ERNDIM MPS Pilot Study
REPORT 2010

Scheme organisers

Dr. G.J.G. Ruijter
Dr. J.G.M. Huijmans
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
SKML
Streekziekenhuis Koningin Beatrix
Beatrixpark 1
7101 BN Winterswijk
Netherlands
e-mail: c.w.weykamp@skbwinterswijk.nl

1. Introduction

In 2010 the Mucopolysaccharidosis (MPS) scheme was organised for the first time by Erasmus Medical Centre (Rotterdam, NL) and SKML, the Dutch organisation for quality assurance in medical laboratories (Winterswijk, NL) following a request by the ERNDIM SAB. A survey was performed in 2008/2009 among members of SSIEM, ERNDIM and ESGLD to establish interest in an MPS scheme. We received a positive response from 82 laboratories, which made us decide to start a pilot scheme in 2010.

Samples

As for other qualitative schemes the MPS pilot scheme requires patient samples. Several laboratories have donated samples in 2009 and 2010, for which they are gratefully acknowledged. We currently have sufficient samples in stock to be able to organise the scheme for 2 more years. We therefore need new patient samples in order to continue the scheme. 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.

Shipment of lyophilised samples would be the cheapest and easiest way to organise the scheme.
Before the scheme was started, we have first tested the effect of lyophilisation on GAG in urine.
Four different authentic urine samples were investigated: 1 control and 3 MPS samples (types I, III and IV) with GAG concentration 20-400 mg/L.
GAG concentrations were comparable in lyophilized samples and untreated (just frozen) samples (Fig.1).
Qualitative GAG analysis resulted in comparable patterns.
We therefore concluded that lyophilisation did not interfere with quantitative or qualitative GAG analysis and that lyophilized samples could be used in an ERNDIM MPS pilot study.

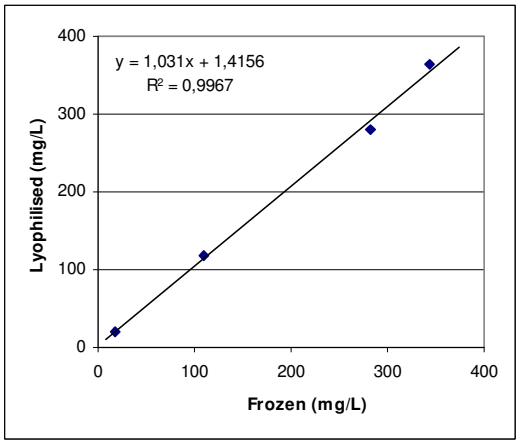

Fig.1. GAG concentration in lyophilized vs. untreated samles

Design of the scheme format and logistics

The Scheme has been designed and coordinated by Dr. George Ruijter and Dr. Jan Huijmans (scientific advisors). Dr. Cas Weykamp at SKML has prepared and shipped the samples (scheme organiser).

In 2010 the scheme consisted of 8 lyophilised urine samples, distributed in February. Four pairs of identical samples were included as described in Table 1. Participants were asked to reconstitute each sample in 5 mL deionised water, 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. 2010 samples

Sample type	Sample no. in set 1 (reporting deadline April 30, 2010)	Sample no. in set 2 (reporting deadline June 30, 2010)
Normal control (f, 13 y)	MPS2	MPS5
MPS III (m, 11 y)	MPS4	MPS6
MPS IV (m, 13 y)	MPS3	MPS8
MPS VI (m, 11 y)	MPS1	MPS7

Reporting

Reporting was done by completing pre-designed forms. Two reporting deadlines were chosen: April 30 and June 30. In addition to results, the reporting forms also included sections to describe methods (first report, April 30 2010) and a question regarding inclusion of oligosaccharide analysis (second report, June 30 2010). Reports were submitted by email to the scheme advisor.

Participants

In 2010 the MPS pilot scheme had 88 participants. On average 80 reports were received per sample (range 79-82). A total of 643 sample reports were received.

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 2) and second to investigate whether relations exists between methods and diagnostic proficiency. The latter will be further studied after the 2011 scheme. Methods were provided by 77 laboratories.

Table 2. Methods

Method for quantitative analysis		**Standard material**		**Method for qualitative analysis**	
DMB	82 %	CS, C4S, C6S	70 %	1-D electrophoresis	69 %
Alcian Blue	8 %	HS	17 %	2-D electrophoresis	17 %
CPC (turbidometric)	6 %	DS	4 %	TLC	13 %
Uronic acid (carbazole)	4 %	Other, Multiple	9 %	Other	1 %

Results of the 2010 samples

Results are summarised in Tables 3 and 4.

The quantitative results revealed a rather large variation in the GAG concentration. Interlaboratory CVs were 25-65 %. In particular the MPS IV sample (MPS3, MPS8) showed large variation. This may be explained in part by the low creatinine concentration and the large variation in this value. Average values of creatinine as well as GAG concentrations correlated well within each sample pair.

Interpretation of quantitative GAG results, i.e. describing results as normal or increased appeared to be fine for most samples with correct results for more than 95 % of the laboratories. Exceptions were the MPS III samples (MPS4, MPS6) with 87 and 89 % of the laboratories interpreting their results as increased compared to their age-matched reference values. Interestingly, out of the 32 sample reports that were not interpreted correctly with respect to quantitative results, 12 did have the correct diagnosis based on electrophoresis/TLC.

Diagnostic proficiency was scored with the following criteria. For the normal sample only 'normal' was considered correct. The MPS III sample was correct with the diagnosis 'MPS III' and partially correct with 'MPS III/normal'. Similarly, the MPS IV sample was correct with the diagnosis 'MPS IV' and partially correct with 'MPS IV/normal'. Finally, the MPS VI sample was considered correct with the diagnosis 'MPS VI' and partially correct with 'MPS I/MPS II/ MPSVI/MPS VII' or combinations thereof as long as MPS VI was among the possible diagnoses mentioned.

On average 11 % of the laboratories did not report a diagnosis. This was mainly due to the fact that these laboratories did not perform qualitative analysis of GAG. Diagnostic proficiency was 90 % for the normal sample, 67% for MPS III, 59 % for MPS IV and 72 % for MPS VI (correct and partially correct scores have been lumped in these numbers). As expected, the MPS IV sample was most problematic.

With regard to the MPS III and MPS IV samples, the majority of the laboratories that did not come to the right diagnosis scored these samples as normal and apparently missed the heparansulfate or keratansulfate in these samples.

Strikingly, many laboratories apparently have the experience that MPS I, II and VI can not be distuingished easily using electropheretic/chromatographic analysis, since they report MPS I/MPS II/ MPSVI instead of only MPS VI.

Upon inspection of the reports, 7 cases of possible sample mix-up were recorded.

We received feedback from one participant with respect to methods. This laboratory did not correctly diagnose the MPS IV samples and investigated the probable causes. They found out that the batch of Alcian blue they used was not suitable to detect keratansulfate. With a different Alcian blue batch of the same manufacturer the keratansulfate showed up. This may be a problem for other laboratories as well and shows that quality control is required for each dye batch (i.e. proof that a batch shows positive results with standards and/or samples from established patients).

Table 3. Results for samples MPS1 to MPS4

Sample ID	**MPS1**	**MPS2**	**MPS3**	**MPS4**
Diagnosis	MPS VI	Normal	MPS IV	MPS III
No. of reports	82	82	82	81
Creatinine (mmol/L)				
Average	1.05	2.45	0.98	2.29
SD	0.29	0.28	0.28	0.39
GAG (mg/mmol)				
Average	61.6	5.5	58.5	23.8
SD	16.8	3.3	38.1	11.2
Quantitative GAG				
Increased (%)	99	3	96	87
Normal (%)	1	97	4	13
Diagnosis				
Correct (%)	27	90	56	64
Part. correct (%)	43	-	5	1
Not correct (%)	18	1	26	25
No diagnosis %)	12	9	13	10

Table 4. Results for samples MPS5 to MPS8

Sample ID	MPS5	MPS6	MPS7	MPS8
Diagnosis	Normal	MPS III	MPS VI	MPS IV
No. of reports	79	79	79	79
Creatinine (mmol/L)				
Average	2.42	2.22	1.03	0.99
SD	0.31	0.32	0.17	0.28
GAG (mg/mmol)				
Average	6.0	23.9	64.0	57.0
SD	3.6	12.0	15.8	25.9
Quantitative GAG				
Increased (%)	5	89	100	95
Normal (%)	95	11	0	5
Diagnosis				
Correct (%)	90	66	32	51
Part. correct (%)	-	3	42	5
Not correct (%)	0	20	16	32
No diagnosis (%)	10	11	10	13

Scoring of results

In the pilot phase of the scheme scoring of results of individual laboratories will not be performed.

Preview of the scheme in 2011

The 2011 scheme will be similar to 2010. We will change from 8 to 6 samples. The main reasons to do this are lack of sufficient suitable samples.

Only 52 % of the laboratories were keen to have oligosaccharide analysis included in the scheme (converting it to a lysosomal scheme rather than an MPS scheme). We therefore decided not to include oligosaccharide analysis in the 2011 scheme.

A number of participants mentioned that some of the samples were too dilute for proper analysis. Again this relates to the lack of sufficiently large sample volumes for almost 100 participants. We will attempt to use samples with higher creatinine/GAG concentration.

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).