**ERNDIM Administration Office**
Manchester Centre for Genomic Medicine
6th Floor, St Mary's Hospital, Oxford Road,
Manchester M13 9WL, United Kingdom.
**Tel:** +44 161 276 6741
**Fax:** +44 161 850 1145
**Email:** admin@erndim.org

**Scientific Advisor**
Mrs Joanne Croft
Dept of Clinical Chemistry
Sheffield Children's NHS Foundation Trust, Western Bank
Sheffield, S10 2TH
United Kingdom
**Tel:** +44(0)114 271 7000 Ext 17267
**Fax:** +44(0)114 276 6205
**Email:** Joanne.Croft@sch.nhs.uk

**Scheme Organiser**
Dr Xavier Albe,
CSCQ
Swiss Centre for Quality Control
2 chemin du Petit-Bel-Air
CH-1225 Chêne-Bourg
Switzerland
**Email:** Xavier.Albe@hcuge.ch

**December 2017**

# Diagnostic Proficiency Testing (DPT) Scheme (United Kingdom) Annual Report 2017

## 1. Scheme Design

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

Note: This annual report is intended for participants of the ERNDIM DPT-UK scheme. The contents should not be used for any publication without permission of the scheme advisor.

## 2. Geographical distribution of participants

Twenty-two laboratories from 7 countries participated in the 2017 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 |
| Netherlands | 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 2017 to 22 participants by CSCQ (Geneva, Switzerland). Submission deadlines were 13th March 2017 (samples A, B and C) and 12th June 2017 (samples D, E and F).

**Table 2:** Schedule for the 2017 scheme

| | |
|---|---|
| Sample distribution | 6th February 2017 |
| Start of analysis of 1st round (samples A, B and C) | 20th February 2017 |
| 1st round – results submission | 13th March 2017 |
| Start of analysis of 2nd round (samples D, E and F) | 22nd May 2017 |
| 2nd round – results submission | 12th June 2017 |
| Annual meeting of participants | 21st November 2017 |
| Annual report 2017 | December 2017 |

## 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: 'Infant presented at Emergency Department. Febrile, query infection. Sample collected after commencing therapy. 3 days old at diagnosis. Male'

**This sample was obtained from a male infant who presented at 3 days of age. The initial blood ammonia was 542 µmol/L. The plasma citrulline concentration was 1401 µmol/L. This patient has Citrullinaemia Type 1 (argininosuccinate synthase deficiency). This was the common sample for all the DPT schemes.**

- **Findings**

  For the analytical score 1 mark was awarded for detecting increased excretion of citrulline and 1 for detecting the orotic acid. For the interpretative score 2 marks were given for Citrullinaemia Type 1 and 1 mark for another urea cycle disorder.

  22/22 participants identified the increased citrulline concentration (of those providing a quantitative result: mean value = 11853 µmol/mmol creatinine, range = 4000 – 19009, n = 16).

  21/22 participants identified the orotic acid (of those providing a quantitative result: mean value = 79.8 µmol/mmol creatinine, range = 30 – 116, n = 11).

- **Conclusions**

  22/22 participants gave Citrullinaemia Type 1 as their primary diagnosis.

- **Further Investigations**

  21/22 - plasma ammonia (urgently)
  21/22 – plasma amino acids
  14/22 - referral to metabolic team
  9/22 – enzyme analysis
  12/22 - genetic testing
  8/22 – sibling testing/genetic counselling

  Others included protein restriction (2/22), emergency regime required (1/22), ammonia scavengers (2/22), blood gases and LFTs (2/22).

- **Comment**

  Proficiency for this sample was very good with an overall proficiency of 99%. Failure to identify the orotic acid was deemed not to be a critical error by the ERNDIM Scientific Advisory Board as the correct diagnosis was still reached due to the identification of the increased citrulline.

***Patient B***

Clinical details provided: 'Presented with cervical kyphosis. 6 years old. Female.'

**This sample was obtained from a 6 year old girl with Mucopolysaccharidosis Type 6.**

- **Findings**
  For the analytical score, 2 marks were awarded for identifying increased dermatan sulphate and 1 mark for identifying increased glycosaminoglycans (GAGs) with recommendation to do electrophoresis.
  For the interpretative score, including Mucopolysaccharidosis Type 6 as either the primary or alternative diagnosis was given 2 marks and Mucopolysaccharidosis (undefined/wrong one) was given 1 mark.
  1 laboratory used an unconventional method for GAG fractionation and did not report on increased dermatan 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*

  21 of 22 participants scored 2 marks for analysis. 1 participant detected heparan sulphate so were scored 1 mark.
  21/22 participants gave a quantitative result for GAGs. Mean concentration = 40.2 g/mol creatinine (SCH ref. range 6.2 – 12.1), range = 30 – 57.

- **Conclusions**
  21/22 participants scored 2 marks for interpretation. The remaining participant stated that Type 6 was unlikely as heparan sulphate was detected; this laboratory scored 1 mark for interpretation.

- **Further investigations**
  21/22 – enzyme analysis
  14/22 – referral (to specialist)
  9/22 – repeat urine sample
  6/22 – sibling testing/genetic counselling
  5/22 – refer for 2D electrophoresis

- **Comment**
  Performance for this sample was good with an overall proficiency of 97.7%.

***Patient C***

Clinical details provided: 'Severe ketosis following a mild illness. Male. 14 months old'.

**This sample was obtained from a patient with β ketothiolase deficiency.**

- **Findings**
  For the analytical score, detecting both increased tiglylglycine and 2 methyl 3 hydroxy butyric acid was scored 2 marks. Reporting increased excretion of only 1 of these metabolites was scored 1 mark.

  B ketothiolase deficiency (as primary or alternative diagnosis) and 2 methyl 3 hydroxy butyryl CoA dehydrogenase deficiency (as primary or alternative diagnosis) were both scored with 2 marks. This was due to the absence of methylacetoacetate in this sample.

  22/22 participants scored 2 marks for analytical.

- **Conclusions**
  22/22 participants scored 2 marks for interpretation. The clinical details provided may have lead most laboratories to conclude B ketothiolase deficiency as the primary diagnosis (17/22).

- **Further investigations**
  11/22 – fresh urine (for repeat organic acid analysis)
  14/22 – acylcarnitines
  15/22 – enzyme analysis
  18/22 – mutation analysis
  10/22 – sibling testing
  13/22 - referral

- **Comment**
  Proficiency for this sample was 100%.

***Patient D***

Clinical details provided: 'Autistic behaviour. Male. 11 years old'
**This sample was obtained from a healthy child (my son).**

- **Findings**
  No significant abnormality detected, performing at least organic acids and amino acids, was scored with 2 points. Concluding no significant abnormality/normal was scored with 2 points.
  21/22 participants scored 2 marks for analysis. All participants reported back normal results on the analyses they performed.
  1 participant scored 1 mark as they did not perform amino acid analysis

- **Conclusions**
  18/22 participants scored 2 marks for interpretation.
  4/22 participants scored 1 mark. Those laboratories who left the diagnosis section blank or who put 'n/a' were scored 1 mark for interpretation, as they had stated 'normal' or 'no significant abnormality' against each of the test results. However, I have been advised by 2 of the other DPT scientific advisors that I have been lenient with my scoring.

- **Further investigations**
  11/22 participants gave no recommendations
  1 participant stated that no further investigations were indicated
  3 participants requested plasma for a full metabolic screen, including plasma amino acids
  2 participants mentioned mutation analysis of genes implicated in autism

  I believe that the most helpful recommendations were those that advised the attending clinician to contact the laboratory/metabolic biochemists to discuss further testing options based on other clinical information.

- **Comment**
  EQA samples should be treated like patient samples. The diagnosis section has to be completed e.g. No abnormality detected by the tests performed. Please note that in the future, where the interpretation section is left blank, 0 marks will be awarded.

***Patient E***

Clinical details provided: 'Early loss of primary dentition. Female. 4 years old.'

**This sample was obtained from a child with Hypophosphatasia. Further clinical details provided by the laboratory who obtained this sample were premature loss of 2 lower incisors at 2.5 years of age and a few months later. At 3.5 years of age no further teeth loss. X ray of knees – no abnormality detected.**

**Alkaline phosphatase = 158 and 116 IU/l (ref range 250 – 850).**

**PLP = 105 µmol/L (ref 12 – 97)**

**Diagnosis: mild hypophosphatasia**

- **Findings**

  19/22 participants detected the increased phosphoethanolamine and were scored 2 marks. Of those that provided a quantitative result, mean = 51.1 µmol/mmol creatinine, range = 35 – 66, n = 16.

  2 participants did not detect the increased phosphoethanolmine and were scored 0 marks for this sample.

  The remaining participant scored 1 mark for analytical as they stated that phosphoethanolamine was not above the cut off by their LC-MSMS screen, though it was higher than other samples in the batch and would recommend quantitative analysis.

- **Conclusions**

  20/22 participants scored 2 marks for interpretation. 19/20 gave hypophosphatasia as the primary diagnosis. The remaining laboratory gave hypophosphatasia as an alternative diagnosis and stated that 'urine phosphoethanolamine is not a good test for diagnosis of hypophosphatasia' (this was the lab who scored 1 mark for analytical)

  The other 2 laboratories did not detect the phosphoethanolmine and scored 0 for this sample.

- **Further investigations**

  (Excluding the 2 participants who did not detect PEA)
  19/20 - serum/plasma alkaline phosphatase (low in hypophosphatasia)
  15/20 - serum/plasma pyridoxal 5 phosphate
  14/20 - mutation analysis of the *ALPL* gene
  4/20 – skeletal survey
  9/20 - family studies

  Suggestions on who to refer the patient to varied – metabolic, endocrinology and metabolic bone were all mentioned.

  Enzyme replacement therapy was also suggested by 2 participants

- **Comment**

  There were no critical errors for this sample. At the Scientific Advisory Board meeting in 2016 it was decided that this condition is not eligible for critical error as Hypophosphatasia is difficult to diagnose by urinary phosphoethanolamine analysis alone.

***Patient F***

Clinical details provided: 'Presented with jaundice and hepatomegaly. Sample collected while treatment commencing. Male. 6 months old'.

**This sample was obtained from a 6 month old infant who was diagnosed with Tyrosinaemia Type 1. The treatment was NTBC.**

- **Findings**
  The detection of succinylacetone/succinylacetoacetate was scored with 2 marks. Concluding Tyrosinaemia Type 1 was scored with 2 marks. 20/22 participants detected succinylacetone and scored 2 marks. 11 of these 20 also identified hydroxy keto heptanoate. The remaining 2 laboratories did not detect succinylacetone and scored 0 marks.

- **Conclusions**
  All the participants who identified the succinylacetone gave the correct diagnosis.

- **Further investigations**
  (Excluding the 2 participants who did not detect succinylacetone)
  14/20 – plasma amino acids
  11/20 – mutation analysis of the FAH gene
  3/20 – enzyme analysis in fibroblasts/leucocytes
  13/20 – refer to a metabolic clinician/team (most said immediate/urgent)
  6/20 – test siblings
  Many other suggestions:
  Repeat urine for organic acids
  Serum alpha feto protein
  Renal function and liver function tests including coagulation studies
  FBC
  Urine Alpha aminolaevulinic acid (ALA)
  NTBC as treatment or monitoring levels of NTBC
  Quantitative succinylacetone (urine, DBS and plasma all suggested)

- **Comment**
  Failure to detect succinylacetone in this sample was deemed to be a critical error by the Scientific Advisory board. Overall proficiency for this sample was 90.9%.

## 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 2017.

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 2017 ERNDIM Scientific Advisory Board (SAB) meeting.

Following the SAB meeting in November 2017 it was decided that any laboratory failing to identify succinylacetone in Sample F would receive a critical error for this sample. This applies to 2 laboratories in this scheme.

**Educational samples**

Samples may be classed as 'educational' in exceptional cases, e.g. when the metabolite pattern in a sample is particularly challenging and diagnosis is hard to reach or when non-standard methods are required. The Scientific Advisory Board decides whether a sample is classed as educational. When a sample that has been classed educational in an earlier survey is circulated again, it will be scored routinely and cannot be educational for a second time.

There were no samples classed as educational for the DPT UK scheme in 2017.

## 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 | |
| **1** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **2** | 4 | 4 | 4 | 3 | 4 | 4 | **23** |
| **3** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **4** | 3 | 4 | 4 | 3 | 4 | 0 | **18** |
| **5** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **6** | 4 | 4 | 4 | 4 | 0 | 4 | **20** |
| **7** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **8** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **9** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **10** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **11** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **12** | 4 | 4 | 4 | 4 | 4 | 0 | **20** |
| **13** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **14** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **15** | 4 | 2 | 4 | 4 | 4 | 4 | **22** |
| **16** | 4 | 4 | 4 | 3 | 4 | 4 | **23** |
| **17** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **18** | 4 | 4 | 4 | 3 | 4 | 4 | **23** |
| **19** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **20** | 4 | 4 | 4 | 4 | 3 | 4 | **23** |
| **21** | 4 | 4 | 4 | 4 | 4 | 4 | **24** |
| **22** | 4 | 4 | 4 | 3 | 0 | 4 | **19** |

## 8. Proficiency per sample

| Sample | Diagnosis | No of returns | Analytical performance (%) | Interpretative proficiency (%) | Total (%) |
|---|---|---|---|---|---|
| **A** | Citrullinaemia Type 1 | 22 | 97.7 | 100 | **99** |
| **B** | Mucopolysaccharidosis Type 6 | 22 | 97.7 | 97.7 | **97.7** |
| **C** | B ketothiolase deficiency | 22 | 100 | 100 | **100** |
| **D** | Healthy child | 22 | 97.7 | 90.9 | **94.3** |
| **E** | Hypophosphatasia | 22 | 88.6 | 90.9 | **89.8** |
| **F** | Tyrosinaemia Type 1 | 22 | 90.9 | 90.9 | **90.9** |

## Acknowledgements

I would like to thank John Hamilton, Mary-Anne Preece and Stuart Moat for organising the collection of samples used in the scheme this year.

I would also like to thank Mrs Jennifer Watkinson for her work in cataloguing samples and testing them for suitability.

Yours sincerely

Mrs Joanne Croft BSc,MSc
Clinical Scientist
Scheme Organiser