Homogeneity of EQA samples – requirements according to ISO/IEC 17043

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ISO/IEC 17043 – Homogeneity (I)

- Criteria for suitable sample homogeneity must be available
- Inhomogeneities must be considered when participants‘ performance is evaluated
- Assessment of homogeneity must be conducted with appropriate statistical designs

ISO/IEC 17043 – Homogeneity (II)

- PT provider shall use a statistically random selection of a representative number of PT items from the whole batch of test material

- Homogeneity assessment should be performed after the PT items have been packaged in the final form before distribution to participants

ISO/IEC 17043 – Annex B.5 Demonstration of proficiency test item homogeneity

- Demonstration of „sufficient homogeneity“ with valid statistical methods is required

- Application of procedures described in ISO 13528 and IUPAC International Hamonized Protocol

ISO 13528 – homogeneity - method

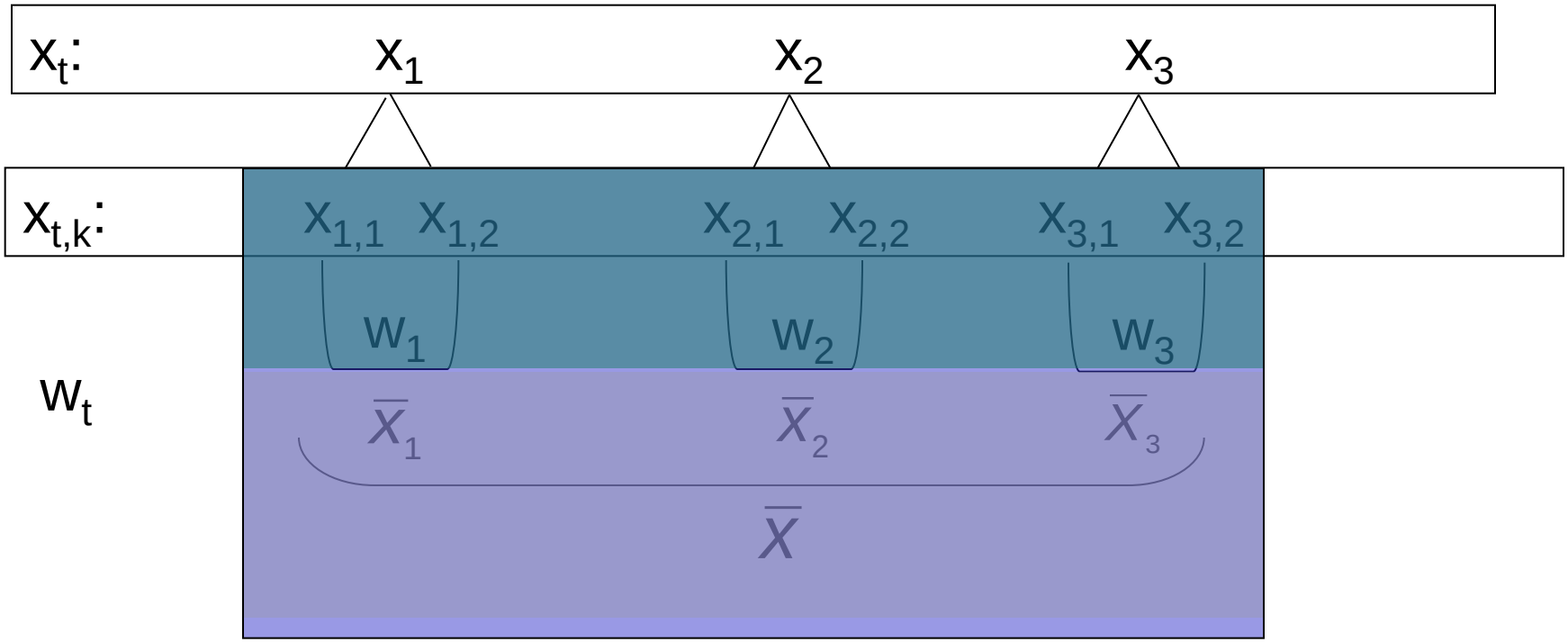
1. Preparation and packaging of the samples
2. Random selection of at least 10 samples per batch ($g \geq 10$)
3. Preparation of two test portions (subsample) from each sample
4. Samples are measured in random order under repeatability conditions
   - Measurements are done from one laboratory
   - Not all measurands, but those which are sensitive to heterogeneity

ISO 13528 – homogeneity - criterion

- Comparison of the standard deviation s_s (between the samples) with the standard deviation for proficiency assessment (SDPA)
- The samples may be considered to be adequately homogeneous if:

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

Scheme of homogeneity testing



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

Within-sample standard deviation: $s_w = \sqrt{\sum w_t^2 / (2g)}$

Between-sample standard deviation: $s_s = \sqrt{s_x^2 - (s_w^2 / 2)}$

ISO 13528 – homogeneity – criterion not fullfilled

- Sample preparation procedure must be examined if improvements are possible
- Heterogeneity is to be included in the standard deviation of SDPA
- Between-sample SD increases SDPA of z-score calculation (usage of z‘-score)

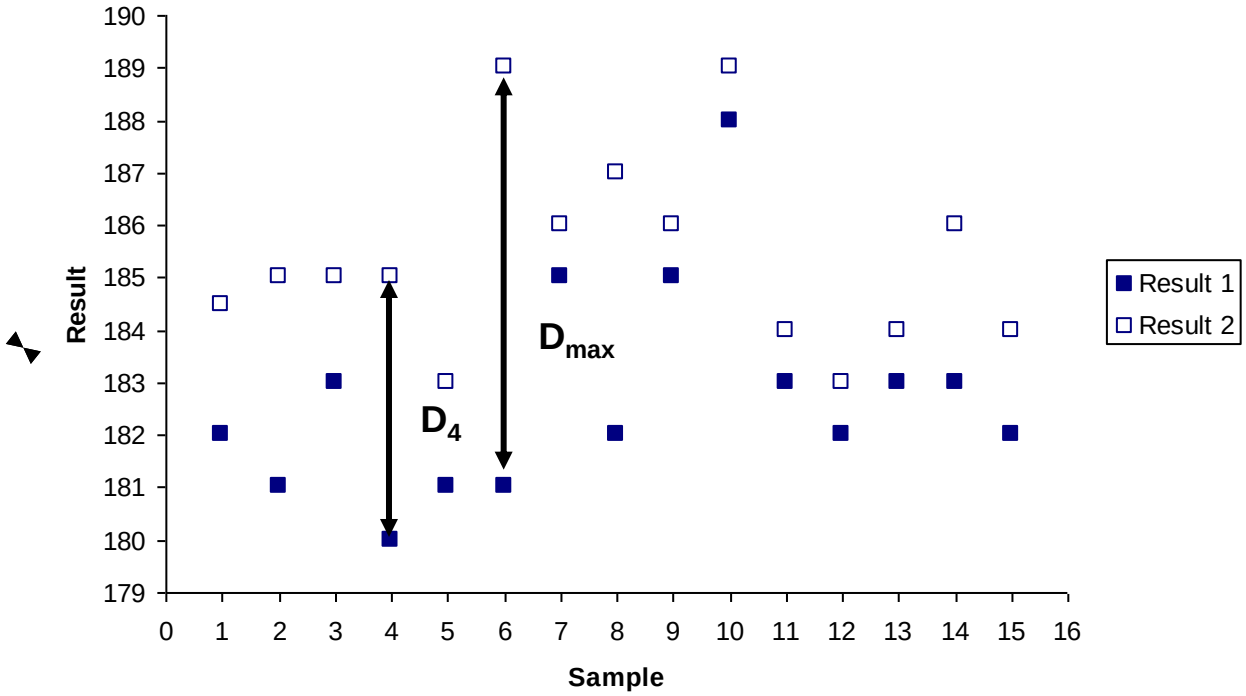
$$\hat{\sigma} = \sqrt{\hat{\sigma}_1^{\,2} + s_s^{\,2}}$$

Note: not if SDPA is derived from the data of the participants

IUPAC – homogeneity – additional remarks

- Method must allow a sufficient precise and satisfactory estimation of s_s
- Check the data for noticeable problems
  - Visual: plot the results against the sample number
  - Trends or discontinuity
  - Excessive rounding
  - Outlying results within the samples
    - Cochran-test: Determination of extreme differences within the samples

IUPAC – homogeneity – Cochran-Test (I)



1. Calculate the sum of square S_{DD} of the m differences: $S_{DD} = \sum_{i=1}^{m} D_i^2$

2. Calculate the ratio of the largest squared difference to this sum of squared differences:

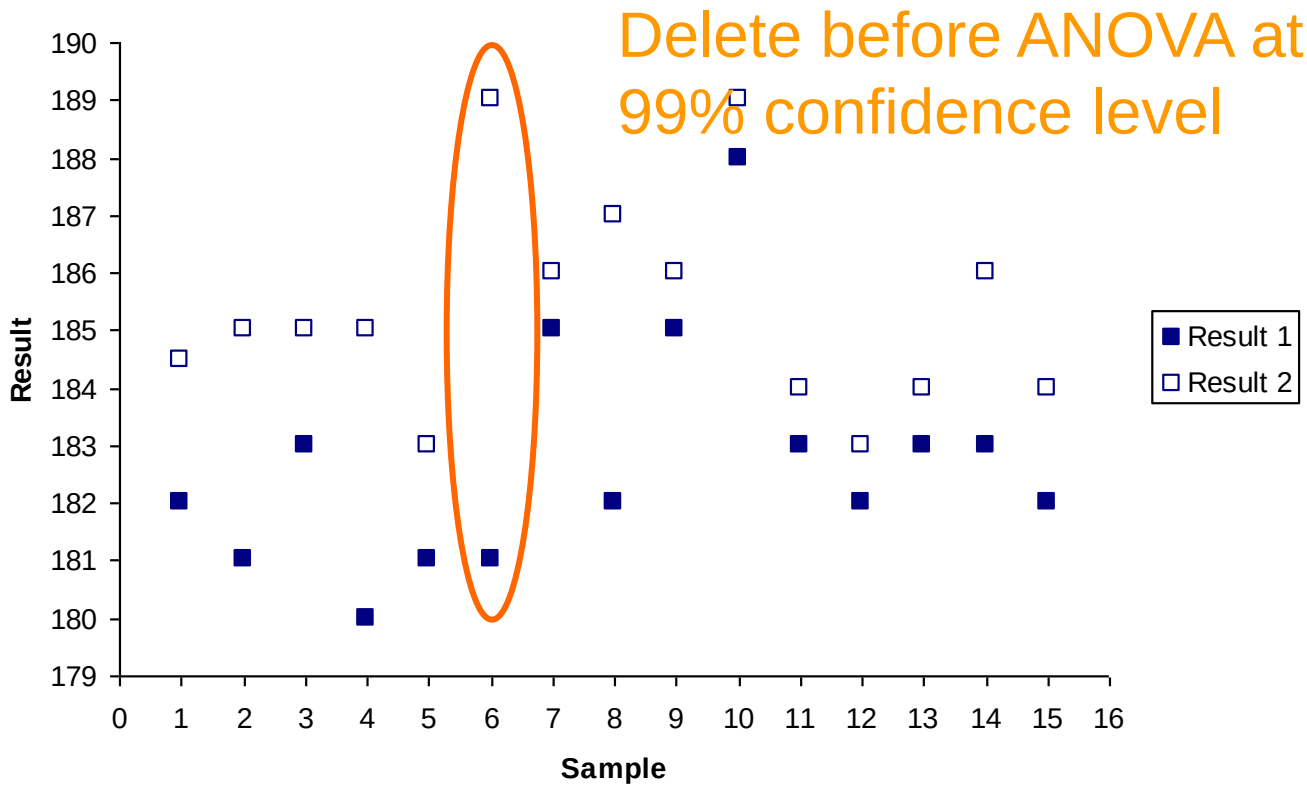
$$C = \frac{D^2_{max}}{S_{DD}}$$

IUPAC – homogeneity – Cochran-Test (II)

3. Compare the ratio with the appropriate critical value from tables

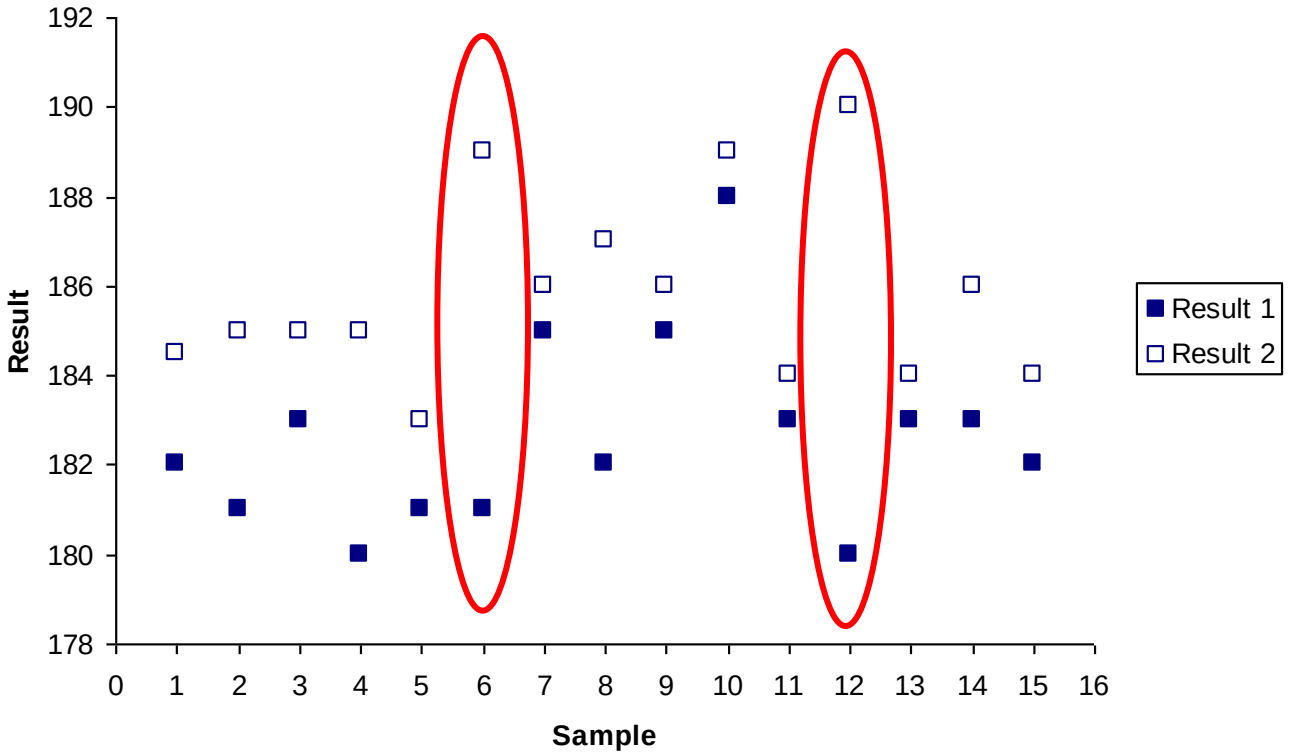
m	95%	99%
7	0,727	0,838
8	0,68	0,794
9	0,638	0,754
10	0,602	0,718
11	0,57	0,684
12	0,541	0,653
13	0,515	0,624
14	0,492	0,599
15	0,471	0,575
16	0,452	0,553
17	0,434	0,532
18	0,418	0,514
19	0,403	0,496
20	0,389	0,48

IUPAC – homogeneity – Cochran-Test (III)



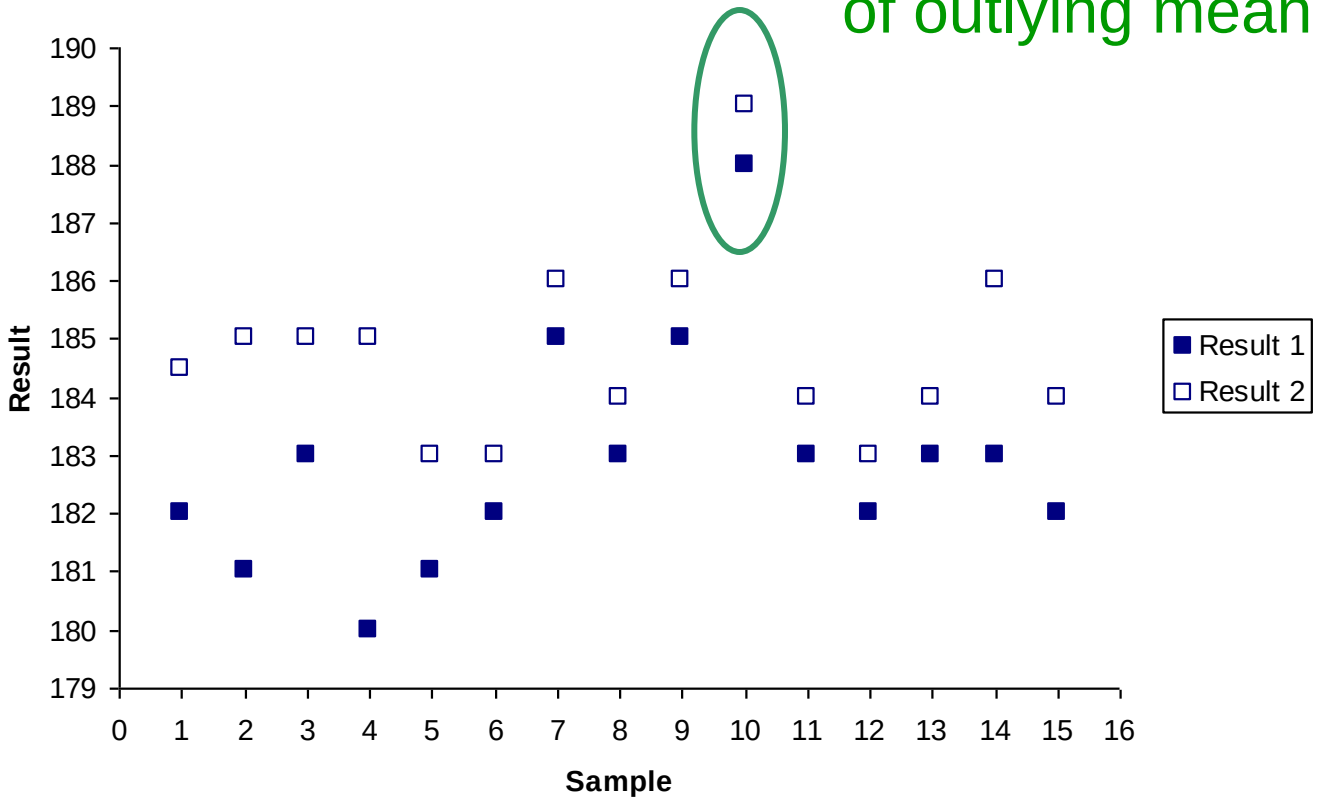
IUPAC – homogeneity – Cochran-Test (IV)

Delete complete data set



IUPAC – homogeneity – Cochran-Test (V)

No elimination in the case of outlying mean value



IUPAC – homogeneity – additional remarks

- If everything is ok:
  - Estimation of the variances resulting from the analysis and the samples ⇒ ANOVA

IUPAC – test for significant homogeneity – ANOVA (I)

- Sum of the squared differences: $s_{an}^2 = \sum_{i=1}^{m} D_i^2 / 2m$

  „equivalent“ to within-sample standard deviation

- Variance V_s of the sums S_i: $v_s = \sum_{I=1}^{m} (S_i - \bar{S})^2 / (m-1)$

  „equivalent“ to the standard deviation of the sample average

- Sampling variance: $s_{sam}^2 = (V_s / 2 - s_{an}) / 2$

  „equivalent“ to between-sample standard deviation

- Allowable sampling variance: $\sigma_{all}^2 = (0{,}3\sigma_p)^2$

IUPAC – test for significant homogeneity – ANOVA (II)

Critical value c for the significance test (F-test):

$$c = F_1 \sigma_{all}^2 + F_2 s_{an}^2$$

m*	20	19	18	17	16	15	14	13	12	11	10	9	8	7
F_1	1,59	1,6	1,62	1,64	1,67	1,69	1,72	1,75	1,79	1,83	1,88	1,94	2,01	2,1
F_2	0,57	0,59	0,62	0,64	0,68	0,71	0,75	0,8	0,86	0,93	1,01	1,11	1,25	1,436

m* is the number of samples that have been measured in duplicate

Homogeneity test has been passed, if:

$$s_{sam}^2 < c$$

Free software...www.aqsbw.de

Homogeneity check (ISO 13528 Annex B)

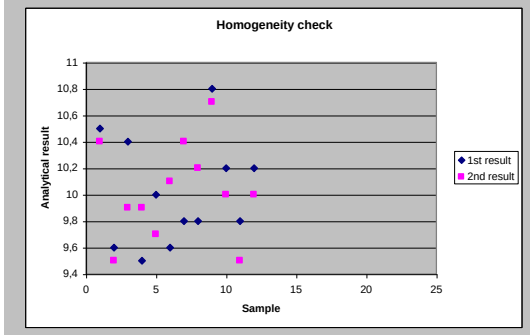
Example from ISO 13528

Sample t	value#1, $x_{t,1}$	value#2, $x_{t,2}$	sample average (B.4), $x_{t.}$	between-test-portion ranges (B.5), w_t
1	10,5	10,4	10,45	0,1
2	9,6	9,5	9,55	0,1
3	10,4	9,9	10,15	0,5
4	9,5	9,9	9,7	0,4
5	10	9,7	9,85	0,3
6	9,6	10,1	9,85	0,5
7	9,8	10,4	10,1	0,6
8	9,8	10,2	10	0,4
9	10,8	10,7	10,75	0,1
10	10,2	10	10,1	0,2
11	9,8	9,5	9,65	0,3
12	10,2	10	10,1	0,2
13				
14				
15				
16				
17				
18				
19				
20				

number of samples g	12
general average (B.6) $\bar{x}_{..}$	10,02083333
STD of sample averages (B.7), s_x	0,340092456
within-samples STD (B.8), s_w	0,247487373
between-samples STD (B.9), s_S	0,291612549

Expected standard deviation for proficiency assessment $\hat{\sigma}$ 1,14

Homogeneity ok



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Homogeneity check (Intern. Harmonized Protocol Appendix 1)

Example from ISO 13528

Sample t	value#1, xt,1	value#2, xt,2	D=a-b	S=a+b	$D^2=(a-b)^2$	$(S_i-\bar{S})^2$
1	10,5	10,4	0,1	20,9	0,01	0,736736
2	9,6	9,5	0,1	19,1	0,01	0,886736
3	10,4	9,9	0,5	20,3	0,25	0,066736
4	9,5	9,9	-0,4	19,4	0,16	0,411736
5	10	9,7	0,3	19,7	0,09	0,116736
6	9,6	10,1	-0,5	19,7	0,25	0,116736
7	9,8	10,4	-0,6	20,2	0,36	0,025069
8	9,8	10,2	-0,4	20	0,16	0,001736
9	10,8	10,7	0,1	21,5	0,01	2,126736
10	10,2	10	0,2	20,2	0,04	0,025069
11	9,8	9,5	0,3	19,3	0,09	0,550069
12	10,2	10	0,2	20,2	0,04	0,025069
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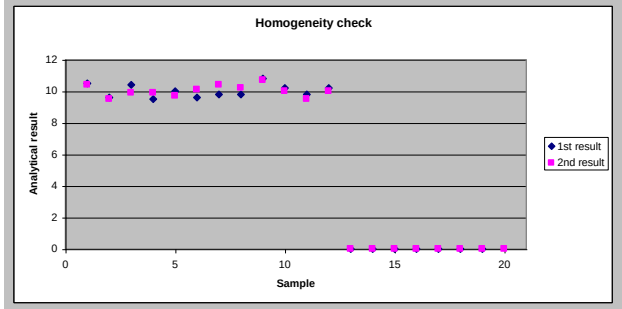

number of samples 12

Cochran test procedure for duplicate results

D_{max}^2	0,36	
S_{DD}	1,47	
C	0,24489796	
critical value (99%)	0,653	**no outlying pair**
critical value (95%)	0,541	**no outlying pair**

Test for significant inhomogeneity

s_{an}^2	0,06125
$\bar{S}$	20,0416667
V_S	0,46265152
s_{sam}^2	0,08503788

Expected standard deviation for proficiency assessment $\hat{\sigma}$ 1,14

Allowable sampling variance	0,116964	critical F_1	1,79
critical value	0,26204056	critical F_2	0,86

Homogeneity OK

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Example (quantitative): Serum (C-reactive protein)

Homogeneity testing		
		Internal code
Material	Serum	
Device	Fuji dri-chem 3500i	656
Parameter	CRP (C-reactive protein)	700
Parameter type	Quantitative	
Method	Fuji	719
Prepared samples	2500	CR
Tolerance	11% ← ?	
Code	13-06	

	Duplicates	
Sample	D.1	D.2
1	370,000	400,000
2	360,000	410,000
3	380,000	410,000
4	430,000	390,000
5	400,000	420,000
6	420,000	390,000
7	430,000	380,000

ISO_IUPAC_Serum_13-06.xlm

- STD of sample averages $\mathbf{s_x}$ < within-sample STD $\mathbf{s_w}$
- Standard deviation of analytical method is around 7%
- Is this sufficiently precise ?

Example (quantitative): Serum (Creatine kinase)

		Internal code	
Material	Serum		
Device	Fuji dri-chem 3500i	656	
Parameter	CK-MB (Creatine kinase)	32	
Parameter type	Quantitative		
Method	Fuji		
Prepared samples	50		
Tolerance	25%	← ?	
	13-09		
		Duplicates	
	Sample	D.1	D.2
	1	44,00	42,00
	2	45,00	41,00
	3	43,00	39,00

- Number of samples is too low
- Recommendation: Decide from the visual plot if it is fit for the purpose or take more samples

ISO_IUPAC_Serum_creatine.xls

Example (semi-quantitative): Urine (Protein)

Homogeneity testing		
		Internal code
Material	Urine	
Device	Urisys 1800	577
Parameter	Protein	135
Parameter type	Semi-quantitative	
Method	Strips	409
Prepared samples	550	U2
Code	12-04	
	Measurement	
Sample	D.1	
1	+++(2-5g/L)	
2	+++(2-5g/L)	
3	+++(2-5g/L)	

- Number of samples is definitly too low
- Evaluation of homogeneity only by comparison of the results possible
- No statistical tool available

Example (qualitative): Urine (Opiates)

Homogeneity testing	
Material	Urine
Device	Tox/see
Parameter	Opiates
Parameter type	Categorical
Prepared samples	200
Code	13-10
	Measurement
Sample	D.1
1	Positive
2	Positive
3	Positive

- No statistical method available
- Comparison of the expected property value
- Quantification by chemical analyses possible ?
- Conventional test for homogeneity possible ?

Example: copper in soya flour

Sample	Value 1	Value 2
1	12,1	10,4
2	9,6	9,5
3	10,4	9,9
4	9,5	9,9
5	10	9,7
6	9,6	10,1
7	9,8	10,4
8	9,8	10,2
9	10,8	10,7
10	10,2	10
11	9,8	9,5
12	10,2	10

$\sigma = 1,14$

ISO_IUPAC_soya_flour.xls

- outlier increases the critical value
- elimination is required, otherwise the result is too much influenced by the „analytical precision“
- After elimination of the outlier, the samples are considered to be homogeneous
- ISO 13528 is more sensitive in this case

Thank you for your attention !