Where are we now?

A report from the MetBioNet Amino Acid questionnaire

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Overview

- Aim of questionnaire was to look at current practice amongst labs analysing amino acids
- What methods and protocols are used and what do we get out of it all
- Questionnaire sent out to all labs in MetBioNet and those who were registered for UKNEQAS amino acid scheme when it last ran

Overview

- Received 37 replies; 12 from labs using qualitative screening techniques only, 21 from labs with quantitative methods available (4 informed us that they no longer did any amino acid analysis)
- Analysed data from the two groups separately

Qualitative Screening Only Group

Overview:

- Majority screening both urine and plasma, 25% only urine
- All use 1D or 2D tlc;

  For plasma, 56% use 2D tlc, 44 % use 1D

  For urine, 67% use 2D tlc, 33% 1D
- Many using marker solutions, donated plasma from normal individuals, and abnormal samples run blind as QC/QA
- Workloads range between 10-100 samples per year for plasma and 40-139 samples per year for urine
- 60% of labs use clinical scientists to interpret and report results, 20% use BMS staff, 20% use combination of both.
- Number of diagnoses is very low

Qualitative Only Group cont..
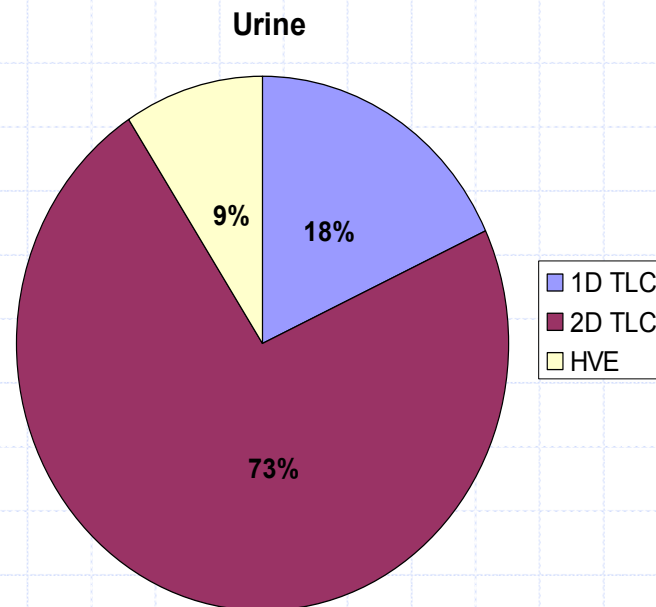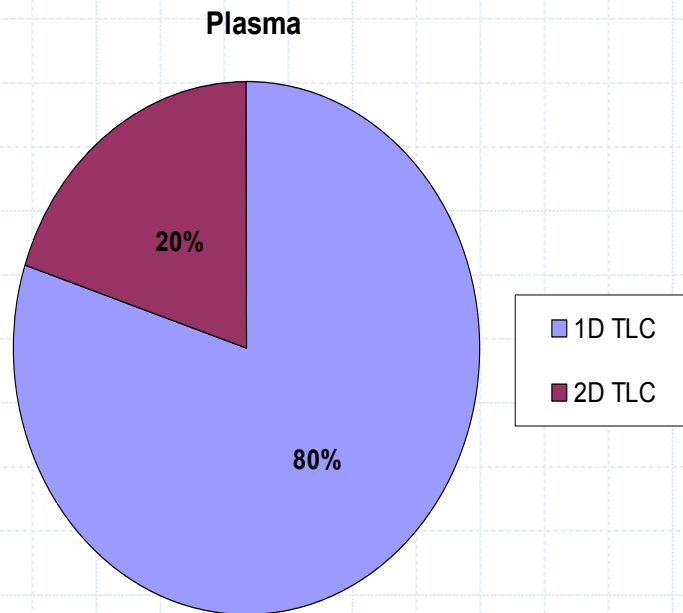
◆ Issues/Conclusions

-Great need for an EQA scheme for these labs

-Some labs have very small workloads, can they maintain the necessary expertise?
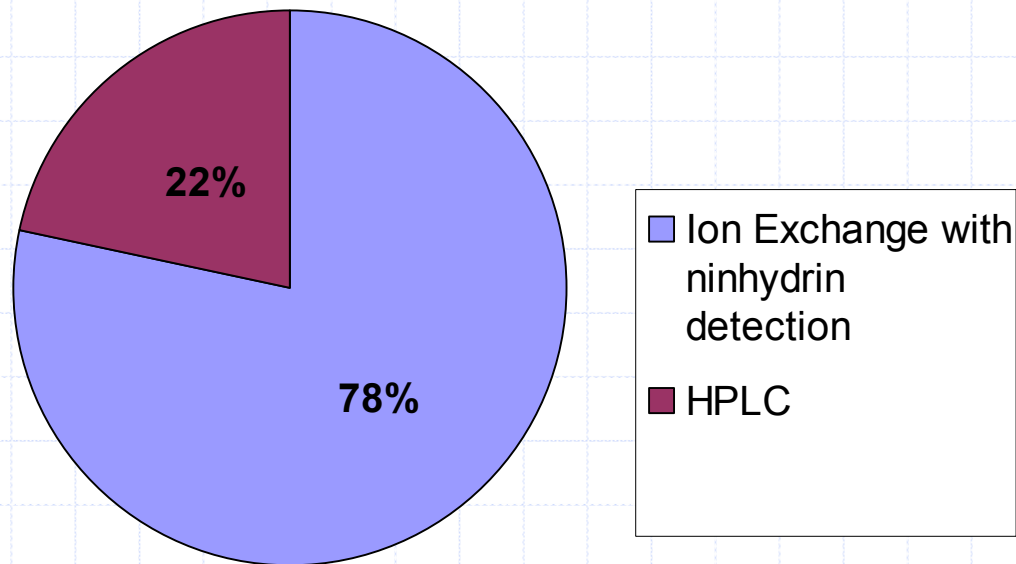
Quantitative Laboratories

◆ Question 1a: Do you pre-screen samples by a qualitative method?

Pie chart:
- Plasma & Urine: 19%
- Urine Only: 24%
- Plasma Only: 5%
- No Pre-screening: 52%
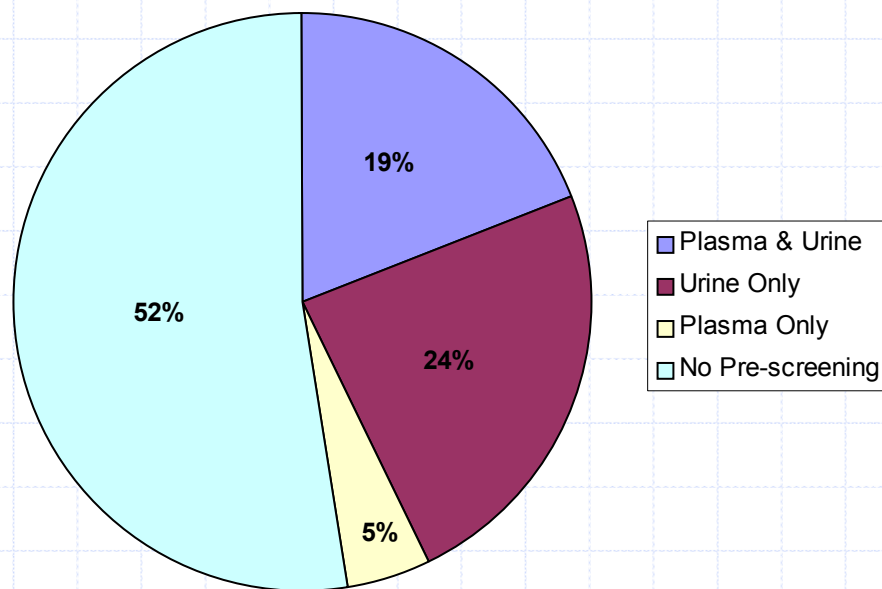
Question 1b: If applicable, what analytical technique is used for screening?

Plasma

20%

80%

- 1D TLC
- 2D TLC

Urine

9%

18%

73%

- 1D TLC
- 2D TLC
- HVE

Question 2: Please indicate your main method of quantitation


- Ion Exchange with ninhydrin detection
- HPLC

22%

78%

◈ Those who specified a make of ion exchange analyser are using Biochrom20/30 or Jeol AminoTac

◈ HPLC users who specified are using RP-HPLC, with PITC derivatisation and UV detection.

Question 3: Do you use an internal standard to calculate results?

◆ 95% Yes.

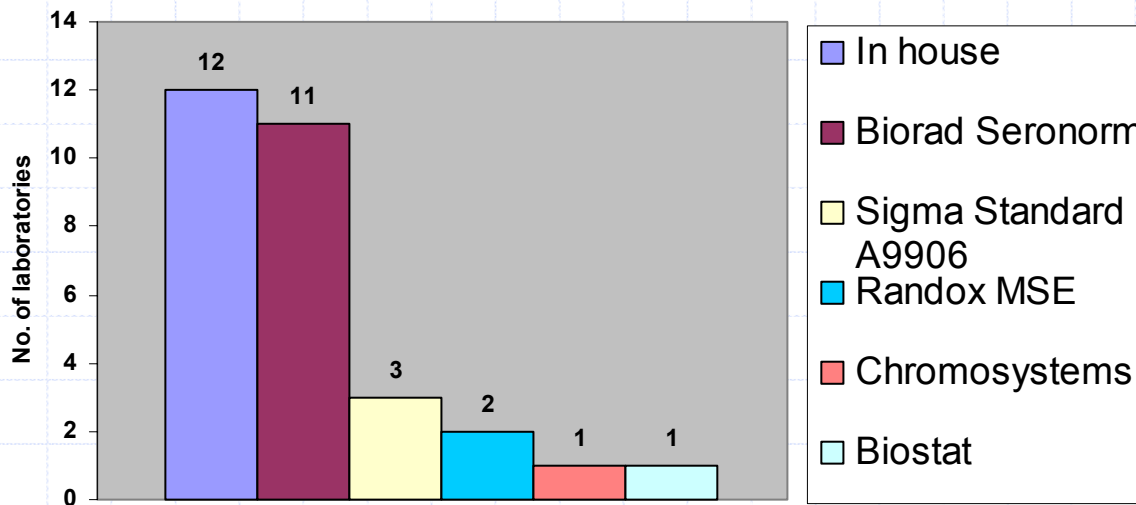The other 5% (one lab) intends to with new analyser

Internal Standards Used
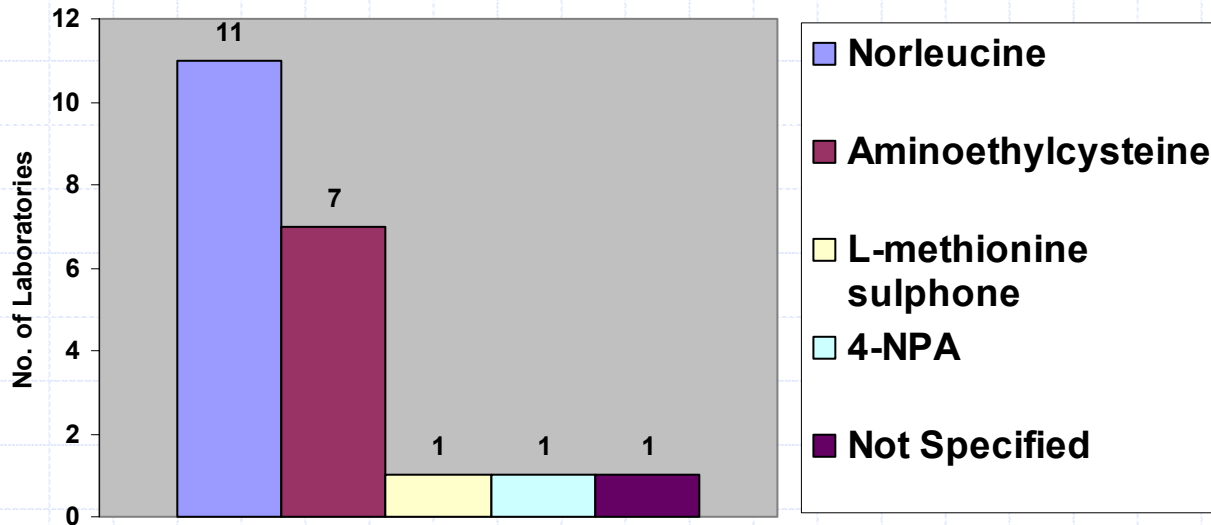
Question 4: Do you use an internal quality control?

- Yes = 85%, No = 10%, No response = 5%
- Most labs using some form of in-house preparation or commercial control spiked with amino acids

Quality Control Used

Legend
In house
Biorad Seronorm
Sigma Standard A9906
Randox MSE
Chromosystems
Biostat

Values (No. of laboratories): In house = 12, Biorad Seronorm = 11, Sigma Standard A9906 = 3, Randox MSE = 2, Chromosystems = 1, Biostat = 1

Question 5: Do you participate in an external quality assessment scheme?

- 19/21 laboratories take part in the ERNDIM scheme
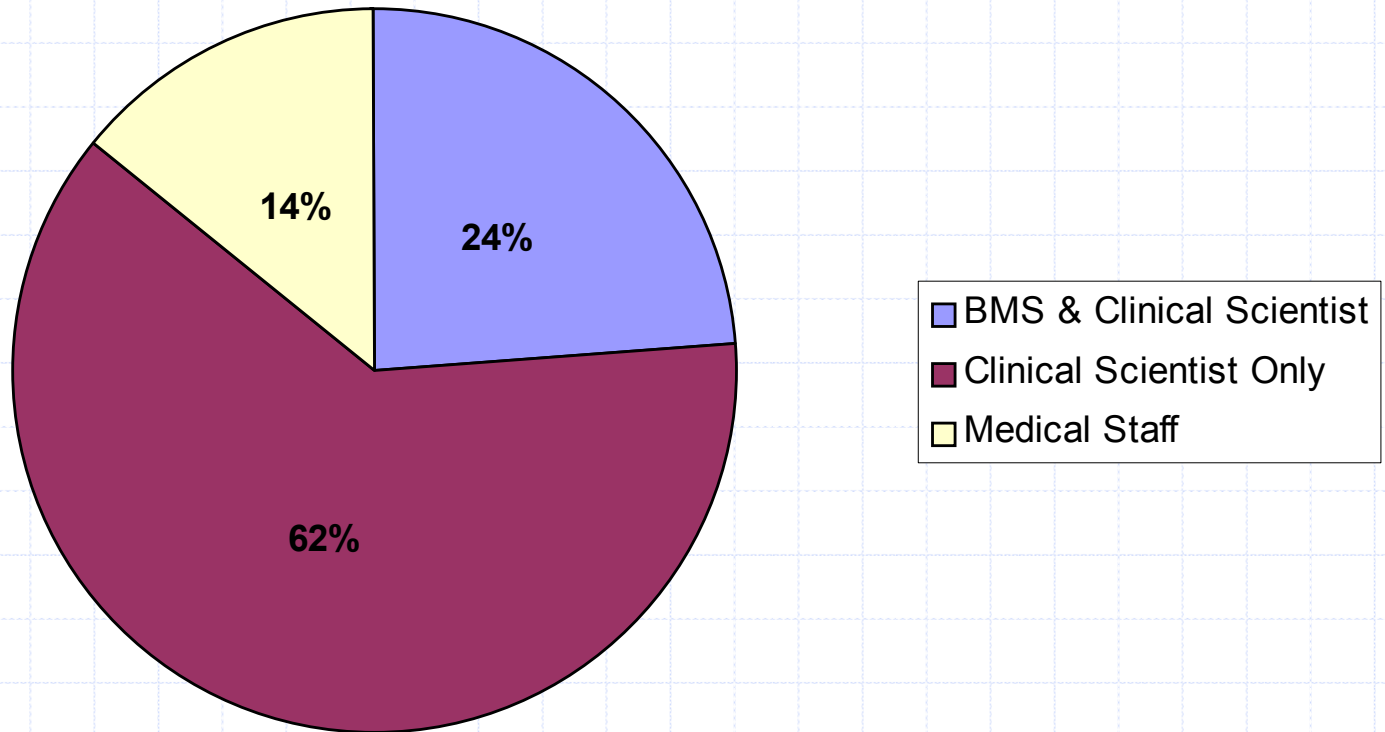- 7/21 mentioned the UKNEQAS scheme (currently defunct)

Question 6: Do you always request paired urine and plasma samples, or will you analyse single requests?

◆ All laboratories will analyse single requests

◆ 2 laboratories stated that they ask for paired samples

Question 7: Which grade of staff identifies and quantitates peaks?

Legend:
- BMS & Clinical Scientist
- Clinical Scientist Only
- BMS Only
- MTO

Values: 26%, 9%, 56%, 9%
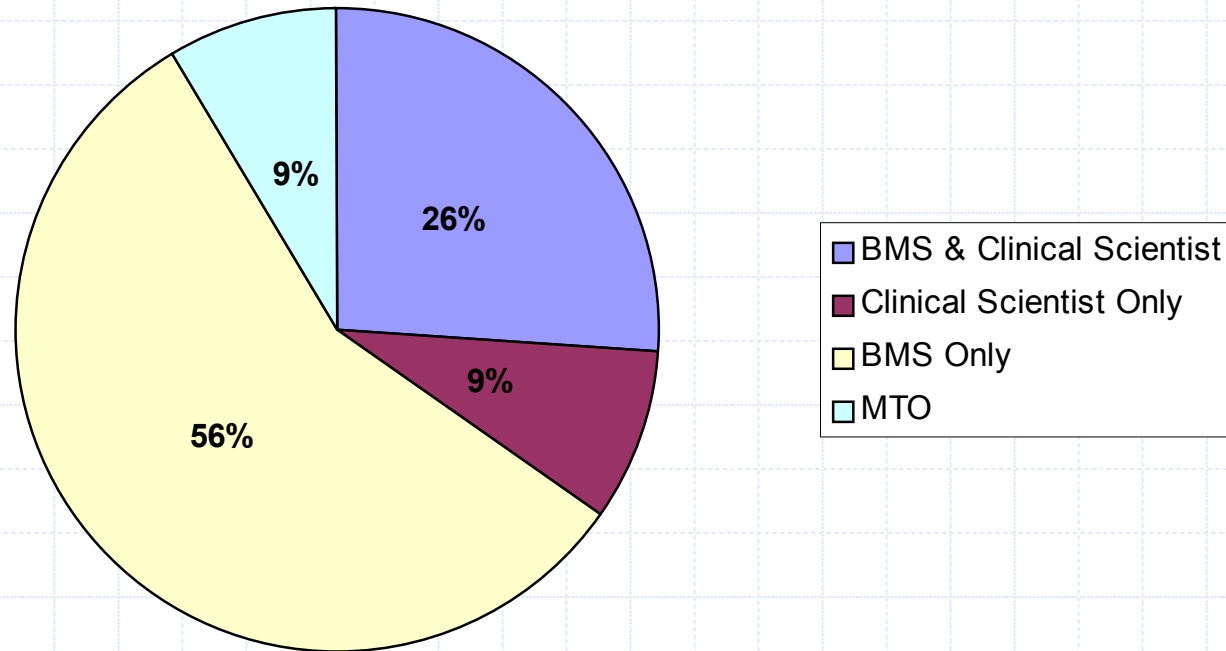
Question 8: Which grade of staff interprets and reports results?

24%

14%

62%

- BMS & Clinical Scientist
- Clinical Scientist Only
- Medical Staff

Question 9a: Do you use age-related reference ranges?
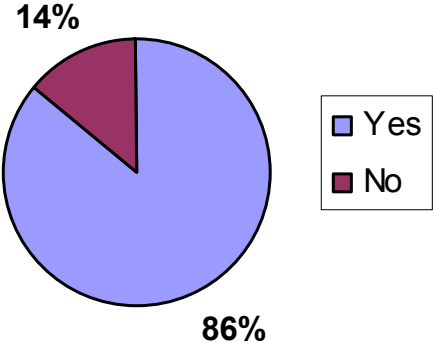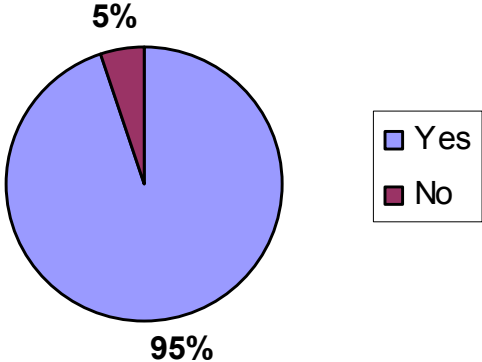
Plasma

14%

86%

- Yes
- No

Urine

5%

95%

- Yes
- No

Question 9b: What is the source of these reference ranges?

Plasma

- 24% — Derived from local data
- 43% — Literature Based
- 33% — A Mixture

Question 9b: What is the source of these reference ranges?

Urine

- 29% — Derived from local data
- 29% — A Mixture
- 42% — Literature Based

Legend:
- Derived from local data
- Literature Based
- A Mixture
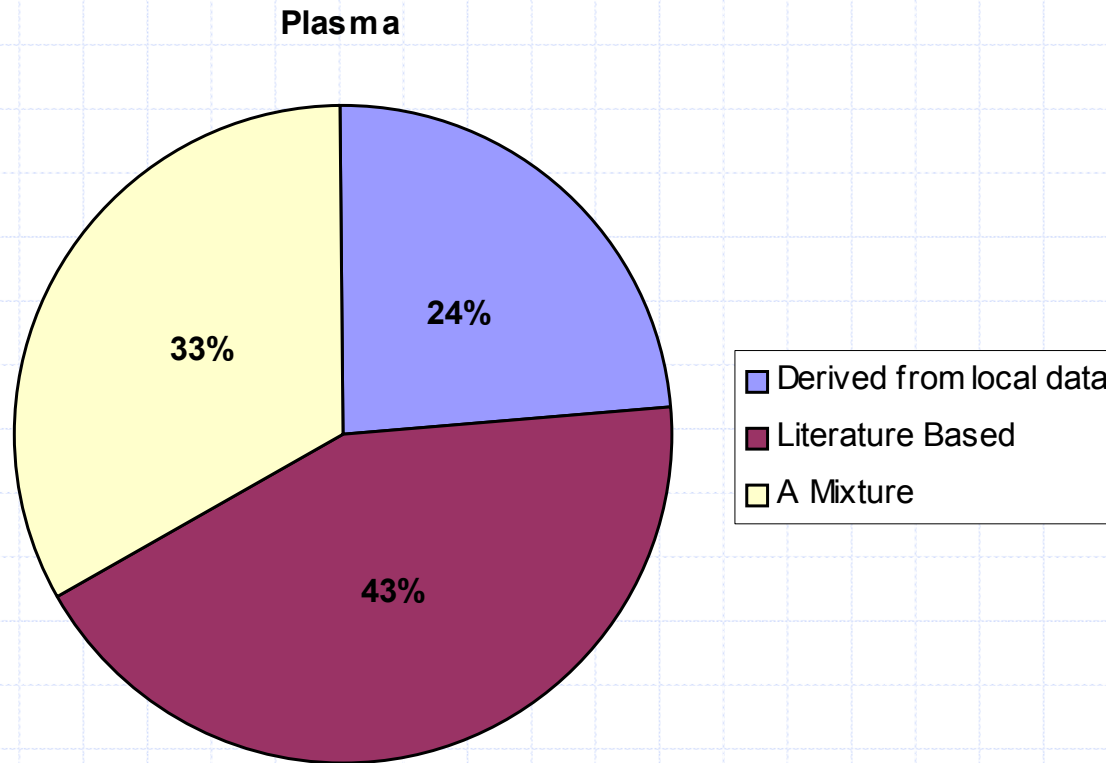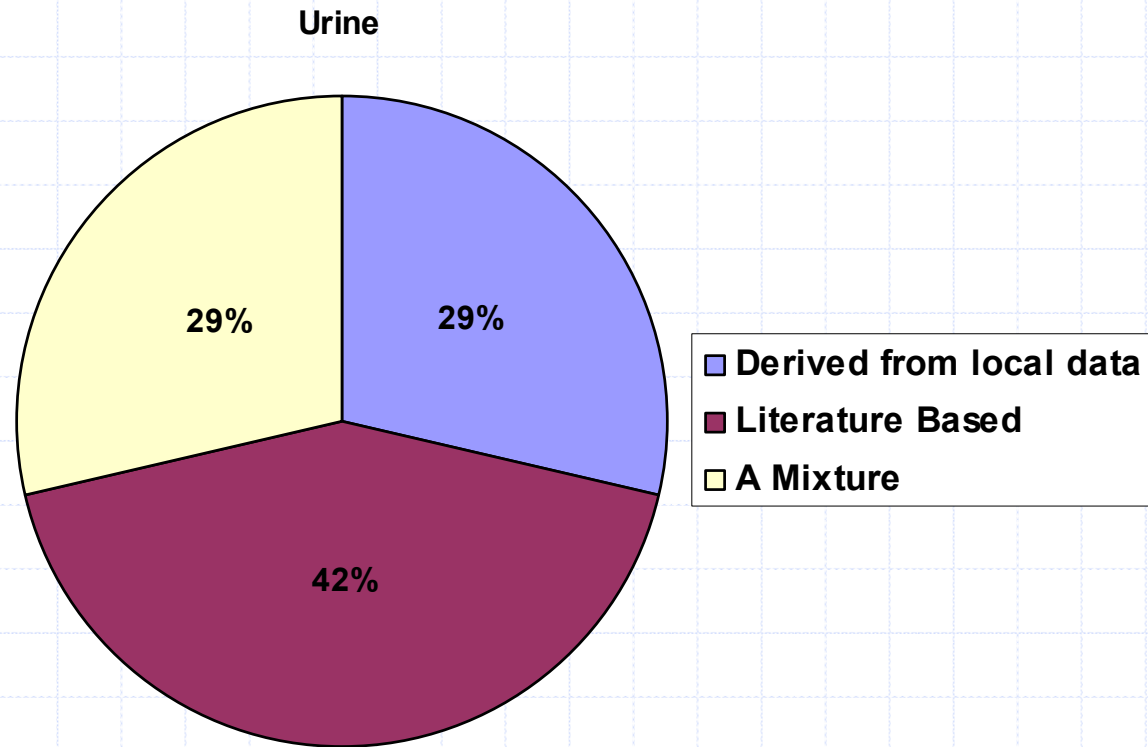
Reference Range Comparison

- 16/21 labs provided their reference ranges for comparison
- The ranges are very variable and come from a variety of literature sources and local data
- Very difficult to do any meaningful analysis of the reference ranges as there is such a difference in the way age groups are subdivided
- Examples of urine reference ranges

  e.g. 1; 0-1m, 1-6m, 6-12m, 1-2y, 2-4y, 4-7y, 7-10y, 10-13y, >13y

  e.g. 2; 0-3m, 3m-2y, 2-14y, >14y

- The "adult" grouping starts anywhere between 13 and 21 years
- Urine ranges are generally subdivided in to more age groups than plasma
- 2/16 labs distinguish between fasting and non-fasting plasma samples

Comparison of Adult Reference Ranges: Urine (µmol/mmol creatinine)

Amino Acid	Mean of lower limit	Range of lower limit	Mean of upper limit	Range of upper limit
Alanine	15	0-35	90	41-110
Arginine	1	0-1.2	8	3-30
Citrulline	0	0	5	2-10
Glutamate	0	0	37	12-300
Glutamine	21	0-45	109	68-300
Glycine	45	0-68	321	173-1050
Histidine	27	0-47	193	146-328
Isoleucine	1	0-0.9	5	4-10
Leucine	2	0-4	12	9-20
Lysine	7	0-10	65	17-190
Methionine	3	0-6	16	10-29
Ornithine	4	0-4	10	5-30
Phenylalanine	2	0-7	19	10-28
Serine	19	0-21	87	50-390
Tyrosine	3	0-10	28	20-52
Valine	3	0-4	16	10-30

Comparison of Adult Reference Ranges: Plasma (µmol/L)

Amino Acid	Mean of lower limit	Range of lower limit	Mean of upper limit	Range of upper limit
Alanine	183	112-243	568	419-778
Arginine	35	12-63	135	89-198
Citrulline	13	8-20	53	33-86
Glutamate	14	2-46	139	41-428
Glutamine	424	100-723	823	645-1079
Glycine	122	81-185	401	236-553
Histidine	41	10-81	133	89-220
Isoleucine	32	6-53	104	79-159
Leucine	69	30-101	197	159-264
Lysine	100	40-165	264	198-378
Methionine	11	5-20	45	25-80
Ornithine	34	20-117	141	77-279
Phenylalanine	35	20-65	101	61-160
Serine	74	60-106	206	114-290
Tyrosine	32	19-57	99	72-120
Valine	121	90-161	319	252-566

Reference range comparison cont…

- Presume the wide range in reference ranges for glutamate and glutamine is due to whether allowance is made for conversion of glutamine to glutamate or not
- Clearly there are major difference in the reference ranges used by different labs but do they have any affect on interpretation?

Question 10: Can you give approximate workload data?

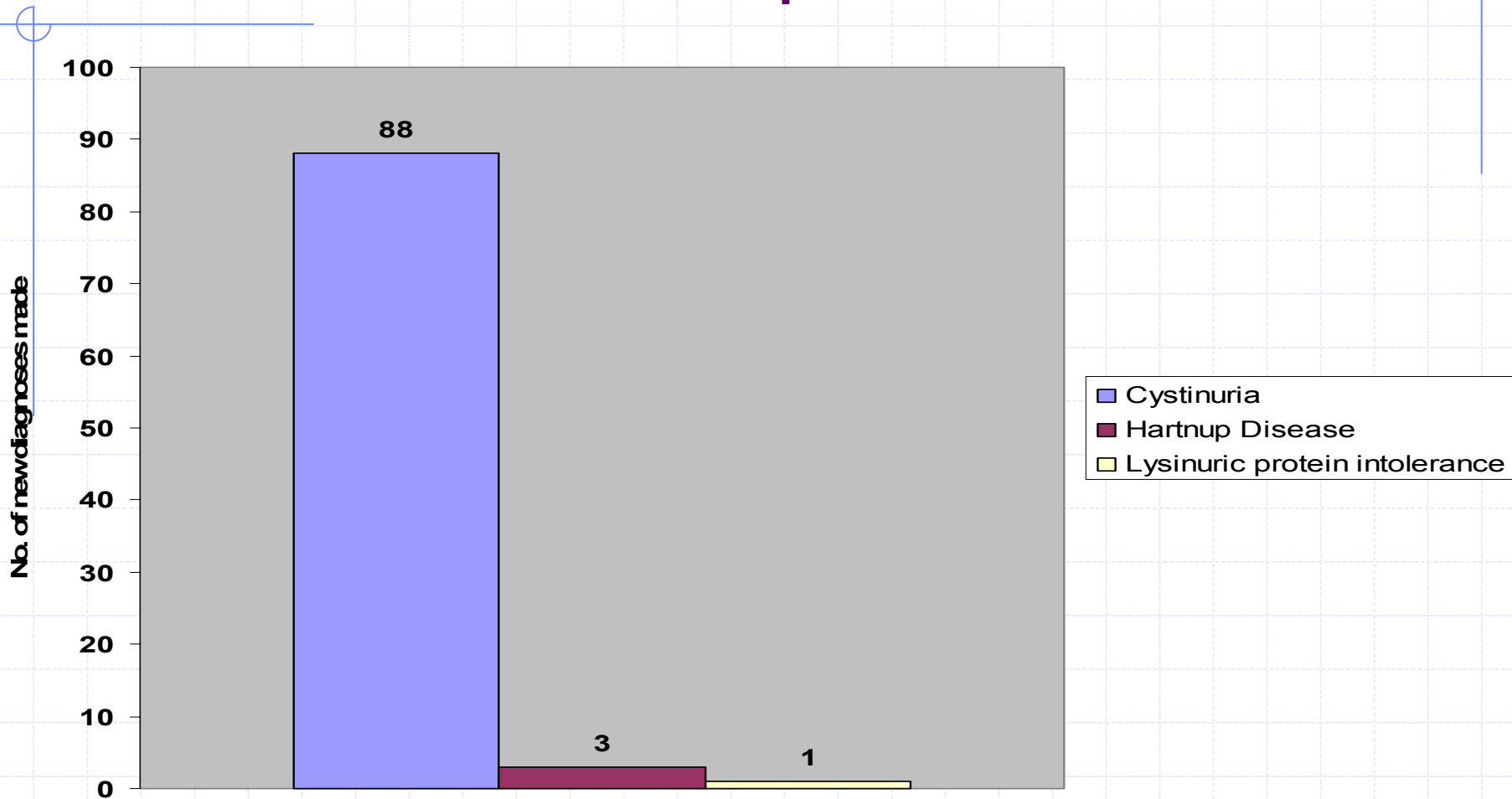|	Mean	Range
Qualitative Plasma:	462	90-1300
Quantitative Plasma:	618	20-2982
Qualitative Urine:	990	150-2100
Quantitative Urine:	414	34-850

Question 11: New Diagnoses Achieved 2002-2004

- Asked for information on disorders where amino acids analysis plays a part in diagnosis (rather than just amino acid disorders)
- Data collected is somewhat incomplete
- 81% of labs provided full or partial data
- Suspect a number of patients have been counted twice (due to transfers or confirmatory testing elsewhere)
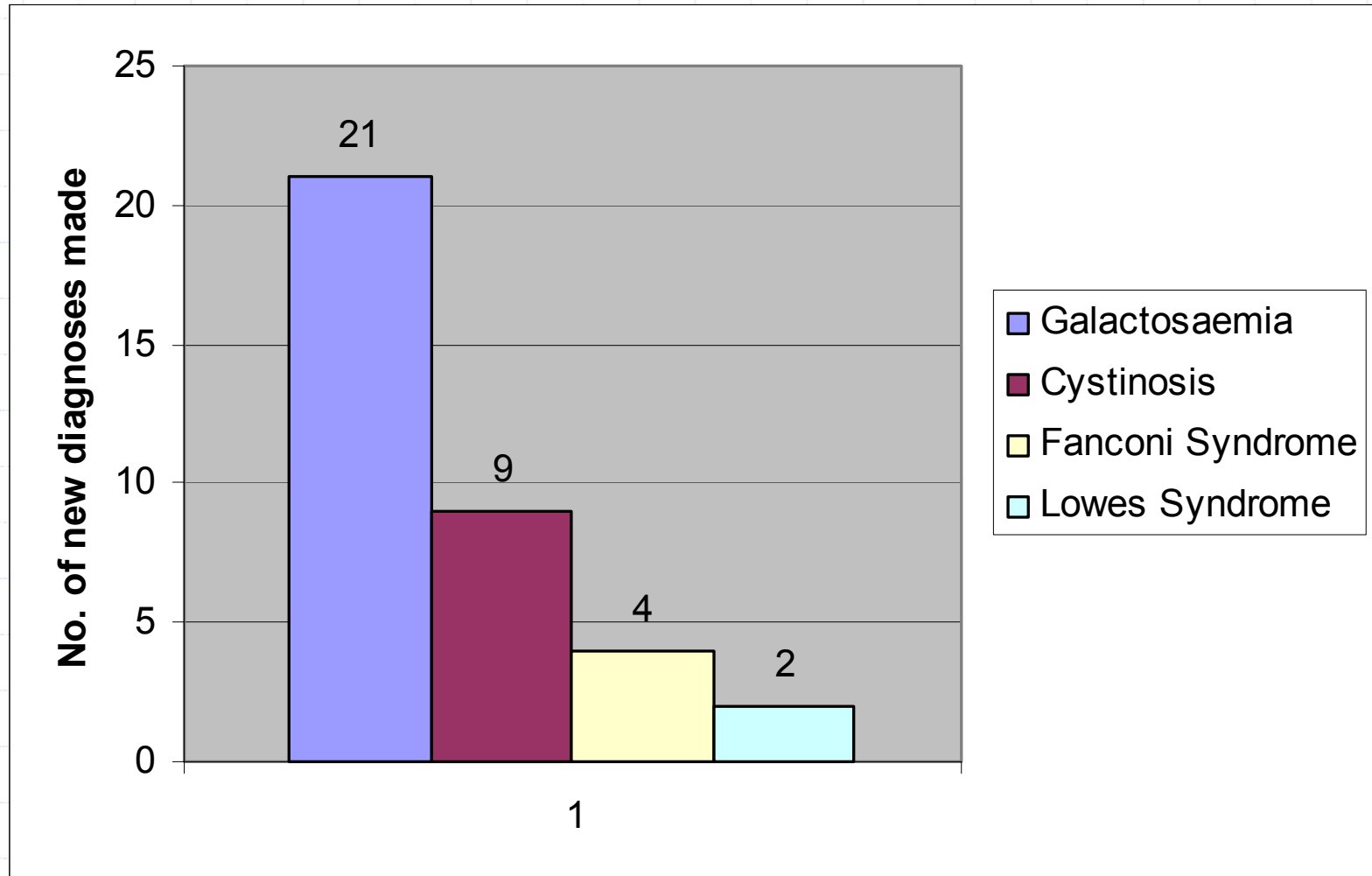- However data does give some insight in to numbers of diagnoses and frequency of individual disorders
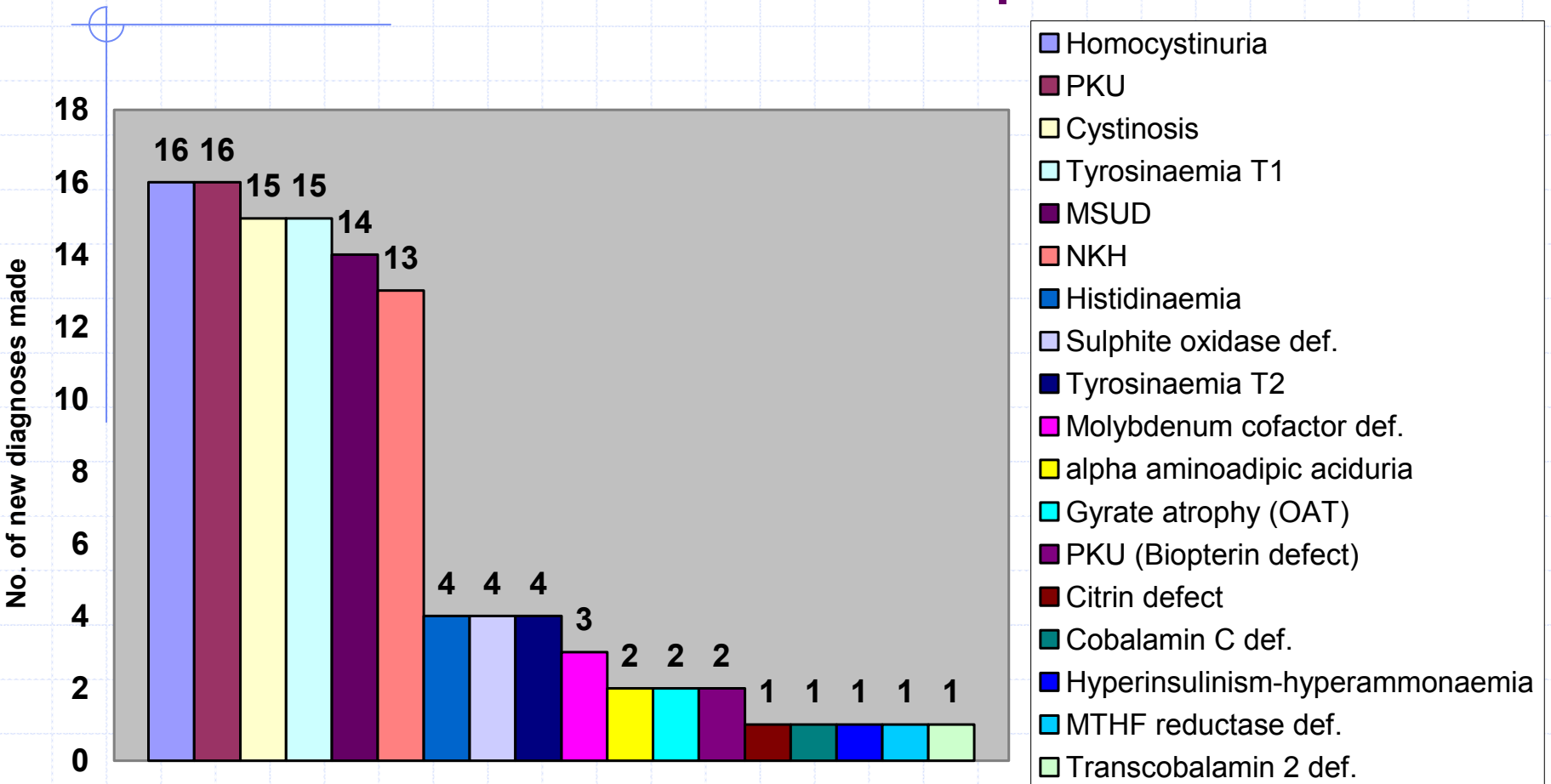
Urea Cycle Disorders
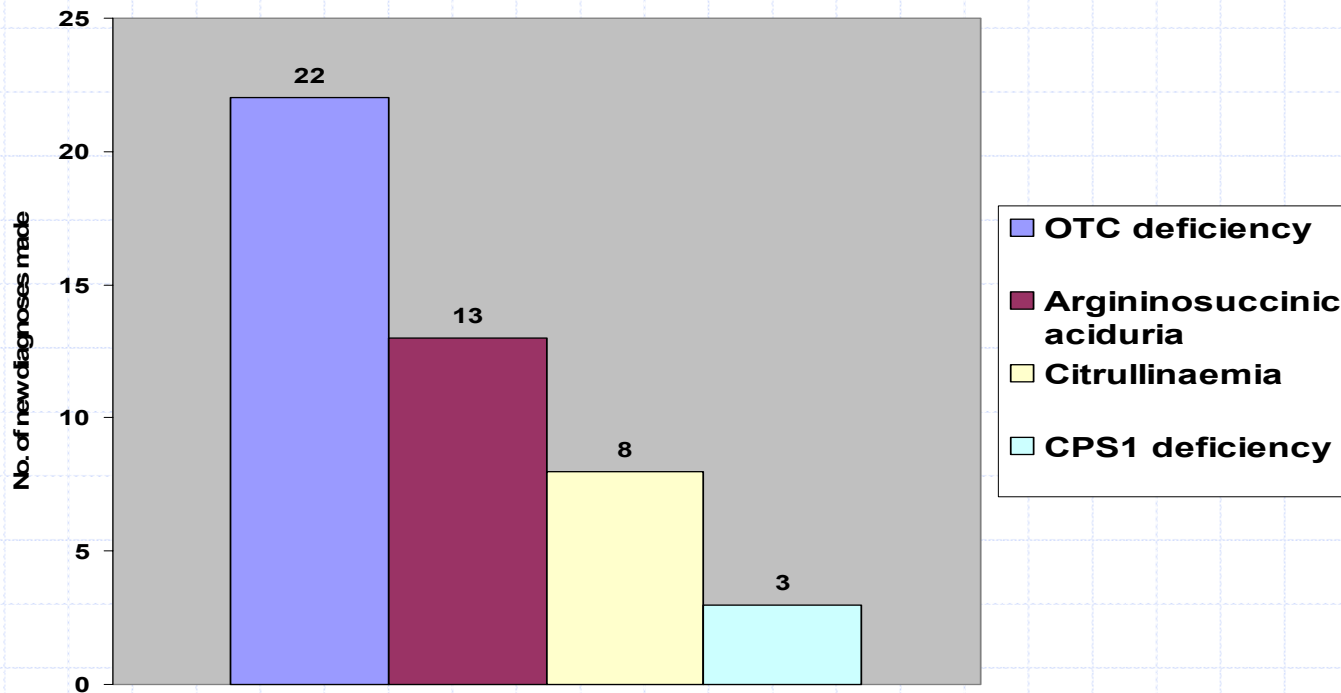
Amino Acid Transport Disorders

Generalised Amino Aciduria Attributable to a Defined Cause

Other Aminoacidopathies

No. of new diagnoses made

Legend:
- Homocystinuria
- PKU
- Cystinosis
- Tyrosinaemia T1
- MSUD
- NKH
- Histidinaemia
- Sulphite oxidase def.
- Tyrosinaemia T2
- Molybdenum cofactor def.
- alpha aminoadipic aciduria
- Gyrate atrophy (OAT)
- PKU (Biopterin defect)
- Citrin defect
- Cobalamin C def.
- Hyperinsulinism-hyperammonaemia
- MTHF reductase def.
- Transcobalamin 2 def.

Values: 16, 16, 15, 15, 14, 13, 4, 4, 4, 3, 2, 2, 2, 1, 1, 1, 1, 1

How many samples do we have to analyse to make a diagnosis?

♦ A very approximate estimate would be that 1 in 350 samples yields a diagnosis or diagnostically useful information

Acknowledgments

◈ Many thanks to Graeme Chalmers (Sheffield Grade A trainee) who did most of the work on this questionnaire

◈ Many thanks to everybody who replied to the questionnaire