ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Diagnostic Proficiency Testing (DPT) Scheme (United Kingdom) Annual Report 2015

1. Scheme Design

The scheme has been designed and planned by Prof Jim Bonham and Mrs Joanne Croft as Scientific Advisor/Scheme Organiser and deputy Scientific Advisor/Scheme Organiser, respectively, both appointed by and according to procedures laid down by the ERNDIM Board.

2. Geographical distribution of participants

Twenty-three laboratories from 7 countries participated in the 2015 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	16
Czech Republic	1
Australia	1

3. Samples and shipment

All samples are obtained following local ethical and consent guidelines. Two sets of three samples (numbered 15.1 to 15.6) were dispatched together in March 2015 to 23 participants by CSCQ (Geneva, Switzerland). Submission deadlines were 30th April 2015 (samples 15.1, 15.2 and 15.3) and 22nd June 2015 (samples 15.4, 15.5 and 15.6).

Table 2: Schedule for the 2015 scheme

Sample distribution	31st March 2015
Start of analysis of 1st round (samples 15.1, 15.2 & 15.3)	7th April 2015
1st round – results submission	30th April 2015
Start of analysis of 2nd round (samples 15.4, 15.5 & 15.6)	1st June 2015
2nd round – results submission	22nd June 2015
Annual meeting of participants	1st September 2015
Annual report 2015	April 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-three 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 15.1

Clinical details provided: 'Behavioural problems since childhood'. Sample collected at age 28 years.

This sample was obtained from a 28 year old male who had been found to have homocystinuria when he was 5 years old. However, unfortunately this patient has never been genotyped and the cause of his homocystinuria has not been definitively established.

- **Findings**
  22/23 participants identified increased homocystine. A quantitative result was provided by 19/23 laboratories (mean = 45 mmol/mol creatinine, range = 14.5 – 63.0).
- **Conclusions**
  Identification of homocystine scored 2 marks for analytical proficiency, as the cause of the homocystinuria is not definitely known.
- **Further Investigations**
  Recommendations to follow up with plasma total homocysteine, plasma amino acids (including methionine), folate and Vitamin B12 are appropriate. Many laboratories provided more than one possible primary diagnosis, with many suggestions for other possible diagnoses. These included cystathionine beta-synthase deficiency (n = 12), methylenetetrahydrofolate reductase (MTHFR) deficiency (n = 17), cobalamin disorders (n = 13) and folate deficiency (n = 6).
- **Comment**
  Proficiency for this sample was good with only 1 laboratory receiving 0 marks. Failure to identify homocystine in this sample was deemed by the ERNDIM Scientific Advisory Board to be a critical error (see page 5 – Scoring of results).

Patient 15.2

Clinical details provided: 'Normal psychomotor development. Phlebitis at 30 years (under oestrogens). Diagnosis at 34 years.'

This sample was obtained from a patient with Cystathionine beta-synthetase deficiency and was the common sample for all the DPT schemes.

- **Findings**
  As with sample 15.1, homocystine was detected in this urine sample by 22/23 participants. A quantitative result was provided by 19/23 laboratories (mean = 36.0 mmol/mol creatinine, range 17.4 – 97.0).
  A quantitative methionine value was provided by 17/23 laboratories (mean = 19.5 mmol/mol creatinine, range 5.3 – 33). Of the remaining 6 laboratories, 4 did not mention methionine, 1 stated that it was increased and 1 stated that it was normal. In total, 14/23 laboratories reported increased methionine concentration.
  All laboratories reported a normal organic acid profile with only 1 laboratory reporting an increased methylmalonic acid concentration (but stated that there was no obvious organic acid disorder present).
- **Conclusions**
  Laboratories who suggested cystathionine beta-synthetase deficiency (or classical homocystinuira) as the primary or alternative diagnosis were scored 2 marks for interpretation and recommendations. Primary diagnoses, as given by the participants, included classical homocystinuria, homocystinuria, suspicion of homocystinuria, CBS deficiency, MTHFR deficiency, uncertain, secondary cause and requires further investigation.

  Other possible diagnoses included folate deficiency, MTHFR deficiency, cobalamin defects, vitamin B12 deficiency (though less likely as methylmalonic acid not increased).

Only 14/23 laboratories included classical homocystinuria/CBS deficiency amongst their list of diagnoses. This does not include those labs who gave the diagnosis as 'homocystinuria' or 'suspicion of homocystinuria' (a further 7 labs).

- **Further investigations**
  Recommendations included plasma total homocysteine (20/23), plasma amino acids (including methionine) (21/23), folate (15/23) and Vitamin B12 (11/23). Other recommendations included genetic analysis of the MTHFR gene, genetic analysis of the CBS gene and enzyme assay for CBS, full blood count, trial of pyridoxine and investigation of primary relatives.

  Those laboratories who did not specifically mention doing plasma total homocysteine were scored less favourably.
- **Comment**
  Proficiency for this sample was good. Failure to identify homocystine in this sample was deemed by the ERNDIM Scientific Advisory Board to be a critical error (see page 5 – Scoring of results).

Patient 15.3

Clinical details provided: 'Developmental delay. Unexplained epilepsy'

This sample was collected from a normal healthy child of a member of the laboratory staff.

- **Findings**
  22/23 participants did not identify any abnormalities from the tests they undertook on this sample. 1 participant detected increased argininosuccinic acid.
- **Conclusions**
  22/23 laboratories determined that the sample had come from a child with no metabolic condition which could be detected from the tests performed.
- **Further investigations**

  These varied widely from none to a long list of other tests to perform. A number of laboratories stated that they would require more clinical details to guide further testing.
- **Comment**

  Performance for this sample was good with 21/23 laboratories scoring full marks and only 1 laboratory scoring 0 marks. 2 marks were awarded for 'analytical' to those laboratories who did at least 3 tests.

Patient 15.4

Clinical details provided: 'Investigation of hyperlipidaemia'

This sample was obtained from an adult male patient. He was initially investigated in the lipid clinic due to hypertriglyceridaemia. Glycerol kinase deficiency has been confirmed.

This sample was obtained from an adult male being investigated for hyperlipidaemia. Unfortunately the details provided stated that this sample was from a female. We apologise for this error and steps have been put in place to ensure this does not occur again.
Given that glycerol kinase is an X-linked condition the error in the details provided may have caused some laboratories to discount glycerol kinase as a possible diagnosis. However, this has not been the case and all laboratories considered glycerol kinase deficiency as either the primary diagnosis or as a possible diagnosis.

- **Findings**
  All participants identified increased glycerol in this urine sample by organic acid analysis.
- **Conclusions**
  21/23 laboratories gave glycerol kinase deficiency as the primary diagnosis, with 5 laboratories rightly querying this in a female. The other 2 laboratories mentioned glycerol kinase deficiency in the other possible diagnoses.

- **Further investigations**
  12/23 laboratories would ask for a repeat urine to rule out the possibility of contamination. 8/23 laboratories suggested repeating the lipid investigations using a blanked triglyceride method.

  9/23 laboratories suggested molecular analysis of the GK gene and 6/23 suggested molecular analysis of Xp21. Other suggestions included performing a synacthen test/ACTH/cortisol measurement as glycerol kinase deficiency may be part of the contiguous gene syndrome leading to congenital adrenal hypoplasia, and family studies.
- **Comment**
  All laboratories have been scored 4 marks for this sample.

Patient 15.5

Clinical details provided: 'Failure to thrive. Sample taken while on treatment'

This sample was obtained from a 16 year old female with argininosuccinic aciduria (argininosuccinate lyase deficiency). The sample was collected while on treatment with arginine.

- **Findings**
  19/23 laboratories reported an increased excretion of argininosuccininc acid (ASA). 18/23 also noted excretion of orotic acid. 4 laboratories failed to identify an increased excretion of ASA, 2 of which did not perform amino acid analysis on this sample.

  Increased excretion of malonic acid was noted by 19/23 laboratories. The presence of malonate in this sample misled some participants and is difficult to explain.
- **Conclusions**
  19/23 laboratories considered argininosuccinate lyase deficiency as the primary diagnosis. 1 laboratory suggested ornithine transcarbamylase (OTC) deficiency as the cause of the increased orotic acid – this lab did not detect the argininosuccininc acid. The remaining 3 laboratories considered malonic aciduria as the primary diagnosis. 2 of these did not perform amino acid analysis and the third did not detect argininosuccininc acid.
- **Further investigations**
  The main recommendations included plasma amino acids (13/23), plasma ammonia (10/23), mutation analysis of the ASL gene (6/23), family studies/sibling screening (6/23) and enzyme assay (8/23).

  Other recommendations included referral to a metabolic team and repeat organic acids and amino acids. Investigation of the possibility of a dual pathology, including acylcarnitines and mutation analysis of the MLYCD gene was also mentioned.
- **Comment**
  Failure to identify argininosuccininc acid AND orotic acid in this sample was deemed to be a critical error by the ERNDIM Scientific Advisory Board (see page 5 – Scoring of results).

Patient 15.6

Clinical details provided: 'Episodes of back pain.'

This sample was collected from a 12 year old male with Cystinuria.

- **Findings**
  Analytical performance for this sample was excellent with all 23 participants identifying increased concentrations of cystine, arginine, ornithine and lysine.

  21/23 laboratories provided quantitative results for these amino acids (see below, units = mmol/mol creatinine).

  Cystine: mean = 318.3 range = 79 – 1028
  Arginine: mean = 792.5 range = 350 – 2445
  Ornithine: mean = 372.4 range = 167 -1170
  Lysine: mean = 1278.2 range = 653 – 4250

- **Conclusions**
  All laboratories gave cystinuria as the primary diagnosis. 3 laboratories also suggested lysinuric protein intolerance as another possible diagnosis.
- **Further investigations**
  Recommendations included: repeat urine (6/23), plasma amino acids (4/23), mutation analysis (6/23), sibling screening/family studies (8/23), referral to a renal physician (12/23) and referral to a metabolic physician (3/23). Other recommendations included plasma ammonia (from those labs who felt lysinuric protein intolerance was a possibility) and imaging for potential calculi. Only 4/23 labs mentioned hydration and alkalinisation of the urine.
- **Comment**

  Performance for this sample was excellent. Laboratories who scored 3 points did not provide quantitative amino acids results.

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 2015.

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 March 2016 ERNDIM Scientific Advisory Board (SAB) meeting.

Following the SAB meeting in March 2016 it was decided that any laboratory failing to identify homocystine in samples 15.1 and 15.2 would receive a critical error for these samples. As sample 15.2 was the common sample sent to all participants of the DPT scheme, this ruling applies to all laboratories in the scheme. For DPT UK this critical error applies to 1 laboratory.

At the SAB meeting it was also decided that any laboratory who failed to identify increased orotic acid AND argininosuccinic acid in sample 15.5 would receive a critical error. This applies to 2 laboratories.

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 number						Total score
	15.1	15.2	15.3	15.4	15.5	15.6	
1	4	4	4	4	4	4	**24**
2	4	4	4	4	4	4	**24**
3	4	3	4	4	1	4	**20**
4	4	4	4	4	4	4	**24**
5	4	4	4	4	4	4	**24**
6	4	3	4	4	4	4	**23**
7	4	4	4	4	4	4	**24**
8	4	4	4	4	0	4	**20**
9	4	3	4	4	4	4	**23**
10	4	4	4	4	4	4	**24**
11	4	4	4	4	3	4	**23**
12	4	4	4	4	4	4	**24**
13	0	2	4	4	0	4	**14**
14	4	4	4	4	2	4	**22**
15	4	3	4	4	4	4	**23**
16	4	4	4	4	3	4	**23**
17	4	3	4	4	4	4	**23**
18	4	4	3	4	4	4	**23**
19	4	3	4	4	4	3	**22**
20	4	4	4	4	4	4	**24**
21	4	4	4	4	4	3	**23**
22	4	3	4	4	4	4	**23**
23	4	3	0	4	3	4	**18**

8. Proficiency per sample

Sample	Diagnosis	No of returns	Analytical performance (%)	Interpretative proficiency (%)	Total (%)
15.1	Homocystinuria	23	96	96	**96**
15.2	Cystathionine beta-synthetase deficiency	23	89	89	**89**
15.3	Healthy child	23	93	96	**95**
15.4	Glycerol kinase deficiency	23	100	100	**100**
15.5	Argininosuccinic aciduria	23	80	85	**83**
15.6	Cystinuria	23	96	100	**98**

Yours sincerely

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Scheme Organiser

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