ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Diagnostic Proficiency Testing (DPT) Scheme (United Kingdom) Annual Report 2016

1. Scheme Design

The scheme has been designed and planned by Mrs Joanne Croft as Scientific Advisor/Scheme Organiser appointed by and according to procedures laid down by the ERNDIM Board.

2. Geographical distribution of participants

Twenty-two laboratories from 7 countries participated in the 2016 scheme, for details see the table below.

Table 1: Geographical distribution of registered participants

Country	Number of participants
Ireland	1
Malaysia	1
New Zealand	2
Spain	1
United Kingdom	15
France	1
Australia	1

3. Samples and shipment

All samples are obtained following local ethical and consent guidelines. Two sets of three samples (labelled A to F) were dispatched together in February 2016 to 22 participants by CSCQ (Geneva, Switzerland). Submission deadlines were 14th March 2016 (samples A, B and C) and 13th June 2016 (samples D, E and F).

Table 2: Schedule for the 2016 scheme

Sample distribution	1st February 2016
Start of analysis of 1st round (samples A, B and C)	22nd February 2016
1st round – results submission	14th March 2016
Start of analysis of 2nd round (samples D, E and F)	23rd May 2016
2nd round – results submission	13th June 2016
Annual meeting of participants	6th September 2016
Annual report 2015	December 2016

4. Submission of results

Laboratories were asked to analyse the sample sets at intervals during the year as if they were separate circulations. All twenty-two laboratories returned results for all 6 samples.

All submitted results are treated as confidential information and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting.

5. Samples

Patient A

Clinical details provided: 'At the age of 5 years this boy was referred for the first time to a paediatric nephrologist because of urolithiasis. At ages 7 and 10, again renal stones were found. At the time of the urine collection, he was 10 years old and in good health. He used no medication, had a normal diet and adequate renal function.'

This sample was obtained from a 10 year old boy with Primary Hyperoxaluria Type 2. Primary Hyperoxlauria Type 2 has been confirmed in this patient by mutation analysis of the GRHPR gene (homozygous for c.103delG). This was the common sample for all the DPT schemes.

- **Findings**
  21/22 participants identified increased excretion of glycerate. 11/22 participants detected increased excretion of oxalate. Of the 11 laboratories who did not detect increased excretion of oxalate, 7 suggested measuring oxalate by a specific assay. Only 2 laboratories provided a quantitative oxalate result in the UK DPT scheme (412 and 234 mmol/mol creatinine).

- **Conclusions**
  21/22 laboratories gave Primary Hyperoxlauria Type 2 as their primary diagnosis. 1 laboratory provided no diagnosis.

- **Further Investigations**
  Recommendations included follow up with quantitative urine oxalate in a 24 hour acidified sample (16/22). Other recommendations were mutation analysis of the *GRHPR* gene, referral to paediatric urology/renal team, renal stone analysis and sibling investigations.

- **Comment**
  For the analytical score 1 mark was awarded for detecting increased excretion of oxalate and 1 for increased excretion of glycerate. This is in line with the other DPT schemes. It is recognised that oxalate extraction is variable in organic acid analysis, an issue discussed at the participants meeting in Rome in September 2016. However, the marking scheme remains as outlined.

  Proficiency for this sample was good with only 1 laboratory receiving 0 marks. Failure to identify both increased oxalate and glycerate in this sample was deemed by the ERNDIM Scientific Advisory Board to be a critical error (see page 5 – Scoring of results).

Patient B

Clinical details provided: 'Dysphagia, dysarthria and spastic quadrepesis. On treatment. Sample collected from a 65 year old female'.

At the time of collecting the sample, this patient was a 65 year old lady with a diagnosis of primary lateral sclerosis with symptoms of dysphagia, dysarthia and spastic quadrepesis. She had been started on Sinemet (Co-Careldopa).

- **Findings**
  All laboratories identified the increased excretion of homovanilate and vanilyl lactate. This was scored with 1 mark for each metabolite (or 2 marks for 'increased dopamine metabolites').

- **Conclusions**
  Only 2 laboratories did not suggest that the patient was on L-DOPA therapy. 3/22 laboratories stated that aromatic amino acid decarboxylase deficiency was unlikely due to the age of the patient. Other diagnoses provided included guanidinoacetate methyltransferase (GAMT)

deficiency due to the low creatinine concentration and non-ketotic hyperglycinaemia (NKH) based on the increased glycine concentration.

- **Further investigations**
  This included recommendation to review the drug history – if on L-DOPA no need for any further investigations. Laboratories who recommended follow up with CSF amino acids or neurotransmitters were scored poorly due to the invasive nature of this test.

- **Comment**
  Proficiency for the analysis was excellent for this sample with all laboratories identifying the increased dopamine metabolites.

Patient C

Clinical details provided: 'History of recurrent skin infection and skin ulcers. Mental retardation.'

This sample was obtained from a boy with Prolidase deficiency. 8 year old boy with suspicion of immunodeficiency. Had a history of recurrent severe infections since the neonatal period: sepsis, skin ulcers and upper respiratory tract infection – leading to hearing problems with secondary mental retardation.'

- **Findings**
  2 marks were awarded to laboratories who identified glycyl-proline or dipeptides or an increase of glycine and proline after hydrolysis. 0 marks were awarded to laboratories who deemed the sample to have deteriorated or who identified no significant abnormality on amino acid analysis. 18/22 laboratories correctly identified this as a sample from a patient with prolidase deficiency and scored 4 marks for this sample. The other 4 participants scored 0 marks for this sample.

- **Conclusions**
  The 18 laboratories who scored 2 marks for analysis all correctly identified this as being a sample from a patient with Prolidase deficiency.

- **Further investigations**
  These included enzymatic analysis of prolidase in red blood cells, mutation analysis of the PEPD gene, referral to a specialist metabolic physician and family studies.

- **Comment**
  It was judged at the November SAB meeting that this sample is not eligible for critical error due to the difficult nature of identifying Prolidase deficiency.

Patient D

Clinical details provided: 'Global developmental delay. Sample taken while on treatment'

This sample was obtained from a patient with Lysinuric Protein Intolerance. Unfortunately there is no further clinical information available.

- **Findings**
  Laboratories were awarded 1 mark for identifying increased lysine concentration and 1 mark for detecting orotic acid. Analytical performance for this sample was good (93%) with only 1 laboratory missing the increased lysine and 2 laboratories missing the orotic acid. No laboratory missed both analytes.
  21/22 laboratories performed quantitative amino acid analysis, with 20/21 providing the quantitative result for the lysine (mean = 1057.8 mmol/mol, median = 1079, range = 167.0 – 2767.0).

6/22 laboratories performed quantitative orotic acid analysis (median = 238.5 mmol/mol, range = 30.0 – 500.0).

- **Conclusions**
  20/22 participants correctly identified this as Lysinuric Protein Intolerance. 1 laboratory gave ornithine transcarbamylase deficiency and another argininosuccinic acid lyase deficiency as the diagnosis.

- **Further investigations**
  These included an urgent blood sample for ammonia analysis, plasma amino acids, referral to a metabolic clinician, mutation analysis of the *SLC7A7* gene, protein restriction, citrulline substitution, enzyme studies, ferritin and LDH analysis.

- **Comment**
  All laboratories gave a urea cycle defect as the diagnosis, therefore no laboratories receive a critical error for this sample. It may be possible that the wide range in lysine and orotic acid results is due to errors in units used when inputting the results on-line.

Patient E

Clinical details provided: 'Regression in psychomotor development. Sample taken while on treatment.'

This sample came from a patient with Mucopolysaccharidosis Type IIIC. Since the age of 7 years this male child had regression in psychomotor development. He was diagnosed at 13 years of age. This sample was obtained at the age of 19 years while receiving treatment.

- **Findings**
  Identification of increased heparan sulphate was scored 2 marks. Identification of increased glycosaminoglycan concentration with recommendation to perform glycosaminoglycan fractionation was scored 1 mark.
  21/22 laboratories performed glycosaminoglycan quantitation. Median result 20.0 g/mol creatinine, range = 0.87 – 54.5. 17/21 laboratories classed this as elevated or grossly elevated, 2 as normal and 2 did not state.
  1 laboratory used an unconventional method for GAG fractionation and did not report on increased heparan sulphate but the correct diagnosis was reached so have been awarded 4 marks for this sample (see below for reference for method used).
  High throughput determination of urinary hexosamines for diagnosis of mucopolysaccharidoses by capillary electrophoresis and high-performance liquid chromatography. Coppa GV et al. Analytical Biochemistry. 411 (2011) 32 – 42

- **Conclusions**
  18/22 laboratories correctly classed this as a mucopolysaccharidosis type 3 (MPS III) (received 2 marks for interpretation). 2 laboratories classed it as a MPS but did not state which type. 1 laboratory did not provide a diagnosis (though they did state the sample would be referred for electrophoresis and if found to be an MPS would be referred) and 1 laboratory provided a list of possible MPS disorders which did not include MPS III. Identification of a MPS disorder scores 1 mark for interpretation.

- **Further investigations**
  Enzyme testing in leucocytes to identify subtype of MPS III, repeat urine to confirm findings, check whether the patient is on any heparin therapy, referral to Paediatric Metabolic team for further investigation, diagnosis and treatment, mutation analysis and family studies.

- **Comment**
  It would be interesting to know whether those laboratories who quantitated the glycosaminoglycans and classed this as not increased always follow up with glycosaminoglycan fractionation (as they did with this EQA sample).
  There were no critical errors for this sample.

Patient F

Clinical details provided: 'History of dental problems in childhood'

This sample was obtained from a patient with Hypophosphatasia. 4 year old girl with premature loss of primary teeth and a waddling gait.

- **Findings**
  The identification of an increased concentration of phosphoethanolamine was scored 2 marks. 20/22 laboratories identified increased phosphoethanolamine concentration. 18/22 laboratories performed quantitative amino acid analysis. 12/18 provided a quantitative result (Mean = 58 µmol/mmol creatinine, median = 56, range = 31.0 – 106.0).

- **Conclusions**
  Hypophosphatasia was scored 2 marks and the suggestion to measure alkaline phosphatase was scored 1 mark (if increased phosphoethanolamine was not detected). 19/22 laboratories scored 2 marks for interpretation. 1 laboratory identified the increased phosphoethanolamine but did not provide an interpretation, 1 laboratory did not identify the increased phosphoethanolamine but stated that alkaline phosphatase should be measured as hypophosphatasia has not been excluded and 1 laboratory did not detect any abnormality and did not mention doing alkaline phosphatase.

- **Further investigations**
  Measure plasma alkaline phosphatase, measure pyridoxal 5 phosphate, mutation analysis of the *ALPL* gene, referral to the Metabolic Bone team, bone radiology and family studies.

- **Comment**
  This sample was not eligible for critical error as Hypophosphatasia is difficult to diagnose by urinary phosphoethanolamine analysis.

6. Scoring of results

ERNDIM are being encouraged by the European Society of Human Genetics to harmonise scheme performance assessments with the other European genetic laboratory EQA providers. ERNDIM has defined criteria for critical error (i.e. an error that would be unacceptable to the majority of labs and would have a serious adverse effect on patient management), which has been implemented since the 2014 scheme year for the DPT schemes. The summary of scoring criteria is given below:

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory or misleading (in some instances will be evaluated also as a critical error)	0
I	Interpretative proficiency	Good (diagnosis was established and appropriate further tests were recommended)	2
		Helpful but incomplete	1
		Misleading/wrong diagnosis (will be most likely evaluated also as a critical error)	0

The total score is calculated as a sum of these two criteria. The maximum score that can be achieved is 4 points per sample. Therefore the maximum score available is 24 in 2016.

Scores assigned by the Scientific Advisor and agreed at the Annual Meeting have been reviewed by an independent advisor from another DPT Centre and the scoring was finalized after any possible discrepancies had been resolved at the November 2016 ERNDIM Scientific Advisory Board (SAB) meeting.

Following the SAB meeting in November 2016 it was decided that any laboratory failing to identify increased oxalate and glycerate in Sample A would receive a critical error for this sample. As sample A was the common sample sent to all participants of the DPT scheme, this ruling applies to all laboratories in the scheme. For the DPT UK scheme this critical error applies to 1 laboratory.

7. Detailed scores for submitting laboratories

The total maximum score was 24 points, with 15 or more points being deemed satisfactory.

Anonymised Laboratory number	Sample						Total score
	A	B	C	D	E	F	
UK20	4	4	4	4	4	4	**24**
UK30	4	4	0	4	4	4	**20**
UK40	3	3	4	4	4	4	**22**
UK50	4	4	4	3	4	4	**23**
UK60	4	4	4	4	4	4	**24**
UK70	3	4	4	4	4	4	**23**
UK80	4	4	0	4	4	2	**18**
UK90	2	3	0	4	4	4	**17**
UK100	4	4	4	4	4	4	**24**
UK110	4	4	4	4	2	4	**22**
UK120	3	3	4	4	4	1	**19**
UK130	3	4	4	4	4	4	**23**
UK140	4	3	4	1	4	4	**20**
UK150	3	4	4	4	2	4	**21**
UK160	3	4	4	3	4	4	**22**
UK170	4	4	4	4	2	4	**22**
UK180	4	4	4	4	4	4	**24**
UK190	0	4	4	4	4	4	**20**
UK200	4	2	4	4	4	4	**22**
UK210	3	2	4	4	4	4	**21**
UK220	3	4	4	4	4	4	**23**
UK230	3	4	0	2	2	0	**11**

8. Proficiency per sample

Sample	Diagnosis	No of returns	Analytical performance (%)	Interpretative proficiency (%)	Total (%)
A	Primary Hyperoxlauria Type 2	22	72.7	93.2	**83.0**
B	Patient on L-DOPA	22	100.0	82.0	**91.0**
C	Prolidase	22	81.8	81.8	**81.8**
D	Lysinuric Protein Intolerance	22	93.2	90.9	**92.1**
E	MPS IIIC	22	90.9	88.6	**89.8**
F	Hypophosphatasia	22	90.9	88.6	**90.0**

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Yours sincerely

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Scheme Organiser