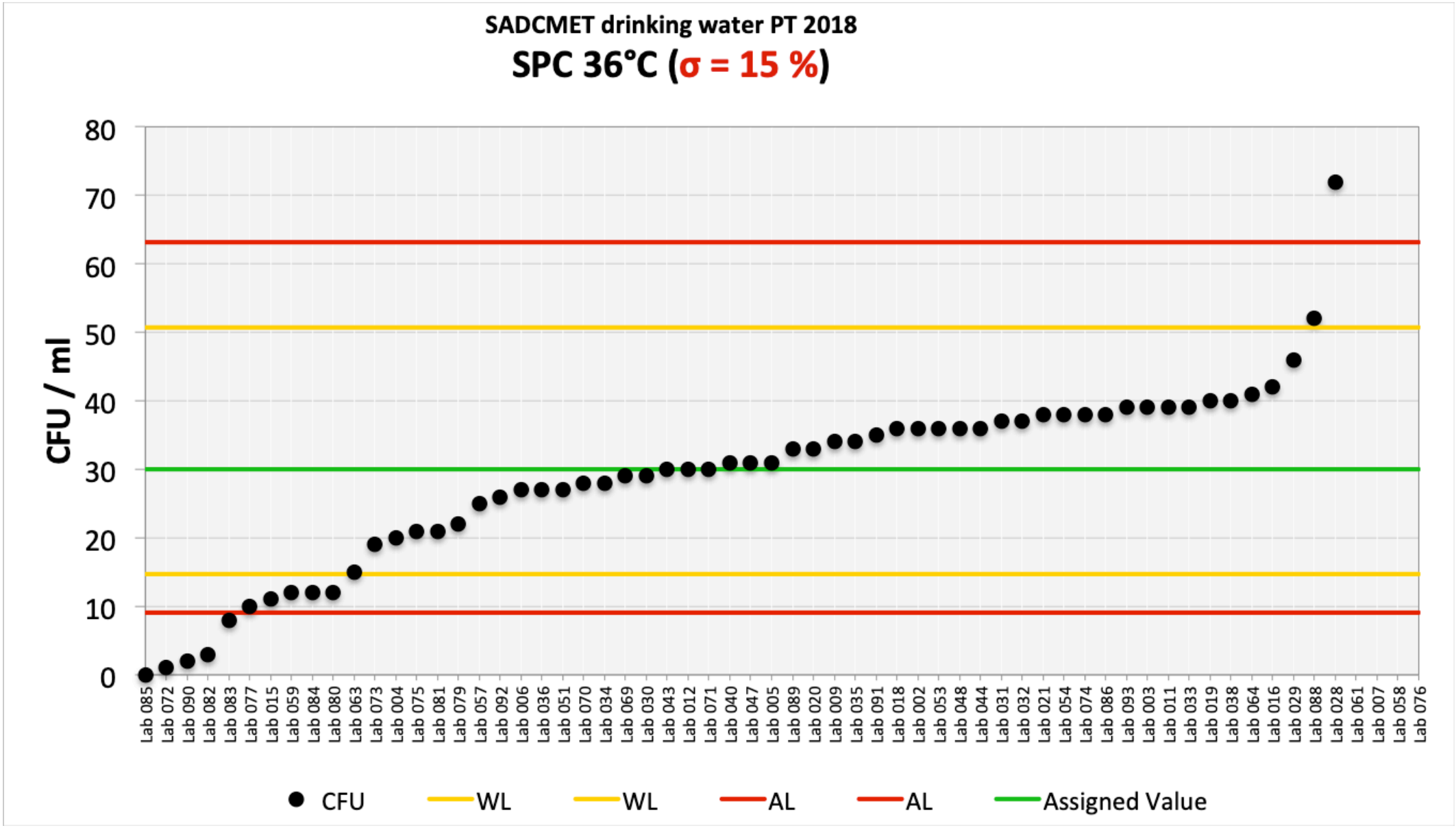

SADCMET drinking water PT 2018
C. perfringens (σ = 20 %)
CFU / 5 ml
160
140
120
100
80
60
40
20
0
Lab 018
Lab 053
Lab 054
Lab 085
Lab 090
Lab 072
Lab 015
Lab 079
Lab 059
Lab 057
Lab 074
Lab 083
Lab 081
Lab 080
Lab 075
Lab 073
Lab 035
Lab 007
Lab 003
Lab 009
Lab 071
Lab 029
Lab 005
Lab 086
Lab 002
Lab 019
Lab 040
Lab 030
Lab 070
Lab 016
Lab 064
Lab 076
Lab 058
CFU
WL
WL
AL
AL
Assigned Value

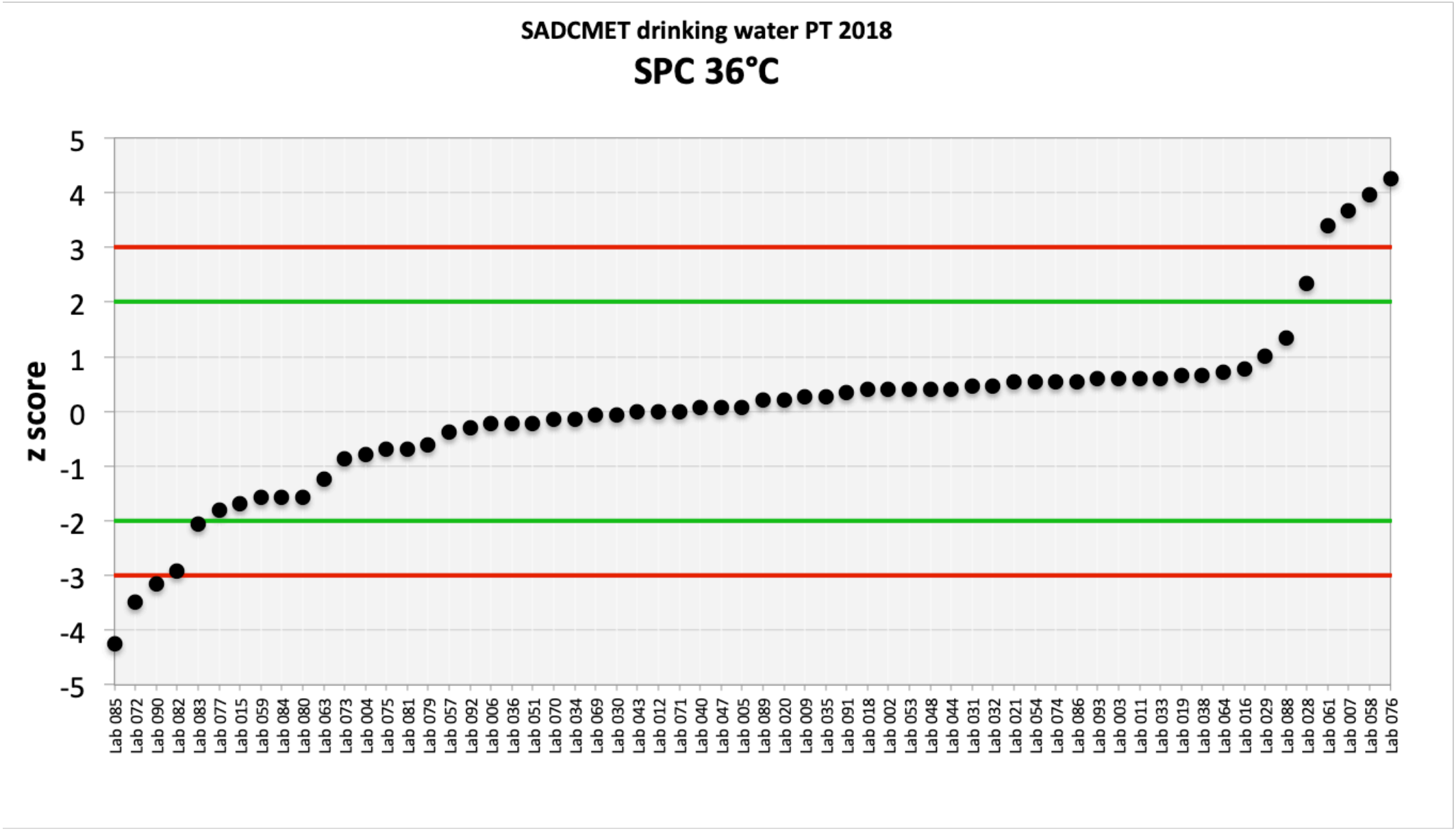

SADCMET drinking water PT 2018
C. perfringens (= 20 %)
CFU / 5 ml
60
50
40
30
20
10
0
Lab 018
Lab 053
Lab 054
Lab 085
Lab 090
Lab 072
Lab 015
Lab 079
Lab 059
Lab 057
Lab 074
Lab 083
Lab 081
Lab 080
Lab 075
Lab 073
Lab 035
Lab 007
Lab 003
Lab 009
Lab 071
Lab 029
Lab 005
Lab 086
Lab 002
Lab 019
Lab 040
Lab 030
Lab 070
Lab 016
Lab 064
Lab 076
Lab 058
CFU
WL
WL
AL
AL
Assigned Value

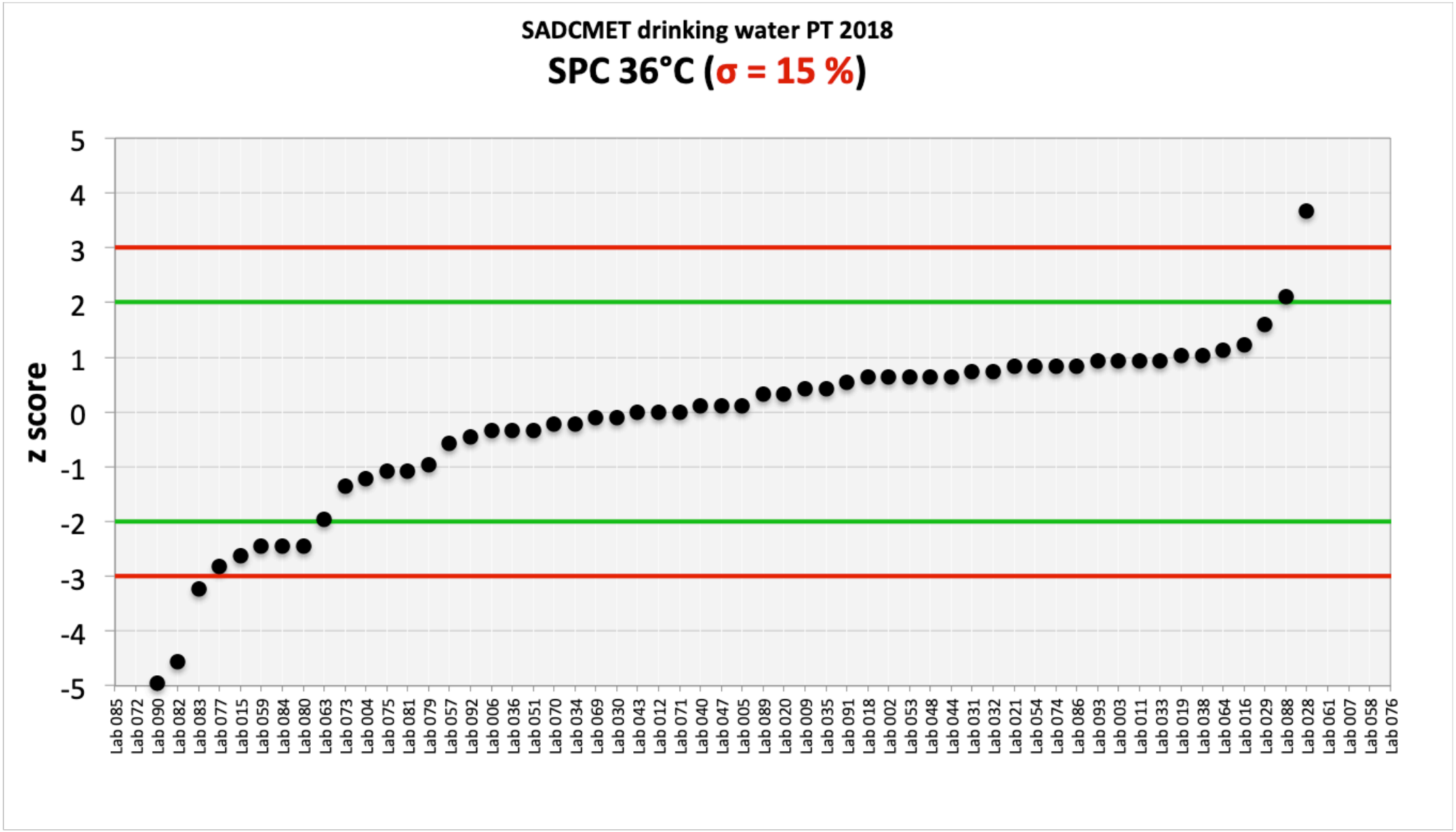

SADCMET drinking water PT 2018
C. perfringens
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 018
Lab 053
Lab 054
Lab 085
Lab 090
Lab 072
Lab 015
Lab 079
Lab 059
Lab 057
Lab 074
Lab 083
Lab 081
Lab 080
Lab 075
Lab 073
Lab 035
Lab 007
Lab 003
Lab 009
Lab 071
Lab 029
Lab 005
Lab 086
Lab 002
Lab 019
Lab 040
Lab 030
Lab 070
Lab 016
Lab 064
Lab 076
Lab 058


SADCMET drinking water PT 2018
C. perfringens (σ = 20 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 018
Lab 053
Lab 054
Lab 085
Lab 090
Lab 072
Lab 015
Lab 079
Lab 059
Lab 057
Lab 074
Lab 083
Lab 081
Lab 080
Lab 075
Lab 073
Lab 035
Lab 007
Lab 003
Lab 009
Lab 071
Lab 029
Lab 005
Lab 086
Lab 002
Lab 019
Lab 040
Lab 030
Lab 070
Lab 016
Lab 064
Lab 076
Lab 058


SADCMET drinking water PT 2018
SPC 36°C (σ = 15 %)
CFU / ml
140
120
100
80
60
40
20
0
Lab 085
Lab 072
Lab 090
Lab 082
Lab 083
Lab 077
Lab 015
Lab 059
Lab 084
Lab 080
Lab 063
Lab 073
Lab 004
Lab 075
Lab 081
Lab 079
Lab 057
Lab 092
Lab 006
Lab 036
Lab 051
Lab 070
Lab 034
Lab 069
Lab 030
Lab 043
Lab 012
Lab 071
Lab 040
Lab 047
Lab 005
Lab 089
Lab 020
Lab 009
Lab 035
Lab 091
Lab 018
Lab 002
Lab 053
Lab 048
Lab 044
Lab 031
Lab 032
Lab 021
Lab 054
Lab 074
Lab 086
Lab 093
Lab 003
Lab 011
Lab 033
Lab 019
Lab 038
Lab 064
Lab 016
Lab 029
Lab 088
Lab 028
Lab 061
Lab 007
Lab 058
Lab 076
CFU
WL
WL
AL
AL
Assigned Value


SADCMET drinking water PT 2018
SPC 36°C (σ = 15 %)
CFU / ml
0
10
20
30
40
50
60
70
80
Lab 085
Lab 072
Lab 090
Lab 082
Lab 083
Lab 077
Lab 015
Lab 059
Lab 084
Lab 080
Lab 063
Lab 073
Lab 004
Lab 075
Lab 081
Lab 079
Lab 057
Lab 092
Lab 006
Lab 036
Lab 051
Lab 070
Lab 034
Lab 069
Lab 030
Lab 043
Lab 012
Lab 071
Lab 040
Lab 047
Lab 005
Lab 089
Lab 020
Lab 009
Lab 035
Lab 091
Lab 018
Lab 002
Lab 053
Lab 048
Lab 044
Lab 031
Lab 032
Lab 021
Lab 054
Lab 074
Lab 086
Lab 093
Lab 003
Lab 011
Lab 033
Lab 019
Lab 038
Lab 064
Lab 016
Lab 029
Lab 088
Lab 028
Lab 061
Lab 007
Lab 058
Lab 076
CFU
WL
WL
AL
AL
Assigned Value


SADCMET drinking water PT 2018
SPC 36°C
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 085
Lab 072
Lab 090
Lab 082
Lab 083
Lab 077
Lab 015
Lab 059
Lab 084
Lab 080
Lab 063
Lab 073
Lab 004
Lab 075
Lab 081
Lab 079
Lab 057
Lab 092
Lab 006
Lab 036
Lab 051
Lab 070
Lab 034
Lab 069
Lab 030
Lab 043
Lab 012
Lab 071
Lab 040
Lab 047
Lab 005
Lab 089
Lab 020
Lab 009
Lab 035
Lab 091
Lab 018
Lab 002
Lab 053
Lab 048
Lab 044
Lab 031
Lab 032
Lab 021
Lab 054
Lab 074
Lab 086
Lab 093
Lab 003
Lab 011
Lab 033
Lab 019
Lab 038
Lab 064
Lab 016
Lab 029
Lab 088
Lab 028
Lab 061
Lab 007
Lab 058
Lab 076


SADCMET drinking water PT 2018
SPC 36°C (σ = 15 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 085
Lab 072
Lab 090
Lab 082
Lab 083
Lab 077
Lab 015
Lab 059
Lab 084
Lab 080
Lab 063
Lab 073
Lab 004
Lab 075
Lab 081
Lab 079
Lab 057
Lab 092
Lab 006
Lab 036
Lab 051
Lab 070
Lab 034
Lab 069
Lab 030
Lab 043
Lab 012
Lab 071
Lab 040
Lab 047
Lab 005
Lab 089
Lab 020
Lab 009
Lab 035
Lab 091
Lab 018
Lab 002
Lab 053
Lab 048
Lab 044
Lab 031
Lab 032
Lab 021
Lab 054
Lab 074
Lab 086
Lab 093
Lab 003
Lab 011
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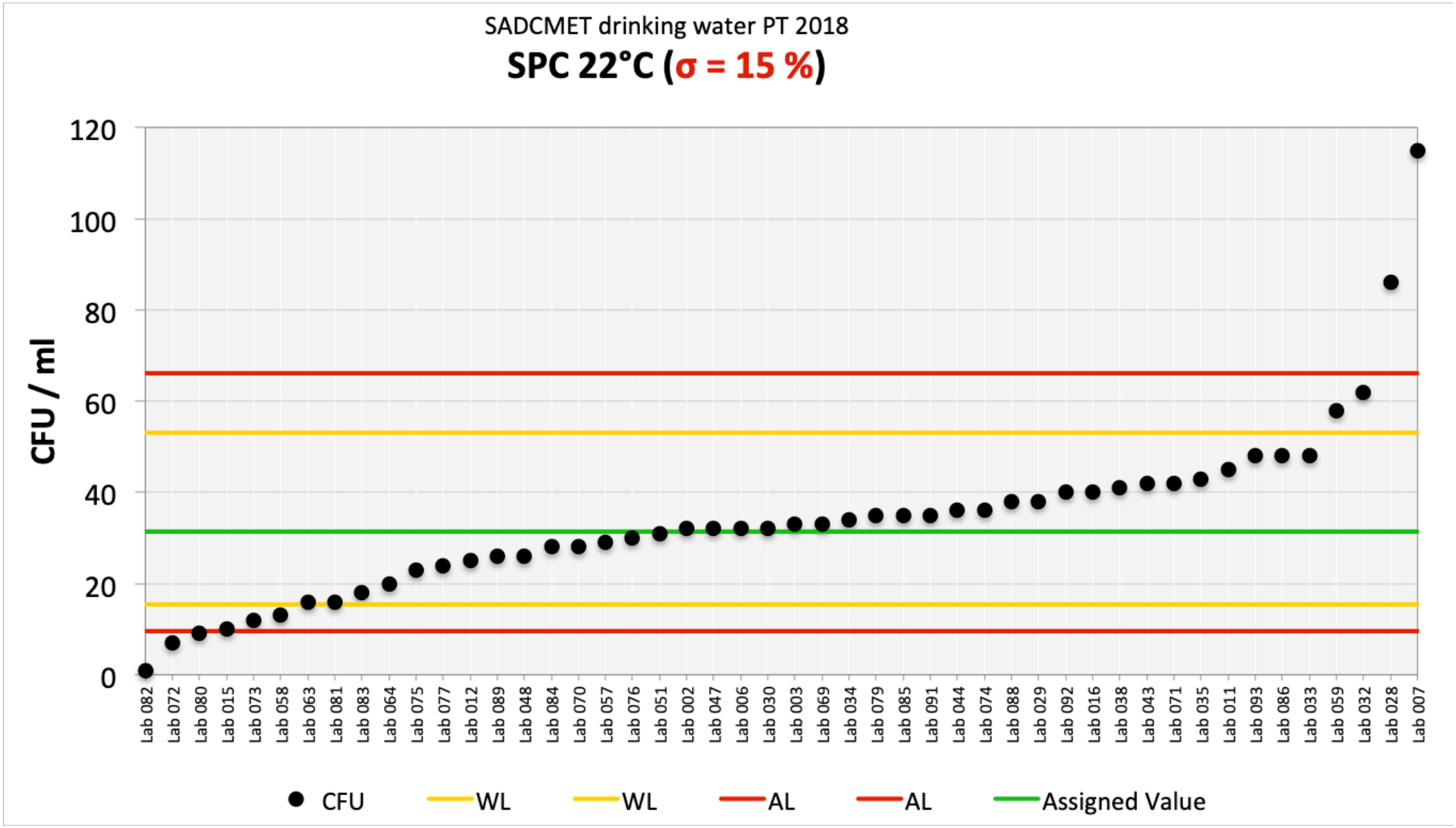
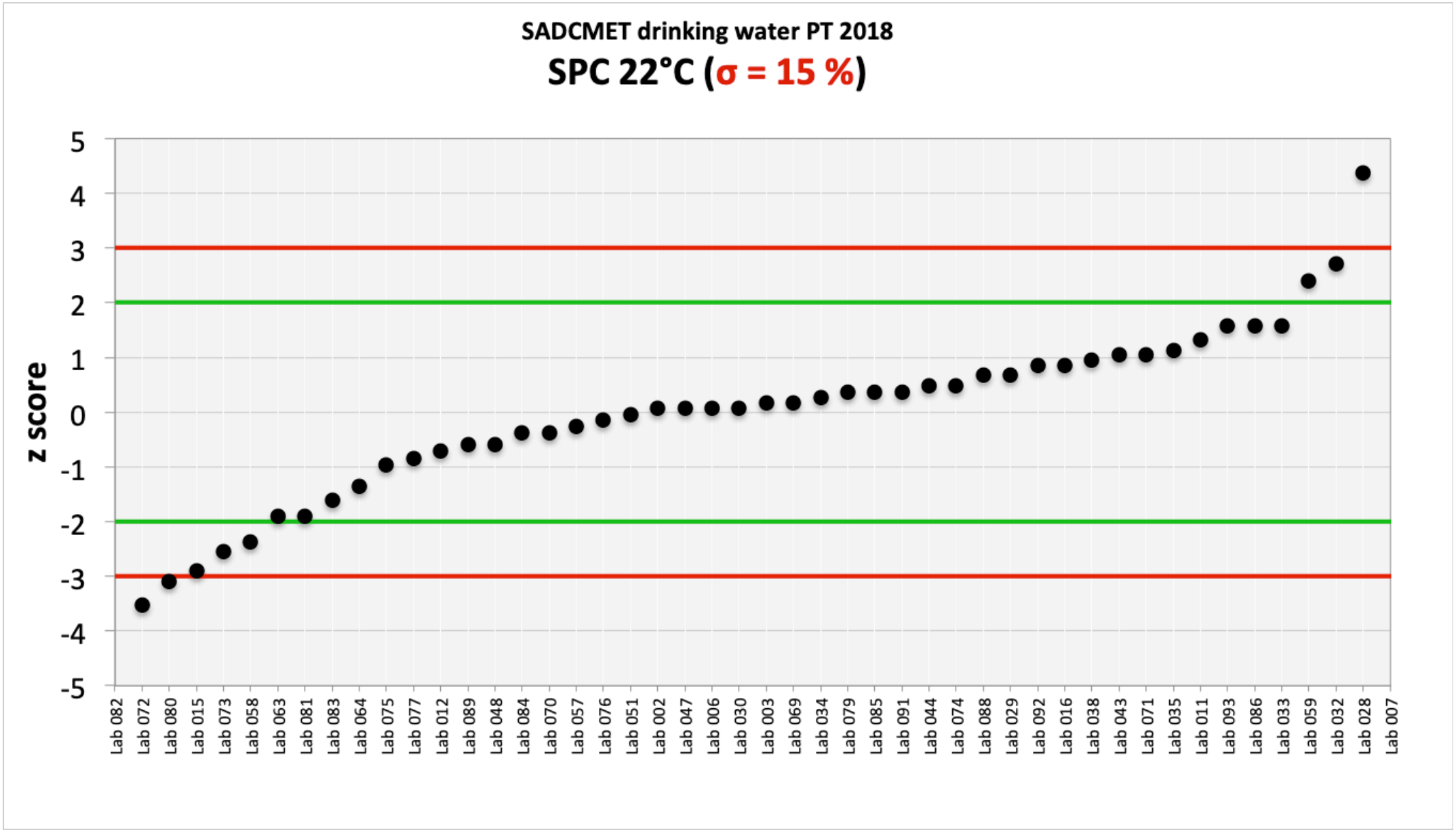
Lab 088
Lab 028
Lab 061
Lab 007
Lab 058
Lab 076

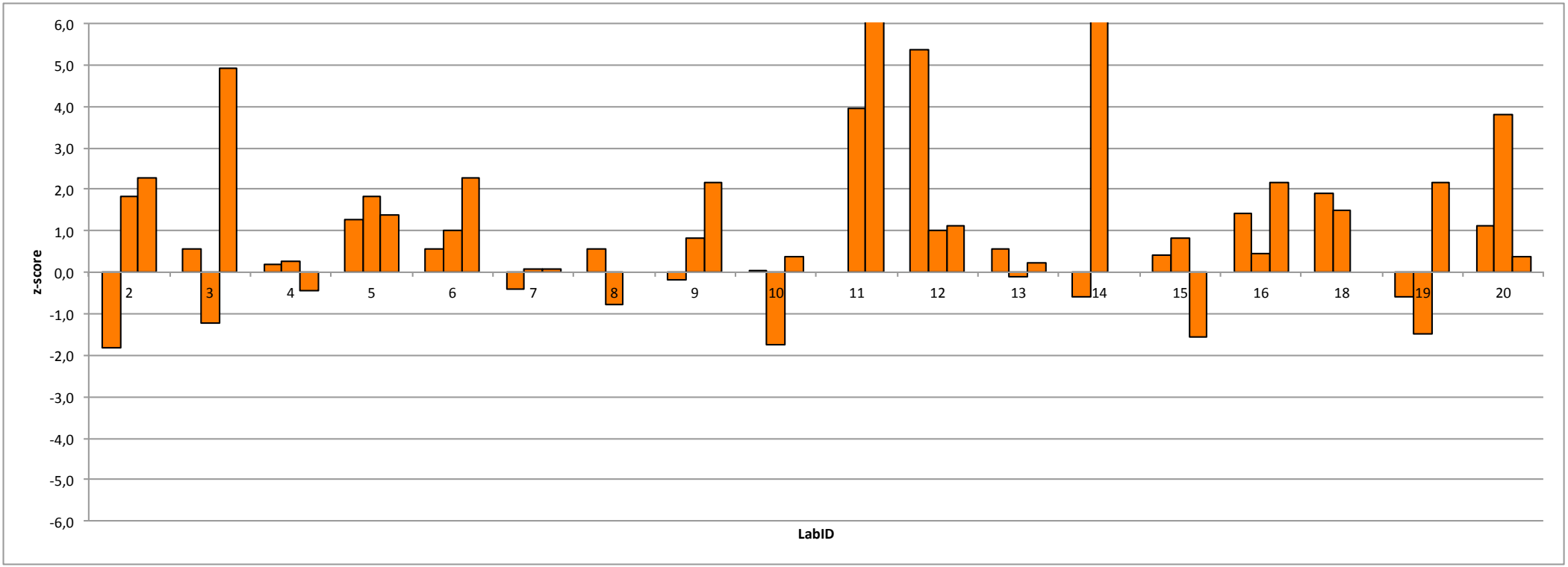

SADCMET drinking water PT 2018
SPC 22°C (σ = 15 %)
CFU / ml
120
100
80
60
40
20
0
Lab 082
Lab 072
Lab 080
Lab 015
Lab 073
Lab 058
Lab 063
Lab 081
Lab 083
Lab 064
Lab 075
Lab 077
Lab 012
Lab 089
Lab 048
Lab 084
Lab 070
Lab 057
Lab 076
Lab 051
Lab 002
Lab 047
Lab 006
Lab 030
Lab 003
Lab 069
Lab 034
Lab 079
Lab 085
Lab 091
Lab 044
Lab 074
Lab 088
Lab 029
Lab 092
Lab 016
Lab 038
Lab 043
Lab 071
Lab 035
Lab 011
Lab 093
Lab 086
Lab 033
Lab 059
Lab 032
Lab 028
Lab 007
CFU
WL
WL
AL
AL
Assigned Value


SADCMET drinking water PT 2018
SPC 22°C (σ = 15 %)
z score
5
4
3
2
1
0
-1
-2
-3
-4
-5
Lab 082
Lab 072
Lab 080
Lab 015
Lab 073
Lab 058
Lab 063
Lab 081
Lab 083
Lab 064
Lab 075
Lab 077
Lab 012
Lab 089
Lab 048
Lab 084
Lab 070
Lab 057
Lab 076
Lab 051
Lab 002
Lab 047
Lab 006
Lab 030
Lab 003
Lab 069
Lab 034
Lab 079
Lab 085
Lab 091
Lab 044
Lab 074
Lab 088
Lab 029
Lab 092
Lab 016
Lab 038
Lab 043
Lab 071
Lab 035
Lab 011
Lab 093
Lab 086
Lab 033
Lab 059
Lab 032
Lab 028
Lab 007

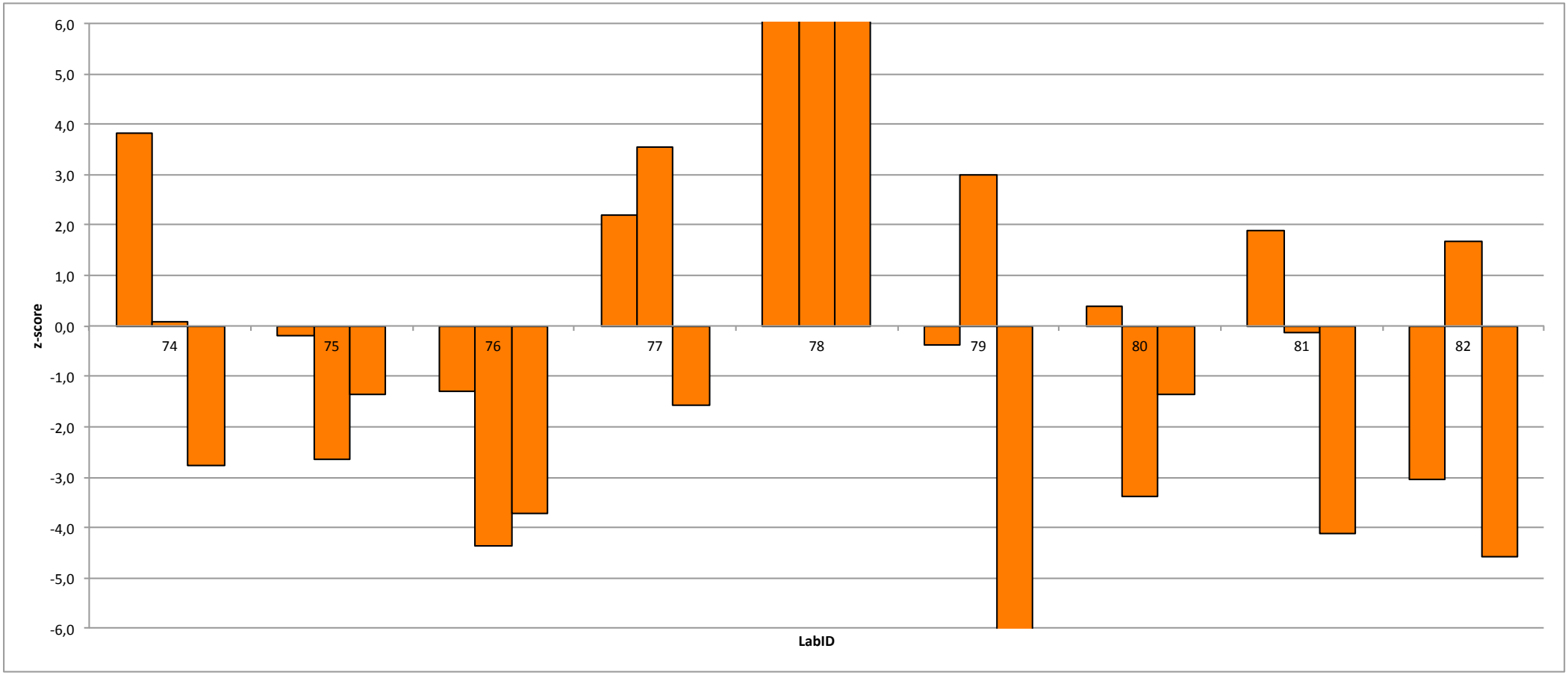
Useful PT results

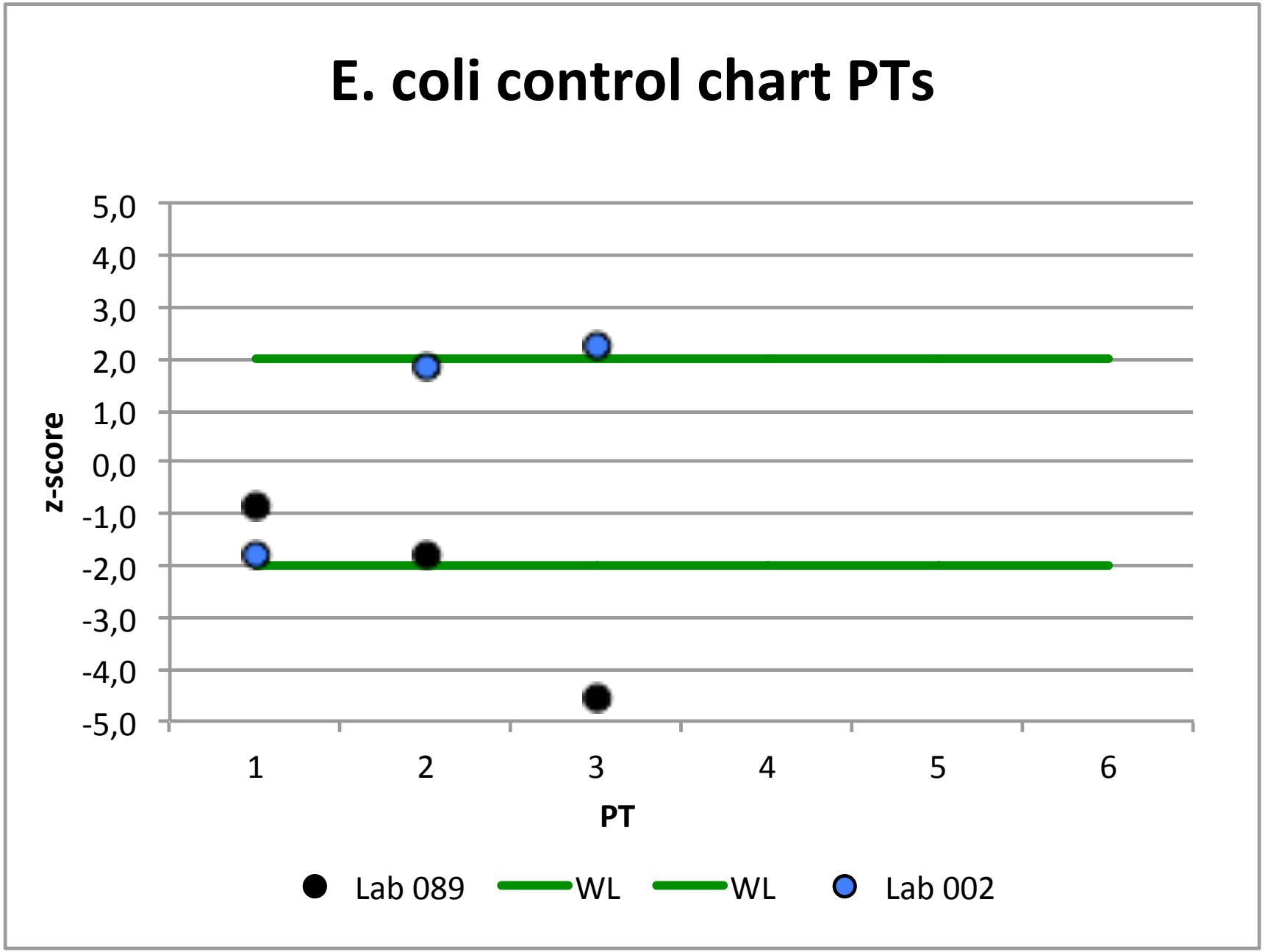
- evaluation of your own performance by comparison with other laboratories (confirmation of competence if used regularly)
- material offered give opportunity for multiple analysis
- compare to other laboratories
- learn about intralaboratory variation (precision)
- (compare different technicians)
- counting
- equipment
- …

Control charts PT
examples for different laboratories



Control charts PT
examples for different laboratories




E. coli control chart PTs
z-score
5,0
4,0
3,0
2,0
1,0
0,0
-1,0
-2,0
-3,0
-4,0
-5,0
1
2
3
4
5
6
PT
Lab 089
WL
WL
Lab 002

Methods E. coli

Z-score	Method & Technique Used	Media Used	Time/Temperature	Confirmation
-6.667	ISO 16649-2:2001, Membrane filtration	TBX Agar	37oC, 24Hrs	
-6.667	ISO 9308-1: 2000, Membrane Filtration	LTTC Agar	37oC, 24hrs	1. Oxidase 2. Tryptone
-6.667	APHA, Membrane Filtration	EMB (Levine) Agar	37oC, 24hrs	
-6.667	ISO 8199:2005, ISO 16649-2, Colilert	E.C Agar	37oC, 24hrs	
-4.588	ISO 9308, Pour plate	TBX	37oC, 24Hrs	Fermentation
-4.588	ISO 9308-1, Membrane Filtration	EMB-agar (BK056)	37oC, 24hrs	Blue black color colonies observed
-4.120	Membrane Filtration	EMB Agar	37oC, 24Hrs	
-4.120	EPA 1304-2002 Membrane filtration	MFC	44oC, 24hrs	EMB Agar
-4.120	MF method	EMB Agar	37oC, 24Hrs	
-3.726	MF Method	m-FC agar broth base	37oC, 24hrs	
-3.379	ISO 9308-1, Filtration	m-Coli Blue	35.5oC, 24Hrs	
-2.777	Membrane filtration	EMB AGAR	37oC, 24Hrs	
-2.777	ISO 9308-1:2014, Membrane Filtration	McConkey agar	37oC, 24Hrs	Indole
-2.018	APHA, MPN	Brilliant Green	44oC, 24Hrs	
-2.018	MF	EMB	37oC, 24hrs	
-1.574	ISO, Membrane Filtration	MLGA	37oC, +/- 36Hrs	Gram stain
-1.574	ISO 9308-1: 2000, *Membrane* Filtration	EMB Agar	37oC, 24hrs	
-1.366	Membrane Filtration	Hi Chrome	37oC, 24hrs	
-1.366	Membrane Filtration	EMB Agar	37oC, 24hrs	
-0.786	Membrane filtration	EMB Agar (Levine)	37oC, 24Hrs	
-0.786	ISO 9308-1, Membrane Filtration	CCA	37oC, 24hrs	McConkey Agar Indole Test
-0.605				
-0.430	ISO 9308-1:2014, Membrane Filtration	MLGA	37oC, 24Hrs	indole test
-0.430	ISO 9308-2:1990 1st ed, Multiple tube (most portable) method	McConkey Broth	44oC, 24hrs	Tryptone water
-0.430	Membrane Filtration	Chromogenic coliform agar	37oC, 24Hrs	Indole
-0.092	ISO 16649-2, pour plate	TBX agar	44oC, 24hrs	indole test
0.070	ISO 9308-1: 2014, Membrane Filtration	BECS 08.01.14	37oC, 24hrs	

Topics of the day

- quality control of microbiological methods

- implementation of a new method in a microbiological laboratory

Quality Control

Quality control (QC) is a **procedure** or set of procedures intended to ensure that a manufactured product or **performed service** adheres to a **defined** set of quality **criteria** or **meets the requirements** of the client or **customer**.

fit for purpose

Quality Control

internal quality control

- method by method
- for the whole laboratory
- for particular compartments/steps of a process or method

- external quality control
  - proficiency testing
  - interlaboratory comparison (2 or more laboratories)

Quality Control

- What is the aim of the exercise (not the only the method)?
- What are the critical steps with the most influence on the outcome of the result?

Microbiological methods

- culture based methods for water analysis
  - sample / subsample
  - Volume measurement
  - inoculation of medium (selective, nonselective)
  - incubation (equipment, time, temperature, humidity…)
  - reading (of plates/MPN..)
  - (confirmation)
  - reporting

working groups

- suggest possible QC measures for each step

	QC
volume	calibration
medium	sterility, positve- and negative controls, quantitative testing (ISO 11133), determination of shelf life
incubation (time, temperature, equipment)	documentation and review of the times and temperatures (traceability of equipment) decontamination of equipment (sterility)
reading / counting	positive and negative controls multiple readings of one plate by same person, different persons…

Working groups

- Define the requirements of one method you want to design the quality control for.
- make a list of the relevant steps
- list the necessary QC measures

Questions

- what are the relevant requirements for your microbiological analysis (law, ordinance, accreditation)
- list regulation
- list matrix (e.g. drinking water, bathing water)
- list parameter (e.g. E. coli)
- list values (e.g. 0/100 ml)
- list methods (e.g. ISO 9301-1:2017)

Implementation of new methods in a microbiological laboratory

- What is the aim / goal of the examination?

Implementation of new methods in a microbiological laboratory

- What is the aim / goal of the examination?

- Working group Scenario: create a dialogue
  - person 1 (decision maker) wants a new method
  - person 2 reacts critical to the requirement and requests more information - perhaps reasoning against the new method
  - record the reasons / important (pros and cons) points and things to consider when implementing a new method (persons 3 and 4)
  - take the most recent method you implemented in your laboratory (alternative: a method that you are about to implement)

ISO 13843:2017

Water quality - Requirements for establishing performance characteristics of quantitative microbiological methods

Contents

1. Scope
2. Normative References
3. Terms and Definitions
4. Basic Concepts
5. Specifications: **some guideline values**
6. Designs for determining performance characteristics of a method
7. Designs for single laboratory verification of a method

Annexes

A. Mathematical models of variation (*informative*)

B. Assessment of the lower limits (*normative*)

C. Assessment of the upper limits (*normative*)

D. Determination of the operational variability in repeatability and intralaboratory reproducibility conditions (*normative*)

E. uncertainty in counting (*normative*)

F. Determination of the operational variability (interlaboratory reproducibility) in a collaborative performance study (*normative*)

G. Glossary of principal symbols (*informative*)

Scope

This document deals with the **characterization of microbiological methods**.

In terms of this document, characterization means the study of parameters that can be measured to describe how the method is likely to perform in a given set of conditions, which can be described as performance characteristics.

The document describes procedures for the determination of performance characteristics which can be used for subsequent **validation** or **verification** of methods.

applies to selective quantitative methods (all types of water)

Definitions (41)

robustness insensitivity of an analytical method to small changes in the procedure

sensitivity fraction of total number of positive cultures or colonies correctly assigned in the presumptive inspection

specificity fraction of total number of negative cultures or colonies correctly assigned in the presumptive inspection

verification performance of a second characterization by a different laboratory to confirm the results of the original characterization

Performance characteristics

Parameter	Definition	required for performance characteristics	required for single laboratory verification
Sensitivity	fraction of the total positives correctly assigned in the presumptive count	X	X
Specificity	fraction of the total negatives correctly assigned in the presumptive count	X	X
False positive	fraction of positive results (e.g. typical colonies) that are subsequentliy shown to be due to non-	X	X
False negative	fraction of negative results (e.g. atypical colonies) shown to be target organisms	X	X
Selectivity	ratio of the number of target colonies to the total number of colonies in the sample volume	X	X
Efficiency	fraction of total colonies correctly assigned in the presumptive count	X	X

Performance characteristics

Parameter	Definition	required for performance characteristics	required for single laboratory verification
Upper limit	upper end of the working range for which the method is useful (i.e. the maximum countable colonies per plate or other detection system)	X	
Repeatability	precision under repeatability conditions (same operator, same…)	X	X
Reproducibility	precision under intralaboratory reproducibility conditions	X	
Robustness	measure of the capacity of a test to remain unaffected by small but deliberate variations in testing conditions (e.g. temperature)	X	
Relative recovery	efficiency with which a method recovers target organisms from a sample when compared with another procedure	X	
Uncertainty of counting	relative standard deviation of replicate counts of the target obtained by repeated counting (plate) under stipulated conditions	X	X

Validation?

Term is not mentioned because it is not clearly defined!!

Initial characterization can be carried out in a single laboratory that needs to have considerable experience in other microbiological methods.

Includes an unambiguous description of the target of interest (such as positive colony or tube)

Verification

- Implementation of a method developed elsewhere into one laboratory.
- Gathering evidence that the laboratory is able to generate performance data similar to those established in the primary characterization
- Natural samples are the optimal test materials and the work need only address those aspects of the method performance that are of interest to the laboratory.

Specification guideline values

Parameter	Definition	guidance value
Sensitivity	fraction of the total positives correctly assigned in the presumptive count	> 90%
Specificity	fraction of the total negatives correctly assigned in the presumptive count	> 80 %
Selectivity	ratio of the number of target colonies to the total number of colonies in the sample volume	results usually not valid if <10 %

How to…

1. Samples need to contain 20-80 CFU (10-60 target organisms)
   - preferably naturally contaminated
2. all colonies (typical and atypical) are counted
3. all colonies are identified by indipendent method (biochemical tests, DNA sequencing…)

at least 20 Samples from different sources

for spiking samples with surface or wastewater at least 3 different sources

		presumptive count		
		+	-	
confirmed	+	a	b	a+b
count	-	c	d	c+d
		a+c	b+d	n

a true positives (confirmed by secondary identification)

b false negatives

c false positives

d true negatives

		presumptive count		
		+	-	
confirmed count	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	n

sensitivity = a / (a+b)	fraction of the total positives correctly assigned in the presumptive count
Specificity = d / (c+d)	fraction of the total negatives correctly assigned in the presumptive count
false positive rate = c / (a+c)	fraction of positive results (e.g. typical colonies) that are subsequentliy shown to be due to non-target organisms
False negatie rate = b / (b+d)	fraction of negative results (e.g. atypical colonies) shown to be target organisms
Selectivity = a / n	ratio of the number of target colonies to the total number of colonies in the sample volume
Efficiency = (a+d) / n	fraction of total colonies correctly assigned in the presumptive count

Repeatability

- 10 replicates analysed under repeatability conditions (same operator, same equipment, same time…)
- minimum of 3 sets
- minimum of 3 sources of target organism

Sample	1	2	3	4	5	6	7	8	9	10
1										
2										
3										

Uncertainty in counting

- Read the same plates repeatedly under uniform conditions (short intervall of time = max 1 hour) (single person or intralaboratory/more than one)
- Randomly selected plates with more than 20 colonies (no unusual plates)
- count 30 plates

Plate	A1	A2	B1	B2	B3	m	s	$u_{rel,L}$	$u^2_{rel,L}$
1									
2									
3									

working groups

Implementation of CCA (ISO 9308-1:2014), TSC (ISO 14189)

- What sources can be used for the 20 samples required for presumptive/confirmed count

		presumptive count		
		+	-	
confirmed count	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	n

- What kind of secondary identification should be used
- what kind of samples would you count for uncertainty of counting

What sources can be used for the 20 samples required for presumptive/confirmed count?

Water types possible containing the target organisms E. coli or C. perfringens respectively)

- surface water (ponds, rivers, wells, lakes)
- borehole water
- waste/sewage water
- bottled water
- secondary quality control material (PT)

What kind of secondary identification can be used?

for E. coli

- biochemical tests (API)
- Oxidase test +Indole+ Gram stain
- Colilert medium used as p/a test in smaller portions for identification of subcultures
- Vitec2
- BGBB
- MALDI-TOF
- target specific PCR

What kind of secondary identification can be used?

for C. perfringens:

- biochemical tests (API)
- Gram stain + sporestain
- Acid Phosphatase
- Ammonium-Hydroxide
- CAMP Test
- MALDI-TOF
- target specific PCR

What sources can be used for the 30 samples required for assessing uncertainty of counting?

Water types possible containing the target organisms E. coli or C. perfringens respectively)

-
- same as for question 1! No need for additional sources.