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Biochemical Genetics Laboratory
Viapath @Guys' Hospital

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Measurement uncertainty

Produced document stating our approach to measurement uncertainty in Biochemical Genetics Lab:

- The patient pathway: measurement uncertainty and existing control measures at each step.
- Different approaches for qualitative and quantitative assays.
  - Calculated MU for GAGs and some plasma enzymes as examples of how to approach MU.
  - Qualitative assays: fishbone diagram identifying causes of uncertainty.
- Included statements commenting on inability to fulfil usual criteria due to nature and frequency of assays performed in BG.
- Majority of tests are specialised assays with no known biological CV or target analytical CV: therefore utility of usual approach is limited.

Quantitative Assays

- There are several methods by which MU can be determined. The main approach which could be used for some tests in Biochemical Genetics would be to calculate MU using quantity values obtained by the measurement of quality control materials under intermediate precision conditions (see figure 1).

- This means that IQC material can be used to calculate MU retrospectively and is based on the assumption that the enzyme activity behaves in the same way in quality control material as in patient samples.

- These measurements should be made under intermediate precision conditions and include as many routine changes as reasonably possible in the standard measurement procedure e.g. substrate lots, operator, reagent lot, pipettes, maintenance visits.
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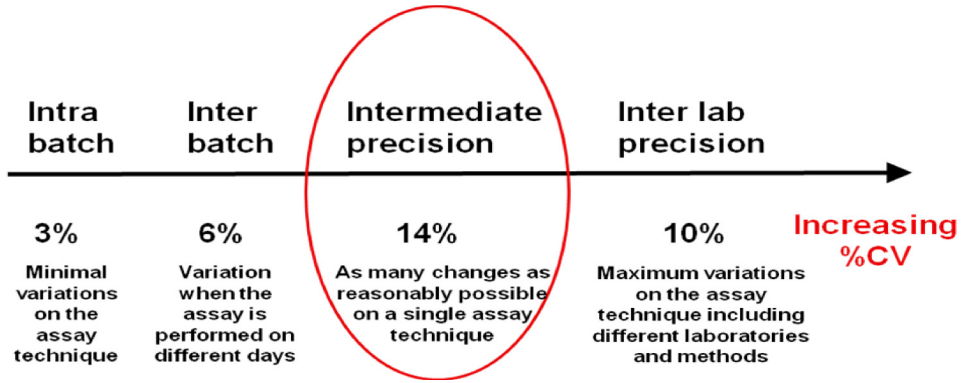
Figure 1. Intermediate precision



Calculation of Measurement Uncertainty

'For quantitative assays, MU should ideally be calculated from a minimum of 100 measurements of QC material performed over a minimum period of 6 months.'

- **Due to the nature and frequency of the assays performed in this department, it will not be possible to fulfil the above criteria.**

  More appropriate to calculate MU with fewer measurements of QC material over a longer period of time e.g. annually (dependent on the assay). This approach would take more random and systematic variations into account over a longer time period to provide a more accurate MU.

- **However, for some assays this may not be possible due to infrequent analysis.**

  These assays are usually run with simultaneous normal and affected controls; results are reported and interpreted in relation to the simultaneous controls.

  Uncertainty, variation and imprecision are therefore considered to be of less significance in these instances.

Some examples :

Prenatal testing on chorionic villus and cultured cells, all other assays in cultured cells, assays where it is known there is considerable substrate batch variation e.g. iduronate sulphatase (for diagnosis of MPS type II).

Results reported with simultaneous controls

All samples run at same time with same reagents under same conditions, and reported with interpretative comment.

Uncertainty, variation and imprecision less significant?

Example report:

Galactose-6-sulphate sulphatase and β-galactosidase levels assayed in leucocytes.

Results (nmol/hr/mg protein)

		Galactose-6-sulphatase	β-galactosidase
Patient		57	771
Normal controls	1	34	521
	2	31	689
Cultured fibroblasts			
Affected MPS IVA		undetectable	2502
Normal control		28	3271

NB: All samples assayed at the same time.

Comment: These normal enzyme levels would make a diagnosis of mucopolysaccharidosis IVA and IVB (Morquio A and B) and GM1 gangliosidosis highly unlikely in this patient.

NB: These results do not exclude the very rare forms of these disorders due to an activator protein deficiency.

Measurement Uncertainty: Plasma lysosomal enzyme assays

Enzyme assays in leucocytes / cells can be complicated and include several processes: for example: specimen prep, protein assay, enzyme assay (also, some assays have more than one step). Therefore started with enzymes we assay in plasma.

The MU for these enzymes has been calculated over a period of approximately 1 year from 29 assays. We can expect to take into account the variations expected in substrate lots, operator variability, reagent lot, pipettes, maintenance visits etc.

Data is derived from IQC samples taken from the same donor at different times, which also takes into account some degree of biological variation.

Example of some MU values in plasma enzymes in our lab:

IQC Expanded uncertainty (95% CI)	α-fuc	α-man	Hex A
Mean (n = 29)	**242**	22	108
1 SD (standard uncertainty)	**23**	4	15.5
*Expanded Uncertainty	**46**	8	31
*Relative Expanded Uncertainty	**19%**	36%	29%
*Expanded uncertainty calculated using a coverage factor k=2 to give confidence level of approx 95%			

So, for **α-fucosidase the MU (95% CI) for an observed activity of 386 nmol/hr/ml would be 386 ± 73**

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Glycosaminoglycans

MU determined using ERNDIM proficiency data

As with the enzyme assays calculation of MU is complicated. MU of the creatinine assay must be taken into account as well as GAG assay.

Found to have a positive bias of 16%.

Also, the lower the creatinine levels the less reliable the result – as we are already aware of and always state on our reports!

Clinical Significance of measurement uncertainty result:

The positive bias may result in a higher number of samples being tested for qualitative GAGs than would be the case with an assay of no statistical bias compared to the proficiency mean. However, this positive bias reduces the likelihood of missing the detection of a mucopolysaccharidosis and an elevated urine GAG result in isolation should not lead to an unnecessary clinical referral.

In our laboratory qualitative (2-dimensional electrophoresis) is performed on all urine samples.

Target Measurement Uncertainty

Once MU has been determined it should be compared with the target value. The target value is a measure of the fitness for intended use of the measurement result; this target is derived from external requirements not from the performance specification of the measuring system. Typically the analytical imprecision should be less than half that of the biological variation'.

In the Biochemical Genetics Laboratory the majority of tests are specialised enzyme assays with no known biological CV or target analytical CV, therefore the utility of the above approach is limited.

- In the majority of cases, we are looking for a specific enzyme **deficiency** (in some cases an elevation – I-cell/ chito).
- Reference ranges are well established and re-assessed regularly.
- A second enzyme, in addition to the one required for testing, is assayed as a control to confirm specimen adequacy (to check for sample deterioration).
- All these assays will also be reported with an interpretative comment

Where appropriate, the decision as to what the target MU should be will therefore be based on local clinical and scientific knowledge and clinical judgement regarding the impact/risk of inaccurate results around clinical decision limits, cut off values and reference intervals..

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Example report: Reference ranges and interpretative comment provided.

Does quoting MU for any of these enzymes provide any extra info for the clinician in this case ?

Probably not.

Biochemical results Lysosomal enzyme activities (nmol/h/ml plasma or serum or /mg white cell protein)

Plasma/Serum:	Patient	Normal Ranges	
I-cell screen (qualitative)	Normal		
Hexosaminidase	1214	438 -- 2047	
Hexosaminidase A	149	100 -- 275	
ß-glucuronidase	99	30 -- 534	
ß-mannosidase	207	61 -- 608	
α-N-acetylgalactosaminidase (nmol/7h/ml)	71	25 -- 123	
α-fucosidase	**367**	**128 – 1077**	**MU = 367 ± 70**
α-mannosidase	60	20.4 – 132	
Aspartylglucosaminidase	70	39 -- 250	
Chitotriosidase	**207***	0 – 170	
White cells:			
Arylsulphatase A	**0.6***	1.8 -- 24.0	
Galactocerebrosidase	4.15	0.3 -- 5.0	
ß-galactosidase	219	78 -- 280	

* **Arylsulphatase A activity is very low**; plasma chitotriosidase activity (a marker of macrophage activity) is slightly elevated. Reduced arylsulphatase A activity is seen in patients with metachromatic leucodystrophy (MLD) but can also be observed in individuals with pseudodeficiency (reduced enzyme activity in vitro but no clinical consequence); DNA testing for pseudodeficiency of arylsulphatase A (PDASA) has therefore been carried out (see page 2).

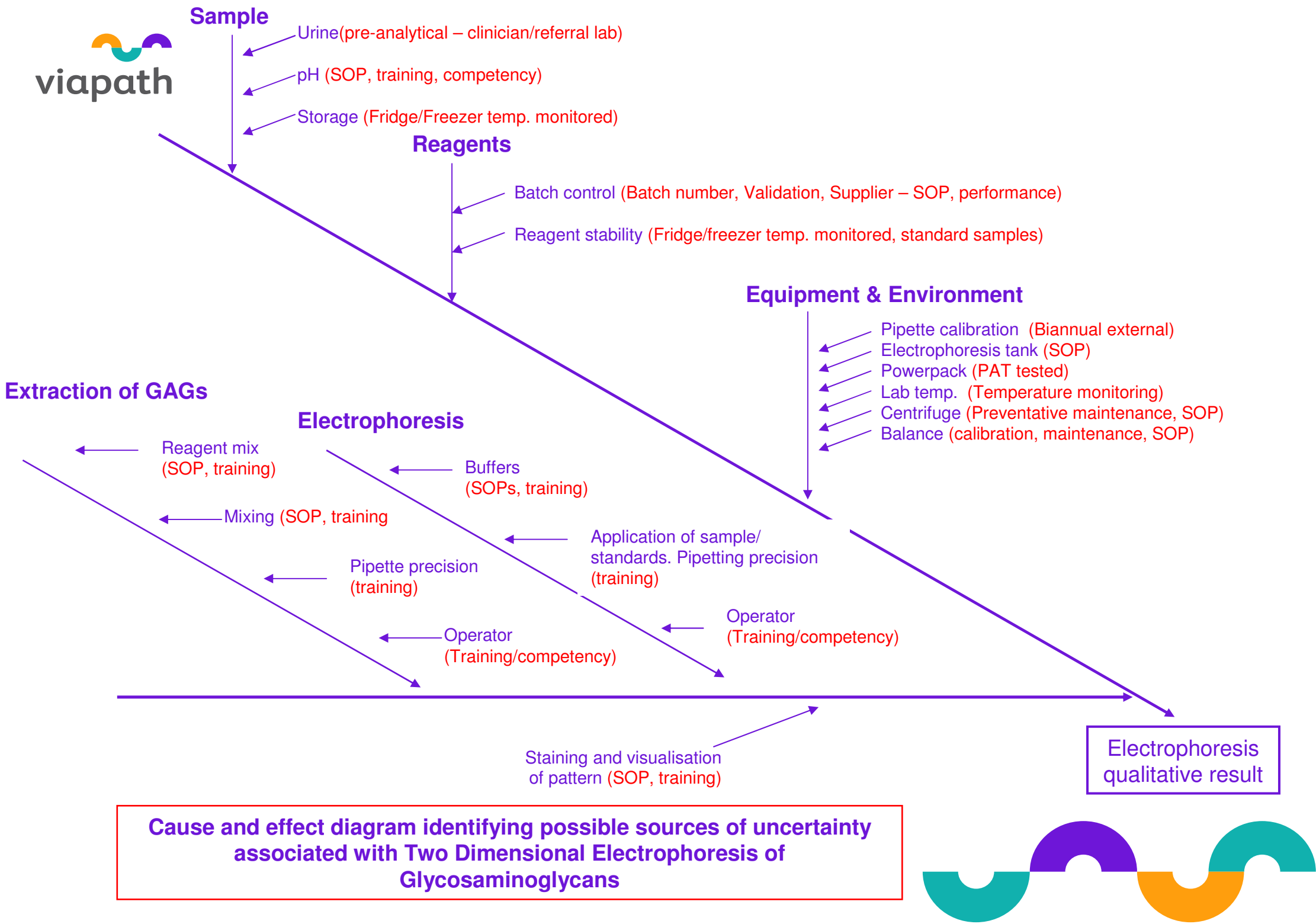
Qualitative Assays

- There is no precision associated with presence/absence or qualitative methods, hence no statistical estimate of uncertainty can be calculated. Nevertheless, sources of uncertainty should still be considered and where possible all sources of variability controlled.

- For qualitative assays each stage of the analytical process will be considered in detail and the critical steps in the process identified and documented (e.g. timing, temperature, reagent volume, weight, method of detection).

- The control measures in place for each critical step will be summarised. A key aspect of this will be ensuring the traceability of important control parameters such as calibrated balance, pipette, thermometer, spectrophotometer etc.

- Biochemical Genetics chose to document this information in the format of **fishbone diagrams**.



Cause and effect diagram identifying possible sources of uncertainty associated with Two Dimensional Electrophoresis of Glycosaminoglycans

Reporting Measurement Uncertainty

- MU allows users of the laboratory to assess the reliability of a test result and may be particularly useful when two results from the same patient are being compared (e.g. if monitoring treatment by ERT etc.).

- However, the Biochemical Genetics Lab has made the decision that MU will not routinely be reported with all enzymology test results.

- Due to the specialist nature of testing in the Biochemical Genetics Laboratory, all results are reported with interpretative comments. Advice is given regarding further or repeat testing (due to abnormal results or sample deterioration). Individual assay SOPs detail these conditions.

- *Any information will be readily available to all users should they request it and will be used by the laboratory to aid interpretation of test results.*

The team !

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