ERNDIM Urine mucopolysaccharides ANNUAL REPORT 2012

Scheme organisers

Dr. G.J.G. Ruijter
Erasmus Medical Center
Dep. Clinical Genetics Ee2422
Lab. Genetic Metabolic Diseases
P.O. Box 2040
3000 CA Rotterdam
Netherlands
e-mail: erndim-mps@erasmusmc.nl

Dr. C. Weykamp
Streekziekenhuis Koningin Beatrix
Beatrixpark 1
7101 BN Winterswijk
Netherlands
e-mail: c.w.weykamp@skbwinterswijk.nl

1. Introduction

In 2010 and 2011 the Urine Mucopolysaccharides (MPS) scheme was organised as a pilot study by Erasmus Medical Centre (Rotterdam, NL) and SKML, the Dutch organisation for quality assurance in medical laboratories (Winterswijk, NL). The scheme has been continued in 2012 as a regular ERNDIM programme: ERNDIM Urine Mucopolysaccharides.

2. Design of the scheme and logistics

The Scheme has been designed and coordinated by Dr. George Ruijter (scientific advisor). Dr. Cas Weykamp at MCA laboratory has prepared and shipped the samples (scheme organiser).
In 2012 the scheme consisted of 6 lyophilised urine samples as described in Table 1. The scheme format was kept identical to that of 2011. Samples were distributed in February. Participants were asked to reconstitute each sample in 5 mL deionised water, to determine creatinine concentration (mmol/L) and GAG concentration (mg/mmol creatinine), to qualify the GAG level according to age-matched reference values (i.e normal or increased), to analyse GAG sub fractions and qualify (i.e. normal or increased CS, HS, DS and KS) and to give the most likely diagnosis.

Table 1. 2012 samples

Survey, reporting deadline	Sample no.	Sample type
2012-1, April 30, 2012	MPS15	MPS IV A (m, 18 y)
	MPS16	MPS II (m, 15 y)
	MPS17	Normal control (m, 4 y)
2012-2, June 30, 2012	MPS18	MPS I Scheie (m, 9 y)
	MPS19	MPS III A (m, 9 y)
	MPS20	MPS II (m, 25 y)

3. Participants

In 2012 a total number of 102 laboratories from many different countries participated in the Urine MPS scheme (Table 2). The number of participants has increased slightly compared to the two years of MPS pilot study. In 2011 the MPS pilot scheme had 89 participants (2010: 88).

Table 2. Number of participants in 2012 per country.

Country	No. of participants		Country	No. of participants
ARGENTINA	1		LATVIA	1
AUSTRALIA	6		LUXEMBOURG	1
AUSTRIA	1		MALAYSIA	2
BELGIUM	4		NETHERLANDS	8
BRAZIL	1		NEW ZEALAND	2
CANADA	2		NORWAY	1
CHINA	3		POLAND	1
COLOMBIA	1		PORTUGAL	3
CROATIA	1		REPUBLIC OF SINGAPORE	1
CYPRUS	1		SLOVAKIA	2
CZECH REPUBLIC	1		SOUTH AFRICA	1
DENMARK	1		SPAIN	3
ESTONIA	1		SWEDEN	1
FRANCE	8		SWITZERLAND	2
GERMANY	6		TURKEY	3
GREECE	1		UK	16
INDIA	1		UKRAINE	1
ISRAEL	1		USA	8
ITALY	3		VENEZUELA	1

4. Samples

As for other qualitative schemes the Urine MPS scheme requires authentic patient samples. Several laboratories have donated samples in the past, for which they are gratefully acknowledged. To be able to continue this scheme we need a steady supply of new patient samples. If you have one or more samples available and are willing to donate these to the scheme, please contact us at erndim-mps@erasmusmc.nl.

5. Reporting

Reporting was done by completing pre-designed forms. Two reporting deadlines were chosen: April 30 and June 30. Reports were submitted by email to the scheme advisor (erndim-mps@erasmusmc.nl).
In addition to results, the first reporting form (April 30, 2012) included a section to describe methods.
In 2012 on average 90 reports were received per sample (range 89-90) from 92 different participants.
In 2011 the average number of reports was 79.

6. Methods

In the first report participants were asked to specify their methods. This question had two aims. First to make an inventory of methods in use (Table 3) and second to investigate whether relations exists between methods and diagnostic proficiency. The latter will be studied later, i.e. when a sufficient number of different samples have been included in the scheme.
Methods were provided by 89 laboratories.

Table 3. Methods reported by participants.

Method for quantitative analysis		Standard material		Method for qualitative analysis	
DMB	83 %	CS, C4S, C6S	65 %	1-D electr (limited resolution)	30 %
Alcian Blue	7 %	HS	27 %	1-D electr (discontinuous)	32 %
Uronic acid (carbazole)	6 %	DS	3 %	TLC	17 %
CPC (turbidometric)	3 %	Glucuronolacton	4 %	2-D electrophoresis	13 %
Azure A	1 %	Multiple	1 %	1-D agarose electrophoresis	2 %
				Multiple	6 %

7. Results of the 2012 samples

Results are summarised in Table 4.

7.1 Quantitative results

Quantitative GAG results were evaluated separately for each method (DMB, Acian Blue, Uronic acid/carbazole, CPC/turbidity). Most participants use DMB (83 %) for quantitative GAG analysis (Table 3). The number of participants using the other 3 methods is small, which prohibits statistically meaningful interpretation. For each sample the average GAG values produced by the DMB, Acian Blue and CPC/turbidity methods were similar, with the exception of lower CPC/turbidity values of samples MPS15 and MPS17 (Fig. 1). These 2 samples had comparatively low GAG concentrations regardless of the method used. This may cause suboptimal GAG precipitation by CPC and lead to underestimated GAG values. The uronic acid/carbazole method uses a different standard for quantification and the results can not be compared directly to the other methods.
Interlaboratory CVs of DMB results were 21-41 % for the 6 different samples with a tendency of lower variation for samples with higher GAG concentrations.

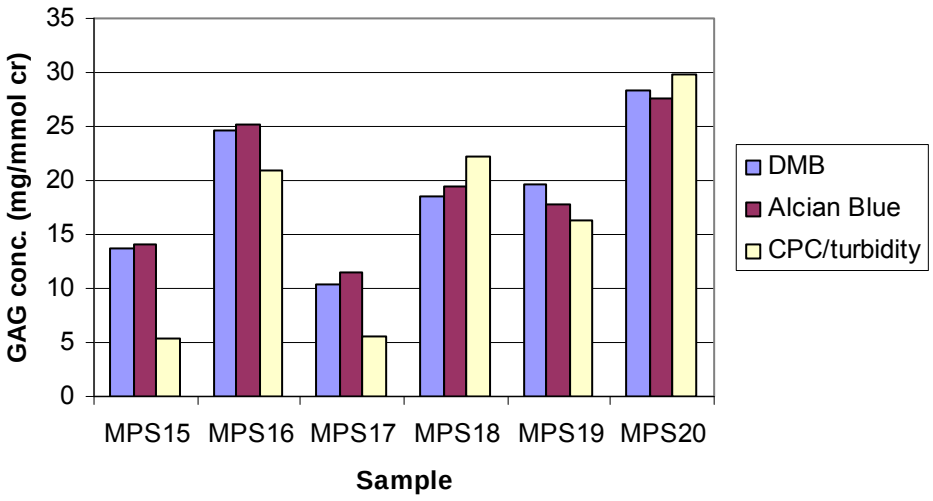

Figure 1. Average quantitative GAG results of different methods.

Interpretation of quantitative GAG results, i.e. labelling results as normal or increased, appeared to be very good for samples MPS16, MPS17 and MPS20 (94 to 99 % correct; Table 4). GAG concentrations apparently were clearly elevated in urine of the 2 MPS II patients (MPS16 and MPS20). A lower level of correct interpretation was obtained for sample MPS15, MPS18 and MPS19 with 85-87 % of the laboratories interpreting their results as increased compared to their age-matched reference values. These 3 urine samples were obtained from a Morquio, a Scheie and a mild Sanfilippo patient.

Apparently, it is more difficult to interpret GAG levels for these relatively mild MPS patients. Amongst the participants who interpreted the quantitative results of samples MPS15, MPS18 and MPS19 as normal, performing qualitative analysis (i.e. electrophoresis or TLC) did result in a considerable number of (partially) correct diagnoses: 16 out of 35 sample analyses.

Table 4. Results of samples MPS15 to MPS20

Sample ID	**MPS15**	**MPS16**	**MPS17**	**MPS18**	**MPS19**	**MPS20**
Diagnosis **Age of patient**	MPS IVA 18 y	MPS II 15 y	Normal 4 y	MPS I 9 y	MPS III 9 y	MPS II 25 y
No. of reports	90	90	90	89	89	89
Creatinine (mmol/L)						
Average	2.24	3.83	2.51	3.63	3.66	4.27
SD	0.50	0.65	0.49	0.54	0.52	0.72
N	88	88	88	87	87	87
GAG (mg/mmol)						
DMB						
Average	13.7	24.6	10.4	18.6	19.7	28.3
SD	4.7	5.2	4.3	5.5	5.7	6.4
Median	13.6	24.3	9.8	18.0	19.4	28.4
n	69	70	69	68	67	68
Alcian Blue						
Average	14.0	25.1	11.4	19.4	17.7	27.6
SD	10.7	9.8	8.1	5.9	7.6	7.7
Median	13.0	28.0	12.0	17.8	17.5	28.6
n	6	7	6	6	6	6
Uronic/carbazol						
Average	7.2	8.0	3.2	5.2	7.3	8.1
SD	9.9	7.5	3.2	5.3	7.0	8.1
Median	2.5	5.3	1.5	3.4	4.7	6.1
n	4	4	3	5	5	5
CPC/turbidity						
Average	5.3	21.0	5.6	22.3	16.3	29.8
SD	2.2	1.4	1.6	5.5	3.6	2.8
Median	5.7	21.2	6.0	24.5	15.0	28.3
n	3	3	3	3	3	3
Quantitative GAG						
Increased (%)	85	99	6	86	87	97
Normal (%)	15	1	94	14	13	3
Diagnosis						
Correct (%)	54	49	83	18	71	47
Part. correct (%)	10	33	3	43	4	33
Not correct (%)	21	9	4	32	15	11
No diagnosis %)	14	9	9	7	10	9

7.2 Qualitative results

For sample MPS15 (Morquio syndrome) 77% of the participants (51/66) reported elevated keratan sulfate (KS). In addition, 16% (11/68) of the participants reported elevated chondroitin sulfate (CS). Galactose 6-sulfate sulfatase, the enzyme which is deficient in MPS IV, is involved in degradation of C6S and C6S may therefore accumulate in MPS IV patients.
In the two MPS II samples MPS16 and MPS20, dermatan sulphate (DS) was reported elevated by the majority (99%) of the participants. Heparan sulphate (HS) was found increased by 78% of the

participants in MPS16 and by 76% in MPS20. Similarly, 97% of the participants reported increased DS in the Hurler sample (MPS18). However, only 37% of the participants reported increased HS in sample MPS18. Apparently, the dermatan sulfate fraction was predominant in this sample, which may have masked heparan sulfate.
Sample MPS19 from a Sanfilippo patient was reported to be abnormal with elevated HS by 90% (69/77) of the participants. Electrophoresis or TLC patterns of sample MPS17 from a healthy infant were interpreted as normal by most of the participants (95%).

7.3 Most likely diagnosis

Diagnostic proficiency for samples MPS16 and MPS20 (both Hunter syndrome) was similar: 82% and 80% respectively. Proficiency was 64% for MPS15 (MPS IV), which is slightly better than obtained for another MPS IV sample circulated in 2010 (59% proficiency). Diagnostic proficiency of the remaining samples was 86% for MPS17 (normal control), 75% for MPS19 (MPS III) and 61% for MPS18 (MPS I). With regard to sample MPS15 (Morquio), the majority of the laboratories that did not come to the right diagnosis scored this sample as normal (15/19). For MPS19 (MPS III) this number was 7/13.
The relatively low percentage of correct diagnoses reported for sample MPS18 (MPS I), is because many laboratories (24 %) diagnosed this sample as MPS VI. As described above, in this sample HS was detected by less than half of the participants, which explains the low diagnostic proficiency. In 2011 another MPS I sample gave identical results. This underscores the difficulty to distinguish MPS I from MPS VI samples on the basis of urine mucopolysaccharide analysis with present technologies.

On average, 10 % of the laboratories did not report a diagnosis (range 7-14 for the 6 different samples). This was mainly due to the fact that these laboratories did not perform qualitative analysis of GAG. An exception was sample MPS15 for which 14 % of the participants did not suggest a diagnosis. With this sample many laboratories reported the absence of bands or the presence of faint bands upon qualitative analysis, which precluded a diagnosis.

8. Scoring of results

In 2012 a scoring system was developed. Similar to other qualitative (proficiency testing) ERNDIM schemes, the maximum score for a sample is 4 points. Points are allocated to diferent elements of the scheme (Table 5).
Qualitative results and diagnostic proficiency of the 2012 samples were scored using the criteria given in Table 6 and 7. These criteria have been set by the Scientific Advisor and have been devised on the basis of (1) for each sample: the type of MPS, (2) current possibilities of routine MPS testing, and (3) actual achievable results for a particular sample.

Table 5. Scoring of results

Item	Description of scoring criteria	Score
Quantitative results	Correct classification of quantitative results (i.e. normal or increased) according to reference values	1
	Incorrect classification of quantitative results	0
Qualitative results	Correct results according to criteria set for the sample as defined by scientific advisor (Table 6)	1
	Incorrect: minimallly required results not reported	0
Diagnostic proficiency	Correct according to criteria set for the sample as defined by scientific advisor (Table 7)	2
	Partially correct	1
	Unsatisfactory or misleading	0
	Maximum total score	4

Table 6. Criteria used for scoring qualitative results of 2012 samples

Sample	To obtain 1 point the report should state (minimally)
MPS15	Increased KS
MPS16	Increased DS
MPS17	Normal results for all GAG types, or increased CS only
MPS18	Increased DS
MPS19	Increased HS
MPS20	Increased DS

Table 7. Criteria for scoring of diagnostic proficiency of 2012 samples

Sample	Diagnoses (or combinations of possible diagnoses) scored as correct - 2 points	Combinations of possible diagnoses) scored as partially correct - 1 point	Not correct - 0 points
MPS15	MPS IV	Normal or MPS IV	Normal Any other (combination of) MPS No diagnosis
MPS16	MPS II MPS I or II MPS I or II or VII	MPS I or II or VI MPS I or II or VI or VII	Normal Any other (combination of) MPS No diagnosis
MPS17	Normal	-	Any (combination of) MPS No diagnosis
MPS18	MPS I MPS I or II MPS I or II or VII	MPS I or II or VI MPS I or II or VI or VII	Normal MPS VI Any other (combination of) MPS No diagnosis
MPS19	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS20	MPS II MPS I or II MPS I or II or VII	MPS I or II or VI MPS I or II or VI or VII	Normal Any other (combination of) MPS No diagnosis

The final decision about scoring of the scheme is made in the Scientific Advisory Board. During its meeting in London on November 29, 2012, the Board decided that satisfactory performance required at least 12 points out of the maximum 24 in this year.
Distribution of scores in 2012 is depicted in Figure 2. In 2012, 84% of the participants achieved satisfactory performance (≥12 points), while 66% had at least 18 points. From the 15 participants that did not accomplish satisfactory performance, 5 obtained a low score due to incomplete submission of results (i.e. 1 survey report submitted instead of 2 reports).
Scores will be sent to individual participants by email.
ERNDIM provides a single certificate for all its schemes with details of participation and satisfactory performance.

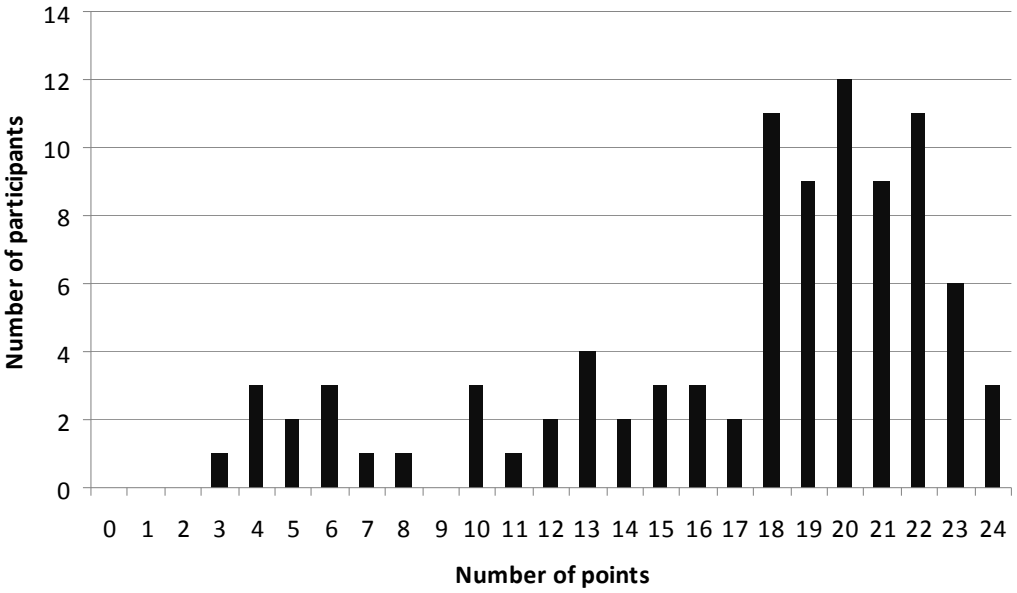

Fig. 2. Distribution of scores in 2012

9. Preview of the scheme in 2013

The format of the MPS 2013 scheme will be similar to 2012.
In 2013 we will start to develop website reporting in collaboration with CSCQ, the Swiss organisation for quality control. The CSCQ has also developed website reporting for the ERNDIM Diagnostic Proficiency Schemes. According to current planning, reporting of the Urine MPS scheme results will still be done by email in 2013.

10. Questions, Comments and Suggestions

If you have any questions, comments or suggestions, please address to the scientific advisor of the scheme, Dr. George Ruijter (erndim-mps@erasmusmc.nl) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).