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# ANNUAL REPORT 2010

## 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. The Amsterdam/Rotterdam scheme historically has participants from the Benelux region as well as from the North-western part of Germany and one participant from South Africa was recently added to the scheme..
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 2010. It is foreseen that website reporting will become operational either in 2011 or in 2012.

Since 20 years this scheme has been run in conjunction with SKML, the Dutch 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 has been transferred to the Winterswijk location in 2010. Accordingly, professor Willems has terminated his activities for the scheme. In order to achieve a more uniform scoring panel throughout the five DPT schemes, the ERNIM Board has instituted a slight change in the rules of scoring by adding a second scoring officer from one of the partner DPT schemes. The external scores will be discussed with the scheme's own scientific advisor(s). In case of the Amsterdam/Rotterdam scheme, additional scores will be made by the scientific advisor of the Basel scheme.

This year's scheme consisted of six urine samples, distributed in January and June; the discussion of the results took place in Istanbul on the occasion of the ERNDIM workshop held at the SSIEM Annual Symposium on 31st August. The meeting, as usual open to participants only, was attended by representatives of most of the participating institutes. George Ruijter, Erasmus Medical Center Rotterdam, chaired the meeting, whereas minutes were taken by Nanda Verhoeven, University medical Center Utrecht, who is acknowledged for this task.
A lively discussion characterized the annual meeting.

## 2 Participants

The 2010 scheme had 19 participating laboratories with the following allocations:

| Country | Number of participants |
|---|---|
| Luxembourg | 1 |
| Belgium | 5 |
| The Netherlands | 11 |
| Germany | 1 |
| 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. This potential drawback has been overcome by sending the samples to the South African participants two weeks prior to the regular shipment.
The pan-European sample was supplied by the colleagues of the Lyon scheme. Its results were discussed at the 2010 Annual Symposium of SSIEM as part of the ERNDIM workshop. It regarded a patient with a lysosomal defect of oligosaccharide breakdown which was reassuringly good analyzed and interpreted.

## 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 |

Participants who failed to achieve satisfactory performance were those who scored less than 18 points out of the maximum 30 in this year. These so-called poor performers have received a warning letter from the Scientific Advisors which is explicitly aimed at improving the participant's performance in the near future.

## 5 Results of individual samples

The following scores were calculated for the 2010 samples:

| sample | Reports | Correct | Partial correct | Diagnosis |
|---|---|---|---|---|
| P | 18 | 18 | 0 | Cystinuria |
| Q | 18 | 1 | 5 | Essential fructosuria |
| R | 18 | 17 | 1 | Methylmalonic aciduria |
| S | 19 | 18 | 0 | Alkaptonuria |
| T | 19 | 18 | 1 | No inborn error |
| U | 19 | 15 | 1 | Sialidosis type 1 |

The total number of reports was 111 out of the 114 which were expected on the basis of the number of registered participants. For all samples,86 out of 111 reports (77%) were correct, considerably better than in 2008 and 2009. The overall performance of the participants in this year's DPT scheme has to be considered adequate.
One of the samples scored quite badly. It came from a patient with essential fructosuria. Not only the clinical information was quite uninformative, but also the detection of fructose in the regularly performed oligosaccharide analysis turned out to be troublesome. Several participants experienced that retrospective appreciation of their original results did reveal the presence of fructose. In this respect the educational value of the DPT scheme has once more been highlighted.

Minutes of the ERNDIM DPT Amsterdam 2010 discussion

Istanbul, August 31 2010, 9.00-10.30

1. Minutes of the meeting in Basel on October 23, 2009
No comments

2. News from ERNDIM
Accreditation of ERNDIM (mainly the office): regular meetings with the EMQN are ongoing. SKML will be accredited by the end of the year.
The ERNDIM board has decided that the ETAC training will take place on October 4$^{th}$ and 5$^{th}$ in Manchester. The laboratory topic includes 3 topics over 2 days: MPS disorders and oligosaccharidoses, purine-pyrimidines and the peroxisomal disorders.

3. Website reporting
Accounts and passwords have been circulated to all participants in June. All participants have the opportunity to practice with the 2009 and 2010 samples. The aim is that everyone will report via the website in 2011 but is not absolutely certain that all deadlines will be met. The process has to be simplified. Comments and suggestions can be sent to George Ruijter.

4. Any other business
Currently 2 ID codes are used in the list of participants: an 'old' SKML number and the ERN number. Please use the ERN number for all future correspondence (including reporting results).
All samples were received in time by the labs in the past year.
Please provide urine samples, minimum 300 mL. This will give you a 20% discount in the year following utilisation of the sample in the scheme. For the common sample, 1.5 L is required.
New ideas for additional tests are welcome. Galactitol, mevalonic acid and urine amino acids were suggested. Oligosaccharides may be included in the MPS scheme. It was suggested that it would be valuable to upload oligosaccharide profiles when the website is in use.

Discussion of the 2010 samples P-Q-R-S-T (U was the common sample).

P. Cystinuria
The DD of metabolic causes of kidney stones is: cystinuria, xanthine DH, APRT, hyperoxaluria types 1, 2, and 3, uric acid overproduction/hyper excretion, orotic acid overproduction and low citrate excretion.

In case tandem MS is used for amino acid quantification, one should be aware of the possibility of cystinuria in case lysine, arginine and ornithine are elevated (cystine is not analysed).
The lactate observed in the urine may have been caused by bacterial contamination of the sample (the pH of the sample was high). Some participants observed the cysteine-homocysteine mixed disulfide. Its elevated level in urine may be explained if transport also occurs via the 'cystine' transporter.
There is a new classification for cystinuria: type A is a defect of SLC3A1, type B is a defect of SLC7A9. Heterozygotes of the B type may develop kidney stones; they regularly have an increased urine excretion of cystine and lysine.

Q Essential fructosuria
Only one participant came to the correct diagnosis. The dipstick fails to detect this amount of fructose. Twelve laboratories performed oligosaccharide analysis, 4 reported elevated fructose, 4 the presence of a hitherto unknown monosaccharide. Five laboratories reported elevated fructose on the basis of sugar analysis.
The fructosuria does not explain the mental retardation. However, most participants felt that metabolic laboratories should diagnose essential fructosuria.

R. Methylmalonic aciduria
This was a relatively straightforward sample to analyse.
How are propionylglycine and methylcitrate quantified, as apparently no standards are commercially available. Mirjam Wamelink will inquire after the origin of the standards (note added by GR: propionylglycine and [3,3,3-$^{2}H_{3}$]propionylglycine can be purchased from Herman ten Brink at the VU medical centre in Amsterdam, methylcitrate from CDN Isotopes, Pointe-Claire, Quebec, Canada)

S. Alkaptonuria
Homogentisic acid was quantified by most labs (median value 4000 mmol/mol; range 306 – 18700). GC or GC-MS may result in values far from the true number. HPLC and colorimetric determination resulted in the following values: HPLC 7639 mmol/mol, colorimetric 6146 mmol/mol.
Homogentisic acid interferes in creatinine determination performed with the enzymatic assay. In alkaptonuric patients, the Jaffe method (colorimetric), HPLC or MS/MS should be used for creatinine quantification. In addition it has been observed that in alkaptonuria urine samples creatinine decreased during storage.
Some labs reported that this sample also contained elevated lactate and 3-methyl-glutaconic acid. Slightly elevated 3-methyl-glutaconic acid may be normal in adult women and there may be a relation to pregnancy (Walsh et al 1997 Lancet 349:776).
This sample was also used in 2000 (patient C). Creatinine averaged 4.5 mmol/L at that time (in 2010: 1.5), exemplifying the continuing apparent decrease of the creatinine value in alkaptonuric urine.
Purines and pyrimidines have been performed by some labs since a male with a HPRT variant has been described with neurological problems after exercise.

T. No inborn error of metabolism.
Macrocephaly DD: L-2 hydroxyglutaric aciduria, glutaric aciduria type I, Canavan disease, respiratory chain defects, GM2, Tay Sachs, alpha mannosidosis, Krabbe disease, mucopolysaccharidosis.
Glutarylcarnitine in urine is a marker for glutaric aciduria type I.

General remark: always try to give advice for follow-up investigations to qualify for 1 point for this item.

The next meeting of this group, discussing the results of the 2011 rounds of testing will be held in Geneva, Switzerland on the occasion of the SSIEM Annual Symposium, provisional date 30th August 2011