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# ANNUAL REPORT 2009

## 1. Introduction

The Diagnostic Proficiency Test (DPT) scheme for inborn errors of metabolism is run by ERNDIM as before and continues to be the ultimate challenge for diagnostic labs throughout Europe. The Nijmegen/Amsterdam/Rotterdam scheme historically has participants from the Benelux region as well as from the North-western part of Germany.
Following the previous announcement of website reporting of the DPT-findings, insufficient progress in developing the system has been made to enable testing of the website in 2009. It is foreseen that website reporting will become operational in 2010.

Since 20 years this scheme has been run in conjunction with SKML, the Netherlands QA-organization for medical laboratories. Due to changes in the SKML-organization, the actual handling of samples will no longer be carried out by the Nijmegen office but will be transferred to the Winterswijk location in 2010. Accordingly, professor Willems will terminate his activities for this scheme. ERNDIM is particularly grateful to professor Willems for his long-standing expert contributions to the development of Diagnostic Proficiency Testing in the area of inborn errors of metabolism.

The scheme consisted of six urine samples, distributed in January and June; the discussion of the results took place in Basel on the occasion of the ERNDIM workshop on 22nd October. The meeting, as usual open to participants only, was attended by several interested colleagues after consent of all participants. G.Ruijter, Erasmus Medical Center Rotterdam, chaired the meeting, whereas minutes were taken by W.Onkenhout, Leiden University Medical Center, who is acknowledged for this task.
A lively discussion characterized the annual meeting with 12 attending participants from The Netherlands, Belgium, Luxembourg, and South-Africa. Unfortunately, no German participants were present.

## 2 Participants

The 2009 scheme had 22 participating laboratories with the following allocations:

| Country | Number of participants |
|---|---|
| Luxembourg | 1 |
| Belgium | 5 |
| The Netherlands | 11 |
| Germany | 4 |
| South-Africa | 1 |

## 3 Logistics of the scheme

Shipment of samples was effected as in previous years by regular mail. As discussed before this may cause some delay for remote laboratories. This was exemplified by the South-African participants, who found out that they had only one week for their analyses. Especially in the era of website reporting with strict deadlines the system must guarantee an equal amount of handling time for each participating laboratory.
The pan-European sample was supplied by the colleagues of the Basel scheme. Its results were discussed at the Basel ERNDIM meeting and can be viewed at the ERNDIM website, section Meetings and Reports. As it regarded a patient with a lysosomal transport defect of sialic acid, a (semi)-quantitative assay of urine sialic acid was needed to arrive at the correct diagnosis. This proved to be a considerable problem in all schemes, including the present scheme. Consensus was achieved on the package of analyses for diagnosing lysosomal storage disorders which must include the analysis of mucopolysaccharides, oligosaccharides, and free sialic acid.

## 4 Scoring of results

| **For each individual sample a score can be achieved for:** | | |
|---|---|---|
| | | **Score** |
| Analytical performance: | Correct results of the appropriate tests | 2 |
| | Partially correct or non-standard methods | 1 |
| | Unsatisfactory or misleading | 0 |
| | | |
| Interpretative performance: | Good (diagnosis was established) | 2 |
| | Helpful but incomplete | 1 |
| | Misleading / wrong diagnosis | 0 |
| | | |
| Recommendations:<br>(for further investigations) | Helpful | 1 |
| | Unsatisfactory or misleading | 0 |
| | | |
| | Total score | 5 |

Poor performers are those participants who score less than 12 points out of the maximum 25 in this year. These poor performers will receive a so-called warning letter from the Scientific Advisor.
At the request of several participants, individual scoring results will be sent to each individual participant.

## 5 Results of individual samples

The following scores were calculated for the 2009 samples:

| sample | Reports | Correct | Partial correct | Diagnosis |
|---|---|---|---|---|
| H | 18 | 11 | 2 | Barth syndrome |
| J | 18 | 9 | 0 | Galactosemia |
| K | 18 | 13 | 4 | SCAD-deficiency |
| L | 21 | 21 | 0 | Non-ketotic hyperglycinemia |
| M | 21 | - | - | Wilson disease |
| O | 21 | 6 | 0 | Sialic acid storage disease |

The total number of reports was 117 out of the 132 which were expected on the basis of the number of registered participants. For patients H, J, K, L and O 60 out of 96 reports (63%) were correct, approximately the same as in 2008.
The scoring routine proved to be extremely difficult this year, mainly attributable to the complexity of the samples. Four out of six samples were not 'straightforward' and will be discussed here.

The Barth syndrome sample (H) had only a moderately increased level of 3-methylglutaconic acid and the interpretation was further complicated by the presence of large amounts of ketones and lactate.
Galactosemia (sample J) in an adult can only be diagnosed by finding increased galactitol in the urine, especially because the patient had a self-chosen lactose-restricted diet (not included in the supplied clinical information). The clinical presentation was by no means classical.
The Wilson sample did not contain any specific marker enabling its diagnosis apart from the increased level of copper. Only very few labs appeared to be able to produce a figure for copper. Moreover, the biochemical profile was further complicated by a moderate methylmalonic aciduria, correctly identified by all labs.
Taken together it was felt appropriate not to include this sample in the calculation of the performance scores.
A diagnosis of Sialic storage disease involves the assay of sialic acid in the urine. It turned out that not all labs had this assay available and those who did not have this assay failed to consult a neighbouring lab.

## 6 Minutes of the Annual Meeting of the ERNDIM DPT (Nijmegen/Rotterdam/Amsterdam) scheme; Basel, 23 October 2009.

**Present**
Rotterdam: Ruijter (chairman), Wijgerde
Amsterdam: Duran (DPT advisor), Abeling, Kulik
Nijmegen: Ruitenbeek, Kluijtmans
Leiden: Onkenhout (minutes)
Utrecht: de Sain
Almelo: Maatman
Maastricht: Bierau
Enschede: van den Bergh
Luxembourg: Hoffmann
Bruxelles: Martens
Louvain: Marie
Potchefstroom (South Africa): Dercksen

**Welcome**
The new chairman George Ruijter welcomes the participants and because of the presence of some new participants everyone is asked to introduce oneself. Unfortunately there are no representatives from VUMC Amsterdam, Groningen and Tilburg from the Netherlands, Germany and several Belgian metabolic centers. At present there are 22 (21) laboratories participating in the DPT scheme.

**Minutes of the meeting in Lisbon, September 2nd 2008**
The minutes were not discussed.

**Information from the Executive Board, Trust Board and Scientific Advisory Board** (Duran)

1. Participants are asked to provide patient's urine samples. When the urine is used in the scheme DPT participation at a discount will be offered. Also interesting urine samples without a definite diagnosis can be used. For the local DPT scheme a volume of >250 ml is required and for the pan-European sample >1000 ml.
2. From 2009 on there will be 2 assessors for each DPT scheme.
3. Because of the need for accreditation of ERNDIM, cooperation with EMQN (European Molecular Genetics Quality Network) has been started.
4. In October 2010 (20-22?) there will be a SSIEM/ERNDIM training for laboratory workers and clinicians in Manchester. Topic to be announced by ETAC.

**Website reporting**

A new website reporting system is designed by Swiss CSCQ, Geneva. There is not much progress, however in the Basel DPT scheme the website reporting has already been implemented. For all the other participants the website is now available for practicing. In 2010 the website reporting will probably not yet be realized for our DPT scheme.

**DPT 2008 results**

Patient **H**: 3-methylglutaconic aciduria type II (Barth syndrome) (22 participants, 18 reported results, 11 correct)

This was a patient with dilated cardiomyopathy. The differential diagnosis includes lysosomal storage disorders, fatty acid oxidation disorders and glycogenoses. 12 of the laboratories performed purines & pyrimidines, however it is not warranted! All labs that found increased 3-methylglutaconic acid had the correct diagnosis. 3-methylglutaconic acid is not commercially available anymore and most labs therefore use an old calibration curve.

| **Organic acids** | 3-MeGlutaconic | 3-Meglutaric | 3-OHisovaleric |
|---|---|---|---|
| Type I hydratase | 200-1000 | 5-10 | 50-4000 |
| Type II tafazzin | 20-150 | ↑ | - |
| Type III OPA3 | 10-190 | ↑ | - |
| Type IV other | ↑ | ↑ | - |

Ethylhydracrylic acid and 3-OH-isovaleric acid can be increased both in Barth syndrome and ketosis. In this patient 3-OH-butyric acid was also increased.

References: Gibson et al, J Ped (1991) (Syndromes of 3-methylglutaconic aciduria); Wortmann et al, Brain (2009) (3-methylglutaconic aciduria type IV).

Patient **J**: Galactosemia (galactose-1-phosphate uridyl transferase deficiency) (22 participants, 18 reported results, 9 correct)

This was an adult patient with a very peculiar presentation (only cataract and retardation and normal galactose in urine). Because of the cataract determination of polyols is indicated. There was no treatment and no aversion of milk(-products). The untreated urine did not contain galactose.

Reference: Reijngoud et al, Tijdschr Kindergeneesk (1993)

Patient **K**: SCAD (short chain acyl-CoA dehydrogenase) deficiency (22 participants, 18 reported results, 13 correct)

Almost all participants found increased ethylmalonic acid and methylsuccinic acid. 2 laboratories reported very slight increase of butyrylglycine.

3 labs reported (mild) MAD as the diagnosis, however in that case other metabolites (acylglycines, 2-OH-glutaric acid) should have been found. Also acylcarnitines in urine can be helpful in establishing the correct diagnosis.

Patient **L**: Non-ketotic hyperglycinemia (NKH) (21 participants, 21 reported results, 21 correct)

This was an easy case. The hallmark was the extremely increased glycine excretion in urine which ranged from 3980-89000 µmol/mmol creatine for the 21 labs.

Patient **M**: Wilson disease (21 participants, 21 reported results, 8? correct)
This was a complicated case because 1) copper is not determined routinely in the metabolic laboratories 2) the patient was treated and 3) additionally an elevated excretion of methylmalonic acid was found which was not known to the organizers(!).
Several labs did not determine copper and diagnosed this patient as methylmalonic aciduria. The amino acid profile shows peaks of penicillamine (often co-eluting with glycine), penicillamine-cysteine disulfide and penicillamine-penicillamine disulfide. Some labs identified the latter peaks as homocysteine-cysteine disulfide and homocystine, respectively and therefore reported homocystinuria with methylmalonic aciduria as the diagnosis.
Neither the copper excretion in a treated patient with Wilson disease is known nor is the copper excretion in a normal person treated with penicillamine.
Because of the above aspects the scientific advisors found it impossible to devise an adequate scoring for this patient and therefore no points were given. However, this decision has no negative consequences for the overall DPT judgment of any of the participants.

Patient **O**: Salla disease (21 participants, 21 reported results, 6 correct)
This is the common sample and is discussed during the plenary ERNDIM meeting in Basel.

**Any other business/Questions**
Duran: DPT results should be reported within 3 weeks, however in practice 4 weeks is also allowed. In the future, when website reporting is used, the deadlines are very strict.
Dercksen: is it possible to send urine samples to South Africa before sending them to the European participants? Duran: we can consider this.
De Sain: the annual report 2008 of the ERNDIM special assays on the website changed afterwards. Duran: you have to contact the scheme organizer Dr. Burlina.

**Next meeting**
The next ERNDIM DPT meeting will take place on August 31, 2010 in Istanbul during the Annual SSIEM symposium.