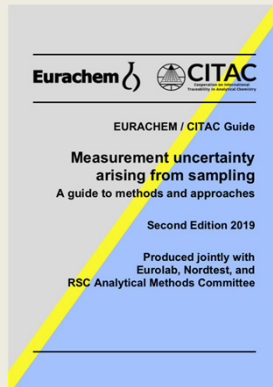
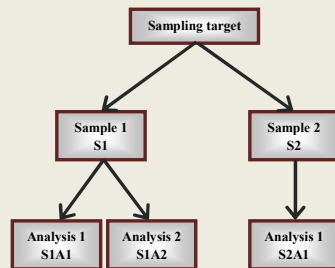


## Using unbalanced designs to reduce the cost of sampling uncertainty estimation



*Eurachem/Eurolab Workshop,  
Uncertainty from sampling and  
analysis for accredited laboratories  
November 2019, Berlin*



Peter Rostron

US University of Sussex



Analytical Methods Trust

## Overview

1. Example of using the **balanced** experimental design to estimate measurement uncertainty including uncertainty from sampling (example A1 from the Eurachem guide)
2. Reducing the cost of uncertainty estimation using the **unbalanced** design
3. Validation of **robust ANOVA** on the unbalanced design (theoretical)
4. Application of the unbalanced design to real data:
  1. Example A1 from Eurachem guide
  2. Example A2 from Eurachem guide
5. Conclusions

## Nitrate concentration in lettuce – Example A1 from Eurachem guide

Nitrate is a **potential** risk to human health

- Toxicity/benefits unclear

- Toxicity:

- 'blue baby' syndrome (1981)

- stomach cancer (1963)

*Both disputed*

- Beneficial effects

- May have antimicrobial effect on gut pathogens (speculative)

- EU threshold  $4000 \text{ mg kg}^{-1}$  (summer) -  $5000 \text{ mg kg}^{-1}$  (winter)<sup>1</sup>

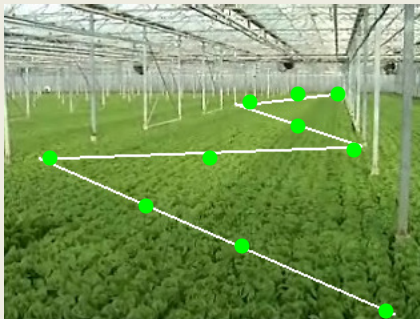
Scope (from guide): Estimate the measurement uncertainty, and contributions from sampling and analysis, for routine monitoring of glasshouse grown lettuce, using a standard sampling protocol



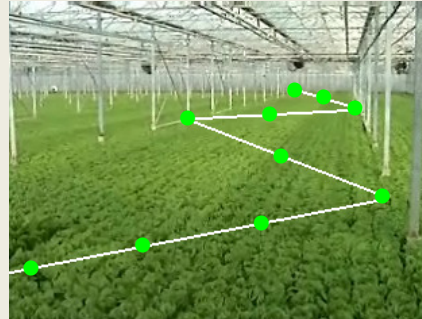
<sup>1</sup> COMMISSION REGULATION (EU) No 1258/2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs <https://eur-lex.europa.eu/eli/reg/2011/1258/oj>

## Nitrate concentration in lettuce – Example A1 from Eurachem guide

- **Sampling target** = 1 bay of lettuce (up to 20,000 heads)
- **Sampling protocol** specifies taking 10 heads to make a single composite sample from each batch (*in 'W' or 'star' design*)



'W' Sampling Design for Lettuce

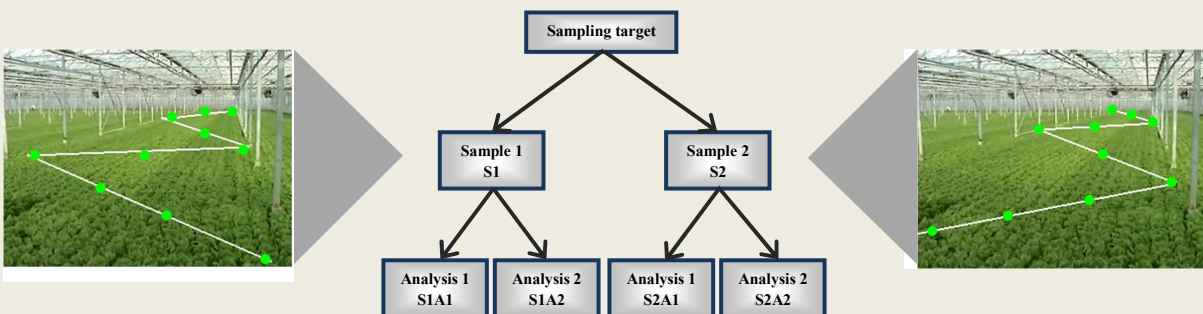


Duplicate is equally likely interpretation of 'W' design



## Nitrate concentration in lettuce – Example A1 from Eurachem guide

Measurement uncertainty was estimated using the balanced design.

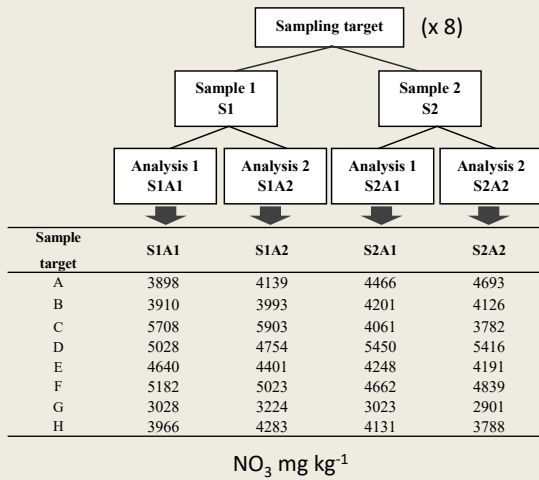


8 bays selected for duplicate sampling

Recommended sampling duplicates @ 10% of the sampling locations in the whole survey  
- Minimum 8

Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

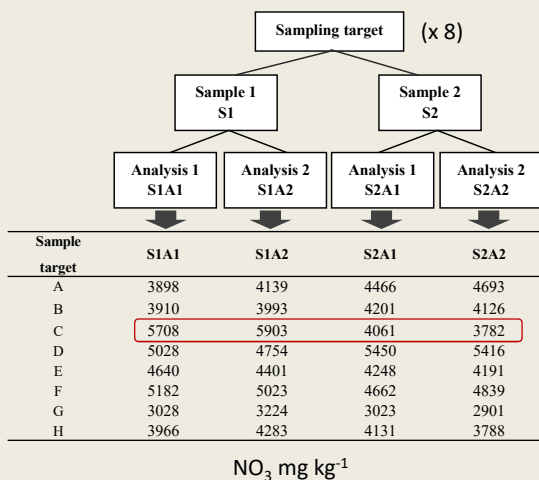
Estimating uncertainty from the sampling/analytical duplicates : **Classical** ANOVA



	Classical ANOVA
$U_{samp}$	24%
$U_{anal}$	7%
$U_{meas}$	25%

Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

Estimating uncertainty from the sampling/analytical duplicates : **Classical** ANOVA



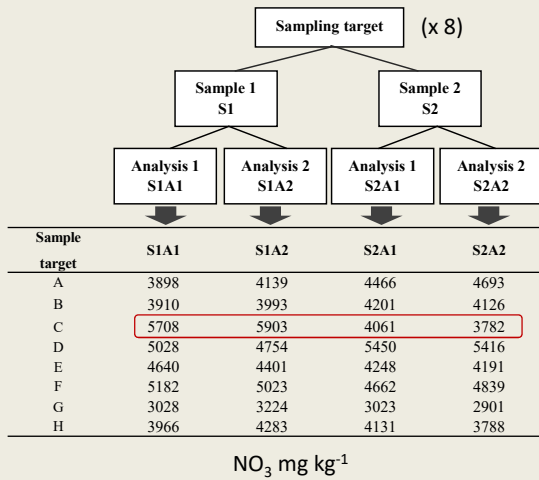
	Classical ANOVA
$U_{samp}$	24%
$U_{anal}$	7%
$U_{meas}$	25%

Visual inspection suggests outlying sampling variance at target C

Use of **Robust** ANOVA down-weights the effect of outlying variances on  $U$  estimates

Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

Estimating uncertainty from the sampling/analytical duplicates : **Robust** ANOVA



	Classical ANOVA	Robust ANOVA
$U_{samp}$	24%	15%
$U_{anal}$	7%	8%
$U_{meas}$	25%	16%

Robust uncertainty more representative of main body of data

Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)<sup>1</sup>
- In practice: Often a small proportion (i.e. <10%) of outlying values exist in the frequency distributions of the analytical, within-sample and between-sample variability<sup>1</sup>
- Robust ANOVA gives more reliable estimate of the variances of the **underlying** populations (See example in Appendix A1 of the Eurachem Ufs guide<sup>1</sup>)
- Computer intensive, iterative process

MS Excel – RANOVA2

CLASSICAL ANOVA					ROBUST ANOVA				
Mean	377.8	No. Targets	10	Mean	297.31				
Total Size	248.89	Standard deviation	136.43	Total Size	218.49				
Standard deviation	197.65	% of total variance	87.46	Standard deviation	179.87				
Expanded relative uncertainty (95%)	85.23	Expanded relative uncertainty (95%)	83.29	Expanded relative uncertainty (95%)	83.83				

<sup>1</sup>Ramsey, M.H., Ellison, S.L.R. (eds.) (2007). Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches Eurachem (2007).

### Robust ANOVA

- **RANOVA2** - Free download from the *AMC Software* page on the *Analytical Methods Committee* section of the website of the *Royal Society of Chemistry* <https://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software>
- Downloaded as ZIP file, includes help text and examples
- Extract all files to new folder
- Specify location of *Ranova2Help.CHM* file before the help system can be used. Press "Activate Help" and follow instructions
- See the included installation notes for more information

Name	Cmp (KB)
BalancedwithID.txt	1
BalancedwithoutID.txt	1
ExtendedwithID.txt	1
Ranova 2 Installation notes.docx	27
RANOVA 2.xlsm	214
Ranova2Help.chm	4,536
Ranova2Help.pdf	5,166
Robust analysis of variance.docx	54
UnbalancedColumn2WithID.txt	1
UnbalancedColumn2WithoutID.txt	1
UnbalancedColumn4WithID.txt	1
UnbalancedColumn4WithoutID.txt	1

RANOVA2 - Robust analysis of variance for balanced and unbalanced experimental designs with 2 samples 6:1-2 analyses

BALANCED DESIGN				BALANCED DESIGN			
Classical ANOVA				Robust ANOVA			
Mean	207.8	No. Targets	10	Mean	207.31	Total Error	0.0000
Total Size	249.39						
Standard deviation	195.65	Std. Target	17.00	Std. Target	179.82	Sampling	11.144
% of total variance	67.86	Analysis	0.50	% of total variance	61.63	Analysis	6.28
Expanded relative uncertainty (95%)	16.29	Measurement uncertainty (95%)	85.80	Expanded relative uncertainty (95%)	62.29	Measurement uncertainty (95%)	31.62
Uncertainty Factor (95%)	1.92		2.6207				

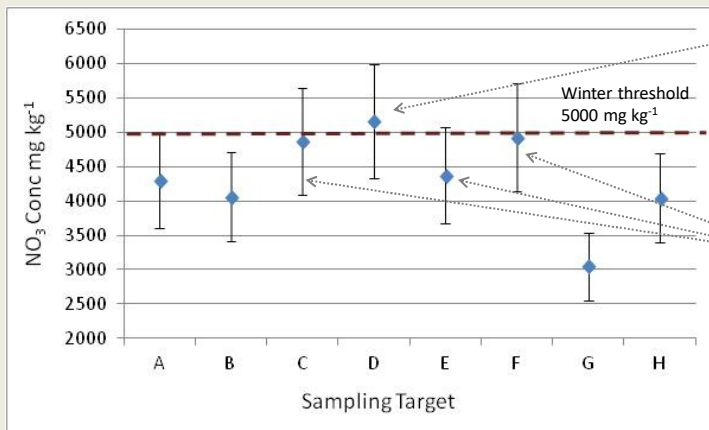
Two pie charts show the distribution of variance: Classical ANOVA (Target, Sampling, Analysis) and Robust ANOVA (Target, Sampling, Analysis).

Buttons: Life existing data, Clear and edit data, Import new data, RUN RANOVA (balanced/unbalanced), Copy results to clipboard, Copy results to file.

<sup>1</sup>Ramsey, M.H., Ellison, S.L.R. (eds.) (2007). Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches Eurachem (2007).

### Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

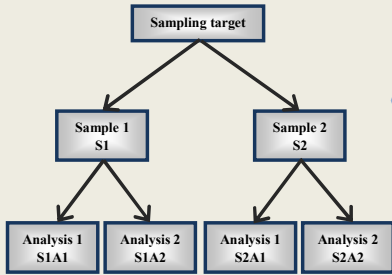
$U_{meas}' = 16\%$  from Robust ANOVA



- 1 possible false positive (sampling target D)
- 3 possible false negatives (sampling targets C, E, F)

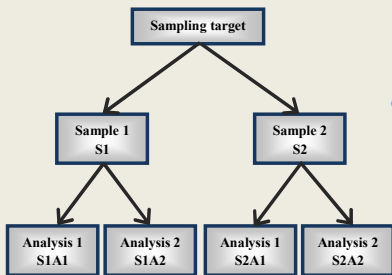
**Robust ANOVA** enables better decisions on probabilistic classification

### Analysing data from the duplicate method



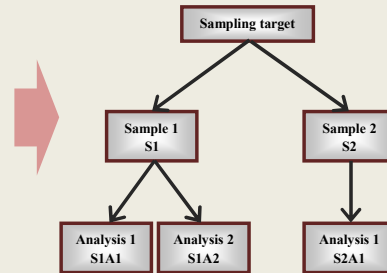
- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

### Reducing the cost of uncertainty estimation



- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

- Alternative **unbalanced** experimental design provides similar uncertainty estimates but with **only 2 additional analyses at each sampling target**
- **Lower cost alternative**



### Unbalanced experimental design

```

graph TD
    ST[Sampling target] --> S1[Sample 1 S1]
    ST --> S2[Sample 2 S2]
    S1 --> S1A1[Analysis 1 S1A1]
    S1 --> S1A2[Analysis 2 S1A2]
    S2 --> S2A1[Analysis 1 S2A1]
        
```

- 2 samples required as per balanced design
- 2 analyses are performed on one sample, one analysis on the other sample
- **Reduces analysis cost of  $U$  estimation by 33%**
  - Sometimes cost is a reason not to estimate  $U$
- **Equalizes number of duplicates** sampling/analysis.
  - In balanced design there are twice as many analytical duplicates, even though sampling uncertainty is often dominant
  - Unbalanced design treats sampling and analysis with **equal importance**
- **RANOVA2** now includes Robust ANOVA on the unbalanced design

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Validation by computer simulation<sup>1</sup>
- 1000 simulated normal datasets (balanced design) were generated for each of 3 seed ('True') values

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1						
Seed 2						
Seed 3						

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.



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- 11 different outlier scenarios were applied to each of these

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		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
Seed 2	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
Seed 3	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

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	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
Seed 2	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
Seed 3	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					

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- Average percentage differences between results of **unbalanced** ANOVA and **balanced** ANOVA are shown in the table

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA (((Unbalanced - Balanced)/Balanced %)				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
Seed 2	Base (None)	0.0	0.0	-0.1	-0.1	-0.3
	Analytical -90	0.3	-0.8	0.5	-6.1	-0.2
	Analytical +100	-0.3	-1.5	-0.6	-6.1	-0.2
	Analytical +200	-0.7	-4.9	-5.8	-6.1	-0.2
	Sampling -90	0.1	-0.6	-1.8	2.9	-0.3
	Sampling +100	0.0	-0.2	-1.2	2.9	-0.3
	Sampling +200	0.0	0.6	0.1	2.9	-0.3
	Geochemical -90	0.0	0.4	0.6	-0.6	-0.3
	Geochemical +100	0.0	0.4	0.5	-0.6	-0.3
	Geochemical +200	0.0	0.4	0.5	-0.6	-0.3
	Combined +100	-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3	Base (None)	0.0	0.2	0.3	0.0	0.2
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Validation by computer simulation<sup>1</sup>
- 1000 simulated normal datasets (balanced design) were generated for each of 3 seed ('True') values
- 11 different outlier scenarios were applied to each of these
- Each resultant dataset was analysed as a balanced design (S1A1,S1A2,S2A1,S2A2) **AND** an unbalanced design (S1A1,S1A2,S2A1)
- Average percentage differences between results of **unbalanced** ANOVA and **balanced** ANOVA are shown in the table
- Median/Mean differences calculated

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA (((Unbalanced - Balanced)/Balanced %)				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
Seed 2	Base (None)	0.0	0.0	-0.1	-0.1	-0.3
	Analytical -90	0.3	-0.8	0.5	-6.1	-0.2
	Analytical +100	-0.3	-1.5	-0.6	-6.1	-0.2
	Analytical +200	-0.7	-4.9	-5.8	-6.1	-0.2
	Sampling -90	0.1	-0.6	-1.8	2.9	-0.3
	Sampling +100	0.0	-0.2	-1.2	2.9	-0.3
	Sampling +200	0.0	0.6	0.1	2.9	-0.3
	Geochemical -90	0.0	0.4	0.6	-0.6	-0.3
	Geochemical +100	0.0	0.4	0.5	-0.6	-0.3
	Geochemical +200	0.0	0.4	0.5	-0.6	-0.3
	Combined +100	-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3	Base (None)	0.0	0.2	0.3	0.0	0.2
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2
Median		0.0	0.2	0.2	0.0	0.0
Mean		-0.1	-0.4	-0.4	-0.9	0.0

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Majority of differences are small (<2%)

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
	Seed 2	Base (None)	0.0	0.0	-0.1	-0.1
Analytical -90		0.3	-0.8	0.5	-6.1	-0.2
Analytical +100		-0.3	-1.5	-0.6	-6.1	-0.2
Analytical +200		-0.7	-4.9	-5.8	-6.1	-0.2
Sampling -90		0.1	-0.6	-1.8	2.9	-0.3
Sampling +100		0.0	-0.2	-1.2	2.9	-0.3
Sampling +200		0.0	0.6	0.1	2.9	-0.3
Geochemical -90		0.0	0.4	0.6	-0.6	-0.3
Geochemical +100		0.0	0.4	0.5	-0.6	-0.3
Geochemical +200		0.0	0.4	0.5	-0.6	-0.3
Combined +100		-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3		Base (None)	0.0	0.2	0.3	0.0
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2
	Median		0.0	0.2	0.2	0.0
Mean		-0.1	-0.4	-0.4	-0.9	0.0

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Majority of differences are small (<2%)
- Maximum percentage differences (-6.1%) were found for the sampling standard deviation with high analytical outliers on Seed 2

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
	Seed 2	Base (None)	0.0	0.0	-0.1	-0.1
Analytical -90		0.3	-0.8	0.5	-6.1	-0.2
Analytical +100		-0.3	-1.5	-0.6	-6.1	-0.2
Analytical +200		-0.7	-4.9	-5.8	-6.1	-0.2
Sampling -90		0.1	-0.6	-1.8	2.9	-0.3
Sampling +100		0.0	-0.2	-1.2	2.9	-0.3
Sampling +200		0.0	0.6	0.1	2.9	-0.3
Geochemical -90		0.0	0.4	0.6	-0.6	-0.3
Geochemical +100		0.0	0.4	0.5	-0.6	-0.3
Geochemical +200		0.0	0.4	0.5	-0.6	-0.3
Combined +100		-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3		Base (None)	0.0	0.2	0.3	0.0
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2
	Median		0.0	0.2	0.2	0.0
Mean		-0.1	-0.4	-0.4	-0.9	0.0

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Majority of differences are small (<2%)
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- Mean and median of the average percentage differences from each scenario are all < 1%

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		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
	Seed 2	Base (None)	0.0	0.0	-0.1	-0.1
Analytical -90		0.3	-0.8	0.5	-6.1	-0.2
Analytical +100		-0.3	-1.5	-0.6	-6.1	-0.2
Analytical +200		-0.7	-4.9	-5.8	-6.1	-0.2
Sampling -90		0.1	-0.6	-1.8	2.9	-0.3
Sampling +100		0.0	-0.2	-1.2	2.9	-0.3
Sampling +200		0.0	0.6	0.1	2.9	-0.3
Geochemical -90		0.0	0.4	0.6	-0.6	-0.3
Geochemical +100		0.0	0.4	0.5	-0.6	-0.3
Geochemical +200		0.0	0.4	0.5	-0.6	-0.3
Combined +100		-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3		Base (None)	0.0	0.2	0.3	0.0
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2
	Median		0.0	0.2	0.2	0.0
Mean		-0.1	-0.4	-0.4	-0.9	0.0

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

### Method validation of **robust** ANOVA on the **unbalanced** experimental design

- Majority of differences are small (<2%)
- Maximum percentage differences (-6.1%) were found for the sampling standard deviation with high analytical outliers on Seed 2
- Mean and median of the average percentage differences from each scenario are all < 1%
- Method validation for the unbalanced design demonstrated. BUT validation was based on duplicate analysis of 100 sampling targets. Unlikely in practice! High cost
- **How does the unbalanced design perform (when compared to the balanced design) in real data scenarios?**

Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
		Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	Analytical +200	-0.7	-5.5	-5.8	-5.1	0.1
	Sampling -90	0.1	-1.1	-1.5	0.4	0.0
	Sampling +100	-0.1	-0.6	-0.9	0.4	0.0
	Sampling +200	0.0	0.2	0.1	0.4	0.0
	Geochemical -90	0.0	0.2	0.2	-0.2	0.0
	Geochemical +100	0.0	0.2	0.2	-0.2	0.0
	Geochemical +200	0.0	0.2	0.2	-0.2	0.0
	Combined +100	-0.2	-0.8	-0.6	-1.9	0.0
	Seed 2	Base (None)	0.0	0.0	-0.1	-0.1
Analytical -90		0.3	-0.8	0.5	-6.1	-0.2
Analytical +100		-0.3	-1.5	-0.6	-6.1	-0.2
Analytical +200		-0.7	-4.9	-5.8	-6.1	-0.2
Sampling -90		0.1	-0.6	-1.8	2.9	-0.3
Sampling +100		0.0	-0.2	-1.2	2.9	-0.3
Sampling +200		0.0	0.6	0.1	2.9	-0.3
Geochemical -90		0.0	0.4	0.6	-0.6	-0.3
Geochemical +100		0.0	0.4	0.5	-0.6	-0.3
Geochemical +200		0.0	0.4	0.5	-0.6	-0.3
Combined +100		-0.1	-0.5	-0.4	-1.4	-0.3
Seed 3		Base (None)	0.0	0.2	0.3	0.0
	Analytical -90	0.1	0.5	0.4	0.7	0.3
	Analytical +100	-0.1	0.5	0.5	0.6	0.3
	Analytical +200	-0.3	0.3	1.7	-2.6	0.3
	Sampling -90	0.1	0.3	0.4	0.0	0.2
	Sampling +100	-0.1	0.3	0.5	0.0	0.2
	Sampling +200	-0.4	-0.3	-0.4	0.1	0.2
	Geochemical -90	0.0	0.2	0.2	0.0	0.2
	Geochemical +100	0.0	0.2	0.3	0.0	0.2
	Geochemical +200	0.0	0.2	0.3	0.0	0.2
	Combined +100	-0.1	0.3	0.4	0.2	0.2
	Median		0.0	0.2	0.2	0.0
Mean		-0.1	-0.4	-0.4	-0.9	0.0

<sup>1</sup>Rostron, P., Ramsey, M.H. (2012) "Cost effective, robust estimation of measurement uncertainty from sampling using unbalanced ANOVA, *Accreditation and Quality Assurance*, 17, 7-14.

Example A1 from the Eurachem guide – UNBALANCED design

- Can ‘simulate’ unbalanced design by removing single columns from the balanced design data
- 4 different **unbalanced** designs possible
- Compare outputs with balanced design



Sample target	<u>1</u>				<u>2</u>				<u>3</u>				<u>4</u>			
	S1A1	S1A2	S2A1	S2A2	S1A1	S1A2	S2A1	S2A2	S1A1	S1A2	S2A1	S2A2	S1A1	S1A2	S2A1	S2A2
A	4139	4466	4693		3898		4466	4693	3898	4139		4693	3898	4139	4466	
B	3993	4201	4126		3910		4201	4126	3910	3993		4126	3910	3993	4201	
C	5903	4061	3782		5708		4061	3782	5708	5903		3782	5708	5903	4061	
D	4754	5450	5416		5028		5450	5416	5028	4754		5416	5028	4754	5450	
E	4401	4248	4191		4640		4248	4191	4640	4401		4191	4640	4401	4248	
F	5023	4662	4839		5182		4662	4839	5182	5023		4839	5182	5023	4662	
G	3224	3023	2901		3028		3023	2901	3028	3224		2901	3028	3224	3023	
H	4283	4131	3788		3966		4131	3788	3966	4283		3788	3966	4283	4131	

Example A1 from the Eurachem guide – UNBALANCED design

Comparison of  $U'$  estimates - unbalanced design scenarios vs balanced design.



	Balanced Design	Unbalanced designs				Average Unbalanced
	Design	1	2	3	4	Unbalanced
$U_{samp}'$ (%)	14.5	14.7	15.6	15.3	13.0	14.7
$U_{anal}'$ (%)	7.6	7.0	7.0	8.1	8.1	7.6
$U_{meas}'$ (%)	16.4	16.3	17.1	17.3	15.3	16.5

Difference between  $U'$  from balanced design and average of 4 unbalanced designs < 1%

Maximum 7% difference in  $U_{meas}'$  using the unbalanced design

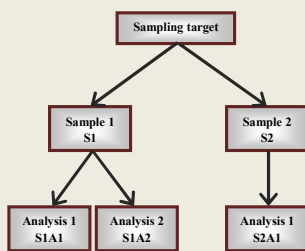
Maximum 10% difference in  $U_{samp}'$  using the unbalanced design

- In this case, using the unbalanced design saves £320 (33%) on total analysis cost

- Sampling cost remains the same

### Example A2 from the Eurachem guide – Lead in topsoil

- Potential housing development site in former landfill (West London) - 9 hectare
- 100 sampling targets in regular grid with soil auger to depth 0.15m
- 10 (10%) designated as duplicate sampling targets



- Same procedure as before applied to evaluate unbalanced design
  - 4 different unbalanced designs analysed from the data
  - Estimated uncertainties compared with balanced design

### Example A2 from the Eurachem guide – Lead in topsoil

Comparison of  $U'$  estimates - unbalanced design scenarios vs balanced design.

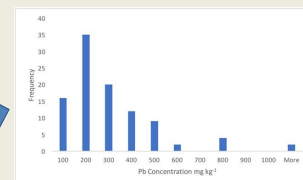
	Balanced Design	Unbalanced designs				Average Unbalanced
		1	2	3	4	
$U_{samp}'$ (%)	<b>83.3</b>	83.3	88.6	77.6	80.2	<b>82.4</b>
$U_{anal}'$ (%)	<b>7.5</b>	8.8	8.9	6.9	6.9	<b>7.9</b>
$U_{meas}'$ (%)	<b>83.6</b>	83.8	89.0	77.9	80.5	<b>82.8</b>

Differences between averages  $\leq$  5%

$U_{samp}'$  dominant factor.  
Maximum difference unbalanced/balanced: 7%

Maximum difference in  $U_{meas}'$  unbalanced/balanced also 7%

- Log-normal distribution: Use of Uncertainty factor  $^FU$  better representation of positive skew in this case,  $^FU = (x/\div) 1.3$



- RANOVA2 also calculates Uncertainty Factor  $^FU$  for the **unbalanced** design

## Conclusions

- Unbalanced design reduces analysis cost of empirical estimation of sampling and analytical uncertainties by 33%
- Robust ANOVA – more reliable estimates of variances of underlying population when outlying variances
- Method validation by computer simulation shows average robust estimates of uncertainty are not significantly different between balanced and unbalanced designs
- In practice differences may occur depending on the magnitude and distribution of outliers
- Savings made using unbalanced design could be used to obtain more sampling duplicates – Further work needed

## Acknowledgements

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Financial Support from The Analytical Methods Trust,  
RSC Analytical Methods Committee



## Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)<sup>1</sup>
- Computer intensive iterative process. To calculate robust mean:
  - Initially estimates:
    - Robust mean  $\mu_r$  = classical mean
    - Robust standard deviation  $\sigma_r$  = median absolute differences
  - Values exceeding  $\mu_r + c \sigma_r$  replaced by  $\mu_r + c \sigma_r$
  - Values less than  $\mu_r - c \sigma_r$  replaced by  $\mu_r - c \sigma_r$ 
    - c typically set to 1.5
- $\mu_r$  and  $\sigma_r$  recalculated, and process repeated until  $\mu_r$  converges to an acceptable level of accuracy

**MS Excel – RANOVA2**

RANOVA 2 - Robust analysis of variance for balanced and unbalanced experimental designs with 2 samples G 1.2 analyses

BALANCED DESIGN					BALANCED DESIGN				
Classical ANOVA					Robust ANOVA				
Mean	377.8	No. Targets	10	Mean	297.37	Mean	210.49	Measure	
Total Sdev	240.79	Bln Target		Total Sdev	210.49	Bln Target		Measure	
Standard deviation	197.55	Sampling	17.89	Standard deviation	179.67	Sampling	123.81	Measure	124.37
% of total variance	47.65	Analysis	4.56	% of total variance	67.63	Analysis	0.26	Measure	22.37
Expanded relative uncertainty (95%)	85.23	Analysis	11.32	Expanded relative uncertainty (95%)	83.29	Analysis	7.50	Measure	83.63
Uncertainty factor (95%)	2.6072		1.12	Uncertainty factor (95%)	2.6272				

% of total variance  
Classical ANOVA

% of total variance  
Robust ANOVA

<b>Edit existing data</b>	Use this to enter or change data in the data input area. New data can be pasted in from another worksheet, using standard Excel copy/paste functions, or entered manually. Results data will be removed.
<b>Clear and edit data</b>	This button clears all existing data before putting the user into data edit mode (as described above).
<b>Import new data</b>	This allows the user to specify a data file to import. Existing data will be cleared. Import data must be contained in a tab-delimited text file. NOT AVAILABLE ON MAC. Use the 'Edit existing data' button and type or paste into the Data input area.
<b>RUN RANOVA (balanced/unbalanced)</b>	Use this command to RUN RANOVA on the data currently displayed in the data input area. First, runs that are completely blank will be removed. The program then automatically detects if the input data is from a balanced or unbalanced design, depending on whether any of the analysis columns contain only null values. In order to successfully run RANOVA, a complete dataset must exist comprising 3 (un-balanced) or 4 (balanced) columns of data with no null or non-numeric values. If a valid full balanced design is in the data input area, then successive button pushes will switch to each of four possible un-balanced designs before reverting back to the original balanced design.

<sup>1</sup>Ramsey, M.H., Ellison, S.L.R. (eds.) (2007). Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches Eurachem (2007).