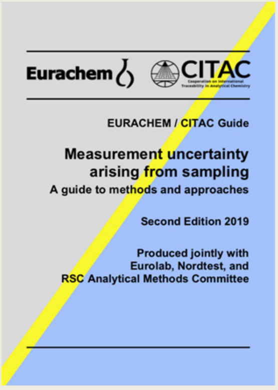
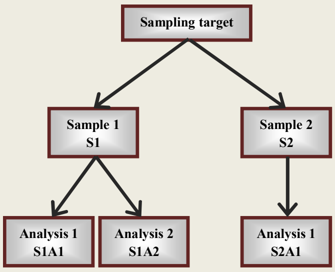
Using unbalanced designs to reduce the cost of sampling uncertainty estimation





Peter Rostron

University of Sussex

Analytical Methods Trust

Eurachem/Eurolab Workshop,
Uncertainty from sampling and analysis for accredited laboratories
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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 mg kg^{-1} (summer) - 5000 mg kg^{-1} (winter)[1]

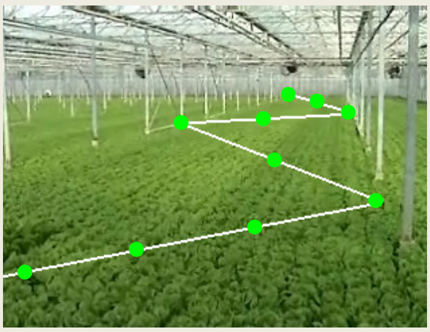
Scope (from guide): Estimate the measurement uncertainty, and contributions from sampling and analysis, for routine monitoring of glasshouse grown lettuce, using a standard sampling protocol

[1] 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)*

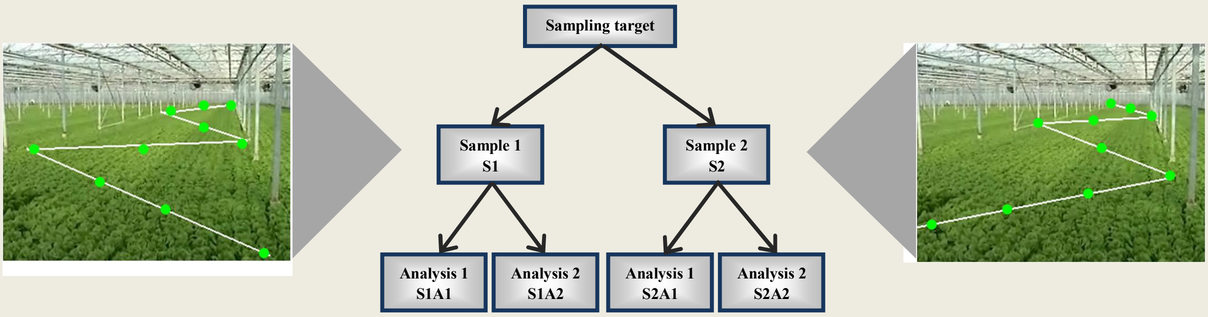
‘W’ Sampling Design for Lettuce



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.

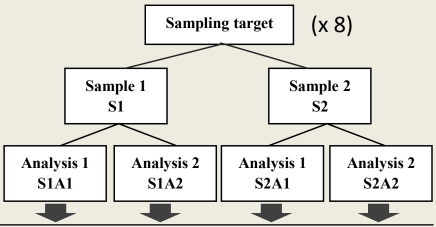


8 bays selected for duplicate sampling

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



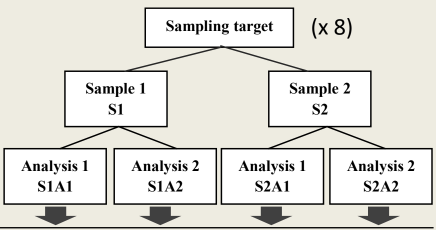
Sample target	S1A1	S1A2	S2A1	S2A2
A	3898	4139	4466	4693
B	3910	3993	4201	4126
C	5708	5903	4061	3782
D	5028	4754	5450	5416
E	4640	4401	4248	4191
F	5182	5023	4662	4839
G	3028	3224	3023	2901
H	3966	4283	4131	3788

NO_3 mg kg^{-1}

	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



Sample target	S1A1	S1A2	S2A1	S2A2
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NO_3 mg kg^{-1}

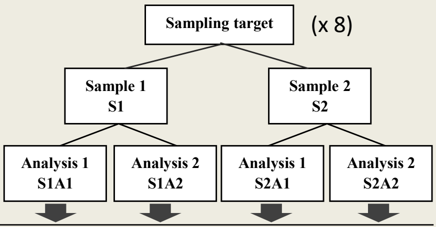
	Classical ANOVA
U_{samp}'	**24%**
U_{anal}'	**7%**
U_{meas}'	**25%**

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



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G	3028	3224	3023	2901
H	3966	4283	4131	3788

NO_3 mg kg^{-1}

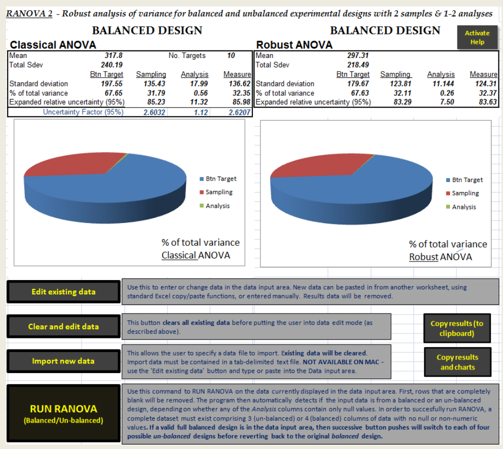
	Classical ANOVA	**Robust ANOVA**
U_{samp}'	24%	**15%**
U_{anal}'	7%	**8%**
U_{meas}'	25%	**16%**

Robust uncertainty more representative of main body of data

Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)[1]
- 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[1]
- Robust ANOVA gives more reliable estimate of the variances of the ***underlying*** populations (See example in Appendix A1 of the Eurachem UfS guide[1])
- Computer intensive, iterative process

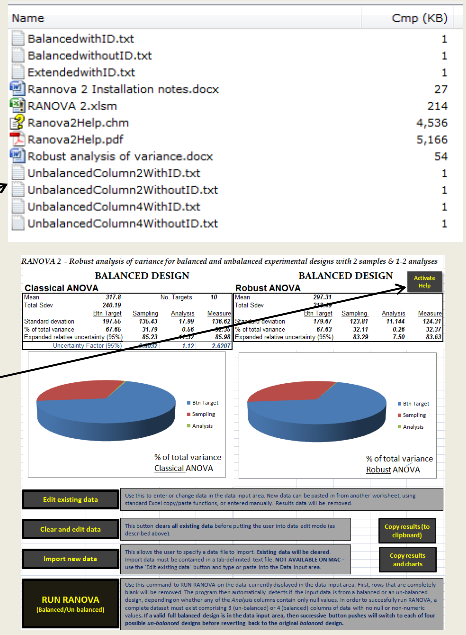
MS Excel – *RANOVA2*



[1]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



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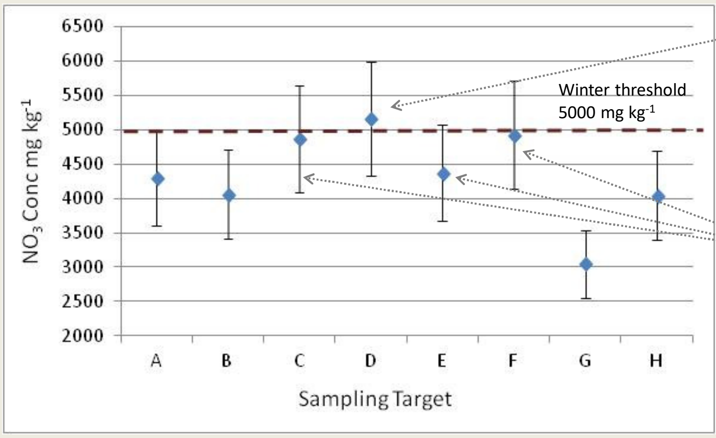
Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

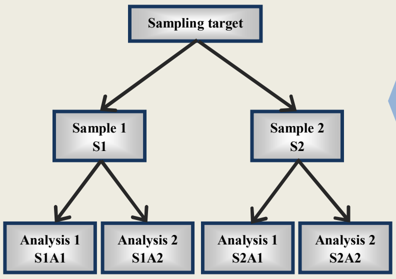
U_{meas}' = 16% from Robust ANOVA



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

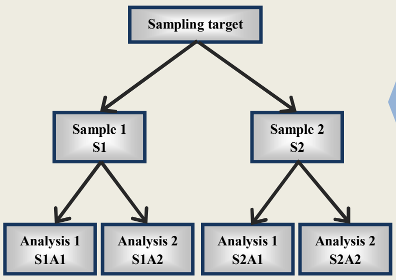
Robust ANOVA enables better decisions on probabilistic classification

Analysing data from the duplicate method



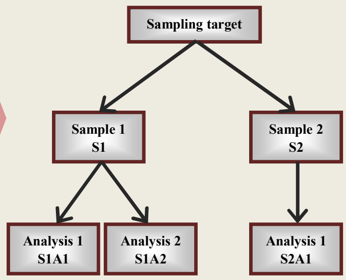
- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

Reducing the cost of uncertainty estimation

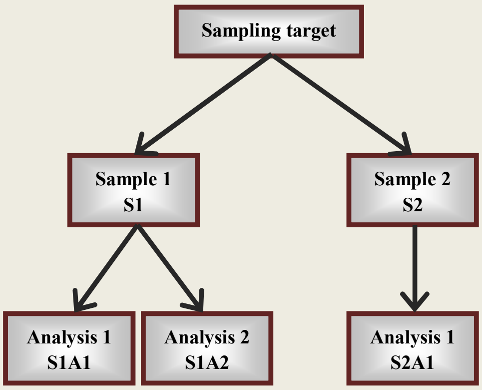


- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

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



Unbalanced experimental design



- 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[1]
- 1000 simulated normal datasets (balanced design) were generated for each of 3 seed ('True') values

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

[1]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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	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
Seed 1	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
Seed 2	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
Seed 3	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					

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	Analytical -90					
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	Analytical +200					
	Sampling -90					
Seed 1	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
Seed 2	Sampling +100					
	Sampling +200					
	Geochemical -90					
	Geochemical +100					
	Geochemical +200					
	Combined +100					
	Base (None)					
	Analytical -90					
	Analytical +100					
	Analytical +200					
	Sampling -90					
Seed 3	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

		Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
Seed params (Refer to Table 2)	Outlier type	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

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- 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

		Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %]				
Seed params (Refer to Table 2)	Outlier type	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***

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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	-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***

[1]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	-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***

[1]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%

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**

[1]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?**

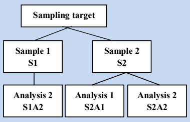
Seed params (Refer to Table 2)	Outlier type	Percent difference U-RANOVA/RANOVA [(Unbalanced - Balanced)/Balanced %] Mean %	Total SD %	Between Target SD %	Sampling SD %	Analytical SD %
Seed 1	Base (None)	0.0	0.2	0.2	0.1	0.0
	Analytical -90	0.3	-1.0	0.1	-5.1	0.1
	Analytical +100	-0.4	-1.8	-0.9	-5.1	0.1
	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	-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**

[1]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

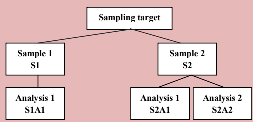
- Can ‘simulate’ unbalanced design by removing single columns from the balanced design data
- 4 different **unbalanced** designs possible
- Compare outputs with balanced design

1



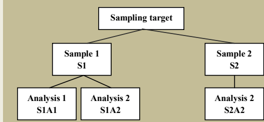
Sample target	S1A1	S1A2	S2A1	S2A2
A		4139	4466	4693
B		3993	4201	4126
C		5903	4061	3782
D		4754	5450	5416
E		4401	4248	4191
F		5023	4662	4839
G		3224	3023	2901
H		4283	4131	3788

2



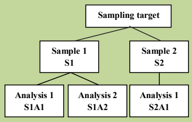
Sample target	S1A1	S1A2	S2A1	S2A2
A	3898		4466	4693
B	3910		4201	4126
C	5708		4061	3782
D	5028		5450	5416
E	4640		4248	4191
F	5182		4662	4839
G	3028		3023	2901
H	3966		4131	3788

3



Sample target	S1A1	S1A2	S2A1	S2A2
A	3898	4139		4693
B	3910	3993		4126
C	5708	5903		3782
D	5028	4754		5416
E	4640	4401		4191
F	5182	5023		4839
G	3028	3224		2901
H	3966	4283		3788

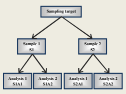
4

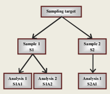


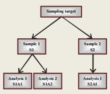
Sample target	S1A1	S1A2	S2A1	S2A2
A	3898	4139	4466	
B	3910	3993	4201	
C	5708	5903	4061	
D	5028	4754	5450	
E	4640	4401	4248	
F	5182	5023	4662	
G	3028	3224	3023	
H	3966	4283	4131	

Example A1 from the Eurachem guide – UNBALANCED design

Comparison of *U′* estimates - unbalanced design scenarios vs balanced design.







	Balanced Design	Unbalanced designs 1	2	3	4	Average 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%

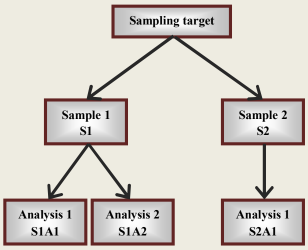
Maximum 7% difference in U_{meas}' using the unbalanced design

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.

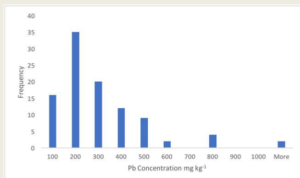
	Balanced Design	Unbalanced designs 1	2	3	4	Average Unbalanced
U_{samp}' (%)	***83.3***	83.3	88.6	77.6	80.2	***82.4***
U_{anal}' (%)	***7.5***	8.8	8.9	6.9	6.9	***7.9***
U_{meas}' (%)	***83.6***	83.8	89.0	77.9	80.5	***82.8***

Differences between averages <= 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 ^{F}U better representation of positive skew in this case, ^{F}U = (x/÷) 1.3



- RANOVA2 also calculates Uncertainty Factor ^{F}U 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

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

amc

Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)[1]

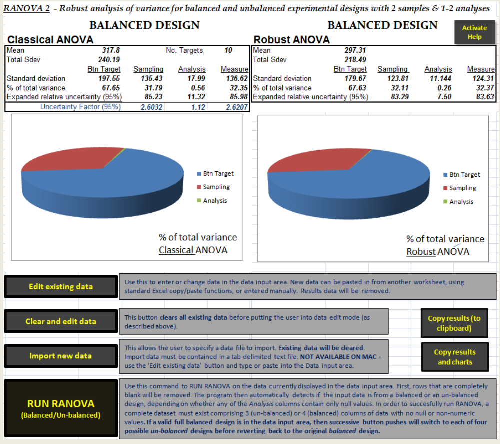
- Computer intensive iterative process. To calculate robust mean:

- Initially estimates:
  - -Robust mean μ_r = classical mean
  - -Robust standard deviation σ_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

- μ_r and σ_r recalculated, and process repeated until μ_r converges to an acceptable level of accuracy

MS Excel – *RANOVA2*



[1]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).