

SADCMET Water PT 2016 Evaluation Workshop

**Microbiology Proficiency Testing Scheme
8th round**

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Topics

- Introduction of my Background
- History of SADC MET Water PT - Microbiology
- **Evaluation of 8th PT round 2016**
 - **ISO Algorithm A and z-scores (PT Statistics)**
 - **parameter specific evaluation**

Governmental Institute of Public Health of Lower Saxony - Germany (NLGA) - PT Scheme

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 l 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	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

Sample Types

LIQUID SAMPLES	FREEZE DRIED MATERIAL
Bacterial solution in a defined mineral medium.	Bacteria adsorbed to a matrix (e.g. milk powder).
Handling exactly like a real sample.	Additional handling steps (resuscitation).
Stability 4-6 days after shipment at <math><10^{\circ}\text{C}</math>.	Stability weeks or months if kept frozen, more stable even at ambient temperatures
Germany, France	UK, Sweden, Australia, ...

Preparation



Shipment



- 800 registered Labs 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
<i>known value</i>	<i>specific test items</i>
certified reference material	definite methods
<i>reference material</i>	<i>analysis, measurement or comparison of the test item alongside a reference material or standard, traceable to a national or international standard</i>
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)
<i>Consensus values from participant laboratories</i>	<i>using statistics... (no details given) with consideration of the effects of extreme values</i>

Assigned value

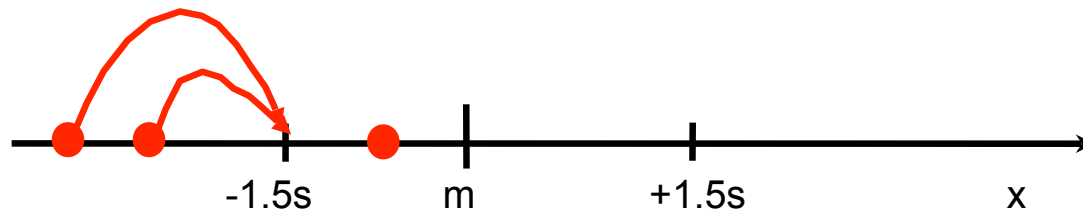
ISO 13528:2009

Statistical methods for use in proficiency testing by interlaboratory comparisons

- details for consensus value from participants results:
 - advantage: easy to realize, 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 $x^* - 1.5s^*$
- extreme values trimmed to $1.5s^*$
- Calculation of Arithmetic mean and SD repeated (*iterative calculation possible*)



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 2016

- 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
- square root transformed results are used in the evaluation of this PT
- **obvious outliers removed**
- 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 25 % depending on the parameter therefore the target value x_{pt} and standard deviation σ_{pt} of the PT were assigned as:

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

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

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

Evaluation procedure

- range:
 - lower warning signal $x_{pt} - 2\sigma_{pt}$
 - upper warning signal $x_{pt} + 2\sigma_{pt}$
- retransformation (square all values)
- rounding to whole numbers (results are counts and there are no parts of bacteria)

Example: Coliform Bacteria

$x_{pt} = 5.085$ calculated by using **Algorithm A** of participants results

$\sigma_{pt} = 1.628$ **32 %** of x_{pt}

$\sigma_{pt} = 0.763$ **15 %** of x_{pt}

calculation of range

lower warning limit (LWL) $x_{pt} - 2\sigma_{pt} = 5.085 - (2 \cdot 0.763) = 3.560$

upper warning limit (UWL) $x_{pt} + 2\sigma_{pt} = 5.085 + (2 \cdot 0.763) = 6.611$

retransformation

rounding

assigned value (CFU/10 ml): $x_{pt}^2 = (5.085)^2 = 25.86 \Rightarrow$ **26**

lower warning limit (LWL): $(x_{pt} - 2\sigma_{pt})^2 = (3.560)^2 = 12.67 \Rightarrow$ **13**

upper warning limit (UWL): $(x_{pt} + 2\sigma_{pt})^2 = (6.611)^2 = 43.70 \Rightarrow$ **44**

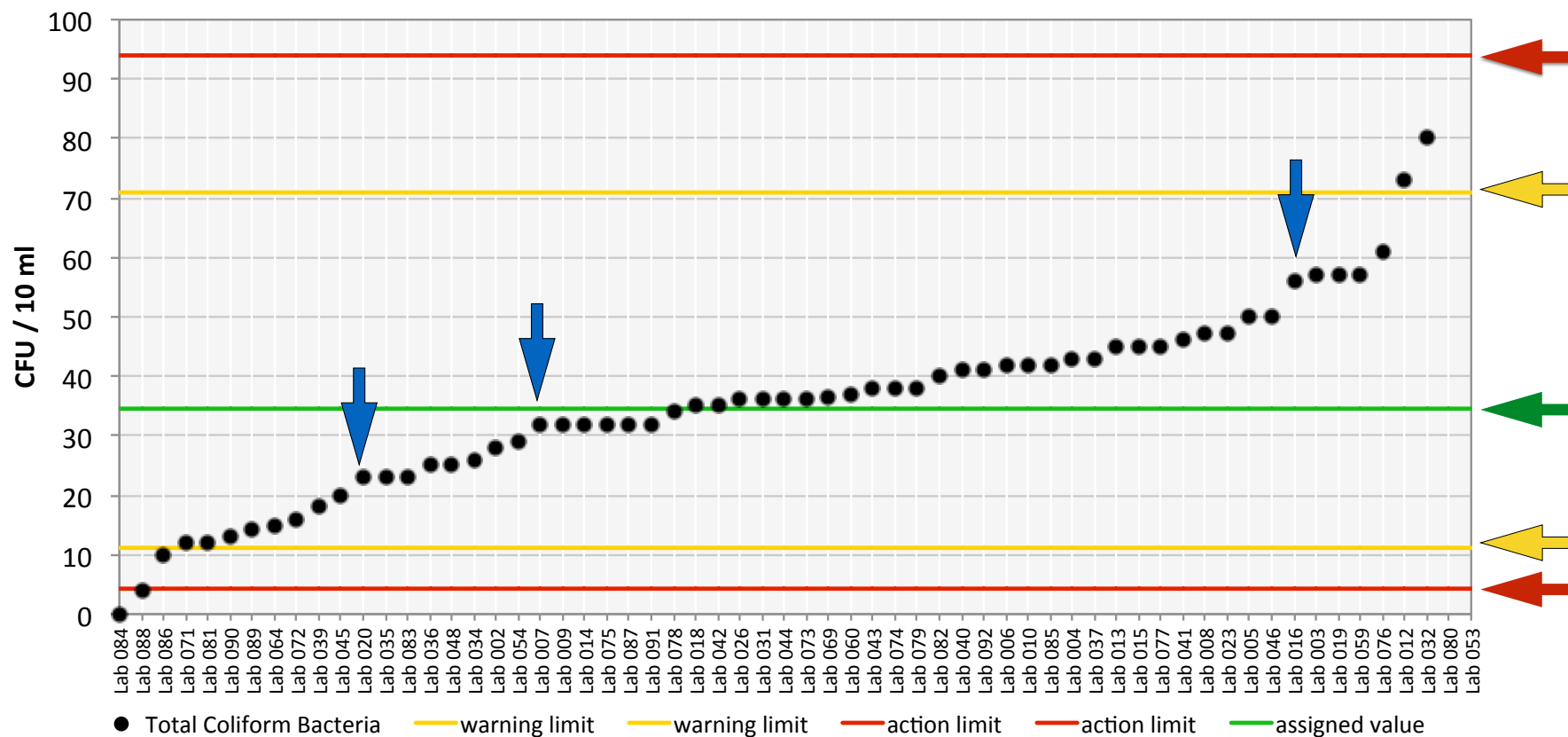
Coliform Bacteria A

n = 59

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	35	35	44	36
rel. standard deviation	23 %	15 %	9 %	20 %
lower warning limit	11	17	30	
upper warning limit	71	58	61	
lower action limit	4	10	24	
upper action limit	94	73	70	

Total Coliform Bacteria A

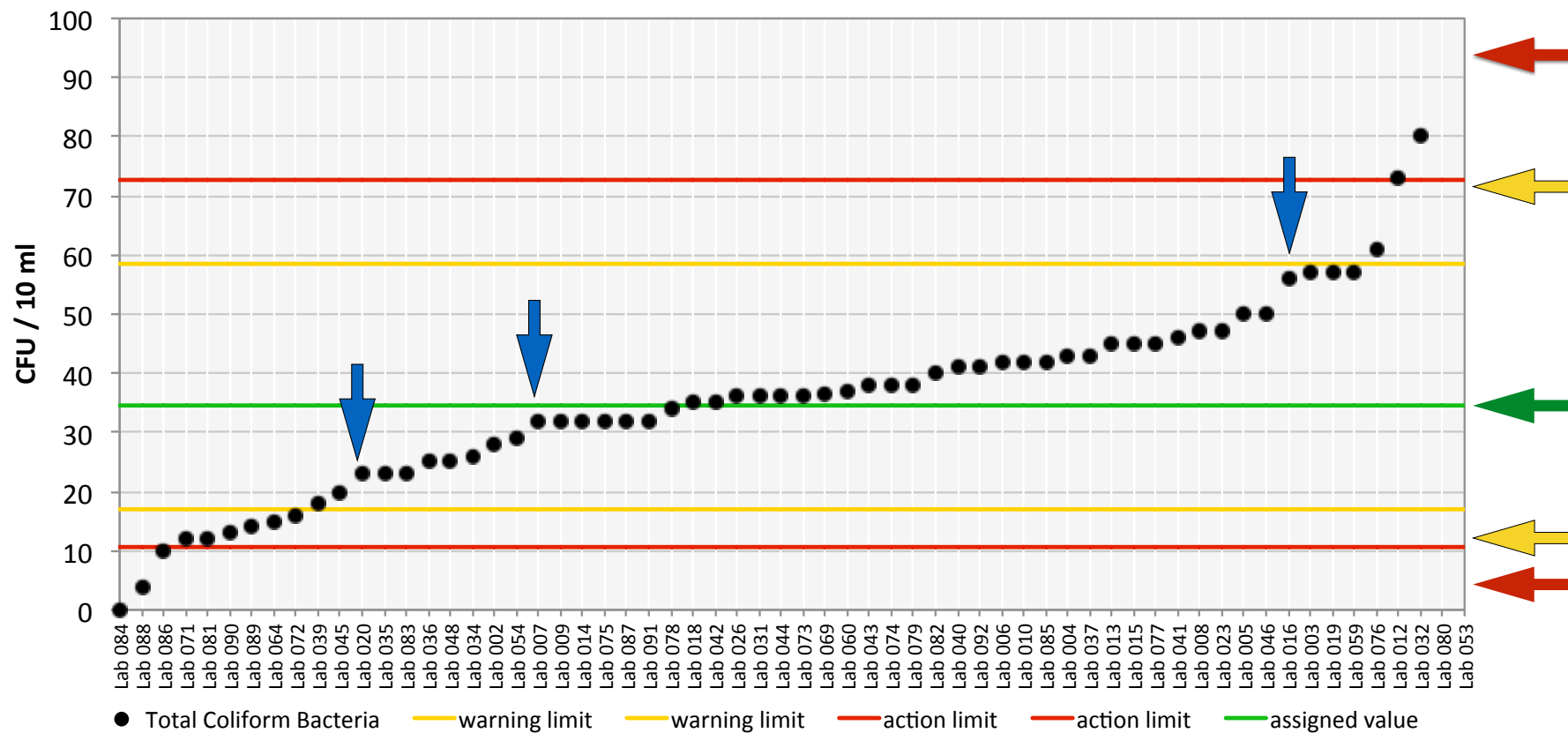
no limitation of standard deviation



Total Coliform Bacteria A

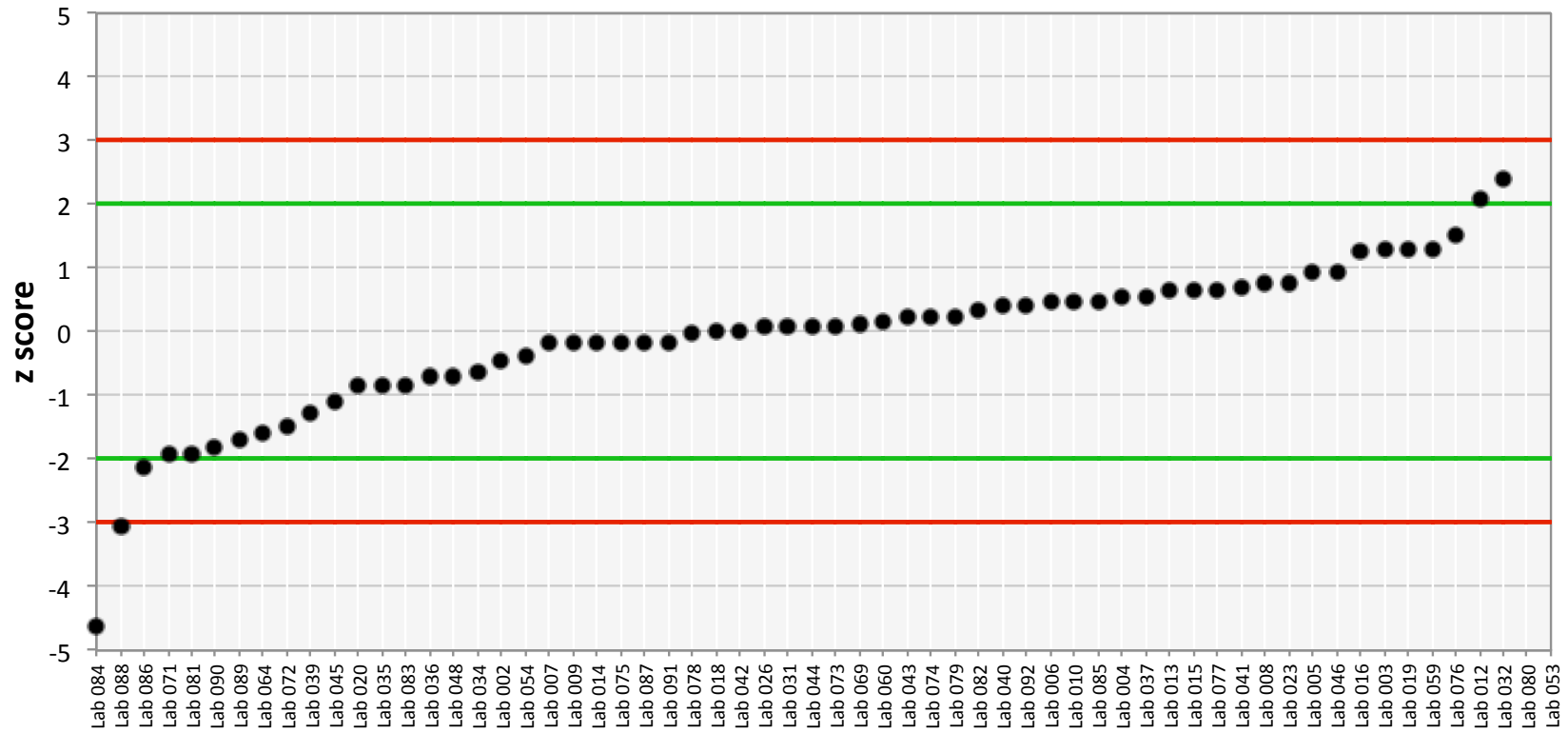
limitation of standard deviation

$$\sigma_{pt} = 15 \%$$



Total Coliform Bacteria

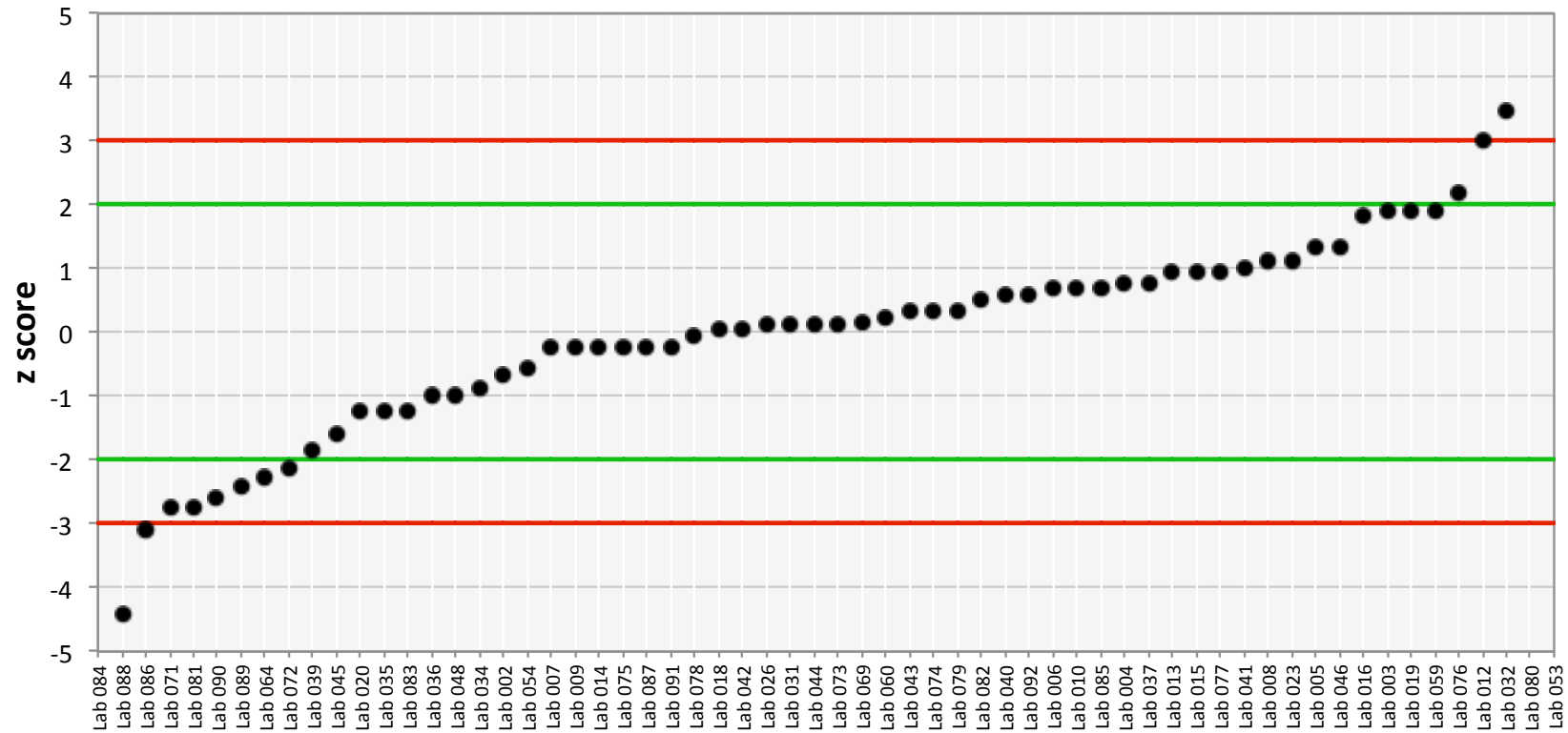
no limitation of standard deviation



Total Coliform Bacteria

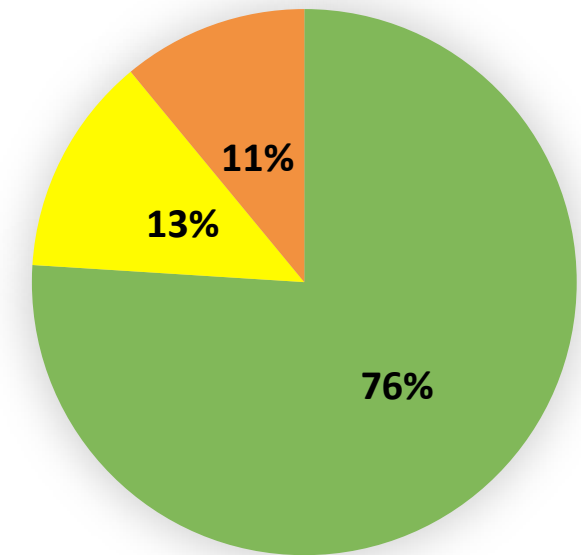
limitation of standard deviation

$$\sigma_{pt} = 15 \%$$



Coliform Bacteria

results	without limitation		with limitation to 15%	
	n	%	n	%
satisfactory	55	89	48	78
questionable	3	4	7	11
unsatisfactory	4	7	7	11
total	62		62	



- Satisfactory
- Questionable
- Unsatisfactory

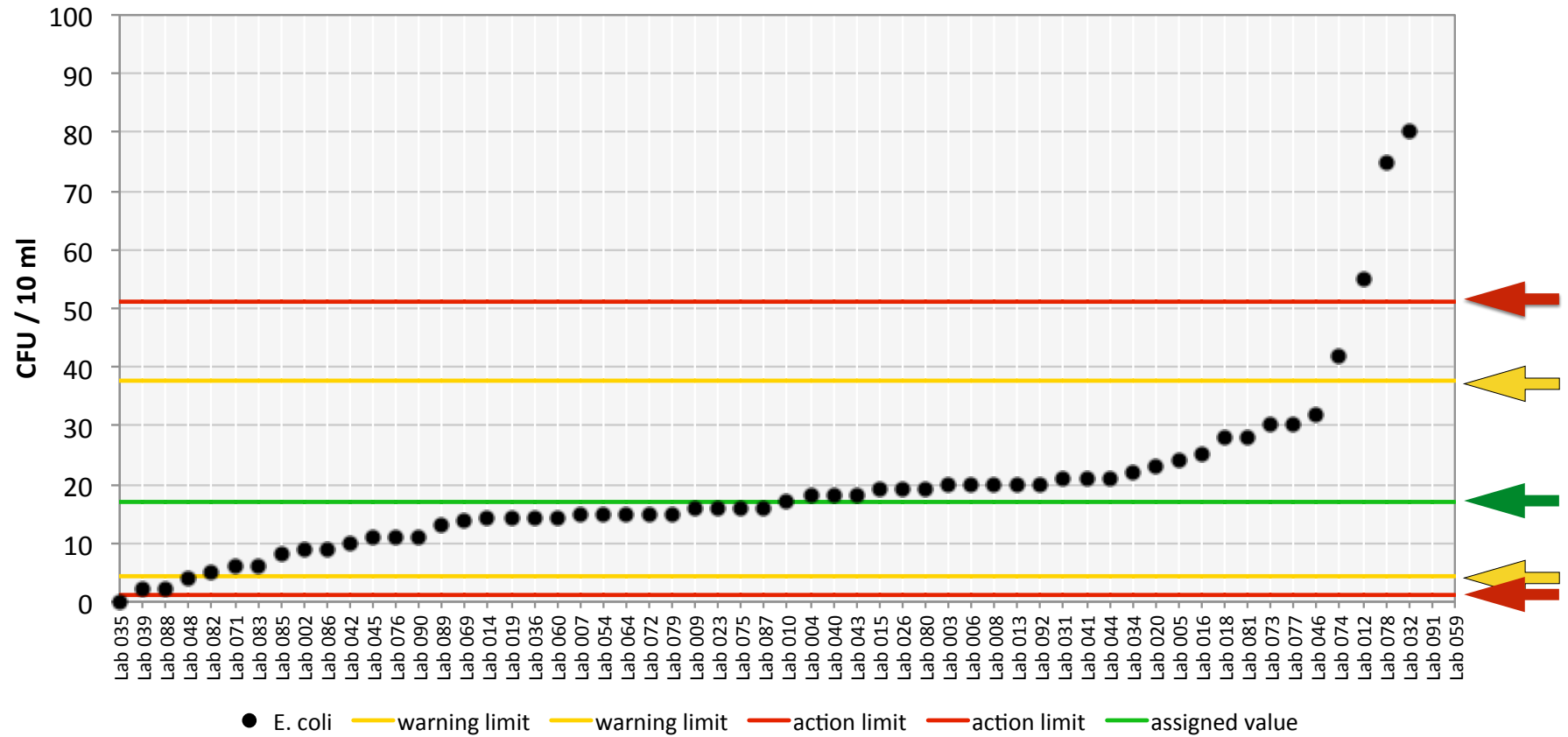
E. coli A

n = 59

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	17	17	19	19
rel. standard deviation	25 %	15 %	10 %	11 % (28 MF)
lower warning limit	4	8	9	
upper warning limit	38	28	29	
lower action limit	1	9	5	
upper action limit	51	32	36	

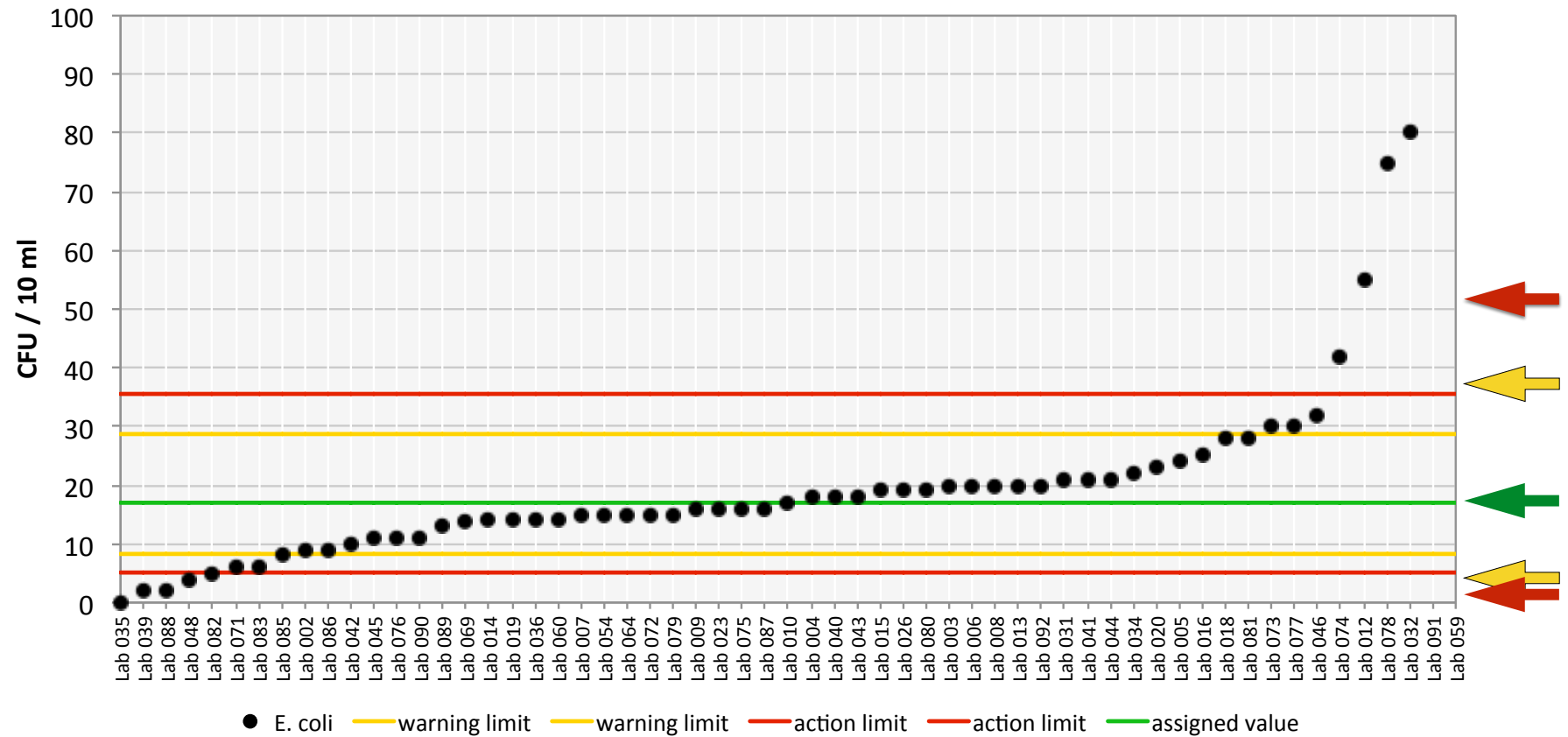
E. coli A

$\sigma_{AlgA} = 25\%$



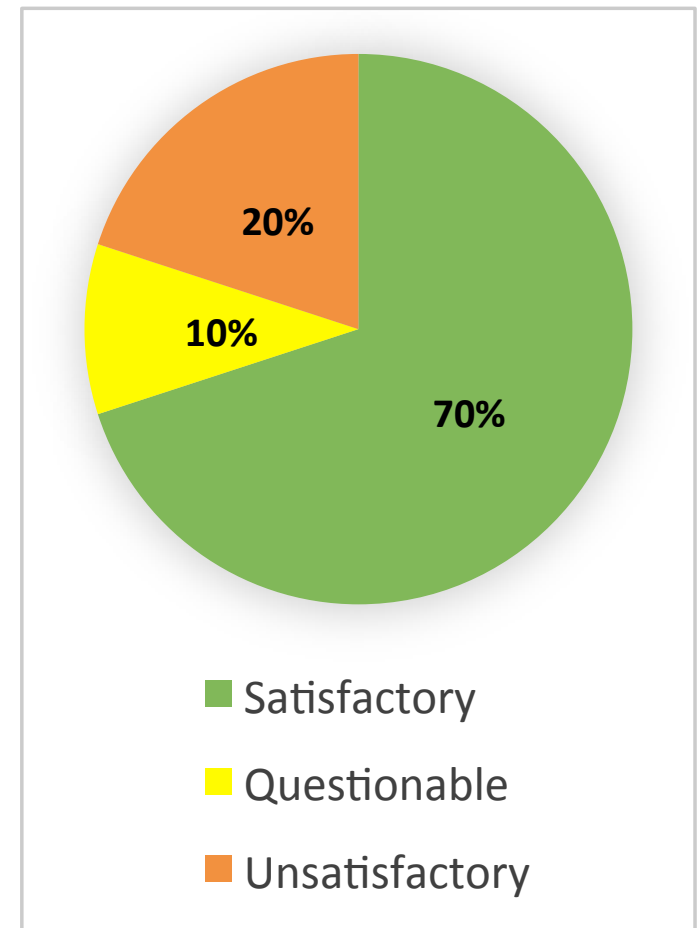
E. coli A

$\sigma_{pt} = 15\%$



E. coli A

results	without limitation		with limitation to 15%	
	n	%	n	%
satisfactory	49	83	42	71
questionable	4	7	6	10
unsatisfactory	6	10	11	19
total	59		59	



E. coli A

- *K. oxytoca* counted as *E. coli* ?
- *K. oxytoca* is indole positive and can grow at 44 °C in confirmation broth but does not produce β -glucuronidase

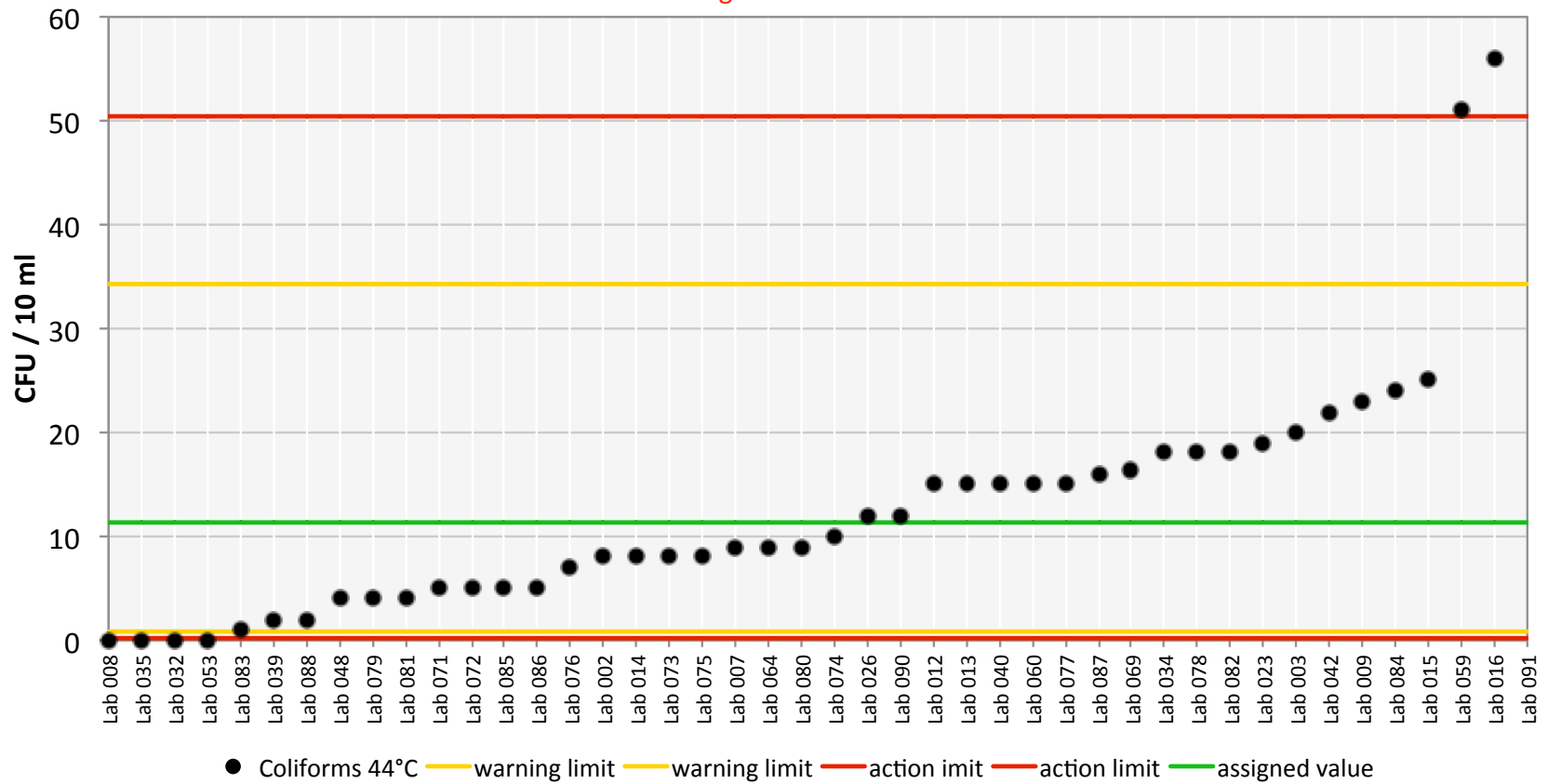
Thermotolerant Coliform Bacteria A

n = 44

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability
assigned value (mean)	11	11	19
rel. standard deviation	37 %	15 %	10 %
lower warning limit	1	6	12
upper warning limit	34	19	28
lower action limit	0	3	9
upper action limit	50	24	32

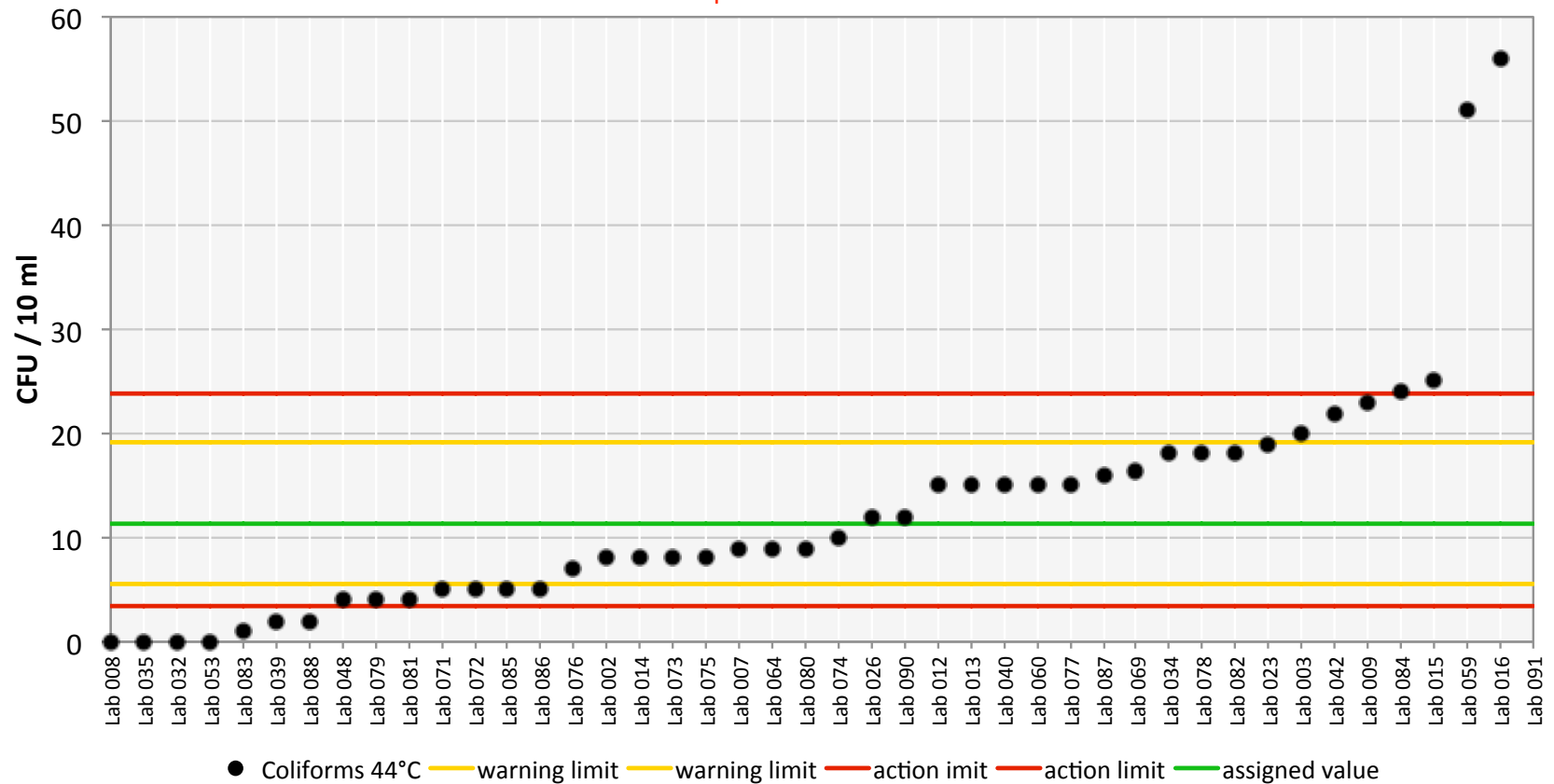
Thermotolerant Coliform Bacteria A

$\sigma_{\text{AlGA}} = 37\%$



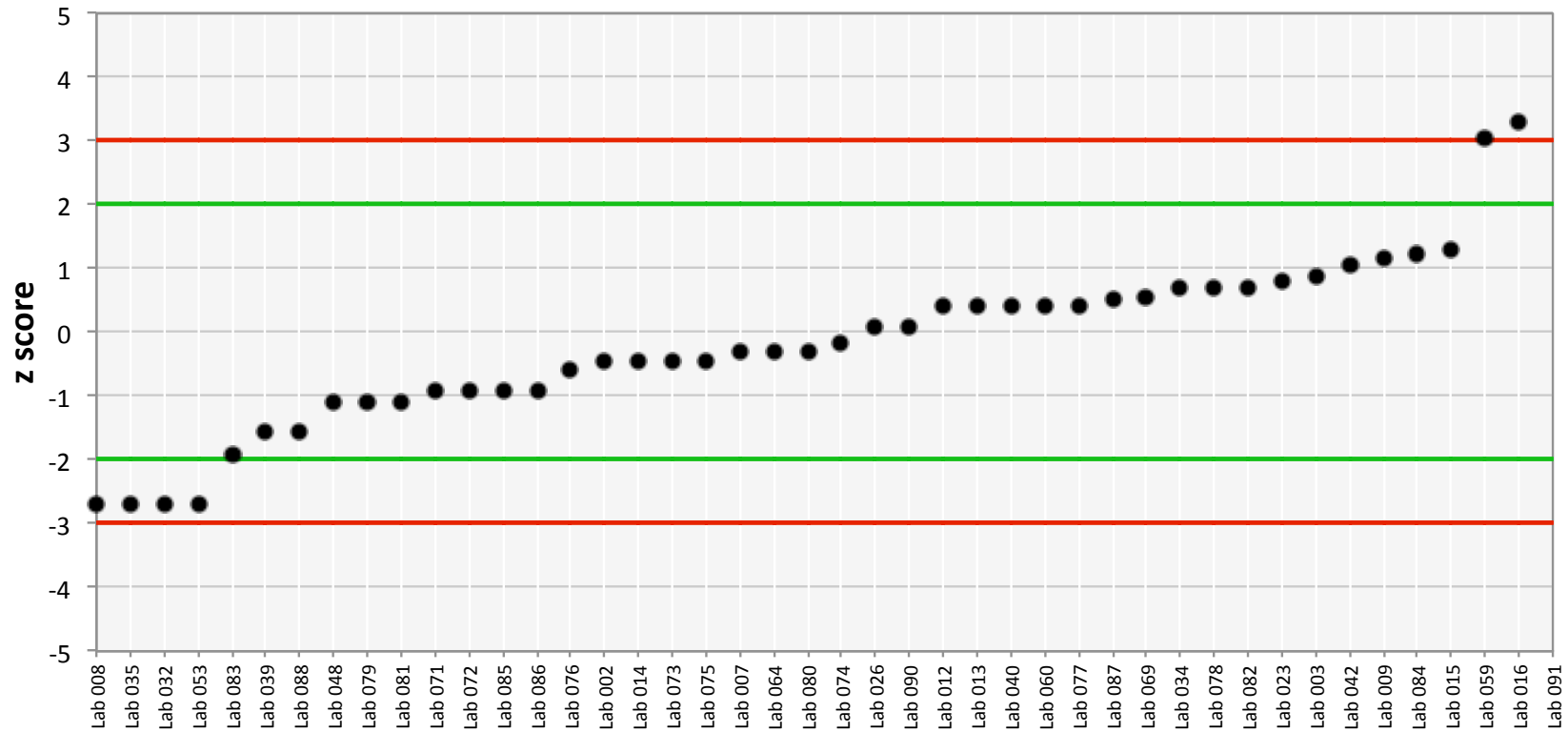
Thermotolerant Coliform Bacteria A

$$\sigma_{pt} = 15 \%$$



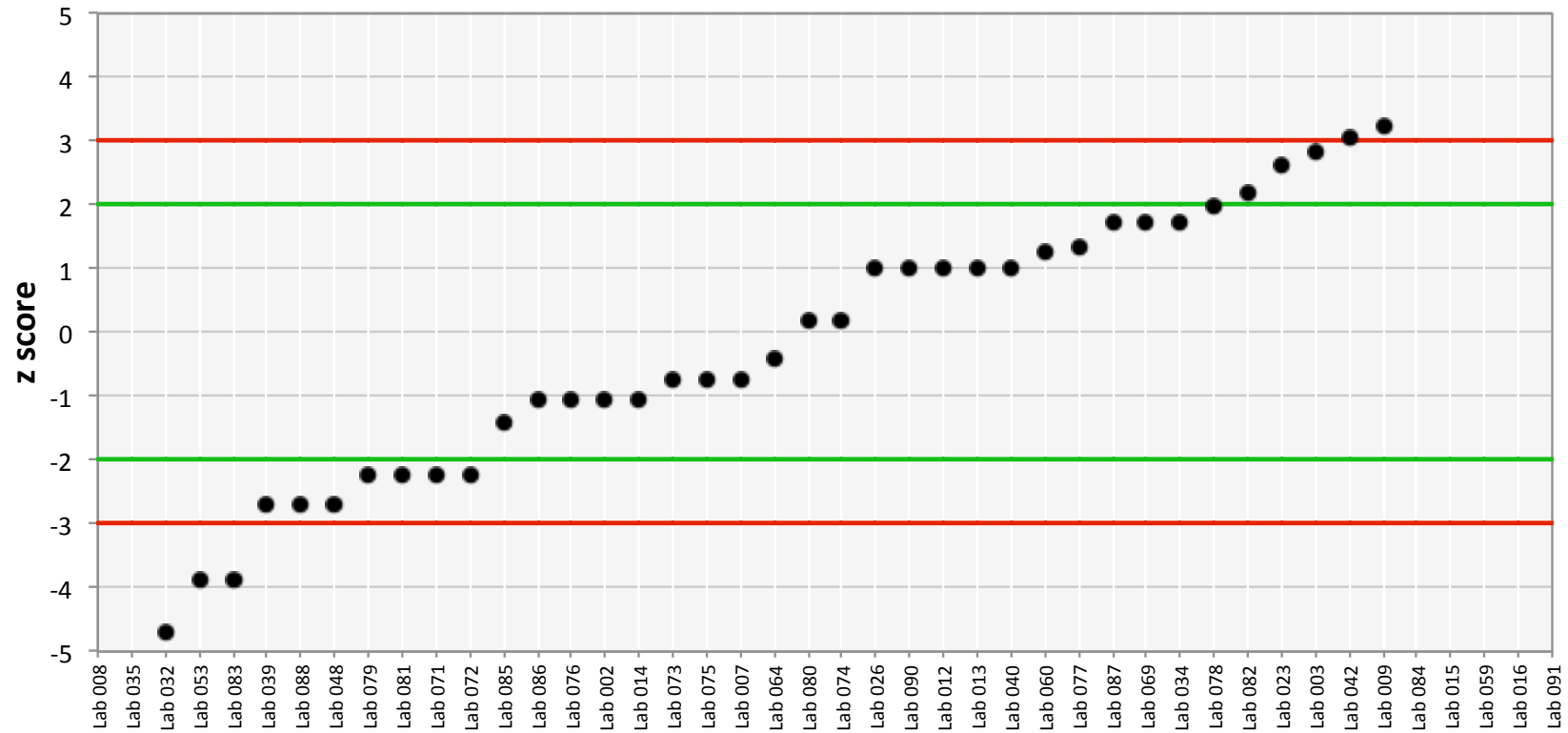
Thermotolerant Coliform Bacteria A

$\sigma_{AlgA} = 37\%$



Thermotolerant Coliform Bacteria A

$\sigma_{pt} = 15\%$



Thermotolerant Coliform Bacteria

	without limitation		with limitation to 15%	
results	n	%	n	%
satisfactory				
questionable				
unsatisfactory				
total				

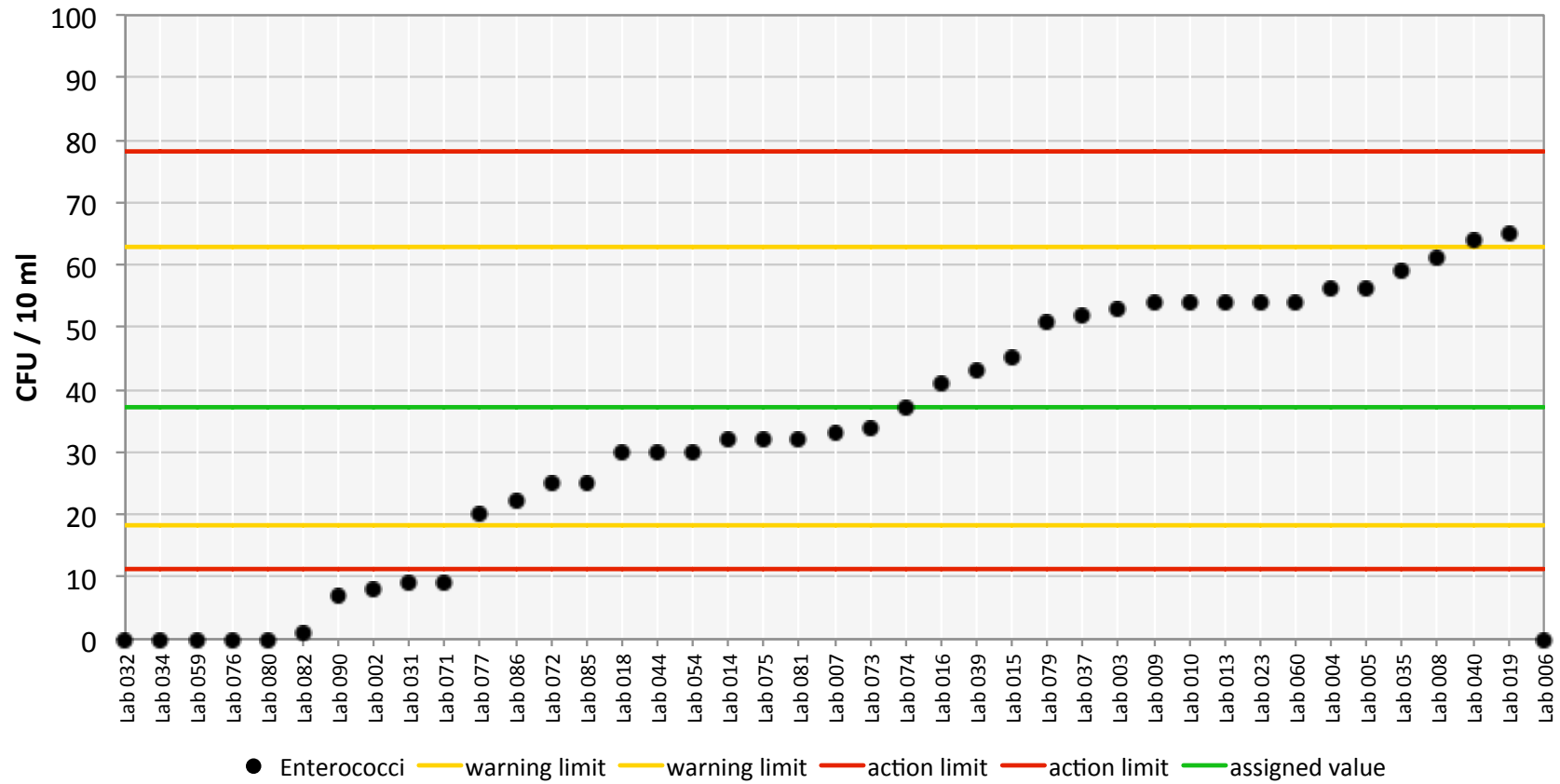
Enterococci A

n = 41

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	37	37	51	43
rel. standard deviation	26 %	15 %	6 %	22 %
lower warning limit	9	18	40	
upper warning limit	86	63	63	
lower action limit	2	11	35	
upper action limit	118	78	69	

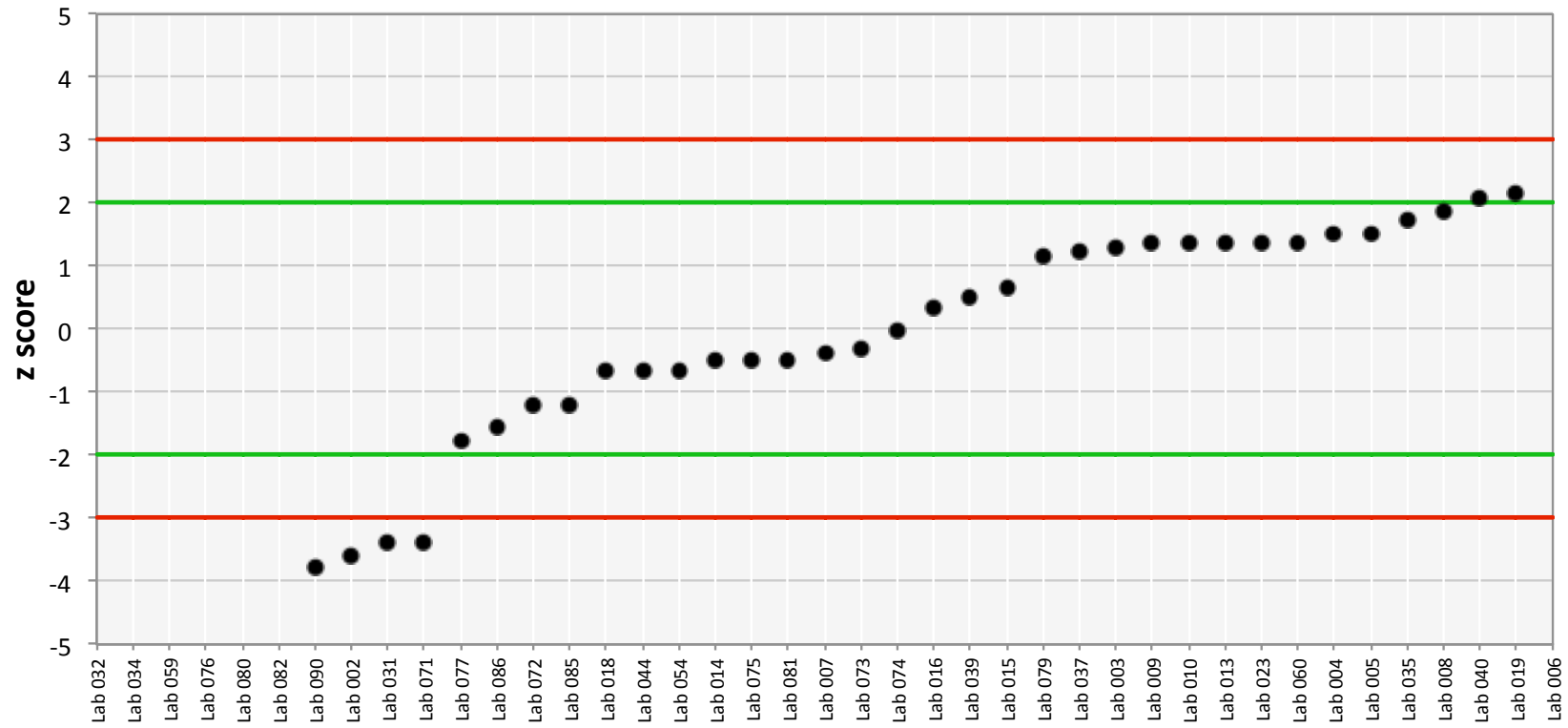
Enterococci

$\sigma_{pt} = 15\%$



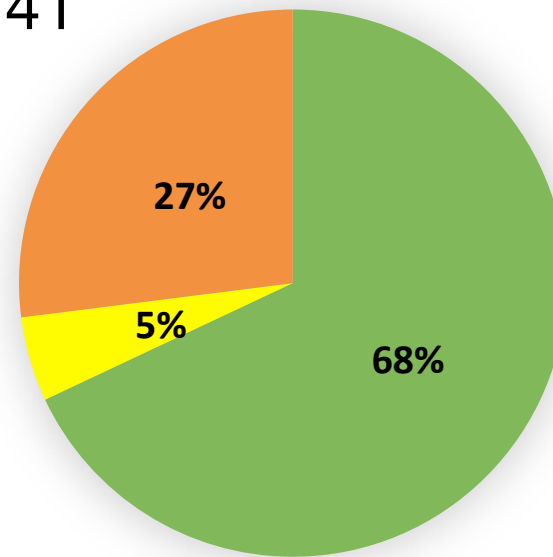
Enterococci

$\sigma_{pt} = 15 \%$



Enterococci A

n = 41



- Satisfactory
- Questionable
- Unsatisfactory

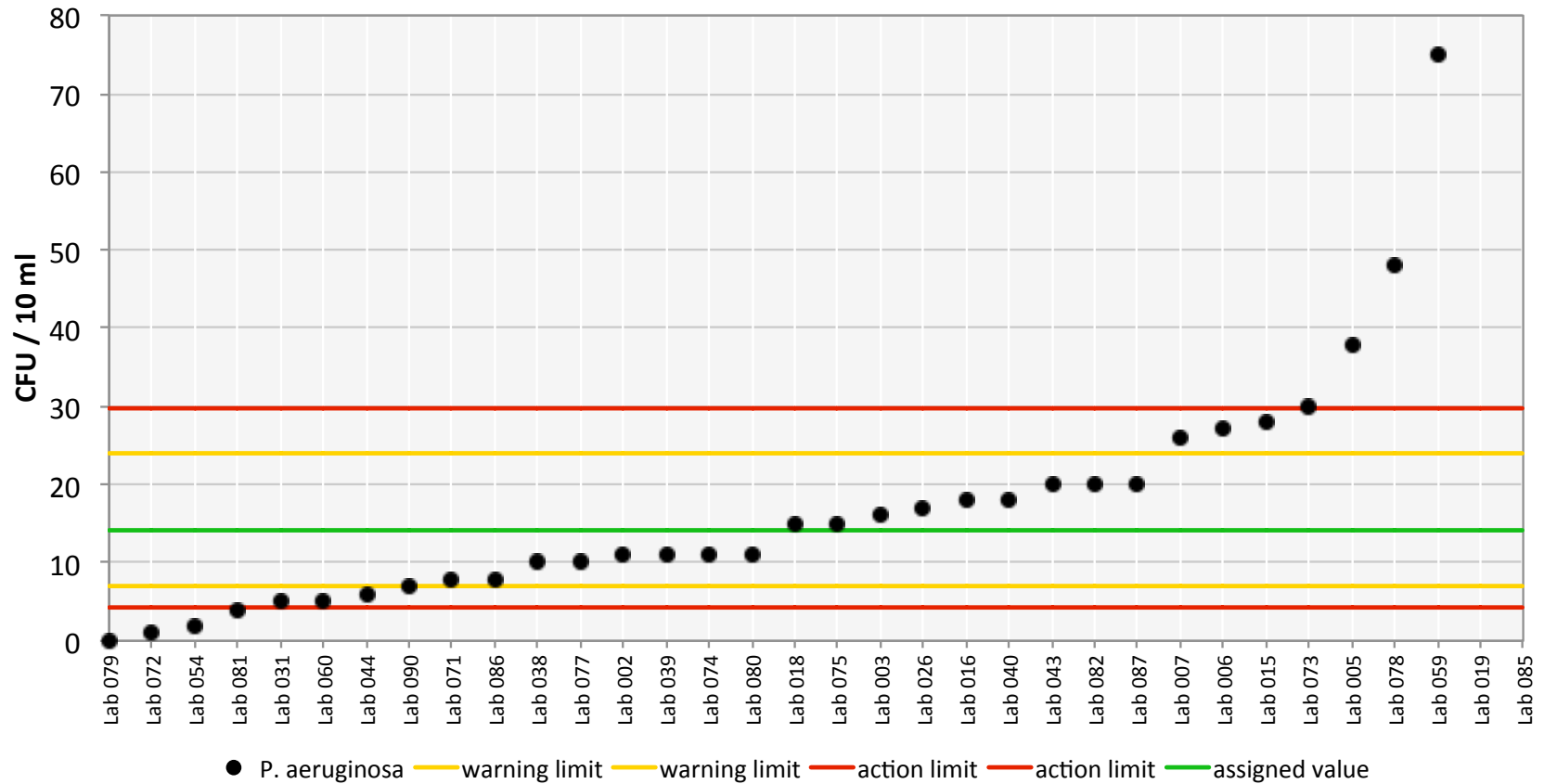
Pseudomonas aeruginosa A

n = 34

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	14	14	22	21
rel. standard deviation	38 %	20 %	12 %	19 %
lower warning limit	1	7	13	
upper warning limit	44	24	33	
lower action limit	0	4	9	
upper action limit	66	30	40	

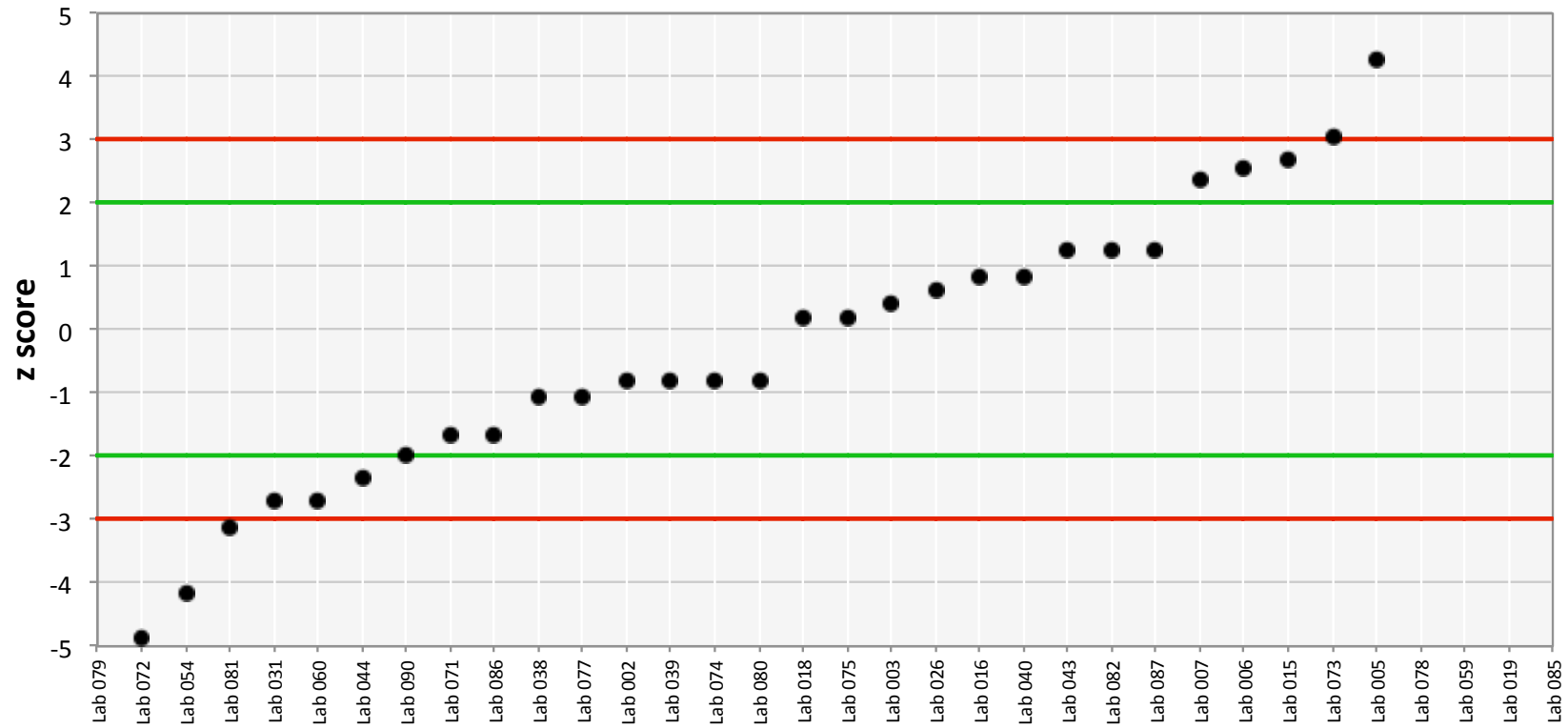
Pseudomonas aeruginosa A

$\sigma_{pt} = 20\%$



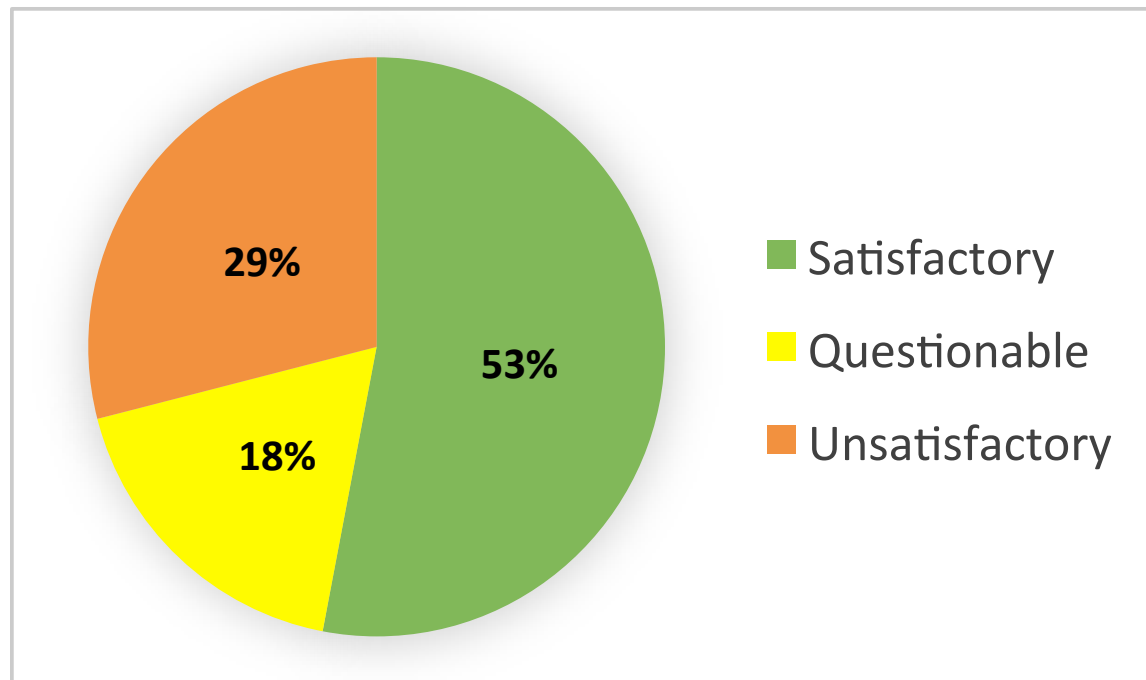
Pseudomonas aeruginosa

$\sigma_{pt} = 20\%$



Pseudomonas aeruginosa A

n = 34



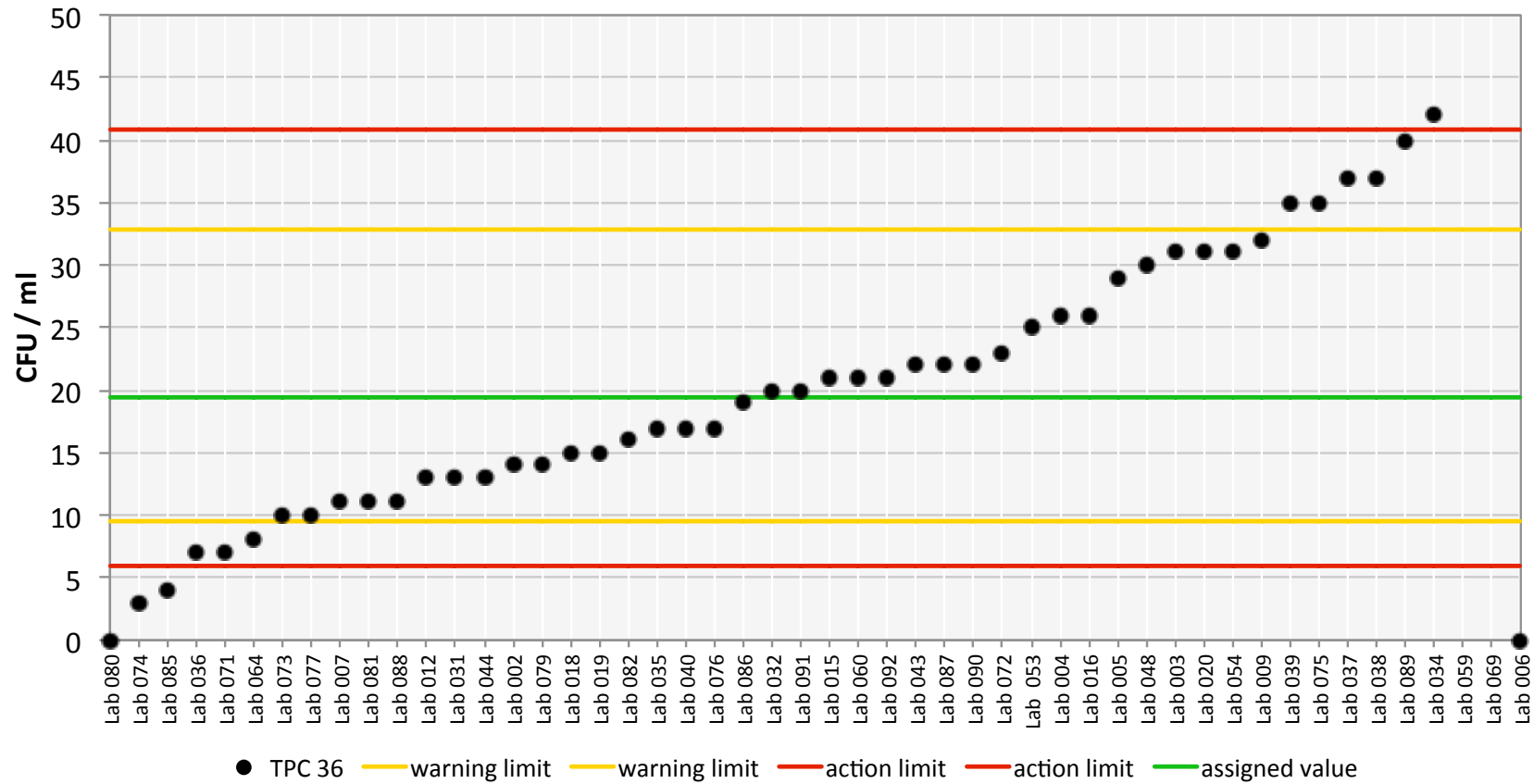
Total Plate Count 36°C A

n = 50

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	19	19	27	28
rel. standard deviation	28 %	15 %	11 %	13 %
lower warning limit	4	10	16	
upper warning limit	47	33	40	
lower action limit	1	6	12	
upper action limit	65	41	47	

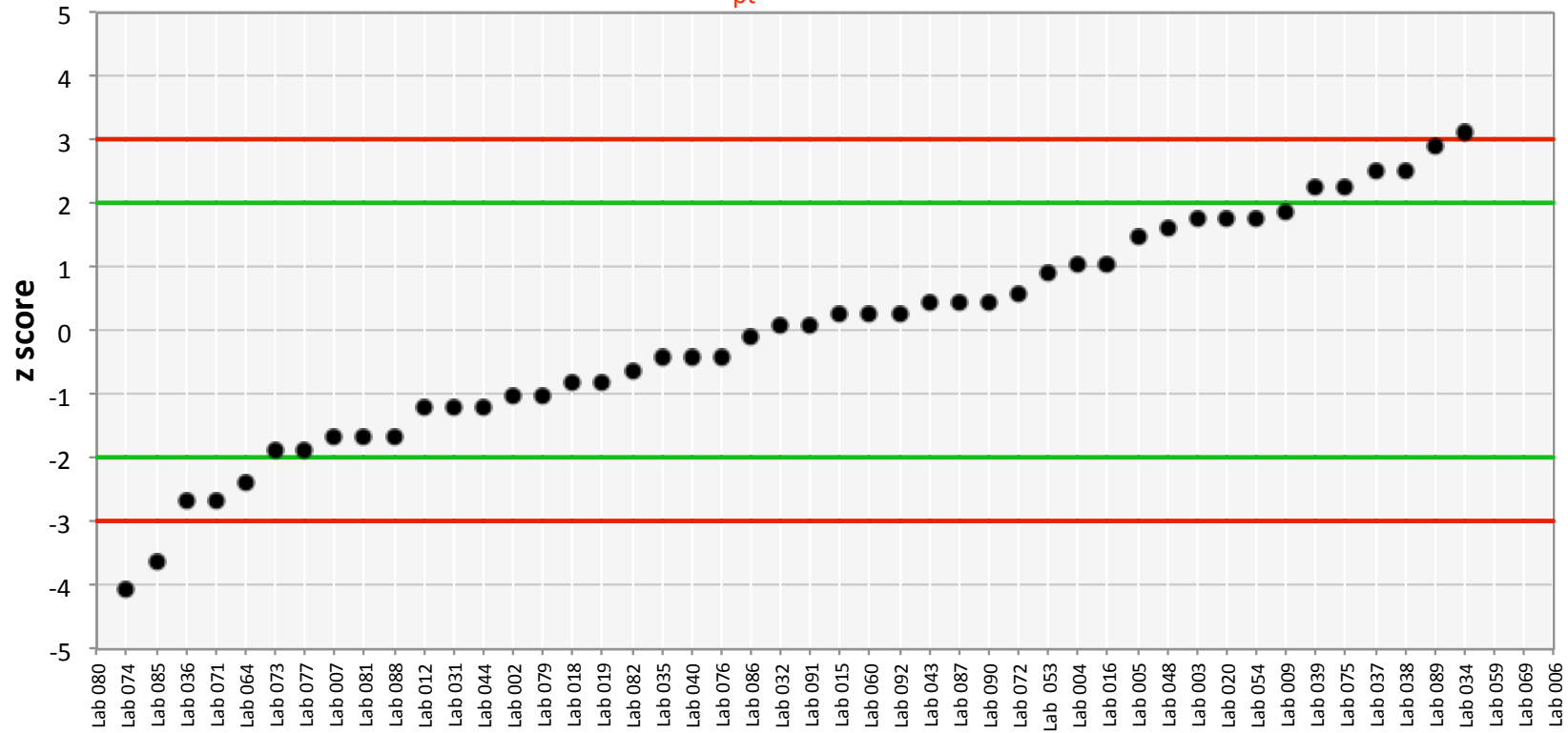
Total Plate Count 36°C

$\sigma_{pt} = 15\%$



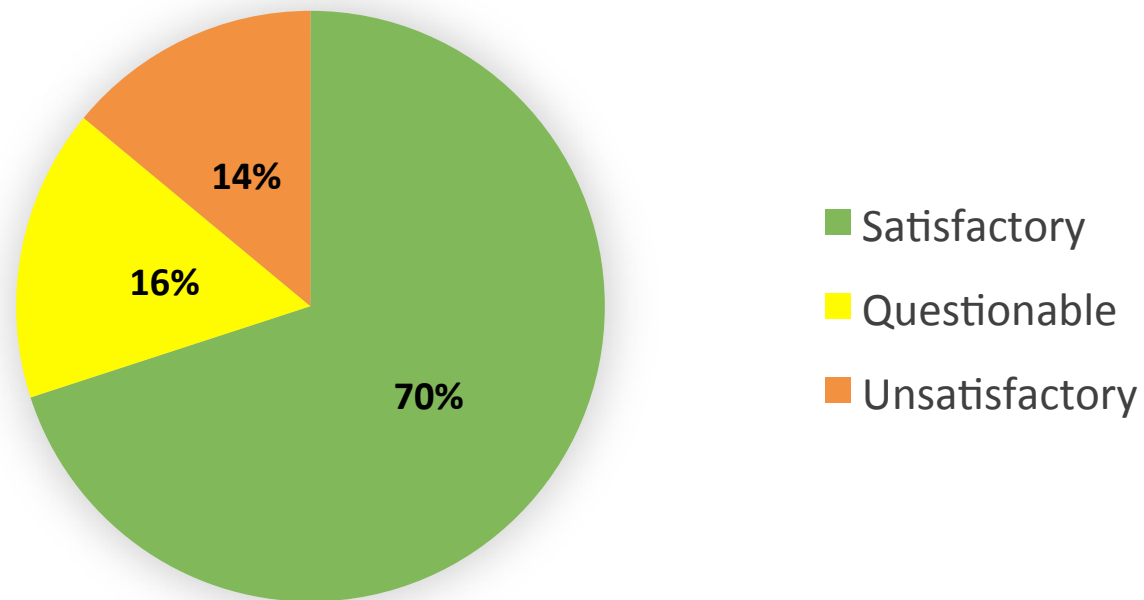
Total Plate Count 36°C

$\sigma_{pt} = 15\%$



Total Plate Count 36°C A

n = 50



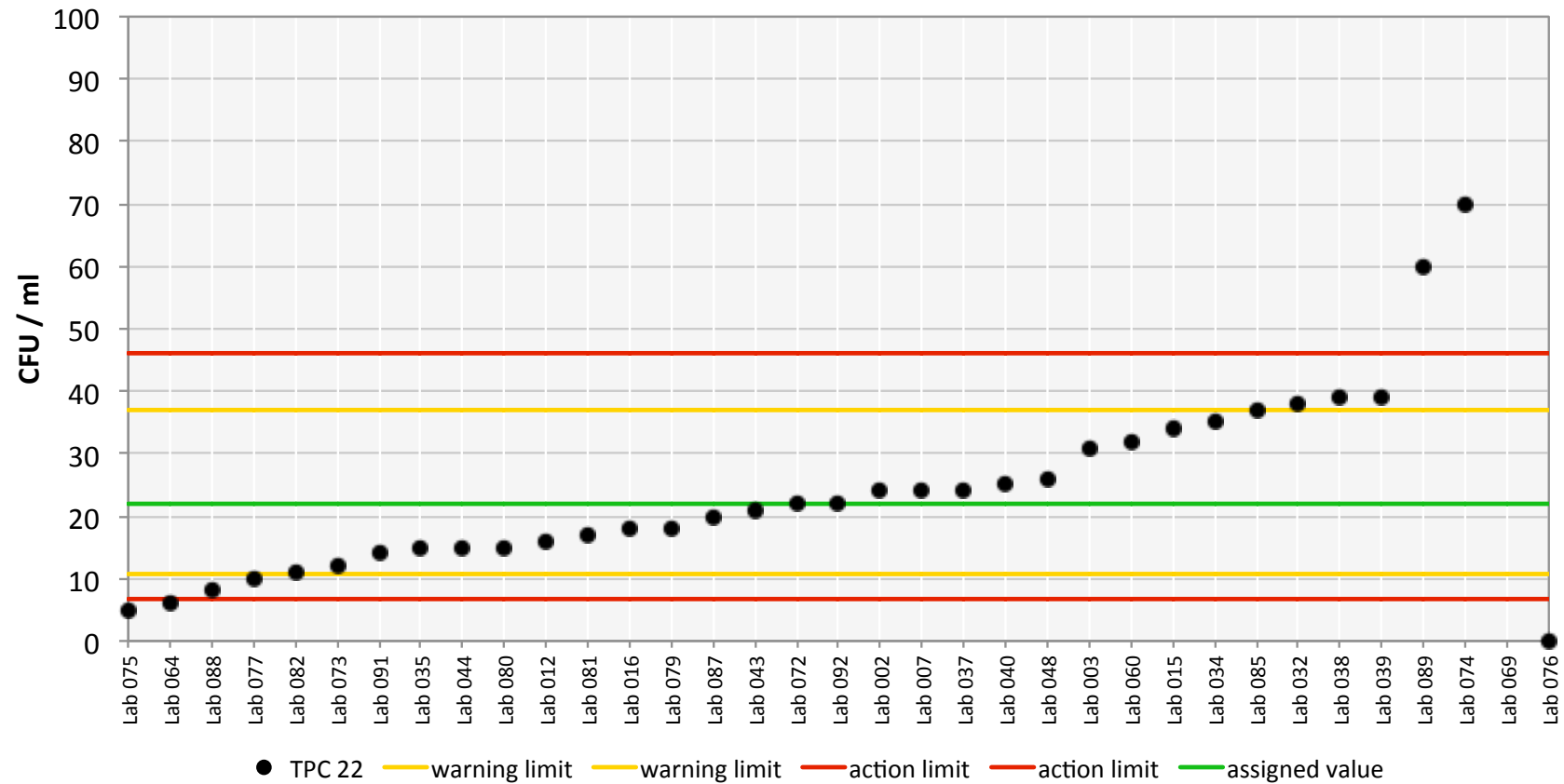
Total Plate Count 22°C A

n = 35

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability	NFA PT
assigned value (mean)	22	22	27	29
rel. standard deviation	28 %	15 %	11 %	13 %
lower warning limit	4	11	16	
upper warning limit	53	37	40	
lower action limit	1	7	12	
upper action limit	74	46	47	

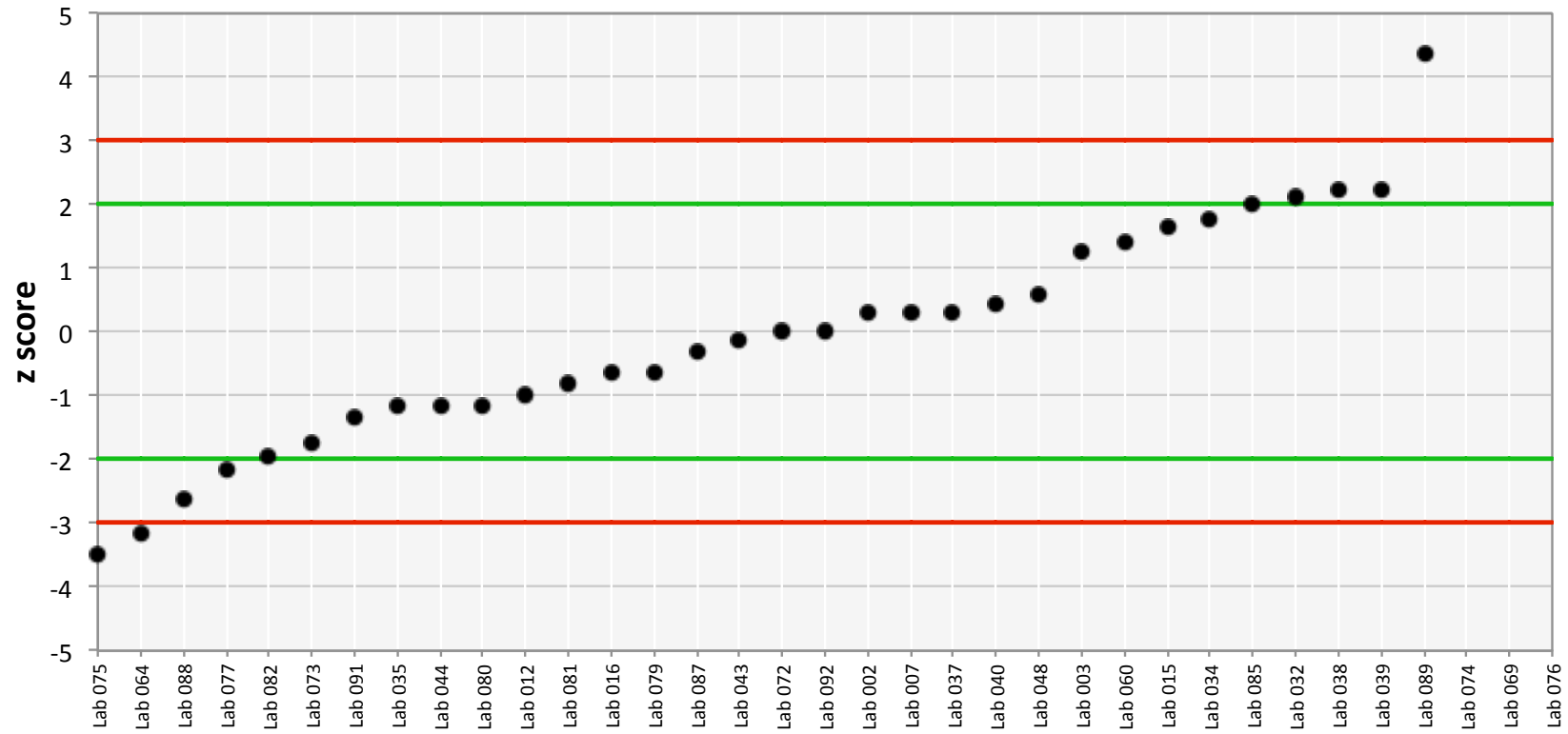
Total Plate Count 22°C

$\sigma_{pt} = 15\%$



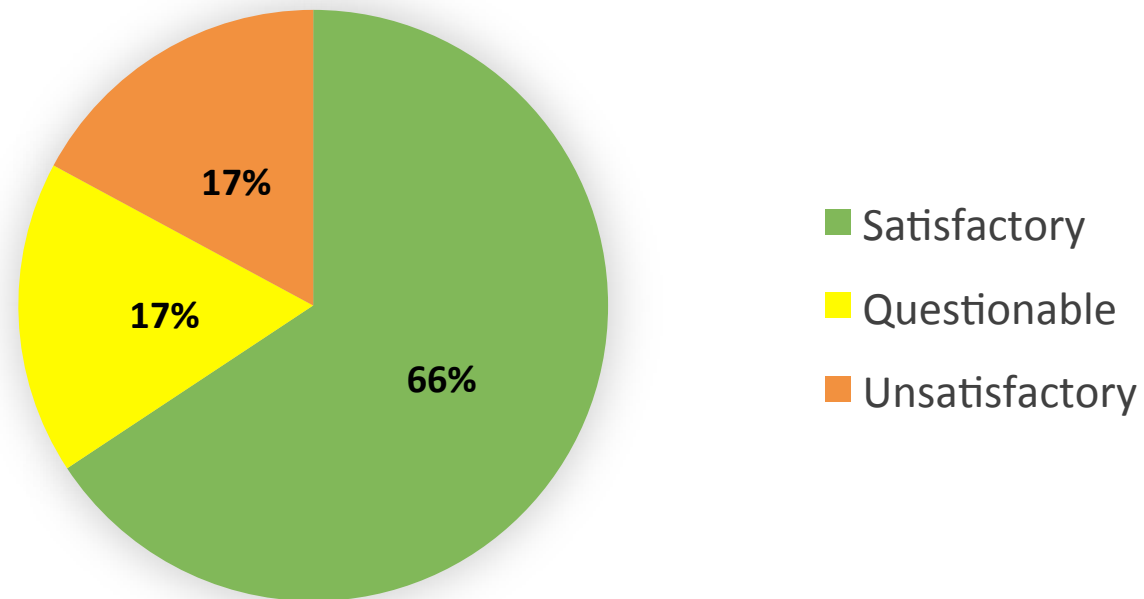
Total Plate Count 22°C

$\sigma_{pt} = 15\%$



Total Plate Count 22°C A

n = 35



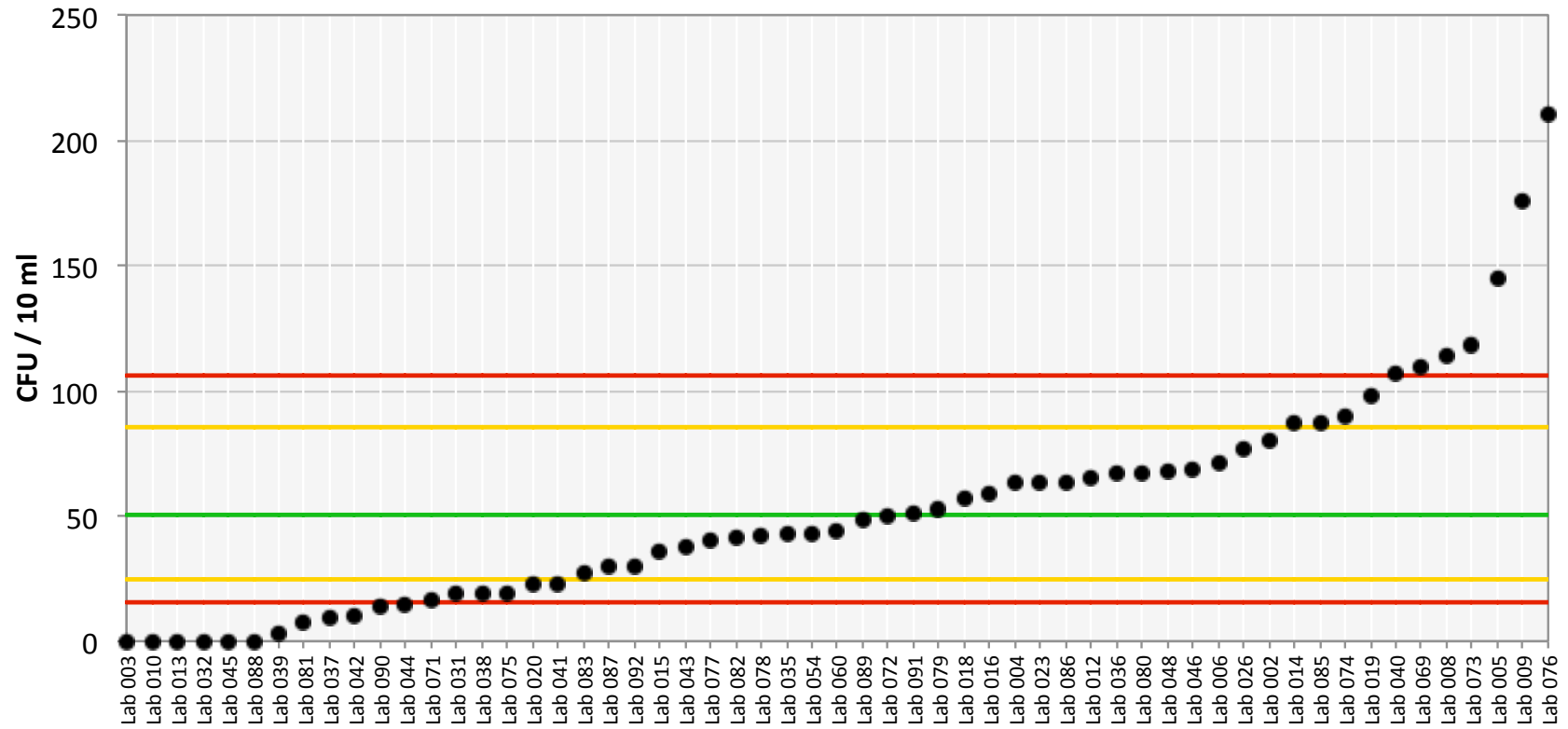
Material B

Microorganism	Strain no.
<i>Citrobacter freundii</i>	SLV-424
<i>Aeromonas hydrophila</i>	SLV-081
<i>C. perfringens</i>	SLV-442

- E. coli, Enterococci and Pseudomonas should have given negative results: no statistical evaluation of these parameters
- instructions for Coliform bacteria were mixed up (10 ml vs 1 ml): no statistical evaluation of this parameter

Total Coliform Bacteria Material B

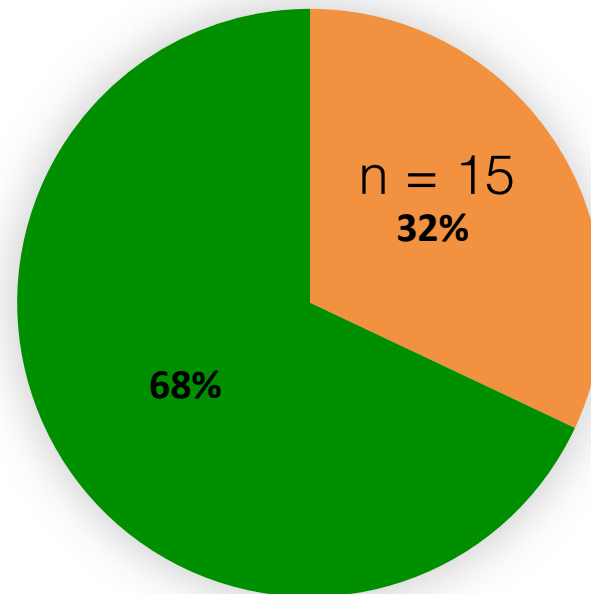
not used for evaluation



E. coli B

n = 49

- methods?
- confirmation steps?



False positives
True Negatives

E. coli B

n = 49

Lab 042	2	-	MPN	Peptone water	24HRS,44oC	
Lab 086	2	-	MF	EMB AGAR	24HRS,44oC	
Lab 073	3	-	MF	EMB AGAR	24HRS,37oC	
Lab 079	3	-	MF	EMB AGAR	24HRS,37oC	
Lab 083	5	-	MF	EMB AGAR	24HRS,44oC	
Lab 071	8	-	MF	EMB AGAR	24HRS,37oC	
Lab 059	12	-		EMB AGAR	48HRS,37oC	
Lab 077	19	-	ISO 9308-1:2000	EMB AGAR	24HRS,37oC	
Lab 074	21	-	MF	EMB AGAR	24HRS,37oC	
Lab 078	22	-	MF	EMB AGAR	24HRS,37oC	
Lab 015	23	-	MF	mLGA	24HRS, 37oC	
Lab 082	23	-	MF	EMB AGAR	24HRS,37oC	
Lab 075	24	-	MF	EMB AGAR	24HRS,37oC	
Lab 076	470	-	MF	mFC AGAR BASE	24HRS,37oC	
Lab 035	<0.3	-	ES ISO 9308-2	LST,BGL,EC	44HRS,37oC	indole
Lab 093	1	-	MF	Tryptone bile agar	24HRS,44oC	indole

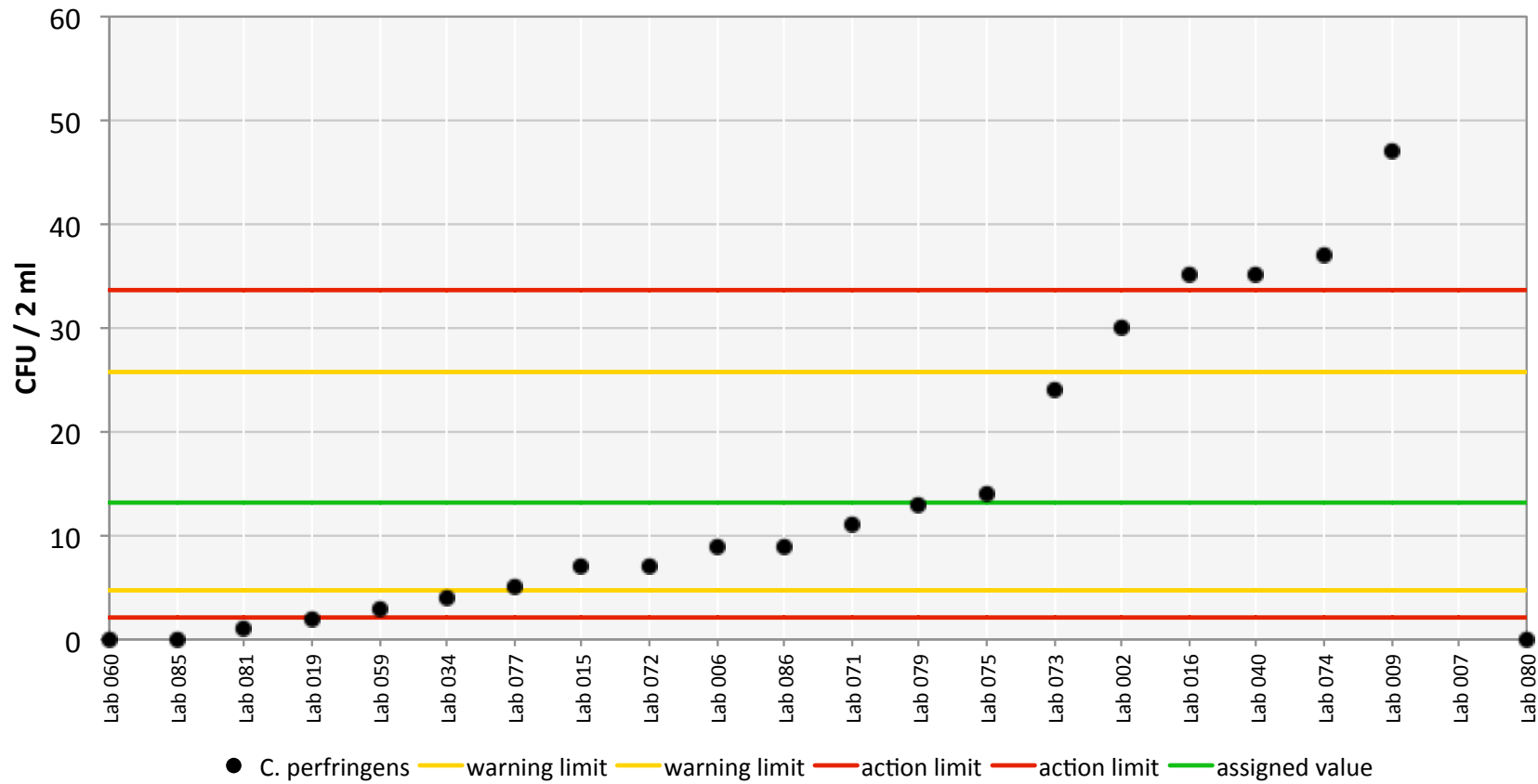
C. perfringens B

n = 22

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability
assigned value (mean)	13	13	40
rel. standard deviation	55 %	20 %	32 %
lower warning limit	0	5	5
upper warning limit	58	26	108
lower action limit	0	2	0
upper action limit	93	34	154

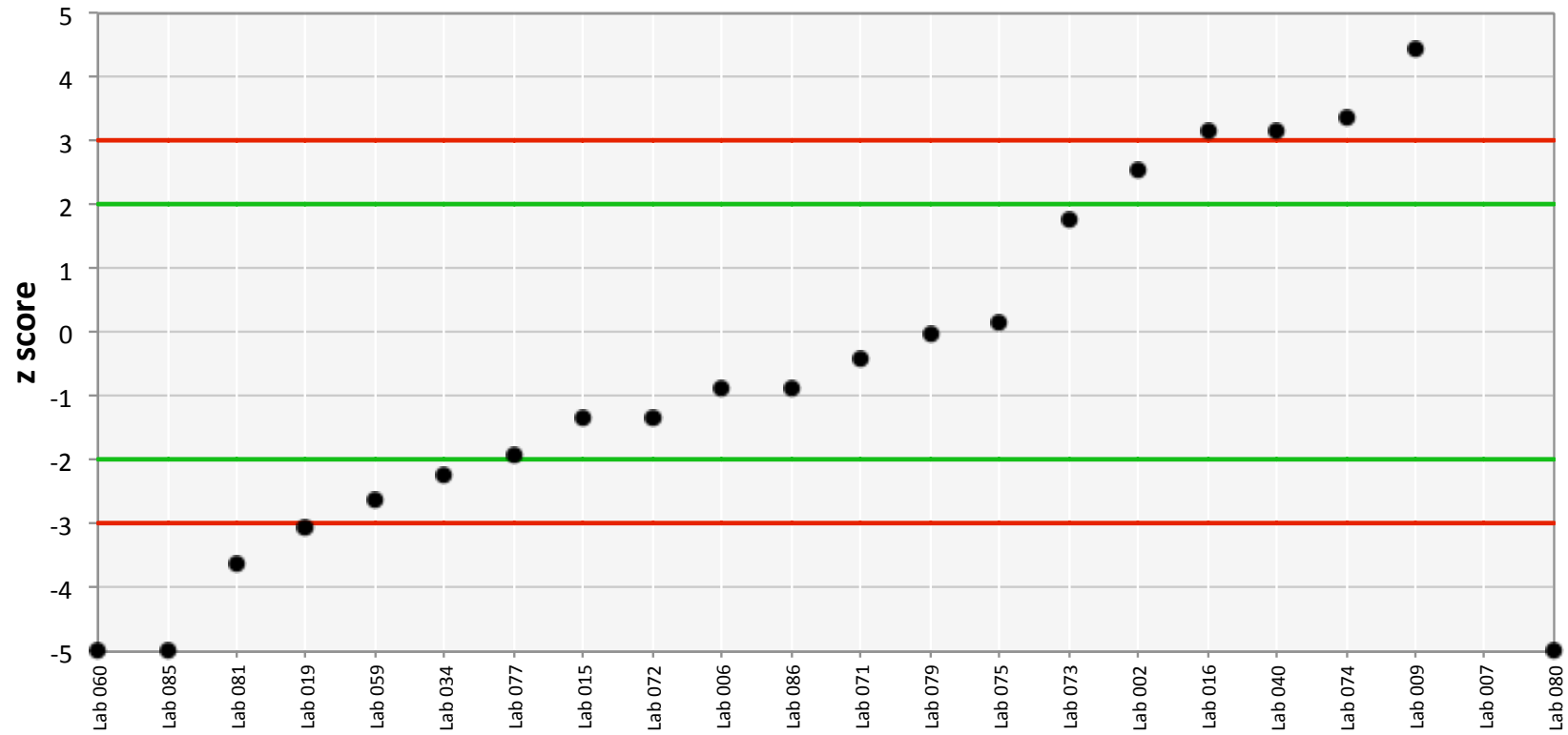
C. perfringens

$\sigma_{pt} = 20\%$



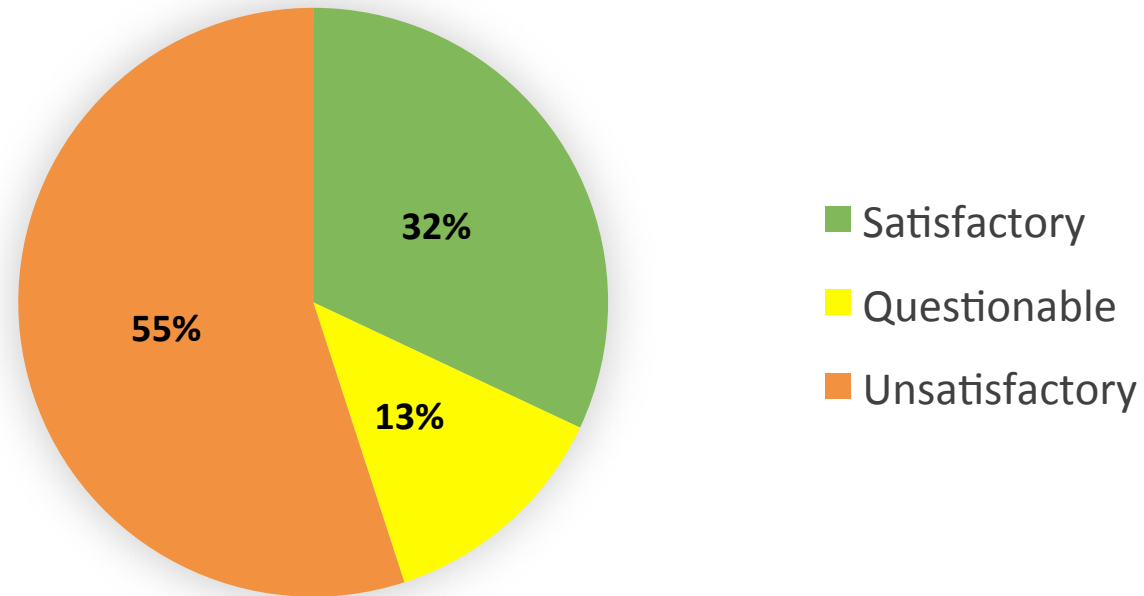
C. perfringens

$\sigma_{pt} = 20\%$



C. perfringens B

n = 22



C. perfringens B

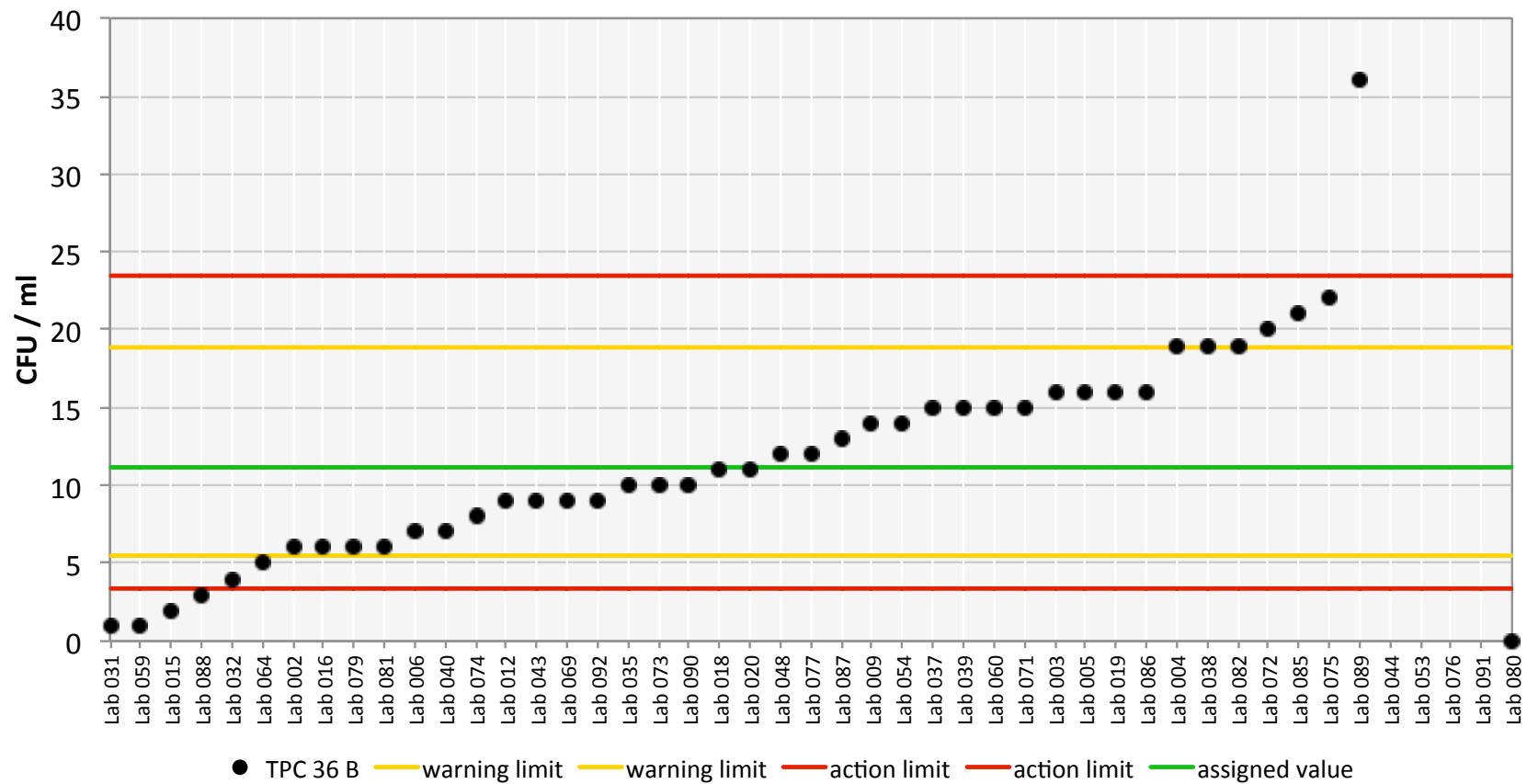
	Result CFU/10ml	Z-Score	Method used	Media Used	Temp/Hours	Confirmation
Lab 060	0	-6,67	ISO 6461-2:1936	TSC agar	48hrs,37oC	
Lab 085	0	-6,67	MF	TSC agar	24hrs,44oC	
Lab 081	1	-4,83	MF	TSC agar	24HRS,37oC	
Lab 019	2	-4,07	ISO 6461-2:1986	Sulphite iron agar	48hrs,37oC	N/A
Lab 059	3	-3,48		SPS agar	24HRS,37oC	
Lab 034	4	-2,99	MF	TSC agar	24HRS,37oC	N/A
Lab 077	5	-2,56	ISO 621-2:1986	TSC agar	24hrs,37oC	
Lab 015	7	-1,80	MF	mCP	24hrs,37oC	Catalase
Lab 072	7	-1,80	POUR PLATE	Gelose TSC agar	44hrs,37oC	
Lab 006	9	-1,15	MF			
Lab 086	9	-1,15	MF	TSC agar	24hrs,37oC	
Lab 071	11	-0,57	MF	TSC agar	24HRS,37oC	
Lab 079	13	-0,04	MF	TSC agar	48hrs,37oC	
Lab 075	14	0,21	MF	TSC agar	44hrs,37oC	
Lab 073	24	2,34	MF	Gelose TSC agar	72hrs,37oC	
Lab 002	30	3,40	ISO 6461-2:1986	Iron Sulphite agar	44hrs,37oC	N/A
Lab 016	35	4,21	ISO 14189:2013	mCP	23hrs,44oC	Ammonium Hydroxide test
Lab 040	35	4,21	ISO 14189:2013	Perfringens TSC agar	24hrs,44oC	Acid phosphatase
Lab 074	37	4,52	MF	TSC agar	48hrs,37oC	
Lab 009	47	5,94	MF	mCP		Ammonium Hydroxide test
Lab 007	190	18,68	MF	mCP	24HRS,37oC	
Lab 080	TNTC	#WERT!	POUR PLATE	TSC agar	24HRS,37oC	

Total Plate Count 36°C B

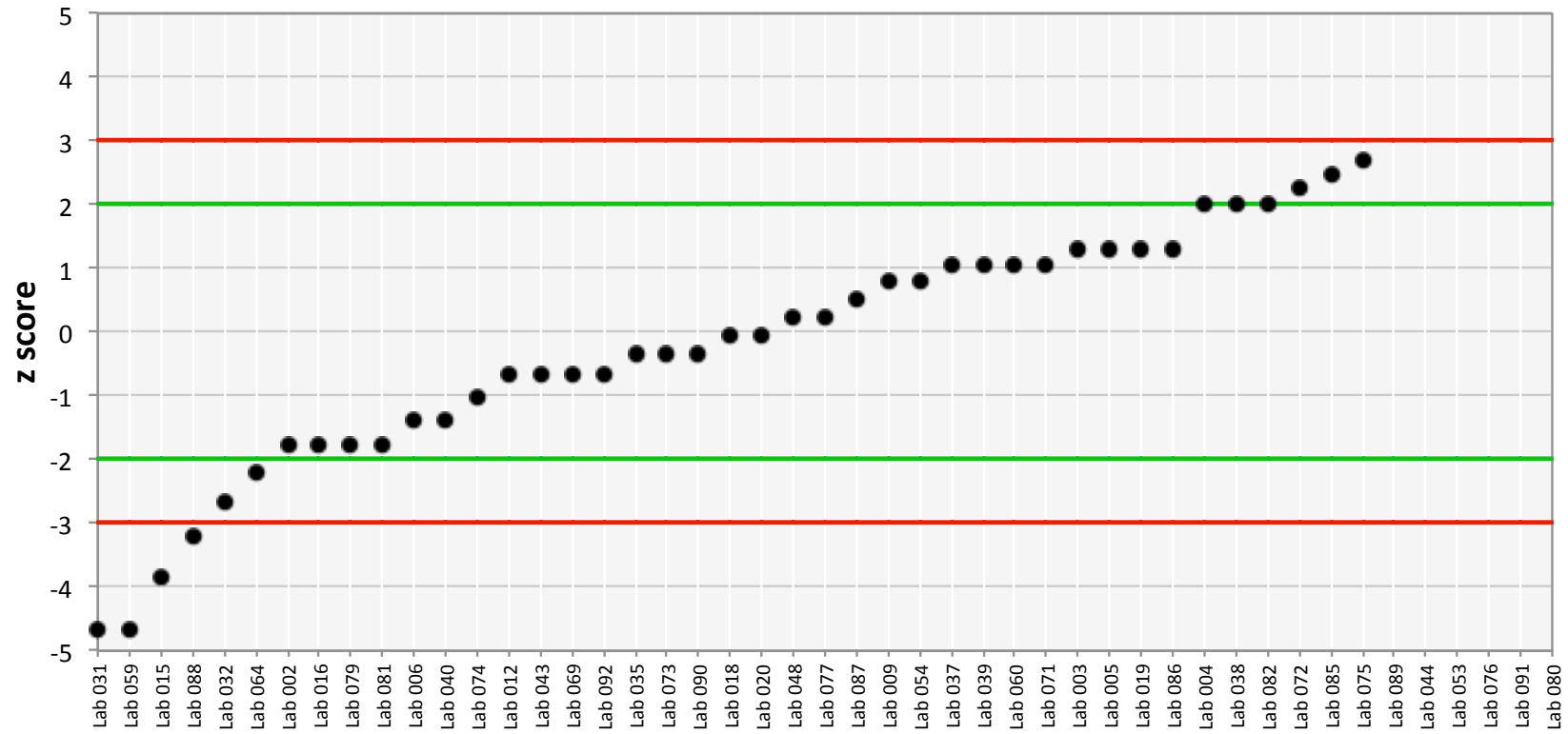
n = 47

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability
assigned value (mean)	11	11	9
rel. standard deviation	30 %	15 %	12 %
lower warning limit	2	5	5
upper warning limit	28	19	15
lower action limit	0	3	4
upper action limit	40	23	18

Total Plate Count at 36°C B

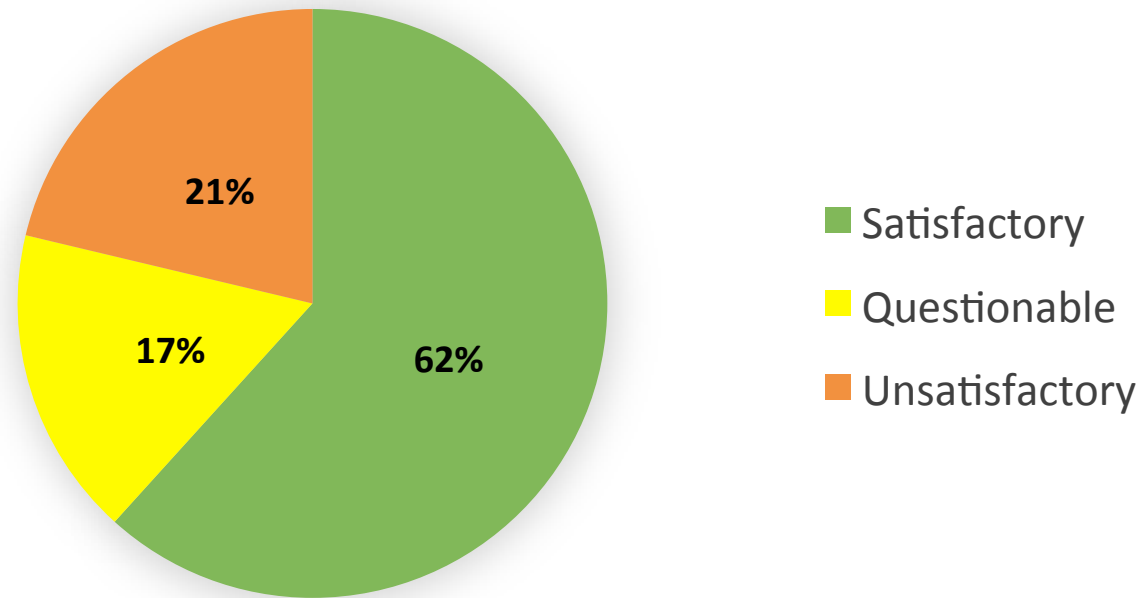


Total Plate Count at 36°C



Total Plate Count 36°C B

n = 22



Total Plate Count 22°C B

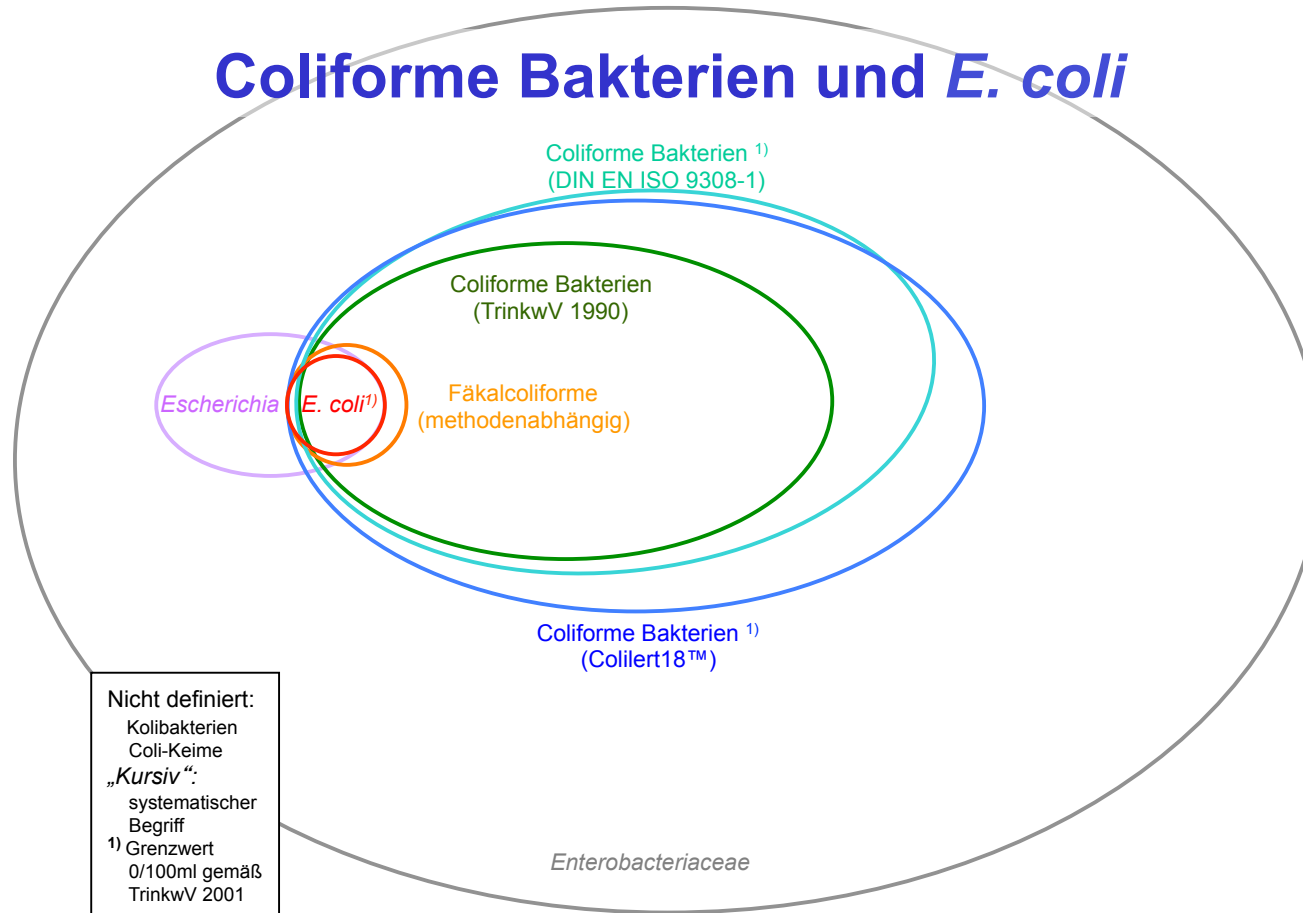
n = 34

CFU / 10 ml	Algorithm A	Algorithm A with limited SD	BOBS stability
assigned value (mean)	12	12	9
rel. standard deviation	25 %	15 %	12 %
lower warning limit	3	6	5
upper warning limit	27	20	15
lower action limit	1	4	4
upper action limit	37	25	18

Conclusions

- Material proves to be suitable
- *P. aeruginosa* material might not be as stable as other parameters
- many different methods used
- high variation in test results
- limitation of standard deviation needed
- high number of results calling for action
- overall outcome of the PT getting slightly better but still
- **much room for improvement**

Coliforme Bakterien und *E. coli*



13. Jahrestagung Trinkwasserringversuche Osnabrück, 29. Februar 2012

Coliform Bacteria

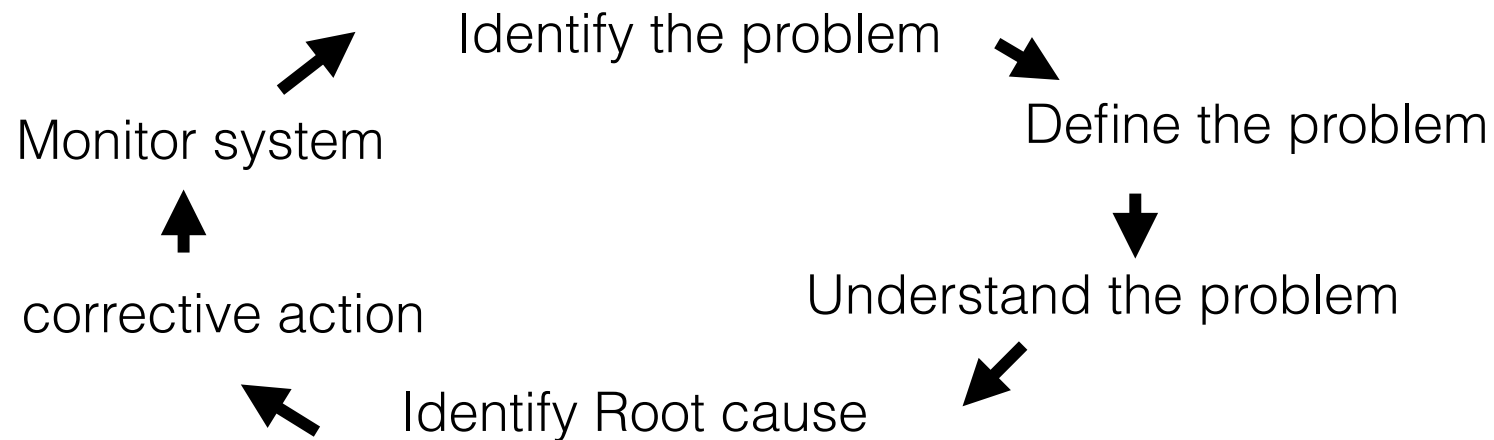
“historic”	ISO 9308-1	Colilert®-18
Escherichia Klebsiella Enterobacter Citrobacter	Escherichia Klebsiella Enterobacter Citrobacter <u>Yersinia</u> <u>Serratia</u> <u>Hafnia</u> <u>Pantoea</u> <u>Kluyvera</u>	Escherichia Klebsiella Enterobacter Citrobacter <u>Yersinia</u> <u>Serratia</u> <u>Hafnia</u> <u>Pantoea</u> <u>Kluyvera</u> <u>Cedecea</u> <u>Ewingella</u> <u>Moellerella</u> <u>Leclercia</u> <u>Rahnella</u> <u>Yokenella</u>

Root Cause Analysis

- problem statements and event descriptions are essential (identify and define the problem)
- brainstorming to describe all factors of influence (not yet finding a solution!) - collaborative/team effort
- getting to the root not staying at the surface
- layers: physical, human, process
- part of a management circle / quality control tool

Different approaches

- 5 why`s
- fishbone diagram/Ishikawa (equipment, process, people, materials, environment, management - effect)
- tree diagram (Symptom, visible problem, first level cause..., root cause)



exercise

- build a fishbone diagram (3 groups)
- create 5 layers of why diagram (3 groups)
- Problem definition: what when where impact on the goal