



# **Use of a ISO 13528 template for quantitative schemes : the issues**

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# ISO 17043 and homogeneity and Stability

- “ ISO 17043 requires that an EQA provider demonstrates sufficient homogeneity and stability of the material using valid statistical methods. The Standard refers the reader to ISO 13528 - Statistical methods for use in proficiency testing by interlaboratory comparisons and the IUPAC International Harmonized Protocol. The procedure described in ISO 13528 Annex B is most suited for large quantitative schemes with little guidance provided for Schemes where there are insufficient number of samples for statistical validity, or where split sample analysis is inappropriate. There is little or no guidance as to the design of suitable homogeneity and stability testing protocols for qualitative analytes.

# Issue 1 . wide method CV

The standard statistical approach for homogeneity and stability includes calculation of the general average, the within sample and between sample standard deviation. The samples are considered to be adequately homogeneous if the between sample sd is  $\leq 0.3 \times$  Standard deviation for proficiency assessment (SDPT)

$t$  represents the sample ( $t = 1, 2, \dots, g$ )  $k$  represents the test portion ( $k = 1, 2$ )

Calculate sample averages as:  $x_{t..} = (x_{t,1} + x_{t,2}) / 2$

and the between-test-portion ranges as:  $w_t = |x_{t,1} - x_{t,2}|$

Calculate the general average:  $\bar{x}_{t..} = \sum \bar{x}_t / g$

the standard deviation of sample averages:  $s_x = \sqrt{\sum (x_{t..} - \bar{x}_{t..})^2 / (g - 1)}$

and the within-samples standard deviation:  $s_w = \sqrt{\sum w_t^2 / (2g)}$

Calculate the between-samples standard deviation as:  $s_s = \sqrt{s_x^2 - (s_w^2 / 2)}$

Homogeneous if  $s_s \leq 0,3 \hat{\sigma}$

# Issue 1 . wide method CV

between-samples standard deviation =

$$s_s = \sqrt{s_x^2 - (s_w^2/2)}$$

If  $SD_{PT} = 0.5$  then  $SD_{allow} = 0.3 \times SD_{PT} = 0.15$

and

If  $S_x = 0.5$  and  $S_w = 0.8$

$$\begin{aligned} \text{Then } S_s &= \sqrt{0.5^2 - 0.8^2/2} \\ &= \sqrt{0.25 - 0.32} \\ &= \sqrt{-0.07} \end{aligned}$$



If  $S_w > S_x$  look at analytical variation of method

If  $S_s$  is negative can't use standard equation use alternative  $SD_{allow}$  which takes into account within sample SD.



# Issue 1 . wide method CV

Calculate SD allow .  $\sigma^2_{allow} = (0,3 \hat{\sigma})^2$

Calculate  $c = F_1 \sigma^2_{allow} + F_2 S^2_w$

F1 and F2 are from standard statistical tables,

If  $ss > \frac{1}{2}c$  then there is evidence that the batch of material is not sufficiently homogeneous

Table B.1 — Factors  $F_1$  and  $F_2$  for use in testing for sufficient homogeneity

<i>m</i>	20	19	18	17	16	15	14	13	12	11	10	9	8	7
<i>F</i> <sub>1</sub>	1.59	1.60	1.62	1.64	1.67	1.69	1.72	1.75	1.79	1.83	1.88	1.94	2.01	2.10
<i>F</i> <sub>2</sub>	0.57	0.59	0.62	0.64	0.68	0.71	0.75	0.80	0.86	0.93	1.01	1.11	1.25	1.43

For 10 samples,  $F_1 = 1.88$  and  $F_2 = 1.01$

$$c = 1.88 \times SD^2_{allow} + 1.01 \times (\text{within sample SD})^2$$

$$c = 1.88 \times 0.15^2 + 1.01 \times 0.8^2$$

$$c = 0.042 + 0.65 = 0.69$$

$$\frac{1}{2}c = 0.345$$

# If Within sample SD > Sample Av SD



	Analyte:	Glucose		Analyte:	Glucose		Analyte:	Glucose	
Batch no: (please enter)	Sample			Sample			Sample		
	Result a	Result b	Sample Ave	Result a	Result b	Sample Ave	Result a	Result b	Sample Ave
Sample 1	2.9	2.3	2.6	2.9	2.3	2.6			
Sample 2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Sample 3	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sample 4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Sample 5	2.7	2.8	2.75	2.7	2.8	2.75	2.7	2.8	2.75
Sample 6	2.8	2.9	2.85	2.8	2.9	2.85	2.8	2.9	2.85
Sample 7	2.9	3	2.95	2.9	3	2.95	2.9	3	2.95
Sample 8	2.2	2.9	2.55	2.2	2.9	2.55			
Sample 9	2.6	2.9	2.75	2.6	2.9	2.75	2.6	2.9	2.75
Sample 10	2.2	3	2.6	2.2	3	2.6			
General Average	2.655			2.655			2.686		
Sum d <sup>2</sup>	1.61			1.61			0.12		
Within Sample SD (SD <sub>diff</sub> )	0.28372522			0.283725219			0.09258201		
SD of sample ave	0.167			0.167			0.195		
<b>1 Between sample SD</b>	#NUM!			0.167414987			0.183873663		
enter WEQAS SD for this level (calculated from precision profile & general average or POCT criteria (in%))	0.183			0.183			0.183		
<b>2 0.3*WEQAS SD</b>	0.0549			0.0549			0.0549		
SD <sup>2</sup> <sub>allow</sub>	0.00301401			0.00301401			0.00301401		
<b>3 z<sub>c</sub></b>	0.29490903								
DATE SAMPLES SENT TO ANALYSER:				Between sample SD (1) must be < 0.3*WEQAS SD (2) or z <sub>c</sub> (3)					
DATE OF ANALYSIS				SD <sup>2</sup> <sub>allow</sub> = (0.3 *WEQAS SD) <sup>2</sup>			c = 1.88*SD <sup>2</sup> <sub>allow</sub> + 1.01*(within sample SD) <sup>2</sup>		

Compare d<sup>2</sup> and remove data points (use Cochran's test if needed).



## Issue 2-Where the $SD_{PT}$ is small

Impossible to achieve  $0.3 \times SD$

- “ Tight performance criteria based on Clinical allowable limits.
- “ Challenging samples near the analytical limits of the assay. In these cases the criterion should be expanded to allow for the sampling errors.



# Issue 3 . Random v Systematic

- “ ISO Guide 34 - systematic sampling in a continuous process may be a better way to detect inhomogeneity rather than random sampling.
- “ Evaluation of the data should include investigation of a trend (or drift) in analysis of the homogeneity measurements, or a trend during the dispensing or production processes.





Analysis sequence of all samples should be random for both approaches to minimise affects of analyser drift

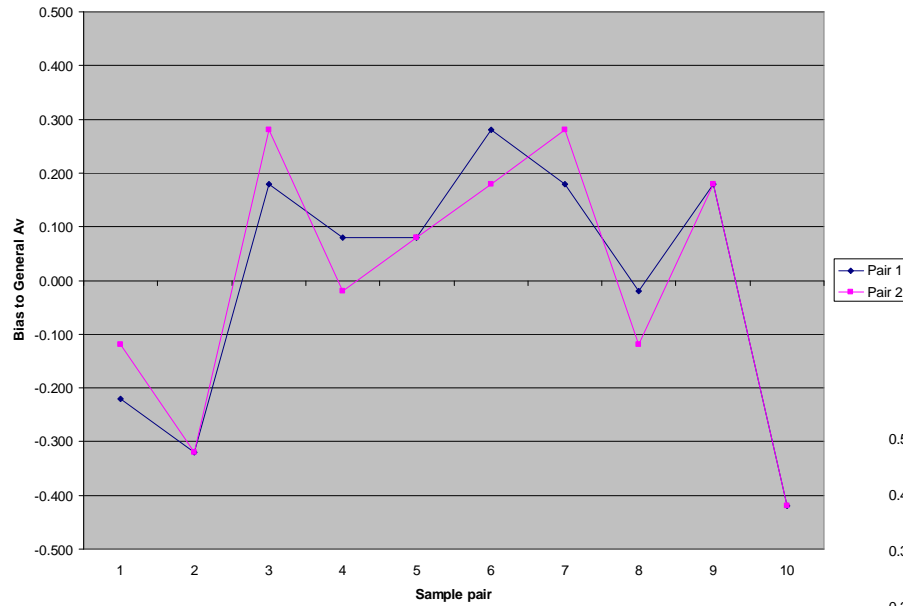
### Random sampling approach

### Systematic sampling approach

	Analyte:	Glucose		Analyte:	Glucose	
Batch no: (please enter)	Sample			Sample		
	Result a	Result b	Sample Ave	Result a	Result b	Sample Ave
Sample 1	2.5	2.6	2.55	2.3	2.3	2.3
Sample 2	2.4	2.4	2.4	2.4	2.4	2.4
Sample 3	2.9	3	2.95	2.5	2.6	2.55
Sample 4	2.8	2.7	2.75	2.7	2.6	2.65
Sample 5	2.8	2.8	2.8	2.8	2.7	2.75
Sample 6	3	2.9	2.95	2.8	2.8	2.8
Sample 7	2.9	3	2.95	2.9	3	2.95
Sample 8	2.7	2.6	2.65	2.9	2.9	2.9
Sample 9	2.9	2.9	2.9	3	2.9	2.95
Sample 10	2.3	2.3	2.3	2.9	3	2.95
General Average	2.720			2.720		
Sum d <sup>2</sup>	0.06			0.06		
Within Sample SD (SD <sub>diff</sub> )	0.05477226			0.054772256		
SD of sample ave	0.238			0.238		
<b>1 Between sample SD</b>	0.23511227			0.235112266		
enter SD for this level	0.27			0.27		
<b>2 0.3*WEQAS SD</b>	0.081			0.081		
SD <sup>2</sup> <sub>allow</sub>	0.006561			0.006561		
<b>3 zC</b>	0.12395435					
DATE SAMPLES SENT TO ANALYSER:				Between sample SD (1) must be < 0.3*WEQAS SD (2) or		
DATE OF ANALYSIS				SD <sup>2</sup> <sub>allow</sub> = (0.3 *WEQAS SD) <sup>2</sup>		

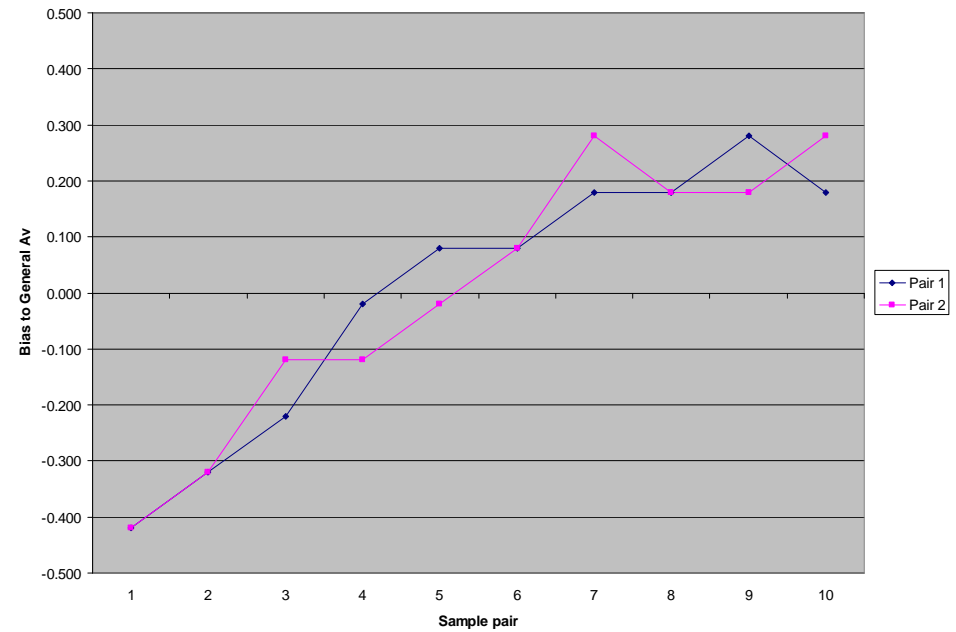


Trend Analysis - Random sampling



Systematic sampling provides greater information than random sampling

Trend Analysis - Systematic sampling



## Issue 4 . Small sample nos.

- “ Use fewer points in ISO 13528 template and use range instead of SD
  - . If  $n = 2,3$  then  $(x_{\text{high}} - x_{\text{low}}) = ss$
  - . If  $n = 4,5,6$  then  $(x_{\text{high}} - x_{\text{low}})/1.5 = ss$
  - . If  $n = 7,8,9$  then  $(x_{\text{high}} - x_{\text{low}})/2 = ss$
  
- “ Alternative approach is to assess the observed  $SD_{PT}$  against the predicted Standard deviation calculated from the Precision profile derived from previous rounds.



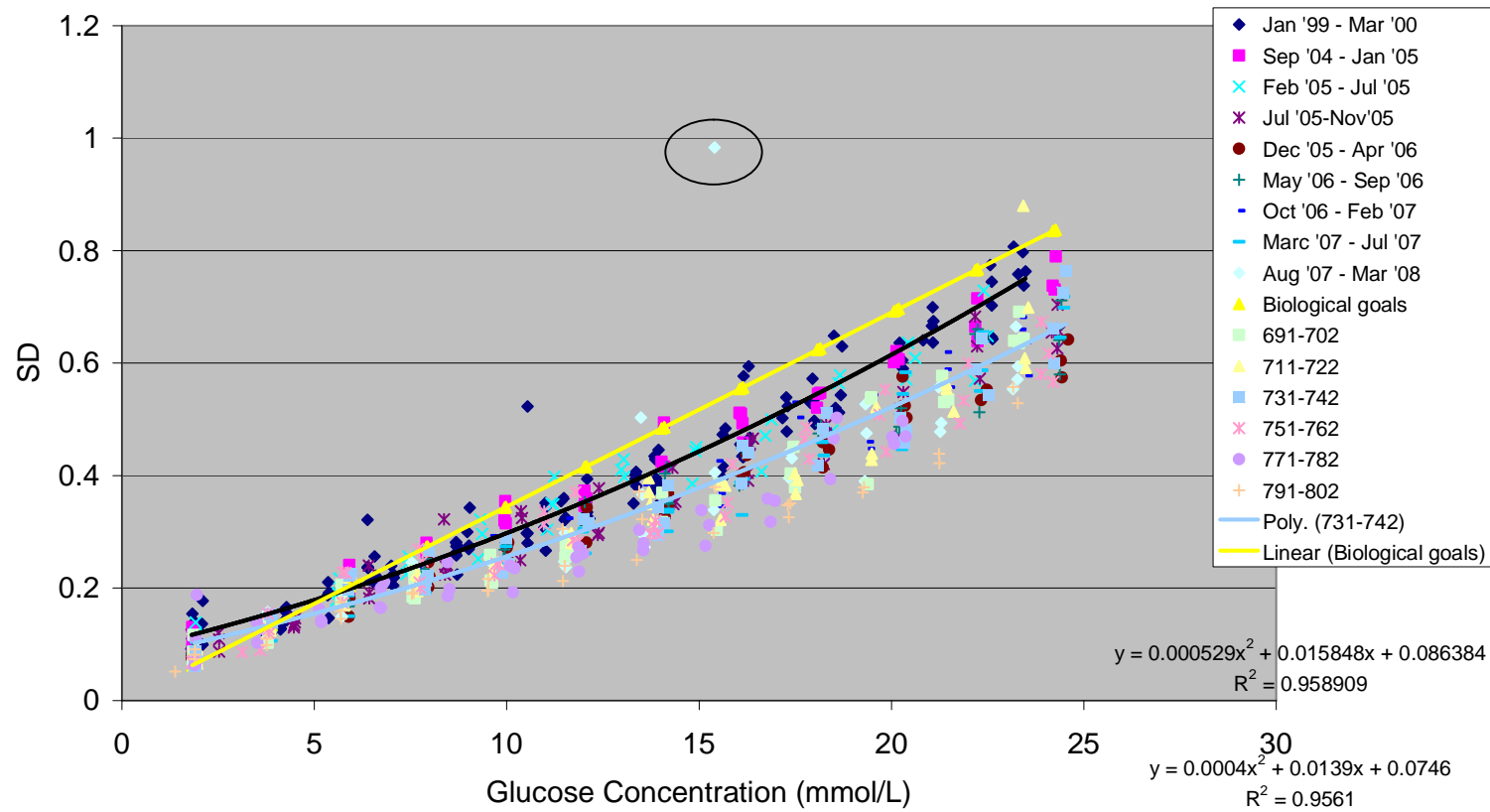
# Use 5 instead of 10 pairs

	Analyte:	Glucose		Analyte:	Glucose	
Batch no: (please enter)	Sample			Sample		
	Result a	Result b	Sample Ave	Result a	Result b	Sample Ave
Sample 1	2.5	2.6	2.55			
Sample 2	2.4	2.4	2.4			
Sample 3	2.9	3	2.95			
Sample 4	2.8	2.7	2.75			
Sample 5	2.8	2.8	2.8			
Sample 6						
Sample 7						
Sample 8						
Sample 9						
Sample 10						
General Average	2.690					
Sum d <sup>2</sup>	0.03					
Within Sample SD (SD <sub>diff</sub> )	0.05477226					
SD of sample ave	0.216					
<b>1 Between sample SD</b>	<b>0.21272047</b>					
enter WEQAS SD for this level (calculated from precision profile & general average or POCT criteria (in%))	0.183					
<b>2 0.3*WEQAS SD</b>	<b>0.0549</b>					
SD <sup>2</sup> <sub>allow</sub>	0.00301401					
<b>3 zC</b>	<b>0.09325416</b>					
DATE SAMPLES SENT TO ANALYSER:				Between sample SD (1) must be < 0.3*WEQAS SD (2) or		
DATE OF ANALYSIS				SD <sup>2</sup> <sub>allow</sub> = (0.3 *WEQAS SD) <sup>2</sup>		



# Compare against previous SD

Precision Profile Glucose



# Assessment criterion for qualitative Schemes

- “ Not covered in ISO 13528
- “ Split sampling procedure could be followed as for Quantitative analytes. The samples could be considered homogeneous if all samples (100% responses) tested gave the same result.
- “ If this is not the case compare the variability of results from the within vial results and the between vial results.
  - . i.e. calculate false negatives/ true positive % for within vial
  - . calculate false negatives / true positive % for between vials
  - . false negatives/ true positive % for between vials / false positive / true positive % for within vial.



# Conclusion

“ Homogeneity and stability testing requires a more pragmatic approach than the procedure described in ISO 13528:2005