Measurement Uncertainty in relation to monitoring of PKU & MSUD

Marjorie Dixon/ Helen Prunty

PKU & MSUD monitoring at GOSH

-Patients post blood spots direct to laboratory

-Samples analysed same day and results given to dieticians

-Blood spot Phe & Tyr analysed by MS/MS

-Blood spot BCAA analysed by UHPLC (UV detection of PITC derivatives)

Analytical Measurement Uncertainty (MU) calculation: the GOSH approach

Calculations are based on iQC data since this should incorporate factors that influence final result which may include:

- Sample preparation
- Calibration of equipment
- Equipment used
- Environmental conditions
- Changes of operator
- Change of reagents, batch no. , kit etc

Data is collected over a sufficient time period to ensure that as many of the above variables are covered and reviewed regularly

(eg. Usually 6mths-1yr – or when necessary due to new equipment etc)

MU calculation: the GOSH approach

The iQC data is used to calculate the imprecision or standard deviation (SD) for the test

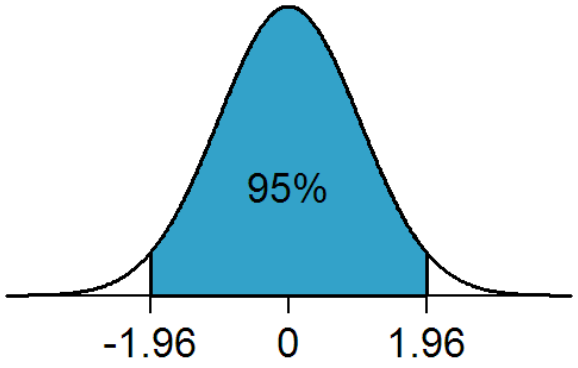
MU = SD x ‘coverage factor’

By using a coverage factor of 1.96 there is a 95% chance that the true result lies within a range covered by

<result value> +/- MU

MU = SD x 1.96

(or as a % MU = CV x 1.96)



Use of MU

1. For a single test result

MU is used to indicate the confidence that the reported value is correct.

2. For serial monitoring

MU can be used to indicate that the difference between 2 results is unlikely to be due to method imprecision alone and is due to normal biological variation or a change in the patient's physiological or pathological condition

Analytical MU- considerations

Analytical MU may be concentration dependent

- it is useful to base iQC values around clinical decision levels

Calculation doesn't account for pre- analytical variation

Blood spot AA results may be affected by variables including

- Quality of blood spot
- Timing of sample
- Sample storage/ transit conditions

Try to identify and minimise these as much as possible

Analytical MU- considerations

MU calculation does not take bias into account

- May need to consider assay bias if using target ranges / decision levels which are not derived from in-house data and are based on different methodologies

Identification of bias

-Blood spot assays are not traceable to international reference standards

-EQA data is available for Phe & Tyr but currently not for BCAA

- Sample swap scheme with Viapath for BCAA

Serial monitoring of patients

Normal within subject biological variation also needs to be considered when deciding if 2 results are significantly different from one another

Biological variation data is available on Westgard website

http://www.westgard.com/biodatabase1.htm

Limitations of biological variation data:

- Only based on one reference paper
- Zoraida Corte and Rafael Venta. Biological variation of free plasma amino acids in healthy individuals. Clin Chem Lab Med 2010;48(1):99–104
- Plasma AA – not blood spot
- Healthy adult population
- Probably not applicable to IMD patients on restricted diets

‘Fitness for purpose’ of an assay

As a general principle:

Acceptable assay performance = $CV_A < 75\% CV_B$

Desirable assay performance = $CV_A < 50\% CV_B$

Optimal assay performance = $CV_A < 25\% CV_B$

Where CV_A = analytical CV and CV_B = within subject biological variation

But:

With the limitations of the available biological variation data how do we prove that our assays are fit for purpose?

Needs to be defined in terms of clinical usage by liaison with clinical teams

Getting the right balance

eed of results

Samples analysed in singlicate

Same day analysis

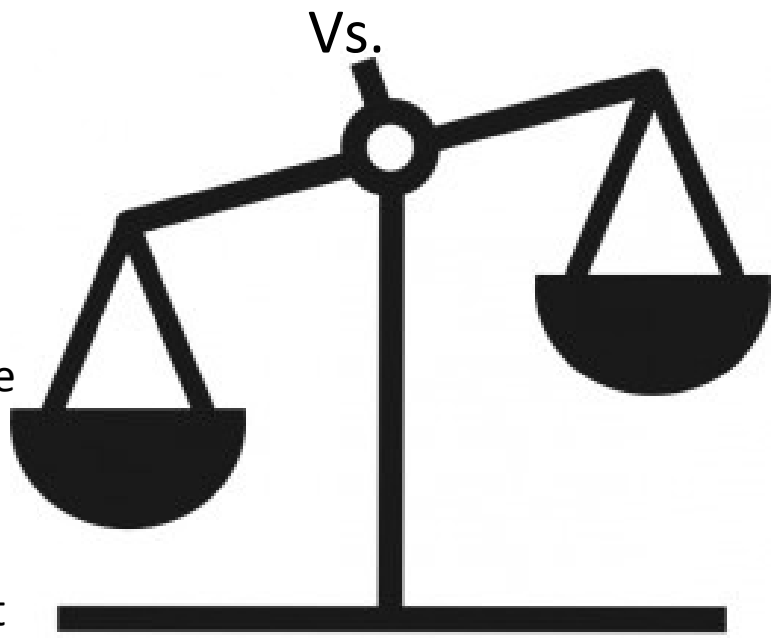
equency of results

Blood spots do not require venepuncture

Able to send more frequently

tient compliance

Blood spots easier and more convenient



Analytical precision

Samples analysed in duplicate/triplicate increases precision but increases time

Plasma AA less variable but samples more difficult to obta

Overall, what is most beneficial to the patient?

Measurement Uncertainty (MU) of Blood Spot BCAA

Analyte	Value at which MU calculated (umol/L)	Analytical CV (%)	MU (SD x 1.96) (umol/L)	MU (CV x 1.96) (%)	Biological CV For PLASMA (%)
Leu	181	7.1	25	14.0	14.8
	367	6.3	51	12.3	
Ileu	120	6.7	16	13.2	15.5
	314	6.1	43	11.9	
Val	261	6.4	35	12.6	10.6
	419	6.0	58	11.7	
Aileu	22	9.0	5	17.6	Not available
	73	6.1	11	11.9	

e.g. for a leucine value of 367:
there is a 95% chance that the true result lies within a range covered by 367 ± 51 (or ± 12.3%)

MU of Blood Spot Phe/Tyr

Analyte	Value at which MU calculated (umol/L)	Analytical CV (%)	MU (SD x 1.96) (umol/L)	MU (CV x1.96) (%)	Biological CV For PLASMA (%)
Phe	38.6	4.6	5	9.1	10.5
	174	4.5	19	8.9	
	700	5.8	114	11.3	
Tyr	31.1	6.3	5	12.4	9.5
	162.7	5.2	20	10.3	
	655.9	5.9	100	11.6	

e.g. for a phenylalanine value of 174:
there is a 95% chance that the true result lies within a range covered by 174 ± 19 (or ± 8.9%)

Calculation of ‘critical difference’

Changes in results can be due to pre-analytical, analytical and biological variation

’Critical difference’ is a mathematical way to determine if a change in concentration between 2 results is significantly different

$$\textbf{Change} > \textbf{Critical Difference} = K \times \sqrt{(CVa^2 + CVb^2)}$$

Where: K = a factor dependent on the probability level selected

(For $p<0.05$ the value of K is 2.77.)

CVa is the coefficient of analytical variation

CVb is the coefficient of within subject variation

However, this calculation does not take pre-analytical variation into account

Calculation of ‘critical difference’

Example calculation for leucine at value around 367

(if biological variation (CVb) is assumed to be the same as for PAA)

Change > Critical Difference $= K \times \sqrt{(CVa^2 + CVb^2)}$

$= 2.77 \times \sqrt{(6.3^2 + 14.8^2)}$

$= 45\%$

ie. a change of > ± 165 in the leucine value would have to occur before the results could be said to be ‘critically’ or ‘significantly’ different

The real life situation

- Not just acting on numbers
- Interpretation is based on expertise and experience
- Results are interpreted in context
- Monitoring is frequent allowing for continual adjustment
-Needs to be some leeway around target values to account for both analytical MU and pre-analytical variation
-Biological variation data is probably not applicable to patients on special diets