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Uncertainty of measurement – practical relevance and the results of the DBS scheme

Prof Jim Bonham
Clinical Director
Sheffield Children's NHS FT

Outline

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- Is MU important for inborn errors ?
- 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?
- The DBS scheme results
- Implications for practice

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 which may lead to variability

Regulatory requirements

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- It is an increasingly important part of accreditation
- The big four in the UK
  - Traceability
  - Measurement Uncertainty
  - Validation and verification
  - Competency
- Accreditation also emphasises the use of independent internal quality controls
- Emphasises the laboratory aspects but we will take a wider view to include:
  - Pre-analytic factors
  - Analytic factors

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. George and 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.

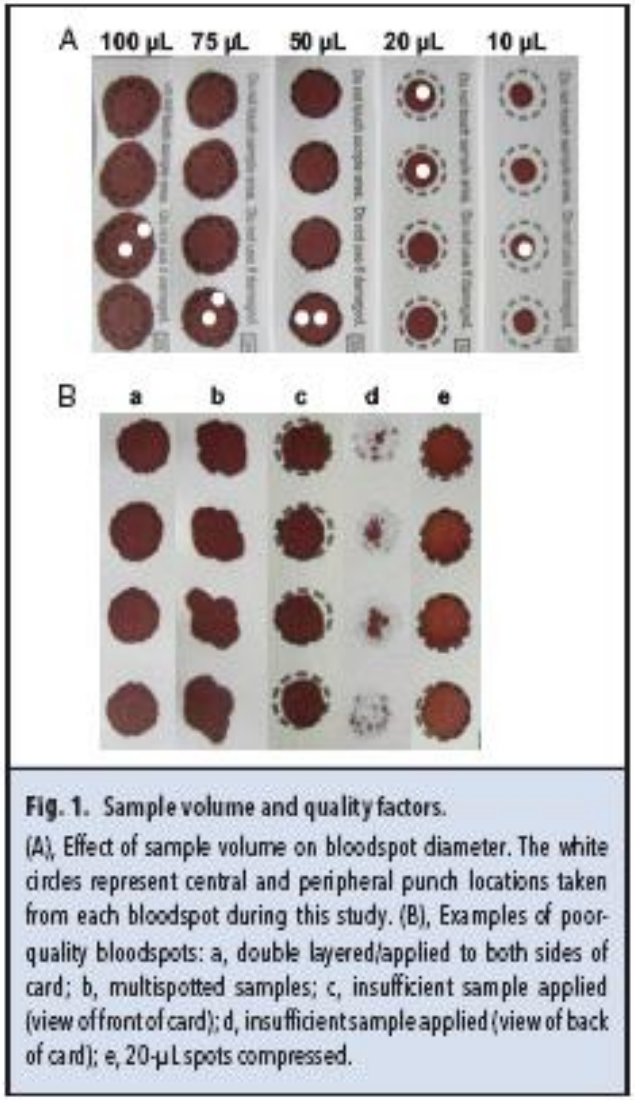

Fig. 1. Sample volume and quality factors.
(A), Effect of sample volume on bloodspot diameter. The white circles represent central and peripheral punch locations taken from each bloodspot during this study. (B), Examples of poor-quality bloodspots: a, double layered/applied to both sides of card; b, 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 648µmol/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.

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

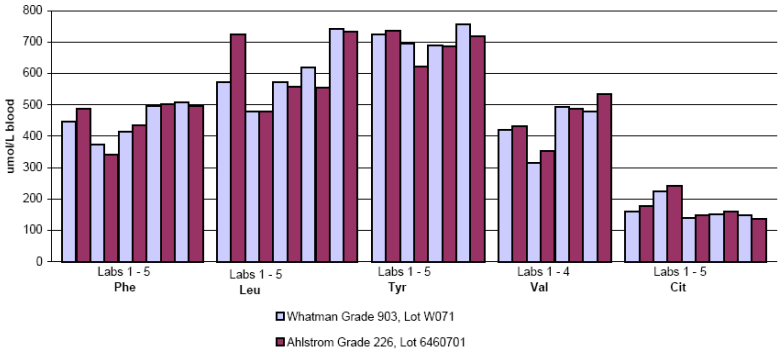
Filter Paper Comparison Study

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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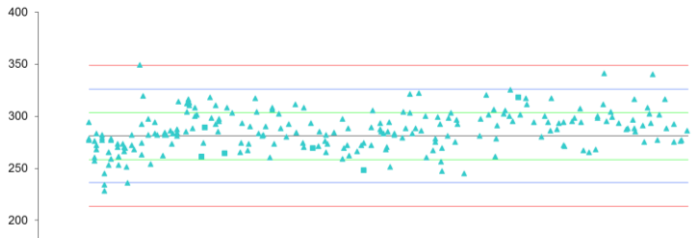
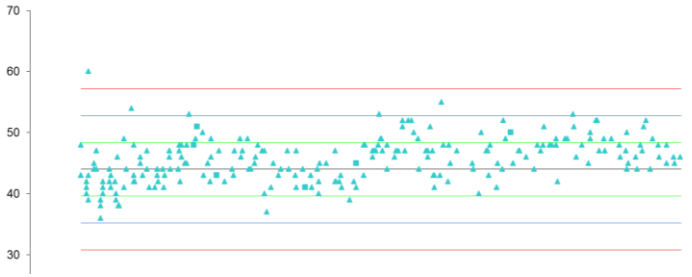
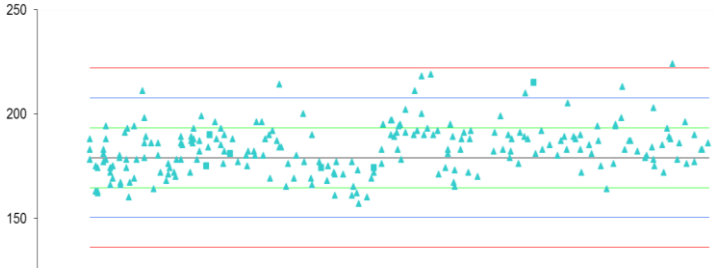
VAL QC2



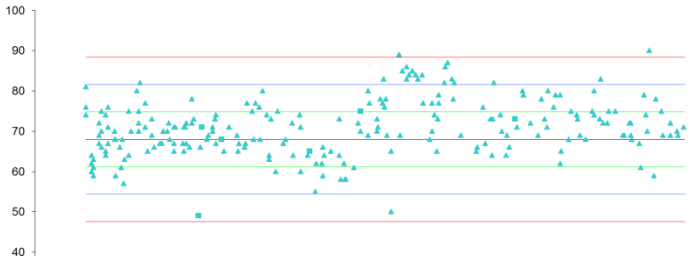
ALLOILE QC



LEU QC



PHE QC



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

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 648 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%)

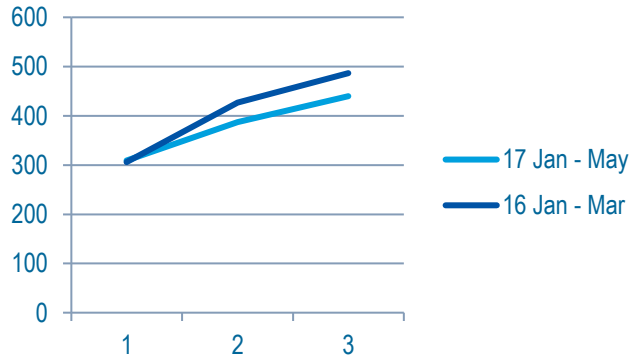
Analytical imprecision

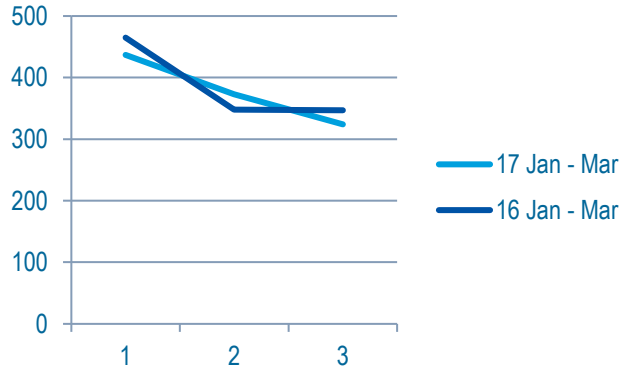
Leu of 400 µmol/L, +/- 56 µmol/L

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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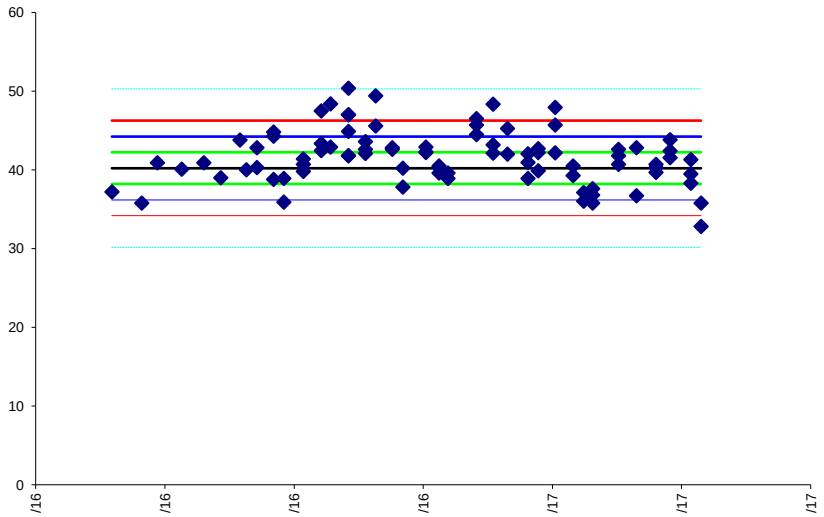
- A tricky issue
  - Guidelines suggest
    - Giving 10 mg/kg/d for 6 weeks
    - Measure Thcys twice before treatment
    - Measure twice on treatment
    - < 50 µmol/L on treatment are clearly responsive
    - A fall of >20% but above 50 µmol/L, may need additional treatment eg betaine
    - A fall 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?

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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 addressed?

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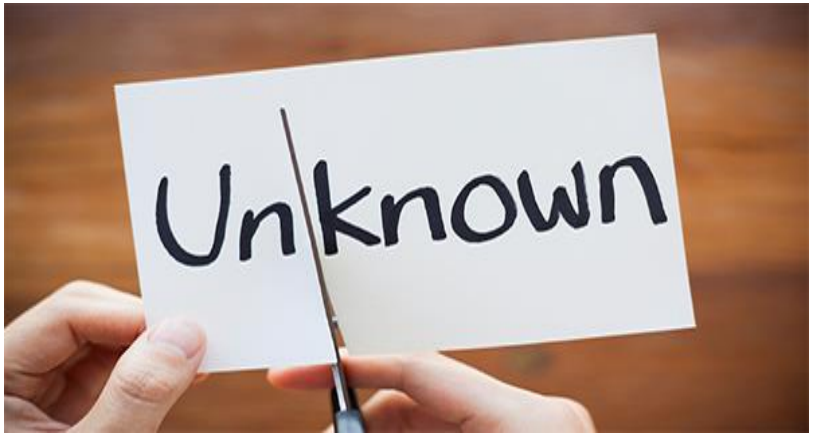
Addressing the issues

- 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
- Continued interlab discussion about performance issues eg at ERNDIM workshops
- The use of independent IQC material
- Shared standardisation of assays



Pilot ERNDIM DBS

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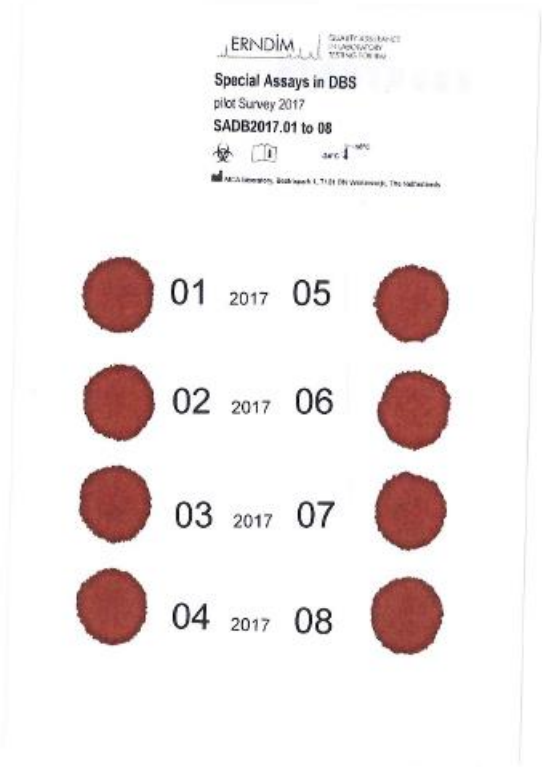
- In June 2017 SKML went to CDC to be trained on DBS preparation
- Stock solutions of citrate/dextrose human blood divided into four aliquots with spikes of different concentration added for:
  - Alloile, Ile, Leu, Val, Phe, Tyr, Hcys
- Each aliquot spotted twice, frozen at -70C and shipped to SKML
- SKML then shipped samples to the 109 labs who subscribed on 11th Sept with a deadline for submission of 3rd Nov. Reports to be issued on 2nd Dec



Annual Report Pilot ERNDIM DBS

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PDF for printing

Analyte	Accuracy (mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (%added analyte)		Data all labs	
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
Number of days between shipment and arrival		10.0		0.0%		0.000		0%	69	68.1%
Alloisoleucine	FR	31.4	FR	11.6%	FR	0.997	FR	96%	48	47.9%
Homocysteine		51.8		9.1%		0.989		124%	30	65.0%
Isoleucine	424	361	2.8%	7.0%	0.997	0.991	120%	93%	50	24.9%
Leucine	610	530	1.6%	6.7%	1.000	0.996	114%	89%	60	29.4%
Phenylalanine	516	447	4.9%	6.1%	0.999	0.997	102%	86%	88	21.1%
Tyrosine	514	429	3.3%	5.9%	0.998	0.995	106%	85%	85	21.0%
Valine	553	408	1.7%	6.4%	0.992	0.989	125%	78%	70	35.4%
Overall	523	283	2.9%	6.6%	0.997	0.869	113%	81%	62	39.1%
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
Analyte	Accuracy		Precision		Linearity		Recovery		Data all labs	

Pilot ERNDIM DBS - messages

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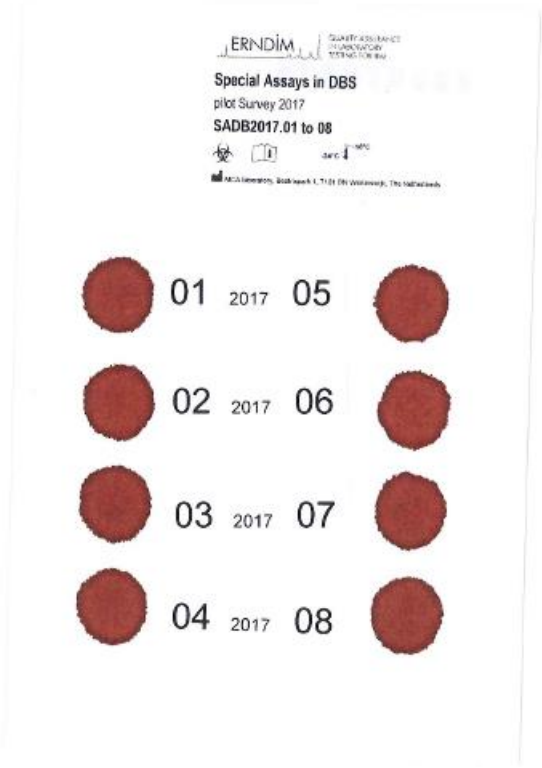
- Submissions range from 30 labs for hcys to 88 labs for phe
- Recovery ranges from 78% for val to 124% for hcys
- Linearity ranges from 0.989 for val to 0.997 for phe
- Within lab precision ranges from 5.9% for tyr to 9.1% for hcys
- Between lab precision ranges from 21% for tyr to 65% for hcys, phe = 21.1%. This suggests poor standardisation and is a traceability issue



Implications of poor between lab agreement

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- MRC guidelines for PKU,
  - 0 – 5 y in PKU, 120 – 360 µmol/L
  - 5 – 18y in PKU, 120- 480 µmol/L
- In reality with the current level of assay performance the confidence around a result of 360 µmol/L within one centre may range from 316 – 404 µmol/L – perhaps OK
- In reality with the current level of assay performance the confidence around a result of 360 µmol/L between centres ranges from 208 – 512 µmol/L (+/- 2SD), with a mean of 447µmol/L, range = 184 – 645 µmol/L – almost certainly not OK
- What do we need to do?
  - Publish the findings – awareness, work with MetabERN
  - Use a traceable standard to improve assay comparability
  - Possibly re-assess clinical guidance