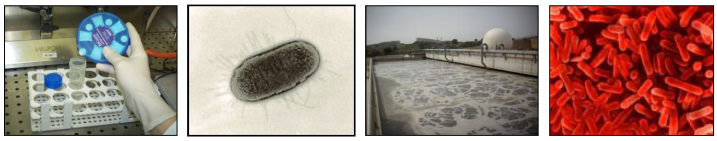


Calculation of the Standard Deviation for Proficiency Assessment in Microbiological Proficiency Testing Schemes

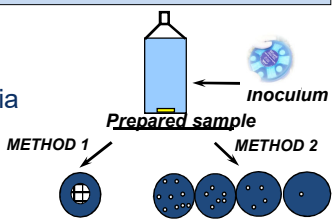
9th Eurachem workshop October-2017

ISO 17025. Section 5.9

"The laboratory shall have quality control procedures in order to assess the validity of tests and calibrations undertaken".

✓ **Internal Quality Control**

- ❑ Quality control of reagents and culture media
- ❑ Control of working conditions
- ❑ Control charts
- ❑ Results quality assessment



✓ **External Quality Control:** Proficiency Testing Schemes (PTs)

Criteria of provider's Selection:

- Technical competence (Accreditation)
- Use of natural matrices
- Number of participants
- Analysis and statistical treatment of results

σ_{pt}: Standard deviation for proficiency assessment

ISO 17043: **σ_{pt} defines the acceptable level of variation between laboratories for a particular test**

It is intended to represent the maximum uncertainty associated with results that would be considered fit for a particular purpose.

- ✓ Quality of PT items: Homogeneity and Stability
- ✓ Performance assessment (*z* score)

σ_{pt}: Standard deviation for proficiency assessment

How can σ_{pt} be calculated?

ISO 13528:

- Robust Standard deviation among labs in the Test
- Experience with previous rounds of PT for the same parameter
- Fixed value
- By perception of experts
- Using the repeatability and reproducibility of the method…

Calculation from a pool of historic data (2005-2013) collected in the frame of 21 different PT rounds

Microbiological limitations

Microbiological limitations

1. **Low uniformity and stability of microbiological samples**
   Over dispersion and agglutination phenomena
   Distributions follow a Poisson or Binomial negative more than a Gaussian model

2. **Microbiological taxonomies are not precise**

3. **Differences in microbiological samples containing the same microorganism (Variability)**
   "Natural" organisms vs Culture Collection organisms

4. **Microbiological analysis depends on the behavior more than on the constitution (Living cells)**
   The number of cells is an approximation of the number of living cells.
   Changes in cellular morphology and in the metabolic activity.

5. **Microbiological methods are not robust**
   Matrix effect (abiotic and biotic factors); Cellular stress; Culture conditions: culture media quality, dilutions, counting, incubation T[a]/time; Analysts training …

Calculation of σ_{pt} in microbiology

1. The coefficient of variation (CV) values were calculated for each microbiological parameters from historical data in each matrix: $\frac{S^*}{X^*} \times 100$

2. The CV values were plotted against the microorganism concentration (assigned value:X*)

3. A mathematical function was obtained for each microorganism.

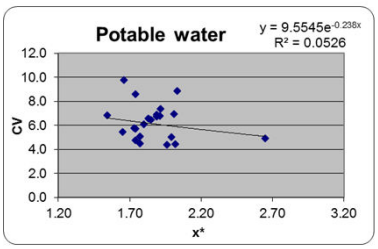
In each new round:

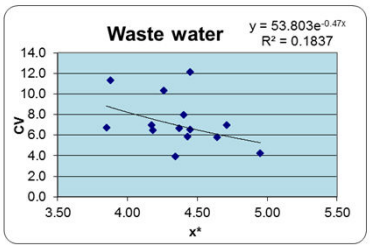
1. Applying the corresponding equation, and considering the obtained X*, the CV value is calculated for each parameter.

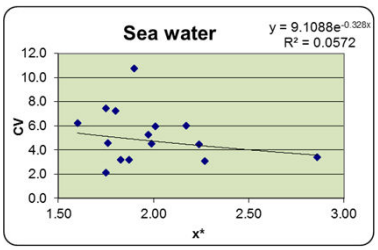
2. Finally, σ_{pt} is calculated following: $\sigma_{pt} = 1.5 \times \frac{CV \times X^*}{100}$

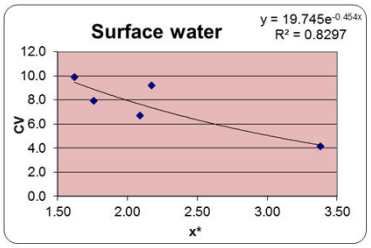
Calculation of σ_{pt} in microbiology

Total coliforms



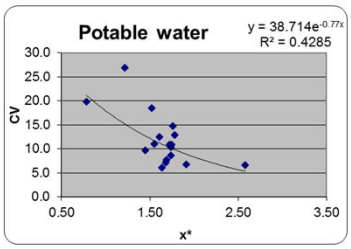


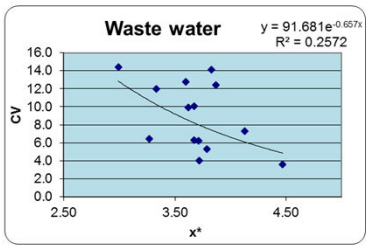


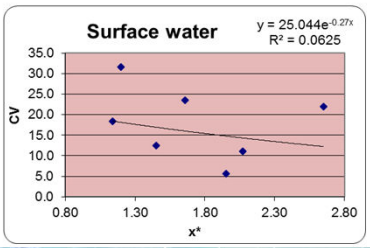


Calculation of σ_{pt} in microbiology

Faecal coliforms

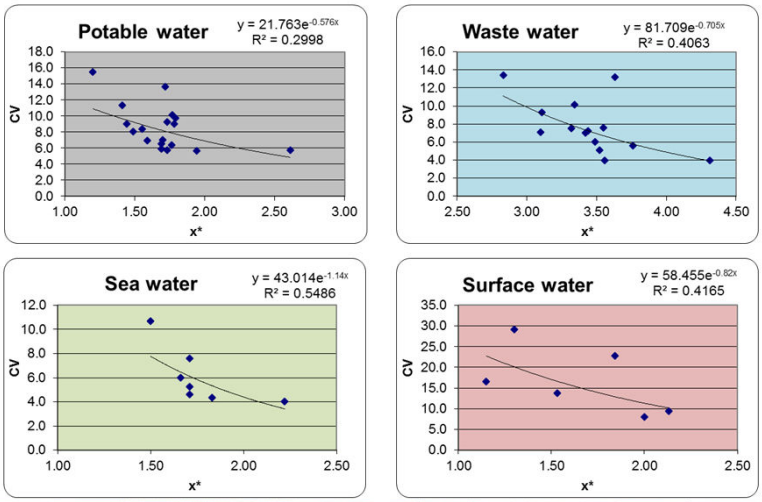






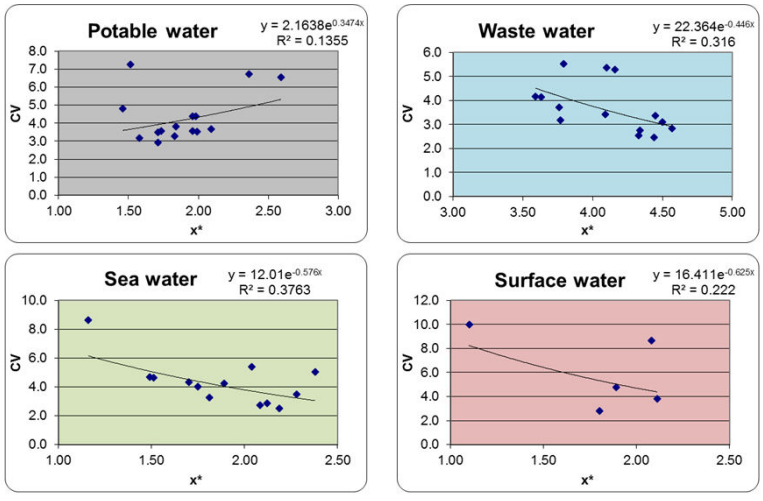
Calculation of σ_{pt} in microbiology

Escherichia coli



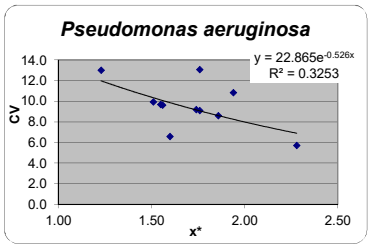
Calculation of σ_{pt} in microbiology

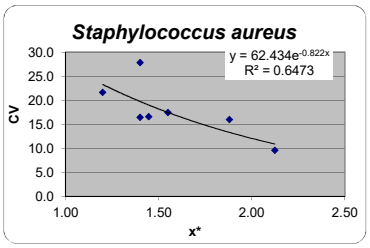
Enterococci

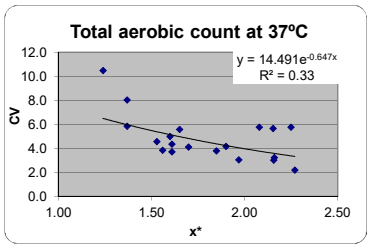


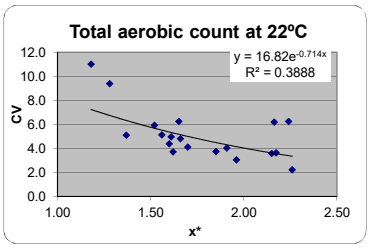
Calculation of σ_{pt} in microbiology

POTABLE WATER (bottled water and swimming pool water)



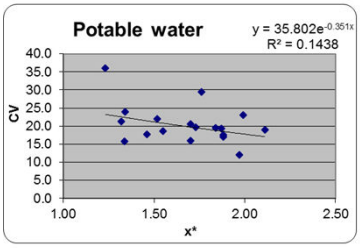


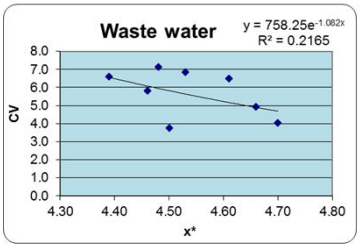




Calculation of σ_{pt} in microbiology

Clostridium perfringens





POTABLE WATER	X* (cfu)	σ_{pt} (log)
Total coliforms	76	0.19
Faecales coliforms	74	0.26
E. coli	<73	0.21
Enterococci	411	0.19
C. perfringens	45	0.39
P. aeruginosa	157	0.23
S. aureus	38	0.41
Total aerobic count at 37ºC	44	0.12
Total aerobic count at 22ºC	43	0.19

WASTE WATER	X* (cfu)	σ_{pt} (log)
Total coliforms	1.34×10^4	0.48
Faecal coliforms	2.78×10^3	0.51
E. coli	9.20×10^2	0.46
C. perfringens	2.28×10^4	0.45
Enterococci	1.80×10^4	0.22

Values of σ_{pt} in Food microbiology

Table A.2 — Standard deviations for coliforms

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
1	vacuum-packed minced beef meat	iv	0,32	0,35	0,11	0,07
3	pastries	iv	0,16	0,23	0,15	0,07
6	fresh beef meat	iv	0,33	0,35	0,05	0,09
10	packed green salad	iii	0,41	0,78	0,33	0,58
12	chicken neck skin	iv	0,15	0,20	0,12	0,06
20	chicken neck skin	iv	0,07	0,12	0,09	0,05
26	raw milk cheese	iv	0,30	0,33	0,09	0,10
29	mechanically separated chicken meat	ii	0,10	0,15	0,07	0,08
30	pastries	iv	0,15	0,19	0,09	0,07
32	fresh pork sausages	iv	0,15	0,31	0,21	0,13
44	raw milk cheese	iv	0,11	0,21	0,10	0,14
45	fresh meat	iv	0,17	0,22	0,10	0,09
58	frozen coconut milk	i	0,12	0,18	0,11	0,08
74	whipped cream	ii	0,07	0,20	0,13	0,13

Table A.3 — Standard deviations for *E. coli*

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
9	raw milk cheese	iv	0,45	0,47	0,10	0,06
16	raw milk cheese	iv	0,09	0,13	0,07	0,07
17	poultry meat (without skin)	iv	0,27	0,35	0,10	0,20
18	raw milk cheese	iv	0,25	0,27	0,07	0,08
19	poultry liver	iv	0,12	0,16	0,09	0,05
35	raw milk cheese	iv	0,13	0,18	0,12	0,03
37	frozen minced beef meat	ii	0,13	0,17	0,10	0,05
47	soya cream	ii	0,13	0,44	0,15	0,39
50	raw milk cheese	iv	0,29	0,30	0,04	0,02
50	raw milk cheese	iv	0,24	0,26	0,08	0,05
51	raw milk cheese	iv	0,13	0,15	0,07	0,02
52	sausage meat	ii	0,08	0,11	0,07	0,03
59	minced meat	ii	0,15	0,19	0,06	0,09

ISO 19036:2006 Microbiology of food and animal feeding stuffs. Guidelines for the estimation of measurement uncertainty for quantitative determinations

Reproducibility standard deviation (S_R):

	Range	Average
Total coliforms	(0.12-0.78)	0.27
E. coli	(0.11-0.47)	0.25
Total aerobic count	(0.09-0.45)	0.24

✓ Other PT provider: σ_{pt}=0.35 log fixed for the majority of microbiological tests.

✓ AEAS: Guide for water laboratory management; Part 1: criteria for quality analysis performance (2016) $S_R \leq 0.2$

Determination of σ_{pt} in microbiological PTs: Examples

Escherichia coli - POTABLE WATER

2017-ROUND I	*Previous Formula* (1999-2004)	*Current formula* (2005-2013)
Nº LABS	126	126
Assigned value (X*)	1.83	1.83
Robust SD (S*)	0.08	0.08
SDPA (σ_{pt})	0.25	0.21
Uncertainty (μ)	0.01	0.01
Outliers	3	3
|Z| ≤ 2	128	128
2 < |Z| < 3	0	0
Z ≥ 3	1	1
Total results	*129*	*129*

Escherichia coli – SURFACE WATER

2014- ROUND I	*Previous Formula* (1999-2004)	*Current formula* (2005-2013)
Nº LABS	34	34
Assigned value (X*)	1.77	1.77
Robust SD (S*)	0.24	0.24
SDPA (σ_{pt})	0.25	0.36
Uncertainty (μ)	0.05	0.05
Outliers	4	4
|Z| ≤ 2	35	35
2 < |Z| < 3	0	2
Z ≥ 3	3	1
Total results	*38*	*38*

Escherichia coli - WASTE WATER

2017-ROUND II	*Previous Formula* (1999-2004)	*Current formula* (2005-2013)
Nº LABS	24	26
Assigned value (X*)	2.49	2.49
Robust SD (S*)	0.15	0.17
SDPA (σ_{pt})	0.28	0.53
Uncertainty (μ)	0.04	0.04
Outliers	6	4
|Z| ≤ 2	25	27
2 < |Z| < 3	1	2
Z ≥ 3	4	1
Total results	*30*	*30*

Conclusions

The use of this approach for the determination of the σ_{pt}:

- Very easy to be performed.
- Takes into account the microorganism, its concentration and the matrix used.
- Fits for the established purposes.
- Ensures that the value used for performance assessment and for homogeneity and stability studies remains realistic and relevant to each microorganism.
- Could be a significant contribution to the improvement of microbiological PT assessment.

THANK YOU FOR YOUR ATTENTION

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