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Uncertain about Measurement Uncertainty?

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Outline

- Brief introduction to measurement uncertainty
- What labs have been busy doing
- Examples of key IMD assays
- Implications for users

Why are we interested?

- Measurement Uncertainty is a ‘hot topic’
- Labs are in the process of transitioning from CPA to UKAS
- Some of the major differences between UKAS and CPA
    - Measurement uncertainty
    - Traceability
    - User engagement

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Measurement Uncertainty

What is it?

"Measurement uncertainty is a parameter, associated with the result of a measurement, (e.g. a test) that defines the range of values that could reasonably be attributed to the measured quantity"

MU for Serum ALP is 300 ± 3 u/L

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Measurement Uncertainty

ISO 15189 5.5.1.4

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

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Progress to date

- For most labs getting to grips with MU has been a significant piece of work
- Understand the concept
- Decide what approach to take
- Implement in the lab
- Document the process
- Establish procedure for monitoring and reviewing MU data
- Decide how to use this information
- If/how/when to communicate with users

ACB Mailbase Discussion

- Mini survey of approach to MU
- How are labs calculating MU?
- Why did you select this method?
- Have you had a UKAS inspection?

- 15 labs responded, 6 had been approved by UKAS
- 7 different sources of 'reference' provided
- 9 labs using QC data to calculate MU
- Conclusion – ACB or RCPath should publish guidance on the best way to calculate MU

Approach at Viapath

- Lack of guidance available
- BMTA meeting, June 2014 - basis of our approach to MU
- Decided that MU and traceability were inextricably linked
- Merit in considering them together
- Produced over arching policy document for department
  - Qualitative approach to generic factors
  - Quantitative approach for individual assay

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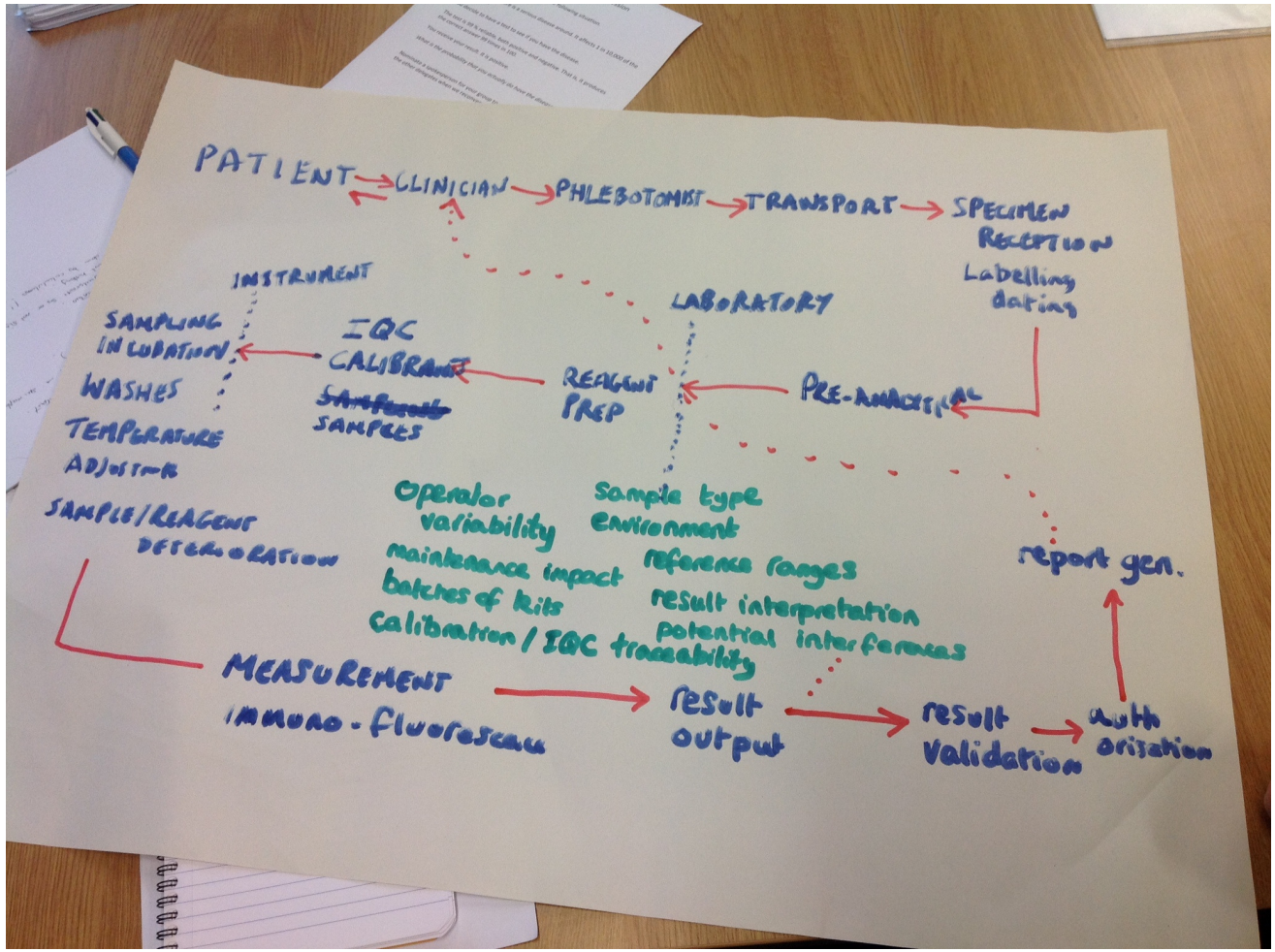
Sources of MU across the sample pathway



Qualitative assessment

Pre Analytical

Step	Measurement Uncertainty	Control measure
Patient	Biological variability Diet Fasting Time of sampling Drugs	User information handbook Website with test information Duty Biochemist for enquiries Audit
Clinician	Order clinically appropriate test Time since last request	As above Smart requesting rules in EPR/LIMS
Phlebotomy	Sample tube Volume	Phlebotomy training/competency Lab liaison Website with test information
Transport	Air tube Porter Royal mail Courier Storage	Maintenance contract SLA with delivery services/courier/etc Sample tracking (barcode confirmation) SOP and competency Audit
Specimen reception	Labelling Aliquoting Booking in Storage	SOPs and competency Visual aids Temperature monitoring

Post Analytical

Step	Measurement Uncertainty	Control measure
Instrument software packages calculate results e.g. QuanLynx	Patient result	Weak control! Method files are not protected. Assay SOP, competency, training See associated Risk Assessment RA-95 Control of instrument software
Numerical Result produced	Accuracy Precision	IQC & EQA Reference ranges/cut off limits Appropriate number of significant figures Determine MU for each assay Monitor MU
Interpretation and authorisation	Subjectivity	Reporting SOP, competency, training, CPD, interpretative EQA schemes, ref ranges, population means, LIMS rules, reflex testing, use of local/national guidelines & protocols, evidence base, MDT
Reporting	Report reaches correct destination in timely fashion	Electronic reporting Failsafe processes Audit Results line/direct dial numbers Communication of results SOP Amending results policy

Analytical

Step	Measurement Uncertainty	Control measure
Analysis of sample	Calibrator value IQC value	Traceability, process for switching to new cal/IQC, patient means, EQA
	Reagents and kits batch variation	Procedure to introduce a new kit/reagent Storage Stock management
	Pipette dispensed volume	Calibration records to UKAS standards In house pipette monitoring records
	Instruments ageing and maintenance	Maintenance (daily, weekly, monthly) Engineer planned maintenance System checks Instrument SOP and training records EQA and IQC
	Environmental fluctuation	IceSpy temperature monitoring, humidity control, air conditioning
	Operator variability	Training – in house end external SOP and competency Examination audit
	Analytical interferences	Method validation SOP Manufacturers guidelines/information

Quantitative Approach to MU

- Documented specific detail for each individual assay (including traceability statement)
- Established target value for MU
- Calculated MU and compared with target
- Regularly monitoring MU
- Investigate assay if MU changes significantly
- Consider if/how/when to communicate info to Users

Target Measurement Uncertainty

- How do we know if performance is acceptable?
- Is our MU comparable with that of other labs?
- Ideally the target value should be derived from external requirements and not just the current performance of the assay
- This is a challenge – few IMD methods have agreed performance goals
- Can compare with published data*
- Only proved useful for plasma amino acids
- Within subject biological variability often not known for less common tests

**** Desirable specification for imprecision which is derived from within subject biological variability Scand J.Clin Lab Invest 1999;59:491-500***

Establishing the target value for MU

- In the absence of published performance specification, alternative approaches include
    - Expert group recommendation
    - External proficiency schemes
    - Horwitz equation

A numerical value for MU

- In addition to describing generic control of uncertainty, a numerical value of MU must be calculated for each assay
- This enables the lab to determine whether the difference in two sequential results from a given patient is significant
- Can also be used to aid interpretation of results close to cut-off values/reference ranges
- Various approaches can be used
- We have opted to use ‘intermediate precision data’

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Glutarylcarnitine (ENBS)

Intra batch	Inter batch	Intermediate precision	Inter lab precision
Minimal variations on the assay technique	Variation when the assay is performed on different days	As many changes as reasonably possible on a single assay technique	Maximum variations on the assay technique including different laboratories and methods

Increasing %CV

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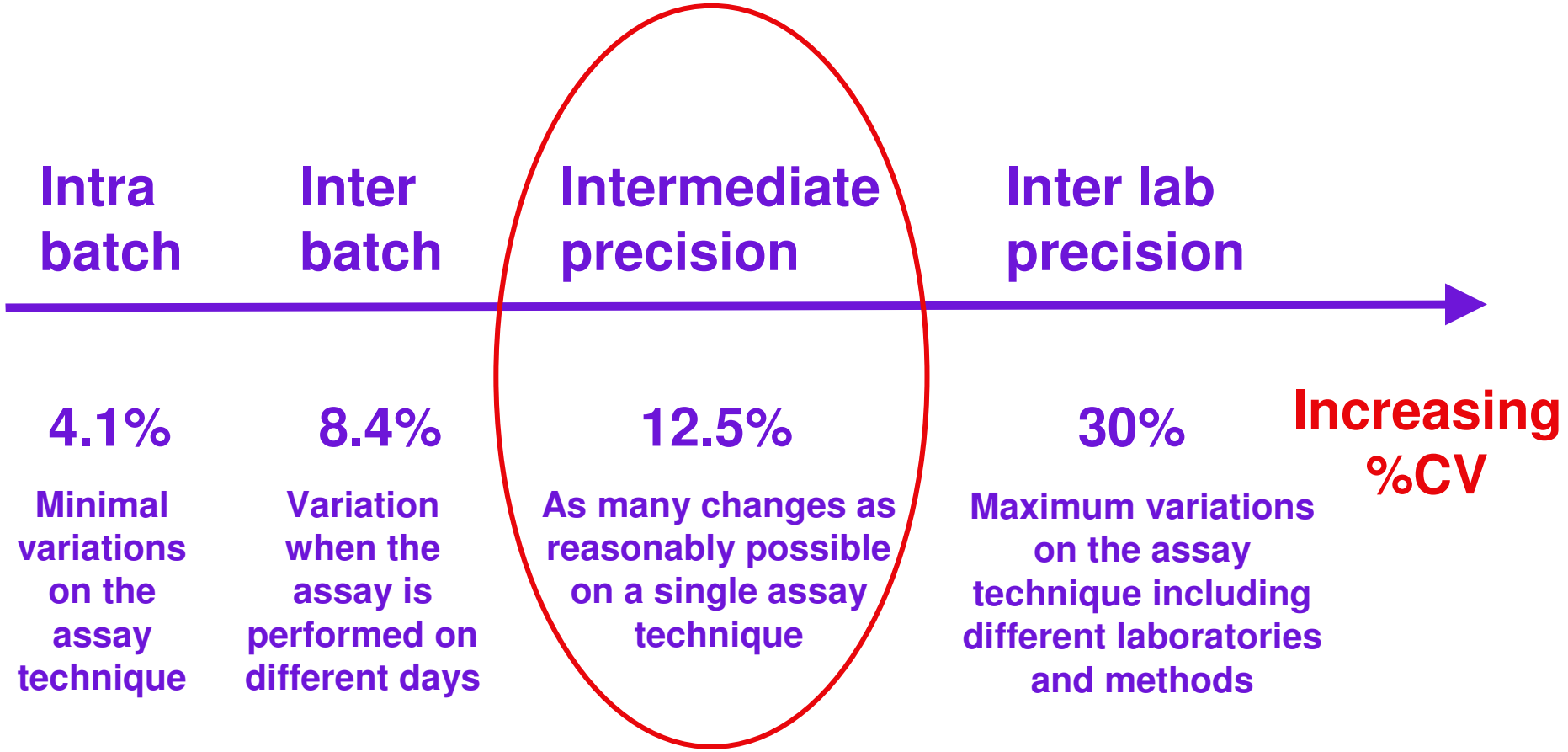
Glutarylcarnitine (ENBS)



Calculation of MU

- Use long term IQC data to calculate MU
- Pool data if using multiple analysers
- **Expanded measurement uncertainty = SD x 2 (95% CI)**
- Recommend using n=100 measurements over minimum period of 6 months
- This is based on guidance from National Pathology Accreditation Advisory Council (NPAAC) to use a ‘statistically valid number of results’
- For many IMD assays, this may not be practical e.g. batch assays
- Logic behind an alternative approach should be documented

Monitoring Uncertainty

- Regular review of MU is a useful way to identify significant changes in bias and/or imprecision
- Block comparison is used to asses bias
- F Test is used to asses variance
- Does **NOT** negate the need for real time IQC and EQA monitoring
- Guidelines state that MU should be recalculated every 6 months
- For many IMD assays this may not be practical

Review of Measurement of Uncertainty

Analyte: PKU Monitoring
Minimum number of samples: 40
Period of assessment: 1/9/15 to 30/11/15
Material assessed: QC
Target MU: conc ±2SDs

Mu calculations: Phenylalanine

QC1	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	135	80	8	5
MU2	10/12/15	127	78	8	5

QC2	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	138	301	35	6
MU2	10/12/15	126	297	29	5

QC3	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	136	1034	115	6
MU2	10/12/15	126	1013	108	5

Mu calculations: Tyrosine

QC1	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	135	71	8	6
MU2	10/12/15	127	71	9	6

QC2	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	138	237	30	6
MU2	10/12/15	126	233	24	5

QC3	Date	Number	Mean	2SD	CV %
MU 1	22/7/15	135	836	103	6
MU2	10/12/15	126	816	96	6

MU within target range: YES

Filename:	MU PKUMON review	Version	2.0
Author:	Rosalind Bray	Issue date	On QPulse
Authorised by:	Farzana Ghoni		

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St Thomas' Hospital

Measurement of Uncertainty review form
SLMM-QR-2
Biochemical Sciences

MU Block Comparison (bias): Phenylalanine

Mu Comparison	Date	QC1	QC2	QC3
MU 1 v MU2	10/12/15	0.15	0.09	0.13

MU Block Comparison (bias): Tyrosine

Mu Comparison	Date	QC1	QC2	QC3
MU 1 v MU2	10/12/15	0.05	0.10	0.14

QC1 MU within acceptance limits (-1.0 to +1.0: Yes
QC2 MU within acceptance limits (-1.0 to +1.0): Yes
QC3 MU within acceptance limits (-1.0 to +1.0): Yes

F test (variance):

Not applicable (all MU comparisons within target range)

Action taken if significant change in bias and/or variance identified or MU is greater than target (including QPulse CAPA reference):

Data reviewed by: Farzana Ghoni **Date:** 11/12/15
Authorised by: **Date:**

Filename:	MU PKUMON review	Version	2.0
Author:	Rosalind Bray	Issue date	On QPulse
Authorised by:	Farzana Ghoni		

Investigating a change in MU

- Monitoring MU will alert us to significant changes in bias and imprecision
- If a significant change is identified the assay must be investigated
- What if anything, has changed recently? e.g. operators, training, IQC material, instrument maintenance, reagents
- Record as a non conformance and refer to Senior Staff meeting
- Consultant Scientist to make judgement on whether assay can continue whilst investigation takes place
- Undertake a critical review of the assay and document

Reporting MU

- When reporting the result, avoid excessive numbers
- The NPAAC recommend using 1 sig fig for uncertainty (use 2 during calculations then round up to avoid error)
- For many tests, 1 sf is adequate e.g. MU of 2.1 becomes 2
- The measurement value is rounded to the same number decimal places as its MU
- For example measurement of 26.9 with MU=4, report as 27 ±4
- Took the decision not to routinely report MU with each test result
- MU is documented by lab and available to any user who requests it
- Pro active approach - initiated discussion via MDT

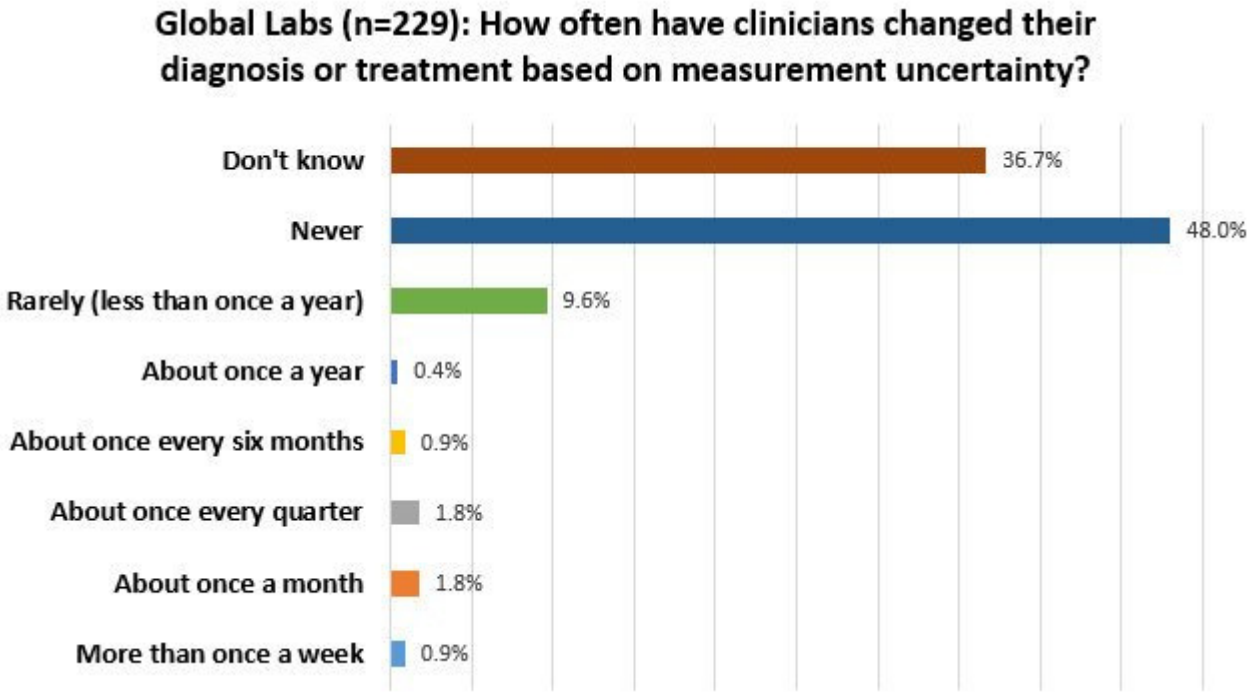
Further Justification?

- Important to document why an assay is clinically acceptable especially when
    - MU is larger than anticipated
    - Assay is not traceable
    - No EQA scheme exists

- Evidence of clinical utility may include
    - In house reference range
    - Patient means
    - Interpretation based on clinical information
    - Interpretation of a profile, not isolated numerical result
    - Report includes interpretative comment

What 90% of labs *DON'T* do with MU

- 2015 Global MU survey by Westgard, 550 responses, 85 countries
- MU is widely calculated but rarely utilised
- 85% of labs that calculate MU don't provide to clinicians unless requested



Plasma Amino Acids

- Used for diagnosis (and monitoring) of a range of disorders
- Diagnosis
  - increases or decreases in one or more amino acids
  - Interpretation based on overall pattern rather than absolute concentration relative to reference range
- Monitoring - Dietary therapy altered on the basis of monitoring result
- All amino acids reports include an interpretative comment
- For some users, no numerical values are reported

Plasma Amino Acids

- MU derived from 2 levels of plasma IQC
- MU is relatively consistent
- Subtle variation between analytes and absolute concentration
- Glutamate has higher MU, expected due to stability
- As concentration increases, MU decreases (marginally)

Plasma Amino Acids

- MU derived from 2 levels of plasma IQC
- MU is relatively consistent
- Subtle variation between analytes and absolute concentration
- Glutamate has higher MU, expected due to stability

- Glycine MU = 165 ± 12 (7%) and 674 ± 41 (6%)
- Citrulline MU = 20 ± 2 (8%) and 334 ± 28 (8%)
- Leucine MU = 128 ± 9 (7%) and 751 ± 41 (5.4%)

- Within desirable specification for imprecision
- Within range predicted by Horwitz (7 – 11%)
- EQA schemes exist (ERNDIM and NEQAS)
- Traceability exists (most AA)

Bloodspot Phenylalanine & Tyrosine

- Used for monitoring PKU and tyrosinaemia patients
- Looking at trends in phenylalanine
- Dietary therapy altered on the basis of monitoring result
- Generally aim for 120 - 360 μmol/L but each child has target range
- If >360 μmol/L, contact family and discuss
  - Has patient been ill?
  - Are they taking the supplement?
- If <120 μmol/L, probable growth spurt and require more protein

Bloodspot Phenylalanine & Tyrosine

- Data derived from 3 levels of bloodspot IQC
- Phe at 81, 304 and 1028 µmol/L
- Tyr at 72, 239 and 834 µmol/L
- MU is consistent for both analytes across analytical range

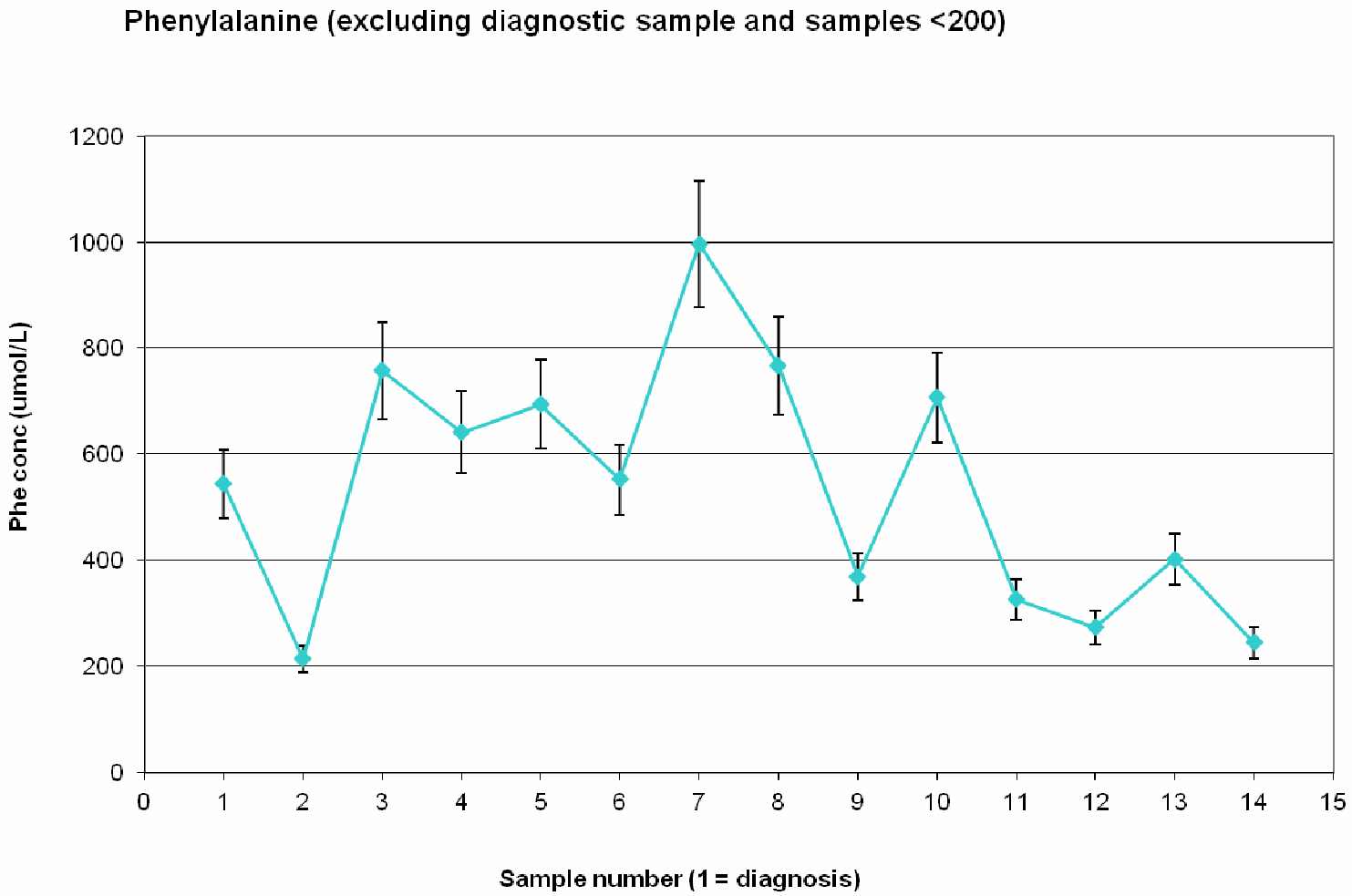
Bloodspot Phenylalanine & Tyrosine

- Data derived from 3 levels of bloodspot IQC
- Phe at 81, 304 and 1028 mmol/L
- Tyr at 72, 239 and 834 mmol/L
- MU is consistent for both analytes across analytical range
- Phe MU = ± 12% and Tyr MU = ± 16%
- Reason for the difference between the two analytes?

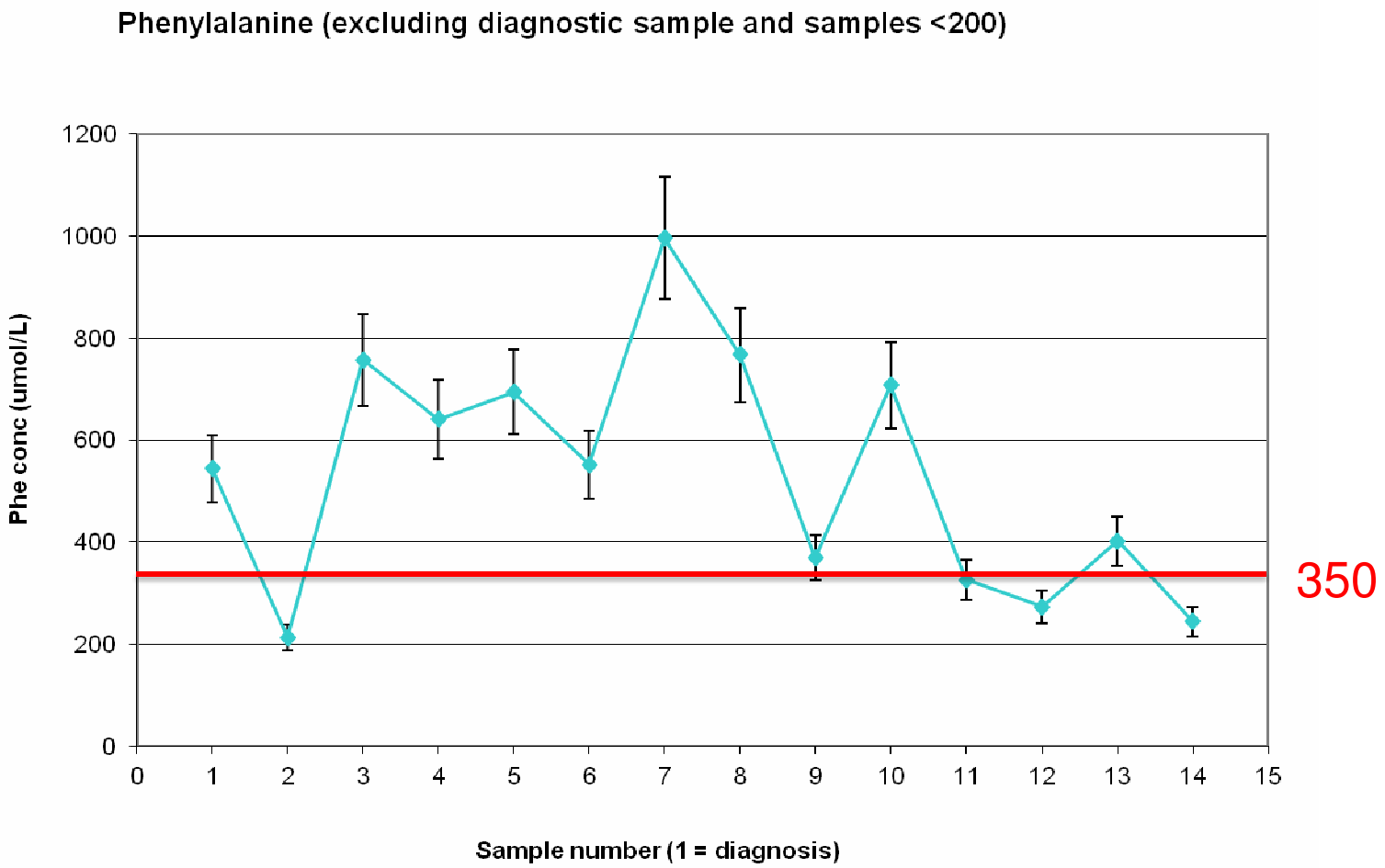
- Phe = 350 ± 42 mmol/L Tyr = 500 ± 80 mmol/L

- No published data on desirable specification for imprecision in blood spots
- Both exceed the range predicted by Horwitz
- EQA scheme exists (NEQAS)
- Assay is traceable (theoretically)

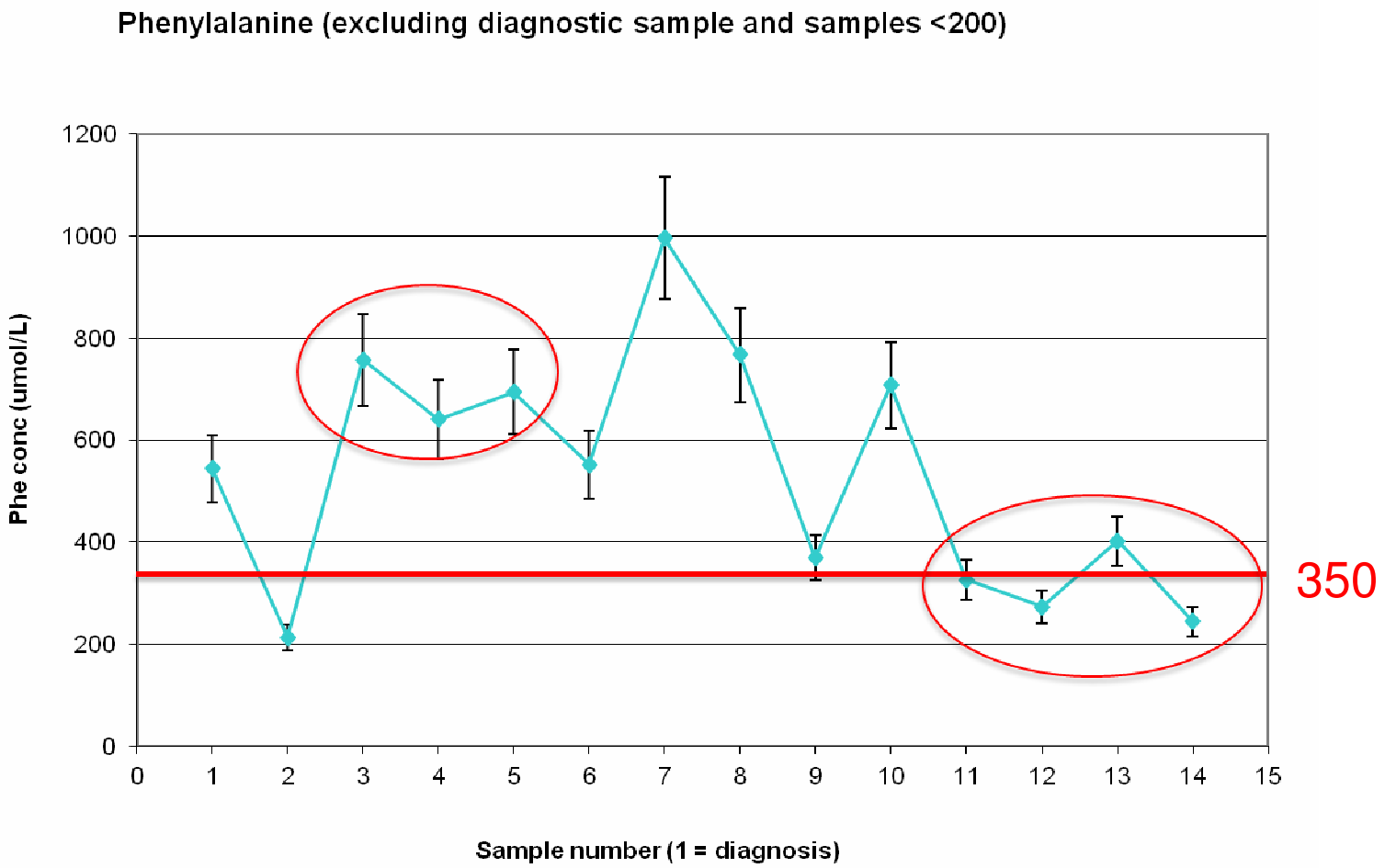
Monitoring trends in Bloodspot Phe



Monitoring trends in Bloodspot Phe



Monitoring trends in Bloodspot Phe



Bloodspot Branched Chains

- Used for diagnosis and monitoring of MSUD
- Looking at trends in concentration with time
- Dietary therapy altered on the basis of monitoring result cf PKU

Bloodspot Branched Chains

- Data derived from 2 levels of bloodspot IQC
- MU is not consistent for analytes across analytical range
- MU increases with increasing concentration
  - internal standard not ideal stable isotope?
  - internal standard concentration?
  - approaching linearity?

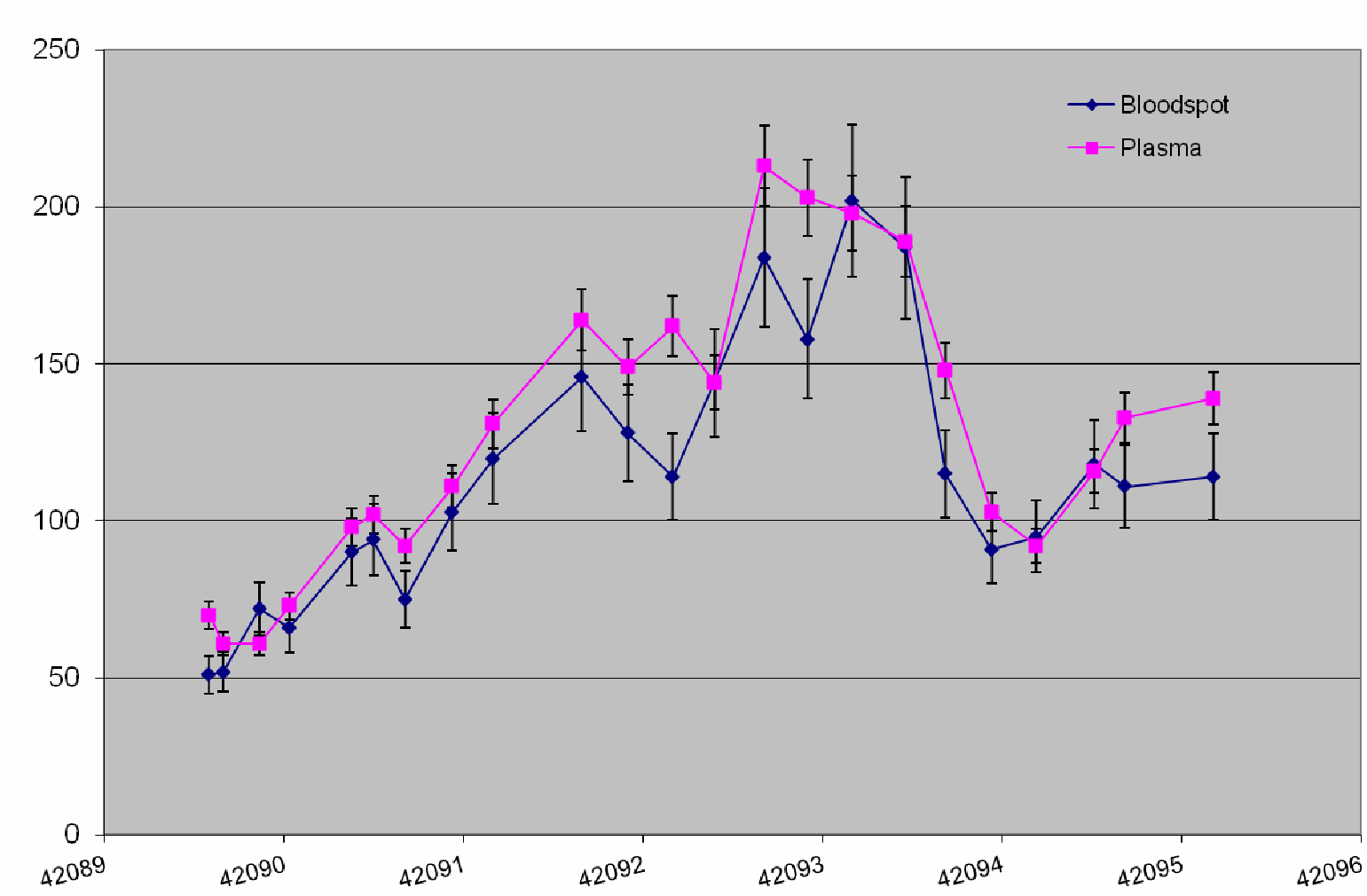
Bloodspot Branched Chains

- Data derived from 2 levels of bloodspot IQC
- MU is not consistent for analytes across analytical range
- MU increases with increasing concentration

- Leu = 171 ± 22 and 1014 ± 122 (± 14% and 12% respectively)
- Iso = 72 ± 10 and 892 ± 116 (± 14% and 12% respectively)
- Val = 270 ± 32 and 1118 ± 246 (± 14% and 22% respectively)
- Allo = 89 ± 12 and 311 ± 54 (± 14% and 18% respectively)

- No published data on desirable specification for imprecision in blood spots
- Both exceed the range predicted by Horwitz
- EQA scheme of limited utility, rely on sample swap
- Assay is traceable (theoretically)

Bloodspot and plasma leucine post Tx



Expanded Newborn Screening

- Screening protocols effectively have MU built in
- Analytical cut off value is 20% below clinical cut-off value
- Any result above analytical cut-off is repeated in duplicate
- Condition suspected result is mean of triplicate is above clinical-cut off

- ?? Evidence base for the protocols
- MU now established and is > 20%
- Common cut-off values in use across England

Calculated MU for Viapath ENBS assays

Analyte	Measurement Uncertainty
Phe	251 ± 46 (18%)
Met	26 ± 5 (19%)
Leu	497 ± 99 (20%)
C5	1.3 ± 0.3 (23%)
C8	0.38 ± 0.1 (26%)
C5DC	0.4 ± 0.1 (25%)

MU derived from long term in-house IQC data from 2 instruments
(n= >100 over 6 month period, MU=2SD)

How do we know if this is acceptable?

Analytical cut off value is 20% below clinical cut off value

No published guidance exists for Target MU

EQA scheme of limited utility due to number of participants

No traceability

Calculated MU for ENBS programme

Analyte	Nominal concentration of IQC umol/L	Mass conc	PRSD (%CV) Predicted between lab variation	HorRat	RSDr Within batch %CV target	MU (2SD)
Leu	409	53 ppm	8.8	***1.30***	5.9	**409 ± 94 (23%)**
Phe	74	26 ppm	9.7	***1.23***	6.5	**158 ± 26 (24%)**
Met	29	12 ppm	10.9	1.00	7.3	**79.5 ± 20 (22%)**
C5	0.19	568 ppb	17.3	0.69	11.6	**2.32 ± 0.56 (24%)**
C8	0.07	163 ppb	20.8	0.43	13.9	**0.57 ± 0.10 (18%)**
C5DC	0.15	132 ppb	21.5	0.69	14.4	**0.46 ± 0.14 (30%)**

Acceptable HorRat is 0.3 – 1.3
Data shown for one level of IQC only

Impact of MU in the lab

- Useful exercise
- Covered all assays – qualitative and quantitative
- Highlights critical steps of assays
- Useful for training - raises awareness of imitations of given assay
- Better use of significant figures when reporting results
- Ensures review of clinical utility of each assay
- Identifies areas for improvement
- Can provide evidence of User Engagement

Impact of MU on Users?

- Useful exercise, raised several questions
- Do we need to review the use of common cut-off values in ENBS?
- Are we over interpreting PKU/BCAA monitoring results?
- Do we need to look at improving bloodspot quality in samples from monitoring patients?
  - Retrospectively audited PKU monitoring samples against NBS guidelines and 50% would have been rejected
  - Patient education has been initiated by Evelina, re-audit in 6 months


Thank You
Acquity
Waters
Xevo TQ-S