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Nijmegen / Amsterdam

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1. Introduction

The Quality Assurance Program (QAP) for Diagnostic Proficiency Testing (DPT) of Inherited Metabolic Diseases is organised by ERNDIM. The DPT centre of Central Europe is one of the four DPT centres of ERNDIM and is located in Nijmegen / Amsterdam (NL). The other DPT centres are located in Sheffield (UK) for the region of Northern Europe, in Lyon (F) for the region of Southern Europe, and, in Prague (CZ) for the region of Eastern Europe. Each DPT centre has approx. 20 participants.
The SKZL is a Dutch QAP-organisation collecting samples of urine from patients with metabolic diseases (participants are obliged to deliver these samples). Twice a year SKZL distributes these samples to the participants of the DPT scheme, evaluates all results, prepares a report and make the reports available to the participants. Once a year a meeting of the participants is organised to discuss the results, to bring faulty results into focus and to discuss recommendations for improvement.

2. Participants

In 2003 the DPT centre Central Europe had 20 participants from Germany, The Netherlands, Belgium and the United Kingdom.

Country	Number of participants
Germany	7
The Netherlands	10
Belgium	2
UK	1

3. Logistics of the scheme

In the DPT scheme 2003 six samples were distributed:

2003.1: patient E, F and G
2003.2: patient H, I and K

Sample E was the common sample which has been distributed by all four DPT centres.

In 2003 samples free from conservatives have been shipped on dry ice. The result form was distributed by e-mail. Participants returned the result form by e-mail.

4. Timetable of the scheme

DPT survey 2003.1:

Shipment of samples:	January 7, 2003
Deadline for returning results:	January 26, 2003
Report of survey	May 8, 2003

DPT survey 2003.2:

Shipment of samples:	June 17, 2003
Deadline for returning results:	July 6, 2003
Report of survey:	September 29, 2003

Meeting of participants: October 3, 2003 (Maastricht, NL)

5. Receipt of samples and results

Survey 2003.1 : 20 participants received the samples and all participants returned their results.
Survey 2002.2 : samples had been sent to 20 participants ; 18 of these returned their results.

6. Scoring of results

The merits of a the scoring system of a DPT scheme were discussed at the September meeting of the Scientific Advisory Board of ERNDIM in 2002. At the end of the year 2002 a final scoring system has been agreed upon. In 2003 the scoring system is introduced as a pilot.

For each individual sample a score can be achieved for:

Analytical performance:	Correct results of the appropriate tests	score: 2
	Partially correct or non-standard methods	score: 1
	Unsatisfactory or misleading	score: 0
Interpretative performance:	Good (diagnosis was established)	score: 2
	Helpful but incomplete	score: 1
	Misleading / wrong diagnosis	score: 0
Recommendations:	Helpful	score: 1
(for further investigations)	Unsatisfactory or misleading	score: 0
		=======
	Total:	score: 5

7. Scores of participants for individual samples

Scores DPT 2003			Samples in 2003 (E - K) Maximum score is 30				
Lab	**E**	**F**	**G**	**H**	**I**	**K**	**total**
1	5	5	5	5	5	3	28
2	5	5	5	5	1	4	25
3	5	5	5	5	5	5	30
4	5	5	5	3	1	5	24
5	5	3	5	2	1	3	19
6	5	5	5	3	1	5	24
7	4	5	5	5	1	5	25
8	5	5	5	3	5	4	27
9	4	5	5	2	2	2	20
10	5	5	5	5	1	4	25
11	3	5	5	3	2	3	21
12	3	5	4	3	3	3	21
13	5	5	5	3	3	5	26
14	3	5	5	5	1	5	24
15	4	5	5	5	2	3	24
16	4	5	5	5	1	3	23
17	5	5	5	5	1	3	24
18	4	5	5	0	0	0	14
19	5	5	5	0	0	0	15
20	3	5	5	3	1	3	20

8. Distribution of total scores for individual laboratories

See table in item 7. In the DPT – centre Central Europe the following was observed.
A score of more than 75% correct diagnoses which means 23 points or more is achieved by 13 / 20 labs. A score below 75% (less than 23 points) was found with 7 / 20 labs. And a score below 50% (less than 15 points) was found in 1 lab.

9. Summary of scores

Sample	Diagnosis	Correct diagnosis
E	Sialidosis	16 / 20
F	Malonaciduria	20 / 20
G	Arginase deficiency	20 / 20
H	Cystinosis	13 / 18
I	Sulphite oxidase deficiency	4 / 18
K	Chronic tubulo-interstitial nephritis of obscure genesis (after kidney biopsy) with secondary Fanconi syndrome	12 / 18

10. Meeting of participants, in Maastricht (October 3, 2003)

Present: Abeling, Dorland, Eyskens, Gennip (chairman), Gerlo, Huijmans, de Jong, Langhans, Lefevere, Poorthuis, Reijngoud, de Sain, Spaapen, Verhoeven, Wamelink and Holtrop (secretary)
Apologies: Duran, Onkenhout, Peters (substituted by Langhans), Sass, Sewell, Starke.

1. Welcome.

The chairman welcomes the participants.

2. Minutes of the meeting held at September 3, 2002 Dublin.

No comments. The minutes are finalised with thanks to Holtrop for preparing them.
An annual report of 2002 will be sent to Reijngoud. **Action: Holtrop**

To the participants it is not quite clear what has to be answered to the question on the report form: "Advice to clinician" ? What are the criteria and how about the scores for this item. This is different for each country. This year no score was given for this item. The scoring system mentioned in item 6 of this annual report is acceptable for the German participants. The criteria have to be discussed in more detail in the Scientific Advisory Board (SAB). **Action: van Gennip**

3. Information

a. Heat inactivation of DPT samples send at room temperature

Heat inactivation of DPT samples is also introduced in the DPT centre Central Europe. In Sheffield no problems have been seen with samples from patients with succinylacetone deficiencies.

The following protocol for heat inactivation is used :
[The protocol that is used is : 1. Add thiomersal 100mg/L of urine ; 2. Heat urine to 56°C for one hour (waterbath) . After this the sample can be send at room temperature.]

b. DPT samples in stock (use of data form)

Delivery of urine samples (300 mL) is highly requested. Detailed information about the sample including clinical information is important to know and to characterize the correct diagnosis for the scientific advisor and the scheme organizer. How the diagnosis is confirmed is very important to report as well.
Participants are requested to use this data form when they sent the obliged sample (every year) to the scheme organiser. The data form is almost identical to the DPT result form. Please fill in the

information about age of collection of the sample, consanguinity, clinical symptomatology, medication, if known, antibiotics, if known, routine biochemical analysis, if known, etc.

Discussion: in the past the clinical information was often misleading or it already gave the diagnosis. From now on the samples, including "old" samples are checked and analysed again by a reference lab before distribution. All information and results are judged by the scientific advisor and the scheme organizer of the DPT-scheme.

c. Introduction of the scoring system

The results of the DPT data reports are judged by the scientific advisor and the scheme organizer. If no result report had been sent or no diagnosis had been mentioned the score is zero. Diagnosis and recommendations is coupled; when one has a wrong diagnosis then the recommendations are wrong as well.
What is the definition of the minimal diagnostic package of a lab for metabolic diseases ? What is state-of-the-art ? E.g. electrophoresis is a standard technique; the dimethylene test and the MPS are included. Suggestions for criteria for the minimal package can be discussed during these DPT meetings as well.

d. ERNDIM certificate (reporting results)

The ERNDIM certificate is distributed to the participants when they report their results and pay the annual fees. The certificate does report anything about performance. The certificate is given for participation and valid for one year.
What about participants who pay the annual fee but who do not send any result forms ? Do they receive a certificate for participation ?

4. Annual contribution of DPT samples

What are the consequences when participants do not deliver the obliged annual sample ?
E.g. 1. send a reminder, 2. send an aggressive reminder 3. stop distribution until obligations are fulfilled, …..

There are enough samples from patients with organic acidurias, but there is a shortage of samples with other abnormalities. For the common sample a volume of 1000 – 1250 mL of urine is needed. How do we get these amounts of urine ? In most cases samples of treated patient are delivered. To achieve a crisis urine is a real problem.
Suggestions:

a. dilution: is no option; diagnoses can be missed and problems are expected with mucopoly-saccharidosis ;
b. mixing samples : is an option. The composition of all the urines used for mixing should be mentioned and the mixed sample has to be analysed before delivery to SKZL.
c. mixing by SKZL : is an option. When the index metabolites, conditions and clinical information are okay then no problems have to be expected.
d. All samples are re-analyzed by SKZL shortly before their distribution.

The lab that delivers samples for the scheme "proficiency testing" is responsible for asking permission from the patient to use its sample for quality control studies.

5. Discussion of the results of the survey 2003-1 (samples E, F and G) and 2003-2 (samples H, I and K).

The result reports had been sent rather late again this year. The results had been evaluated by prof. dr. Willems and by the scientific advisor for the DPT-scheme, dr. van Gennip, as well.

<u>Sample 2003.1 - E : Sialidosis (common sample, distributed in all 4 DPT-centres)</u>
In the DPT-centre Central Europe five participants reported a wrong or no diagnosis. The clinical information was very detailed. For the lab it is important to choose which analytes can be analysed quickly and fast to achieve correct diagnosis.

Several participants notice that the results that they had been reported to SKZL are not included in the overview of the results. Many typing errors have been found as well. Improvement of the quality of the overview of the result is needed. The problem will be discussed with Willems, who has prepared the overview. **Action: van Gennip / Willems**

Previously, it was agreed that certain symbols would be used in the result report. However, in some cases the local printers and / or software did not recognize these signs and no signs had been printed. Advice: use text instead of open or black boxes or arrows

Advice: In general, when you measure abnormalities in a sample you will ask for a second sample. When it is impossible to come to a diagnosis with a certain DPT-sample and normally you should ask for another sample then mention this in the result form.

Sample 2003.1 – F : Malonaciduria

Every participant reported a correct diagnosis.
Analysis of uric acid is difficult because of the technical problems with solubility. The sample has to be pre-treated. Recommendations for the analysis of uric acid are :

[Preanalytical instructions for the analysis of **uric acid** and **oxalate** in urine.

1. Collect 24 hr urine (4°C).
2. Deliver the total sample to the lab (immediately; otherwise freeze at –20°C).
3. Measure the volume of the urine, adjust pH to 7.
4. Heat at 40°C for 20 minutes (ultrasonic bath).
5. Take a sample for uric acid and all other assays.
   ↳ Analyse uric acid according to local procedures. To this purpose a further adjustment of the pH may be necessary.
6. For oxalate assay adjust the pH of the remaining urine to pH 2 – 3 (6 M HCl).
7. Mix well.
8. Store at 4°C overnight.
9. Take a sample and store until analysis (max. 1 week at 4°C, otherwise store at –20°C).
   ↳ Analyse oxalic acid according to local procedures.]

Again some data are not reported in the overview. Advice to SKZL: send a confirmation by e-mail when the result form including attachments has been received. **Action: van Gennip / Willems**

Analysis of malonic acid is difficult in quantitative QC- schemes; a range from 410 – 2170 was found. Co-elution with methylmalonic acid is often found and may be the cause of the variation. Advice : in future a sample like this should be distributed in the DPTscheme again.

Sample 2003.1 - G :Arginase deficiency

The diagnosis had been confirmed enzymatically. Arginine is elevated in plasma. The increase in urine is dependent on the concentrations in plasma. In general, due to time of storage arginine is converted to ornithine. Dihydrothymine is very unusual in these samples. Normally, plasma samples are needed to find a correct diagnosis; arginine deficiency cannot be diagnosed with a sample of urine. OCT and urea cycle disorders were also accepted and scored as correct diagnoses. For further investigations it is recommended to do mutation analysis and after that perhaps a liver biopsy is relevant.

Sample 2003.2 – H : Cystinosis

This diagnosis cannot be made in a urine sample.
Correct diagnoses are: Fanconi syndrome and hyperaminoaciduria.
Gamma-glutamylcycle defect is a wrong diagnosis and the diagnosis "cystinosis" is not possible.
The description of the clinical symptoms is not very clear and might indicate ketonuria, lactic aciduria an respiratory chain defect. Interpretation of this sample is very difficult.

Sample 2003.2 - I : Sulphite oxidase deficiency

The diagnosis sulphite oxidase deficiency is difficult to make in urine; in plasma it is somewhat easier. Suggestions: introduce the analysis of thiosulphate. When a patient has convulsions always analyse thiosulphate.

Sulphite has been disappeared in a pre-heated sample. Analysis of sulphite by urine dip sticks is less sensitve. When dip sticks are used in the diagnostic procedure then test the sticks on a regular basis in standard conditions (quality control) for sensitivity, linearity etc.

Sample 2003.2 - K : Secondary Fanconi syndrome
A generalized aminoaciduria was found. In the overview a FeCl3 test is mentioned. No one in the audience is familiar with this test. Can anyone of the participants explain why this test was used ? Please contact dr. van Gennip about this.

A wrong diagnosis is cystinosis. A mitochondrial defect cannot be excluded, and is not known yet To give an advice to the clinician is very difficult in this case. The right advice is: treatment with cysteine binding compounds.

To improve the DPT scheme : In future the description and analysis of the samples has to be checked by the scientific advisor before distribution. **Action: van Gennip / Willems**

6. Results of the survey for analysis of oxalate, uric acid and creatinine (distributed in 2001)
At the meeting the information was not available.
[Hereby we send you the results of this scheme. About 50% of the participants had reported the results.

Centre ERN/skml	Creatinine mmol/L	Oxalic acid mmol/L	Uric acid mmol/L
ERN078/ 4	NR	NR	NR
ERN016/ 16	3.62	0.250	0.35
ERN073/ 26	3.58	0.234	0.4
ERN074/ 28	3.50	0.25	0.38
ERN082/ 87	3.9	0.214	0.34
ERN072/ 113	3.9	0.33	0.40
ERN132/ 286	3.87	0.238	0.428
ERN065/ 590	NR	NR	NR
ERN054/ 591	NR	NR	NR
ERN087/ 592	NR	NR	NR
ERN116/ 1178	NR	NR	NR
ERN077/ 1189	NR	NR	NR
ERN237/ 1242	NR	NR	NR
ERN120/ 1263	NR	NR	NR
ERN157/ 1264	4.1	0.225	0.40
ERN133/ 1786	NR	NR	NR
ERN017/ 2056	NR	NR	NR
ERN075/ 2098	4.6	NR	0.332
ERNxxx/ 2348	4.33	0.165	NR
ERN238/ 2911	NR	NR	NR
ERN093/ 2948	4.12	0.275	0.370
mean	3.93	0.238	0,378
SD	0.36	0.046	0.034
VC in %	9	19	9

NR : no results received]

7. Any other business
None.

8. Date of the next DPT meeting
The next meeting will be organised on August 31, 2004 in Amsterdam at the SSIEM venue.

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Amsterdam, July 2004
S. Holtrop