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Practical relevance of measurement uncertainty in inborn errors of metabolism

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Outline

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- Is it important for inborn errors ?
- What is measurement uncertainty?
- Regulatory requirements?
- Two clinical scenarios : monitoring and diagnosis
- Sources of uncertainty, dried blood spots : pre analytic
  - Sample quality
  - Filter paper batch changes
- Sources of uncertainty, dried blood spots : analytic
  - Imprecision
  - Analyser to analyser variation
  - Reagent batch changes
- How can MU be assessed and addressed?

Is it important for inborn errors of metabolism ?

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- The diagnostic investigations are often only performed once, often in an urgent situations and are used to make or discount lifelong disorders
- The monitoring results are often used to check compliance against consensus guidelines for control and therefore must be transferable centre to centre – a founding aim of ERNDIM
- We have a responsibility to establish clear case definitions based upon accurate, traceable and reproducible results
- We have a responsibility to help those monitoring patients to understand the strengths and limitations of testing and factors whiich may lead to variability
- An opportunity to describe the plans for a dried blood spot scheme for common metabolites to be piloted in Autumn 2017

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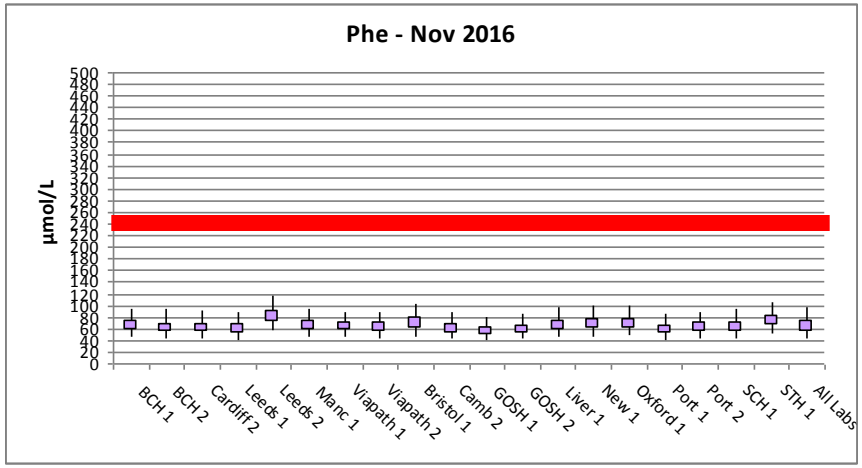

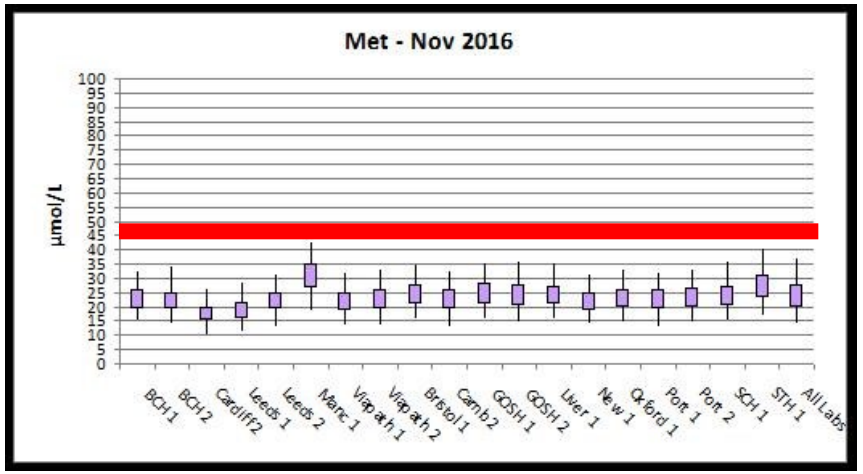

What is measurement uncertainty?

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- In metrology, measurement uncertainty is a non-negative parameter characterising the dispersion of the values attributed to a measured quantity. All measurements are subject to uncertainty and a measurement result is complete only when it is accompanied by a statement of the associated uncertainty. By international agreement, this uncertainty has a probabilistic basis and reflects incomplete knowledge of the quantity value.[1]

JCGM 100:2008. Evaluation of measurement data – Guide to the expression of uncertainty in measurement Joint Committee for Guides in Metrology.

Regulatory requirements

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- An increasingly important part of accreditation
- The big four in the UK
  - Traceability
  - Uncertainty
  - Validation and verification
  - Competency
- Also emphasise the use of independent controls
- Emphasises the laboratory aspects but we will take a wider view to include:
  - Pre-analytic factors
  - Analytic factors

BS EN ISO 15189:2012
Incorporating corrigendum October 2014

Medical laboratories — Requirements for quality and competence (ISO 15189:2012)

5.5.1.4 Measurement uncertainty of measured quantity values

The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty.

NOTE 1 The relevant uncertainty components are those associated with the actual measurement process, commencing with the presentation of the sample to the measurement procedure and ending with the output of the measured value.

NOTE 2 Measurement uncertainties may be calculated using quantity values obtained by the measurement of quality control materials under intermediate precision conditions that include as many routine changes as reasonably possible in the standard operation of a measurement procedure, e.g. changes of reagent and calibrator batches, different operators, scheduled instrument maintenance.

NOTE 3 Examples of the practical utility of measurement uncertainty estimates might include confirmation that patients' values meet quality goals set by the laboratory and meaningful comparison of a patient value with a previous value of the same type or with a clinical decision value.

The laboratory shall consider measurement uncertainty when interpreting measured quantity values. Upon request, the laboratory shall make its estimates of measurement uncertainty available to laboratory users.

Where examinations include a measurement step but do not report a measured quantity value, the laboratory should calculate the uncertainty of the measurement step where it has utility in assessing the reliability of the examination procedure or has influence on the reported result.

Impact in two specific scenarios

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- Monitoring (dried blood spot samples)
  - Conditions such as MSUD, PKU, HCU
  - Measuring Leu, Phe, Thcys using dried blood spots
- Classification of disease (liquid samples)
  - eg Pyridoxine responsiveness in homocystinuria

Monitoring pre analytic: sample quality

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Effect of Dried Bloodspot Quality on Newborn Screening Analyte Concentrations

Roanna S. Georgeand Stuart J. Moat Clin Chem 2016

- (*P*< 0.001). Smaller bloodspots produced significantly lower results (15%–24% for 10µL vs 50µLsample size) for all analytes at all concentrations measured (*P* <0.001).
- Results obtained from peripheral punches were higher than those from a central punch although this did not reach statistical significance for all analytes.
- Compression of bloodspots produced significantly lower results (14%–44%) for all analytes measured
- Insufficient and multispotted samples demonstrated heterogeneous results
- **CONCLUSIONS:** All bloodspots containing 20 µL (bloodspot diameter 8 mm), those in which blood has not fully penetrated the filter paper, and all samples with evidence of compression should be rejected, since there is a risk of producing false-negative results.

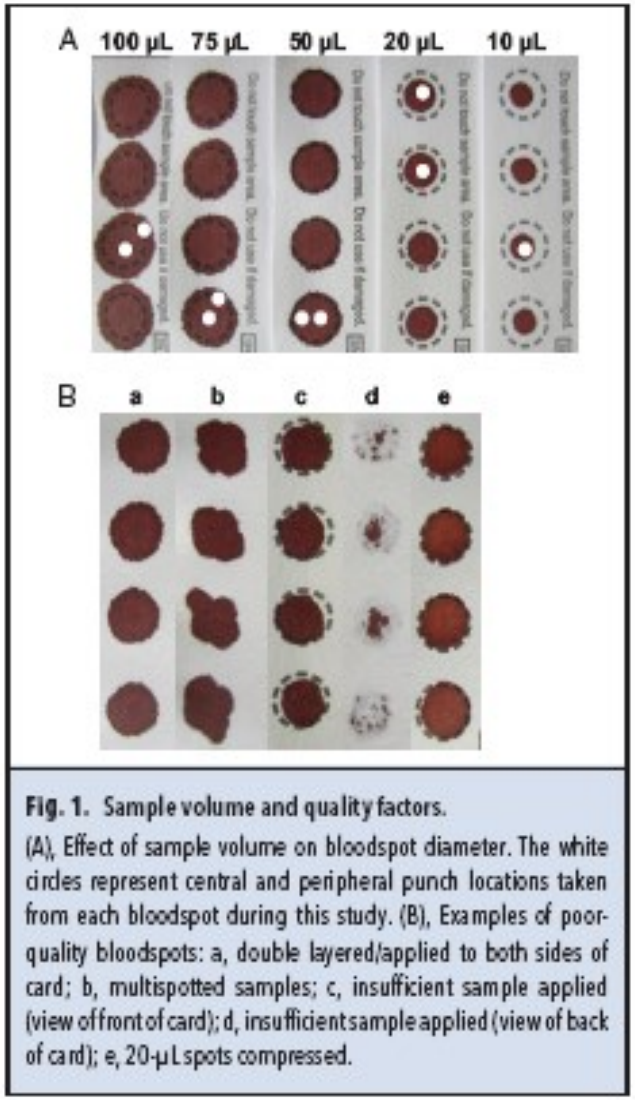

Fig. 1. Sample volume and quality factors.
(A), Effect of sample volume on bloodspot diameter. The white circles represent central and peripheral punch locations taken from each bloodspot during this study. (B), Examples of poor-quality bloodspots: a, double layered/applied to both sides of card; b, multispotted samples; c, insufficient sample applied (view of front of card); d, insufficient sample applied (view of back of card); e, 20-µL spots compressed.

Monitoring pre analytic: sample quality

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- **The effect varies by metabolite**

Leucine in a small spot punched in the centre vs large spot punched at the edge, range: 505µmol/L vs 660µmol/L (ie +/- 13%)

Table 1. Effect of punch location on measured analyte concentration for different sample volumes.[a]

	20 µL			50 µL			75 µL			100 µL		
Analyte	Central	Peripheral	P	Central	Peripheral	P	Central	Peripheral	P	Central	Peripheral	P
Phenylalanine, µmol/L	206 (5.9)	219 (9.2)	<0.001	239 (8.5)	247 (8.2)	<0.05	256 (10.6)	258 (11.3)	NS	265 (8.2)	268 (10.2)	NS
Tyrosine, µmol/L	165 (5.1)	169 (5.9)	NS	183 (6.5)	187 (6.6)	NS	194 (7.9)	193 (7.4)	NS	196 (5.5)	199 (7.3)	NS
Leucine, µmol/L	505 (15.0)	525 (24.7)	<0.05	594 (22.5)	597 (23.3)	NS	635 (26.4)	624 (29.8)	NS	660 (21.7)	648 (25.0)	NS
Methionine, µmol/L	38 (1.1)	39 (1.6)	NS	43 (1.6)	44 (2.3)	NS	46 (2.5)	45 (1.9)	NS	47 (1.4)	47 (1.8)	NS
C8, µmol/L	0.39 (0.01)	0.42 (0.02)	<0.001	0.46 (0.02)	0.47 (0.02)	<0.05	0.48 (0.02)	0.50 (0.03)	NS	0.50 (0.02)	0.52 (0.03)	NS
C10, µmol/L	0.51 (0.02)	0.54 (0.03)	<0.05	0.57 (0.03)	0.60 (0.03)	<0.05	0.61 (0.03)	0.63 (0.04)	<0.05	0.63 (0.02)	0.64 (0.05)	NS
C5DC, µmol/L	0.52 (0.02)	0.53 (0.02)	NS	0.59 (0.02)	0.58 (0.03)	NS	0.62 (0.03)	0.62 (0.03)	NS	0.63 (0.02)	0.64 (0.03)	NS
C5, µmol/L	1.42 (0.04)	1.48 (0.06)	<0.05	1.70 (0.06)	1.70 (0.07)	NS	1.81 (0.07)	1.81 (0.09)	NS	1.89 (0.06)	1.89 (0.08)	NS
TSH, mU/L	NA	NA	NA	11.8 (0.57)	12.6 (0.69)	<0.001	11.9 (0.60)	12.6 (0.87)	<0.001	12.5 (0.60)	12.8 (0.66)	NS
IRT, ng/mL	NA	NA	NA	61 (3.1)	65 (3.1)	<0.05	65 (3.2)	66.2 (3.9)	NS	71 (2.9)	72.1 (4.3)	NS

[a] Data are mean (SD). NA, not analyzed; NS, not significant.

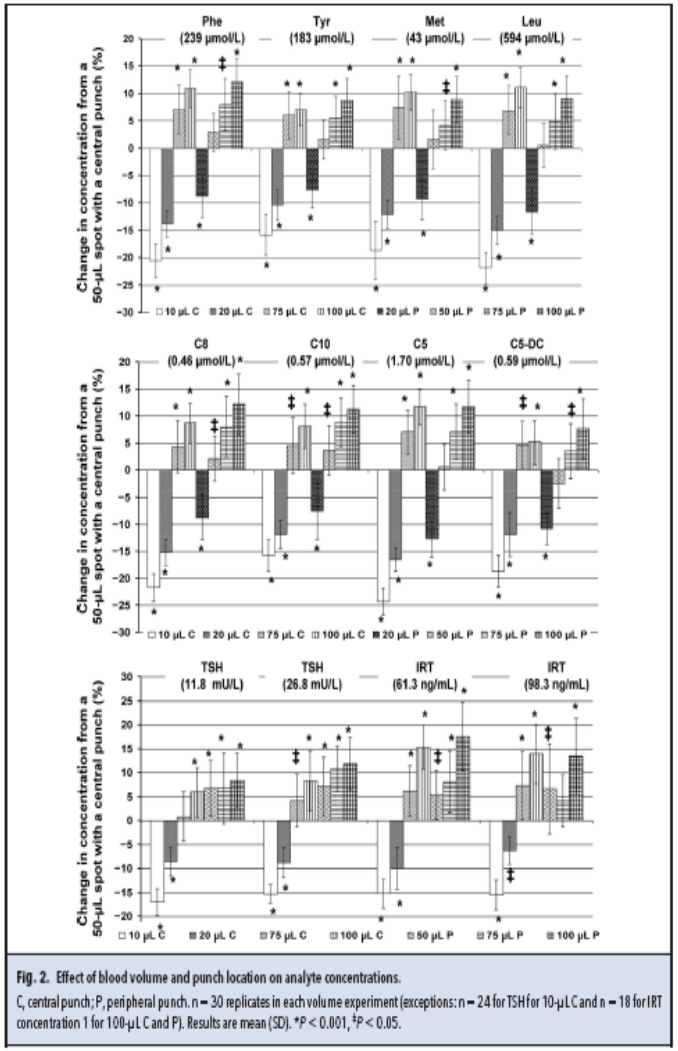

Fig. 2. Effect of blood volume and punch location on analyte concentrations.

C, central punch; P, peripheral punch. n = 30 replicates in each volume experiment (exceptions: n = 24 for TSH for 10-µL C and n = 18 for IRT concentration 1 for 100-µL C and P). Results are mean (SD). *$P < 0.001$, †$P < 0.05$.

Monitoring pre analytic: batch to batch card variation

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- *CDC Filter Paper Comparison Study Report 2009* is a special internal report of the **Newborn Screening Quality Assurance Program**
- The study data indicate that the difference between manufacturers could be at least 4–5% for comparability or, at a minimum, equal to the lot-to-lot variance of a single manufacturer's filter paper products

Range 1.397 – 1.571,
At a Leu of 400: 376 – 424µmol/L
(ie +/- 5.9%)

Newborn Screening Quality Assurance Program

ALTH

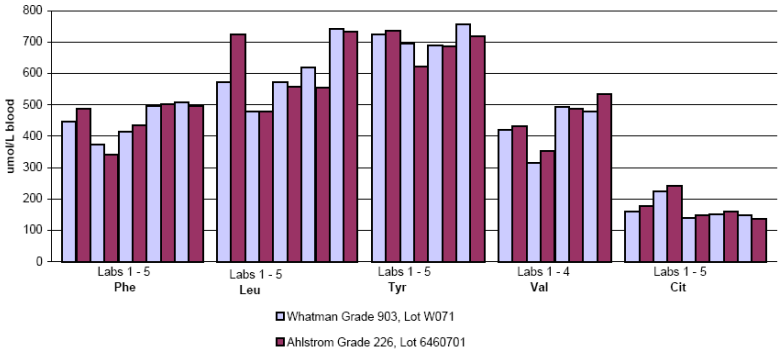
Filter Paper Comparison Study

May 2009

Intact Red Blood Cells (RBC)

Year of Manufacture	Lots	Serum Volume Intact Cell	Mean Serum Volume	SD
1998	W981	1.460	1.474	0.061
2000	W001	1.400		
2001	W011	1.571	n	8
2003	W031	1.510	CV	**4.13%**
2004	W041	1.440		
2005	W051	1.489		
2007	**W071***	**1.397**		
2008	W081	1.521		

Figure 2. Whatman vs. Ahlstrom
Amino Acid - Single Level Multiple-Analyte Specimens
Results per Laboratory (n=4 results per analyte per lab)



Monitoring analytic variation: Imprecision

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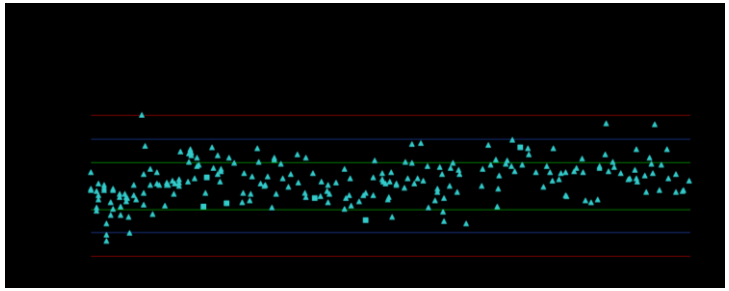

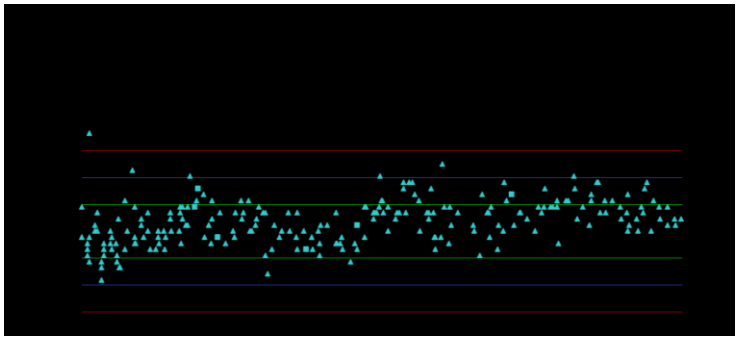

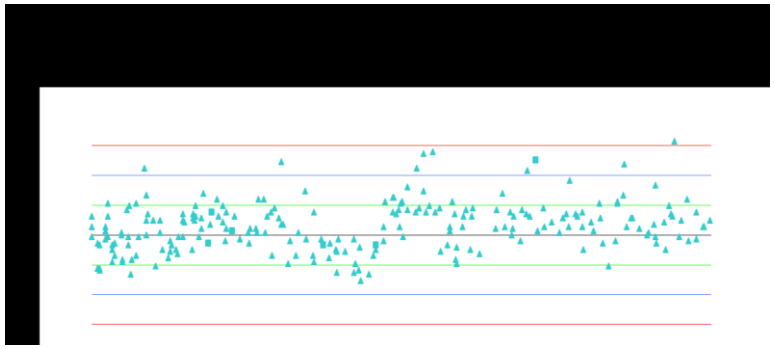

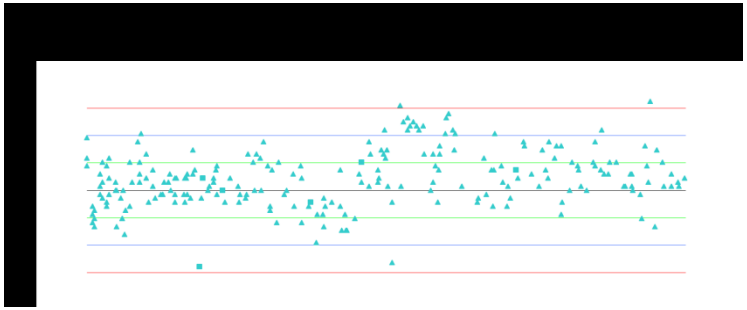

Monitoring analytic variation: Imprecision

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	Running Mean	No	Calculated SD	Calculated CV
VAL	165	62	11.4	6.9
MET	13	62	1.2	9.2
ALLOILE	48	62	3.3	7.0
ILE	50	62	3.5	7.0
LEU	90	62	5.9	6.5
TYR	33	62	2.9	8.8
PHE	45	62	3.5	7.8

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Leu of 400 µmol/L
+/- 56 µmol/L
Range: 344 – 456
(ie +/- 14%)

Range of CV: 6.9 – 9.2 %, 7.6%

Monitoring - taken together

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Blood spot quality and size

A small spot punched in the centre vs large spot punched at the edge, Leu range: 505 µmol/L vs 660 µmol/L (ie +/- 13%)

Filter paper batch change

Range 1.397 – 1.571, serum volume in same size spot

Leu of 400: 376 – 424 µmol/L (ie +/- 5.9%)

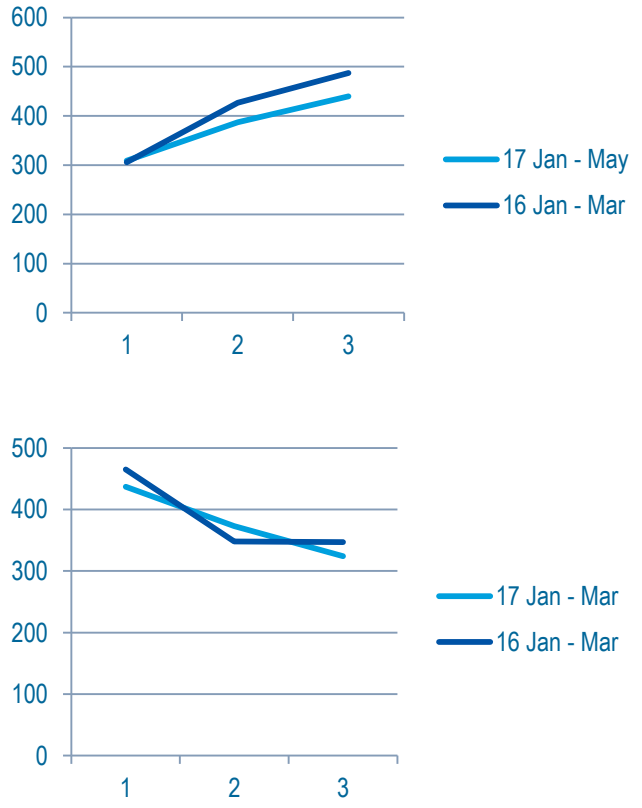
Analytical imprecision

Leu of 400 µmol/L, +/- 56 µmol/L

Range: 344 – 456 (ie +/- 14%)

As independent variables – taken together

The range of Leu at 400 µmol/L may be up to +/- 25% in a real world situation using DBS ie 300 – 500 µmol/L



Are these examples of worsening or improving control?

Determining pyridoxine responsiveness

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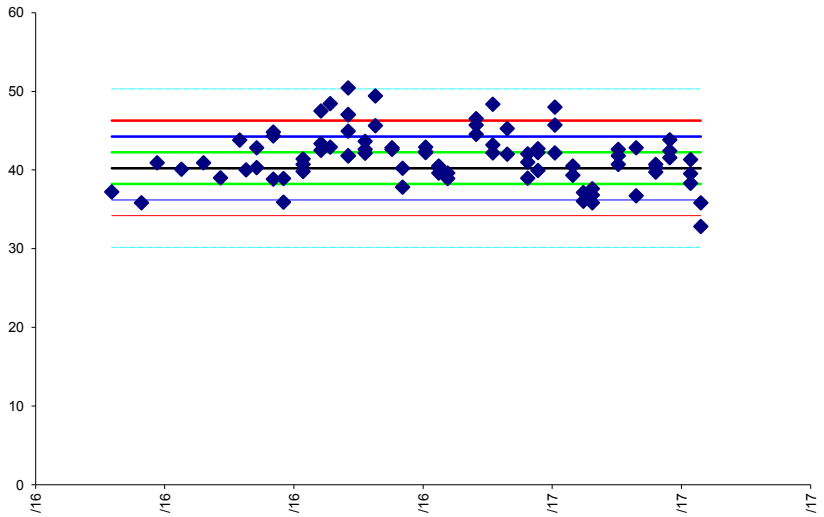
- A tricky issue
  - Guidelines suggest
    - Giving 10 mg/kg/d for 6 weeks
    - Measure Thcys twice before treatment
    - Measure twice on treatment
    - < 50 µmol/L on treatment are clearly responsive
    - A fall of >20% but above 50 µmol/L, may need additional treatment eg betaine
    - A fall of <20%, unlikely to be responsive
- Patient 1
  - Thcys 110 and 100 pre-treatment, 76 and 85 on treatment. Are they a responder?
  - 105 vs 81 - a 23% drop ✓
  - Assuming 5.7% CV at extremes 99 vs 86 – a 13% drop ?
- Patient 2
  - Thcys 70 and 62 pre-treatment, 53 and 44 post treatment. Are they clearly responsive?
  - 66 vs 49 ✓
  - Assuming 5.7% CV at extremes 62 vs 52 ?

	QC2
RUNNING MEAN	41.63
CALCULATED CV	8.13



How can MU be assessed and addressed?

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Assessment

- Within a lab most commonly assessed by retrospective analysis of IQC material, should be independent control material
- If this is not possible eg enzyme assay, then an additive process taking account of the uncertainty intrinsic to each step in the process, such as pipetting, weighing, spectrophotometric measurement etc – these are summed to give MU estimate for the process
- Between labs EQA data has a role in looking at the overall variability – a key role for ERNDIM. This may guide the implementation of guidelines where target values are set
- Population studies can also be valuable

ERNDIM dried blood spot scheme Sept 2017

Analyte	Spike Level 1	Spike Level 2	Spike Level 3	Spike Level 4
Allo ile				
Ile				
Leu				
Val				
Phe				
Tyr				
Total Hcys				
To be investigated				
Met				
Cysteine				
C0				
NTBC				
Succinylacetone				

How can MU be assessed and addressed?

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Addressing the issues

- Awareness, awareness, awareness – within the lab and with the users
- Reporting – but in a sensible and understandable way
- Clear and documented control procedures around tricky areas such as spot quality, batch changes, equipment re-introduction following maintenance, temperature control, reagent storage etc
- Adoption of consistent analytical approaches between labs in a network including common Int Stds for instance in MS/MS
- Continued interlab discussion about performance issues eg at ERNDIM workshops