ERNDIM PROFICIENCY SCHEME (NORTHERN EUROPE)

DEPARTMENT OF CLINICAL CHEMISTRY
Sheffield Children's NHS Foundation Trust
Western Bank
Sheffield
S10 2TH
United Kingdom

Tel: +44(0)114 271 7000
Ext: 17404
Fax: +44(0)114 276 6205

21st August 2013

Dear Colleague

Re: ERNDIM Proficiency Scheme Report - Samples 13.1, 13.2, 13.3, 13.4, 13.5, 13.6

Six samples were distributed to 22 participants. Returns were received from 22 participants for samples 13.1, 13.2 and 13.3 and from 20 participants for samples 13.4, 13.5 and 13.6.

Patient 13.1
3 year old female with short stature.
This sample was obtained from a patient with MPS Type 6 (Maroteaux Lamy; N-Acetylgalactosamine- 4-sulphatase deficiency, arylsulphatase B deficiency).

Findings
9/22 laboratories considered MPS Type 6 their most likely diagnosis, having identified increased excretion of dermatan sulphate on glycosa (GAG) electrophoresis. An additional 3/22 considered MPS 6 a possibility among other MPS disorders.

6/22 laboratories concluded that there was no evidence for a metabolic disorder and 2/22 made no comment..

1 laboratory gave Morquio (MPS Type 4) as their diagnosis. 1 participant gave MPS Type 7 as their likely diagnosis after noting increased dermatan and chondroitin sulphate by GAG electrophoresis.

Conclusions
19/22 laboratories performed a quantitative assay for glycosaminoglycans and obtained a normal result. 1/22 laboratories obtained a grossly elevated GAG result (by DMB colorimetric method). 2/22 laboratories did not perform a quantitative assay. One of these missed the diagnosis as they did not do GAG electrophoresis.
Seven of the 20 laboratories who did do a quantitative assay did not perform a qualitative assay (electrophoresis) presumably due to the normal GAG result. None of these identified an MPS disorder. One of these laboratories stated that a sample should be sent elsewhere for GAG electrophoresis if an MPS disorder is suspected as they do not carry out this test.

13/22 laboratories correctly identified an increased excretion of dermatan sulphate in this sample. One laboratory who did carry out GAG electrophoresis reported a normal result but had used a small sample volume. They stated that a repeat urine would be requested.

Further investigations
All laboratories who correctly included MPS Type 6 in their list of possible diagnoses suggested enzyme studies to confirm the diagnosis. Seven of these suggested referral to a specialist centre or to a consultant in IMD. Only 4 participants suggested testing of siblings. Seven laboratories requested a repeat urine to either confirm their finding or due to the low volume of sample left for use in the electrophoresis.

Comment
This sample came from a 15 year old girl. Our GAG quantitative result (by DMB) = 5.9 mg/mmol creatinine. Our reference range for a 15 year old = 2.0 - 7.6 mg/mmol creatinine making this result normal even for an older patient. Unfortunately it is not known whether this patient was on treatment.
This sample provides evidence that a normal glycosaminoglycan quantitative result does not rule out the possibility of a mucopolysaccharidosis. Where suggestive clinical details indicate MPS as a possibility then qualitative analysis, such as electrophoresis, is indicated.

Patient 13.2
6 year old male with vomiting and unexplained metabolic acidosis
This sample was obtained from a patient who had ingested ethylene glycol.

Findings
21/22 laboratories correctly identified an increased excretion of glycolate with/without oxalate.

Conclusions
19/22 laboratories considered the most likely diagnosis was ethylene glycol ingestion. Two laboratories, who had identified the increased glycolate and oxalate, ascribed these findings to primary hyperoxaluria without considering ethylene glycol ingestion as a possible diagnosis. The remaining laboratory did not identify the key metabolites and reported a lactic acidosis. They did however state that an intoxication cannot be ruled out due to the 'unknown metabolites present' on their organic acid trace.

Further investigations
Many helpful recommendations were provided by those laboratories who gave the correct diagnosis. The most important of which was felt to be urgent communication of the results to the clinician (17/19). Further tests suggested included plasma ethylene glycol analysis, plasma osmolality, anion gap, U+Es and calcium as the patient is at risk of renal failure and hypocalcaemia. For UK laboratories contacting the National Poisons Information Service for further advice and information was suggested. Some laboratories (10/19) mentioned the treatments used in case of ethylene glycol ingestion (ethanol infusion, fomepizole, possible haemodialysis).

Comment
It is concerning that one laboratory failed to identify glycolate and oxalate and that a further two who did identify these metabolites incorrectly interpreted the results. The clinical details “vomiting and unexplained metabolic acidosis” do not immediately suggest primary hyperoxaluria and these indications could have prompted consideration of ethylene glycol ingestion.

Patient 13.3
10 month old female with unexplained dystonia.
This sample was taken from a child with glutaric aciduria Type 1 (Glutaryl-CoA dehydrogenase deficiency)

Findings
22/22 laboratories correctly identified an increased excretion of glutarate and 21/22 identified an increase in 3-hydroxy glutaric acid. Three laboratories also performed acylcarnitine analysis and found increased glutarylcarnitine in the urine.

Conclusions
22/22 laboratories considered that the most likely diagnosis was glutaric aciduria type 1(GA1). Many laboratories commented on increased glycine in this sample, attributing this as secondary to the GA1.

Further investigations
All laboratories gave good suggestions for further investigation. These included mutation analysis(15/22) (also to aid with future pregnancies), enzyme assay in cultured fibroblasts or leukocytes(19/22), plasma or dried blood spot acylcarnitines (17/22), urgent referral to a clinical IMD team for treatment (15/22) and to test siblings (12/22). One laboratory also suggested reviewing the newborn screening result if the child had been screened for GA1.

Comment
It is reassuring that all laboratories identified this as a case of glutaric aciduria type 1 although not all laboratories stated that they would urgently refer this child to a specialist for treatment and only 12/22 would have recommended that siblings should be tested.

Patient 13.4
4 year old male with splenomegaly (known since 6 months of age), failure to thrive, and a special eating behaviour. Sample collected at the age of 17 years during a routine check up while receiving specific treatment.
This sample was taken from a patient with lysinuric protein intolerance. (common sample).

Findings
20/20 laboratories returning results reported an increased excretion of lysine (mean lysine concentration = 380 mmol/mol creatinine, range 158 - 526). Increased arginine was also reported by all laboratories and increased ornithine by 11/20 laboratories. Increased orotic acid was reported by 15 laboratories (range >30 - 92 mmol/mol creatinine).

Conclusions
18/20 laboratories gave lysinuric protein intolerence as their primary diagnosis. Three laboratories also included ornithine transcarbamylase deficiency (OTC) in their diagnostic list. Three participants listed other urea cycle disorders in their possible diagnosis list. One laboratory gave OTC as their primary diagnosis. The remaining laboratory gave arginase or another urea cycle disorder as their primary diagnosis.

Further investigations
15/20 participants recommended measurement of plasma/blood ammonia. 19/20 measurement of plasma amino acids. 8/20 suggested referral to a specialist in IMD.

Comment
This was the common sample. All laboratories detected the increased an lysine concentration in the urine and many detected the increased orotic acid. However, not all participants came

to the correct diagnosis although all did list urea cycle defects amongst their possible diagnoses.

Patient 13.5
12 year old male with cerebellar ataxia.
This sample was taken from a patient with Hartnup disease.

Findings
20/20 laboratories correctly noted increased neutral amino acids in this sample. However, not all ascribed this to Hartnup disease (15/20 participants correctly diagnosed Hartnup disease). 5 participants noted that excretion of proline was not raised - these laboratories gave the correct diagnosis.

Conclusions
15/20 participants correctly diagnosed Hartnup disease. Of these 15, 1 laboratory also included renal tubulopathy/Fanconi, 1 lab included respiratory chain/mitochondrial disorder or Wilson's disease and another laboratory included MPS type 3.
Of the remaining 5 participants who did not give Hartnup as a diagnosis, one suggested maple syrup urine disease, 1 reported that the amino acid results suggested faecal contamination of the sample, 1 laboratory was unable to provide a diagnosis although they did state that the amino acids were abnormal, 1 laboratory suggested this was from a urea cycle disorder patient on treatment and another gave MPS type 3 as their primary diagnosis.

Further investigations
Many participants commented on the fact that this sample showed evidence of deterioration/bacterial contamination (high pH, raised benzoate). Therefore one of the further investigations suggested was a repeat urine sample for amino acids (10/20 labs). Plasma amino acids was suggested by 13/20 participants, with some mentioning the importance of checking for tryptophan deficiency in this condition. Many participants suggested a paired plasma and urine. Mutation analysis (SLC6A19 gene) was suggested by 7/20 participants and testing of siblings by 4/20 laboratories. Referral to a specialist in IMD was suggested by 7 participants. Testing of Vitamin B3 and/or supplementation was suggested by 5 participants. Advice on sun protection was provided by one laboratory. Measurement of serotonin was suggested by one laboratory.

Comment
Many participants commented on the fact that this sample showed evidence of deterioration/bacterial contamination (high pH, raised benzoate). However, this did not impair the ability to come to the correct diagnosis. It is a good educational point that increased excretion of neutral amino acids with normal excretion of proline is indicative of Hartnup disease. This is not a generalised amino aciduria as stated by a few participants. The laboratory who suggested Maple Syrup Urine Disease as the primary diagnosis had noted increased excretion of other amino acids (which were listed in the report) but had obviously not realised the significance of these.

As this condition can be asymptomatic it is disappointing that only 4/15 participants suggested testing of siblings.

Patient 13.6
6 year old female with repeated urinary tract infections.
This sample was taken from a patient with cystinuria.

Findings

20/20 laboratories correctly identified an increased excretion of cystine, ornithine, lysine and arginine.

Amino acid	Mean concentration (mmol/mol creatinine)	Range
Cystine	268	188 - 322
Lysine	1027	648 - 1337
Arginine	348	241 - 433
Ornithine	756	527 - 929

One laboratory did not give quantitative amino acid results but deduced the correct diagnosis from a qualitative assay.

Conclusions
20/20 laboratories considered that the most likely diagnosis was cystinuria.
A few laboratories commented that the cystine concentration was below the threshold required for stone formation but that the concentration of cystine may be higher in a less dilute urine e.g. early morning sample.

Further investigations
There were varying suggestions for further investigations and recommendations. These included genetic testing to differentiate the type of cystinuria - type A (SLC3A1) or type B (SLC7A9), testing of siblings, repeat urine for amino acid analysis (early morning urine as this was a dilute urine sample and the urine cystine may be higher in more concentrated samples; 24 hour urine). Referral of patient to a renal consultant for clinical management (high fluid intake, alkalinisation of the urine, use of penicillamine, mercaptopropionylglycine or captopril).
One laboratory who only suggested measuring plasma concentrations of cystine, ornithine, lysine and arginine scored poorly.

Comment
This seemed a relatively straightforward sample with the correct diagnosis being reached by all those laboratories who analysed it.

Yours sincerely

Prof J R Bonham
Scheme Organiser

Mrs Joanne Croft
Deputy Scheme Organiser