ERNDIM
Urine mucopolysaccharides
ANNUAL REPORT 2013

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1. Introduction

The ERNDIM Urine Mucopolysaccharides scheme has started in 2012 as a regular ERNDIM programme following two years (2010-2011) of pilot study. The scheme is organised by Erasmus Medical Centre (Rotterdam, NL) and SKML, the Dutch organisation for quality assurance in medical laboratories (MCA laboratory, Winterswijk, NL).

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). Sample preparation is performed by lyophilisation of 5 mL aliquots. As in previous years the 2013 MPS scheme consisted of 6 lyophilised urine samples as described in Table 1. The scheme format was kept identical to that of 2011-2012. Samples were shipped by regular mail in February. Details regarding stability of (reconstituted) samples are provided in the sample package. 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. Samples included in the 2013 ERNDIM Urine MPS scheme

Survey, reporting deadline	Sample no.	Sample type
2013-1, April 30, 2013	MPS21	Normal control (m, 8 y)
	MPS22	MPS IIIA (f, 32 y)
	MPS23	MPS II (m, 5 y)
2013-2, June 30, 2013	MPS24	MPS IV A (m, 4 y)
	MPS25	MPS III A (m, 11 y)
	MPS26	MPS I Scheie (f, 38 y)

3. Participants

In 2013 105 laboratories from many different countries participated in the Urine MPS scheme (Table 2). The number of participants has increased slightly compared to 2012 (102 participants).

Table 2. Number of participants in 2013 per country.

Country	no of participants		Country	no of participants
ARGENTINA	1		LUXEMBOURG	1
AUSTRALIA	6		MALAYSIA	2
AUSTRIA	1		NETHERLANDS	7
BELGIUM	4		NEW ZEALAND	2
BRAZIL	1		NORWAY	1
CANADA	3		POLAND	1
CHINA	2		PORTUGAL	3
COLOMBIA	1		REPUBLIC OF	1
CROATIA	1		SLOVAKIA	1
CYPRUS	1		SOUTH AFRICA	2
CZECH REPUBLIC	1		SPAIN	4
DENMARK	1		SWEDEN	1
ESTONIA	1		SWITZERLAND	2
FRANCE	8		TURKEY	3
GERMANY	7		UK	16
GREECE	1		UKRAINE	1
INDIA	2		United Arab	2
ITALY	2		USA	10
LATVIA	1			

4. Samples

The samples used in 2013 were authentic human urine samples, 5 from MPS patients and 1 from a healthy individual. Samples were selected by the Scientific Advisor and tested for suitability in the Scientific Advisor's laboratory (Erasmus Medical Centre, Rotterdam, Netherlands). After preparation by the scheme organiser, one set of samples is checked in the Scientific Advisor's laboratory.
All qualitative ERNDIM schemes require authentic patient samples. Several laboratories have donated samples to the Urine MPS scheme 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).
The first reporting form (survey 2013-1, April 30, 2013) also included a section to describe methods.
In 2013 100 reports were received for samples MPS21 to MPS23 and 98 reports for samples MPS24 to MPS26. Five participant did not submit any report. In 2012 the average number of reports was 90 per sample.
Results submitted were analysed and scored by the scientific Advisor using Excel.

6. Methods for GAG analysis used by participants

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 (Figure 1) 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 99 laboratories. For quantitative analysis the spectrofotometric test using dimethylmethylene blue (DMB) is most common. Other methods, i.e. detection of hexuronic acid by harmine/carbazole or employing Alcian blue or cetylpyridinium chloride (CPC) have smaller numbers of users. Single users reported methods based on Azure A and toluidine blue. One participant stated

application of tandem mass spectrometry to quantify and identify different GAG species. We believe that this novel methodology has great potential in diagnostics of Mucopolysaccharidoses.

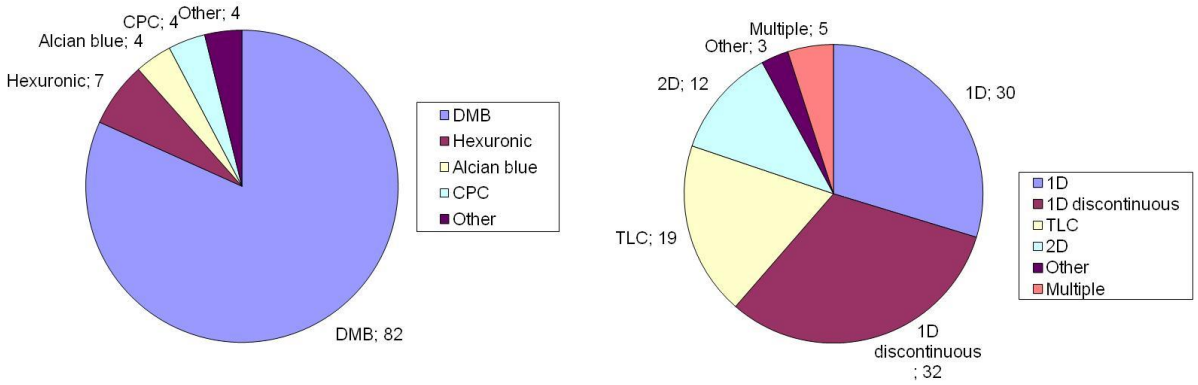

Figure 1. Methods reported by participants (% of total reported).

7. Results of the 2013 samples

Results are summarised in Table 3.

7.1 Quantitative results

Quantitative GAG results were evaluated separately for each method (DMB, Acian Blue, Harmine/carbazole, CPC/turbidity). Most participants use DMB (82 %) for quantitative GAG analysis (Figure 1). The number of participants using the other 3 methods is small, which prohibits statistically meaningful interpretation. Interlaboratory CVs of DMB results were 23-35 % for the 6 different samples.
In the annual report of 2012 we have noted that the CPC/turbidity method may produce too low GAG values in samples with relatively low GAG concentrations. In the 2013 scheme this is also observed in sample MPS21 and MPS22, but not in sample MPS26, which contained predominantly DS.
Interpretation of quantitative GAG results, i.e. labelling quantitative results as normal or increased, appeared to be very good for samples MPS21, MPS23 and MPS25 (97 to 99 % correct; Figure 2, Table 3).

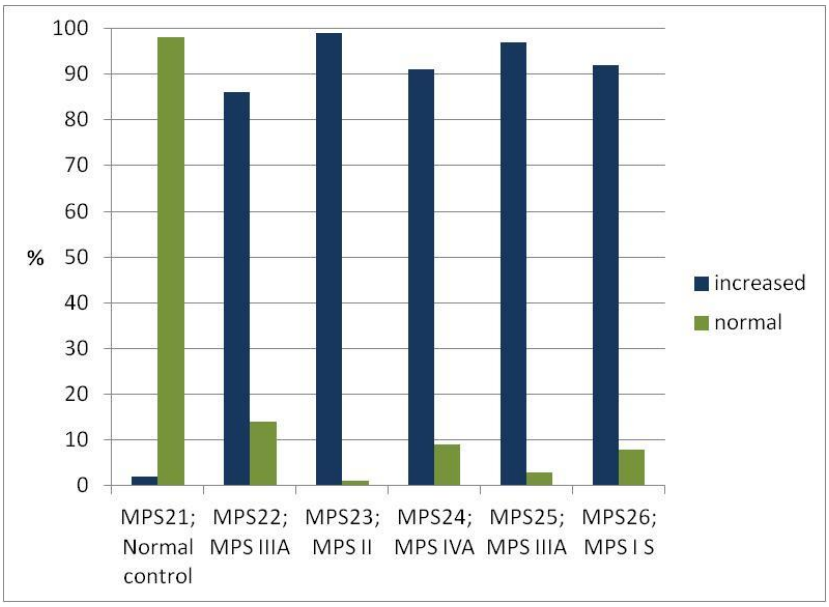

Figure 2. Interpretation of quantitative GAG results.

GAG concentrations apparently were clearly normal in a urine sample of a normal individual (MPS21), or elevated in urine of two MPS patients (MPS23 and MPS25). A slightly lower level of correct interpretation was obtained for samples MPS24 and MPS26 with 91 and 92 % of the laboratories interpreting their results as increased compared to their age-matched reference values. These two urine samples were obtained from a Morquio and a Scheie patient. The sample of a mild (adult) Sanfilippo A patient (MPS22) had the lowest level of correct interpretation of quantitative results (86%). Clearly, it is more difficult to interpret GAG levels for relatively mild MPS patients. Results from previous years show a similar tendency. Amongst the participants who interpreted the quantitative results of samples MPS22, MPS24 and MPS26 as normal, carrying out qualitative analysis (i.e. electrophoresis or TLC) did result in a considerable number of (partially) correct diagnoses: 14 out of 31 sample analyses.
Sample MPS25 was used also in the 2010 pilot scheme (samples MPS4 and MPS6). The median values for quantitative GAG determination of this sample in 2010 and 2013 were very similar: 23.2 and 22.4 mg/mmol creat respectively. Interestingly, interpretation of the quantitative results was much better in 2013 (97% elevated) compared to 2010 (88% elevated). This may be related to changes in the reference values used by the participants to interpret results.

Table 3. Summary of the results reported for samples MPS21 to MPS26

Sample ID	**MPS21**	**MPS22**	**MPS23**	**MPS24**	**MPS25**	**MPS26**
Diagnosis **Age of patient**	Normal 8 y	MPS IIIA 32 y	MPS II 5 y	MPS IVA 4 y	MPS IIIA 11 y	MPS I S 38 y
No. of reports	100	100	100	97	98	98
Creatinine (mmol/L)						
Average	5.15	4.40	4.35	1.26	3.41	3.30
SD	0.42	0.39	0.40	0.13	0.32	0.29
GAG (mg/mmol)						
DMB						
Average	6.7	11.5	70.7	34.3	24.1	12.1
SD	2.3	3.6	19.6	9.1	7.1	2.8
Median	6.6	11.6	70.0	32.6	23.7	12.0
n	78	78	76	73	76	74
Alcian Blue						
Average	10.9	15.2	114.6	43.6	29.0	17.5
SD	4.4	6.7	66.2	20.2	11.8	6.8
Median	10.7	15.5	85.7	49.7	27.1	19.5
n	4	4	4	4	4	4
Uronic/carb						
Average	1.6	3.4	28.4	5.3	6.8	2.0
SD	1.3	3.2	33.4	3.5	4.3	1.3
Median	1.1	2.2	16.1	5.1	5.8	1.8
n	6	6	6	6	6	6
CPC/turbidity						
Average	3.5	6.4	84.3	29.4	23.9	14.9
SD	0.7	2.0	25.3	9.9	10.0	4.5
Median	3.6	6.1	82.3	39.0	21.0	16.0
n	4	4	4	3	3	3
Quantitative GAG						
Increased (%)	2	86	99	91	97	92
Normal (%)	98	14	1	9	3	8
Diagnosis						
Correct (%)	85	70	72	59	88	33
Part. correct (%)	0	1	12	5	0	32
Not correct (%)	6	15	9	30	7	29
No diagnosis %)	9	14	7	6	5	7

7.2 Qualitative results

GAG fractions analysed by electrophoresis or TLC in sample MPS21 from a healthy child were interpreted as normal by most of the participants (97%).
Two MPS III samples were included in the 2013 scheme, MPS22 and MPS25, both from mild Sanfilippo A patients. In the case of MPS22, elevated heparan sulfate (HS) was reported by 88% (71/81) of the participants, while 95% (87/92) reported elevated HS in sample MPS25.
In the MPS II (Hunter syndrome) sample MPS23, dermatan sulfate (DS) was reported elevated by the majority (99%) of the participants. HS was found increased by 93% of the participants in MPS23.
For sample MPS24 (MPS IV; Morquio syndrome) 79% of the participants (56/79) reported elevated keratan sulfate (KS). In addition, 33% (28/84) 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 MPS I-Scheie sample (MPS26), 90% (82/91) of the participants reported increased DS. Only 51% (41/80) of the participants reported increased HS in this sample. Apparently, the dermatan sulfate fraction was predominant in this sample.

7.3 Most likely diagnosis

Diagnostic proficiency was highest for samples MPS21 (normal control; 85% (partially) correct diagnoses), MPS23 (MPS II; 84%) and MPS25 (MPS IIIA; 88%). An MPS disorder (MPS III or IV) was concluded/suspected by 6% of the participants in the normal control sample.
With regard to the two MPS IIIA samples: the lower level of HS detection in sample MPS22 compared to MPS25 (discussed above) was reflected in the lower proficiency accomplished for sample MPS22 (71%) in comparison to sample MPS25 (88%).
Proficiency was 64% for the MPS IV sample MPS24, which is identical to the value obtained for another MPS IV sample circulated in 2012 (sample MPS15). The majority of the laboratories that did not come to the right diagnosis in sample MPS24 scored this sample as normal (23/29).
The relatively low percentage of correct diagnoses reported for sample MPS26 (MPS I Scheie), is because many laboratories (16) diagnosed this sample as MPS VI. As described above, HS was detected by only half of the participants in this sample. In 2011 and 2012 other MPS I samples gave similar results. This once more shows the difficulty to distinguish MPS I from MPS VI on the basis of urine mucopolysaccharide analysis with present technologies.

On average, 8 % of the laboratories did not report a diagnosis (range 5-14 for the 6 different samples). This was only partly due to the fact that these laboratories did not perform qualitative analysis of GAG. Only 5 participants reported ‘no diagnosis’ in the case of sample MPS25 (MPS IIIA) while 14 participants did not suggest a diagnosis sample MPS22 (also MPS IIIA). With the latter sample some 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 different elements of the scheme (Table 4).
Qualitative results and diagnostic proficiency of the 2013 samples were scored using the criteria given in Table 5 and 6. 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.
The final decision about scoring of the scheme is made in the Scientific Advisory Board during the spring meeting. Satisfactory performance required at least 12 points out of the maximum 24 in the 2013 scheme.

Scores have been sent to individual participants by email.
ERNDIM provides a single certificate for all its schemes with details of participation and performance.

Six Performance Support letters will be send for the 2013 surveys. None were sent in 2012.

Starting with the 2014 schemes the concept of 'critical error' will be introduced to the assessment of the DPT schemes (see item 9, Preview of the 2014 scheme).

Table 4. 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 5)	1
	Incorrect: minimallly required results not reported	0
Diagnostic proficiency	Correct according to criteria set for the sample as defined by scientific advisor (Table 6)	2
	Partially correct	1
	Unsatisfactory or misleading	0
	Maximum total score	4

Table 5. Criteria used for scoring qualitative results of 2013 samples

Sample	To obtain 1 point the report should state (minimally)
MPS21	Normal results for all GAG types, or increased CS only
MPS22	Increased HS
MPS23	Increased DS
MPS24	Increased KS
MPS25	Increased HS
MPS26	Increased DS

Table 6. Criteria for scoring of diagnostic proficiency of 2013 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
MPS21	Normal	-	Any (combination of) MPS No diagnosis
MPS22	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS23	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
MPS24	MPS IV	Normal or MPS IV	Normal Any other (combination of) MPS No diagnosis
MPS25	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS26	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

Distribution of scores in 2013 is depicted in Figure 3. In 2013, 92% of the participants that submitted at least 1 report achieved satisfactory performance (≥12 points), while 71% had at least 18 points. In the 2012 scheme the corresponding percentages were 84 and 66. The type of samples (easy or difficult) may affect overall proficiency, but the improved performance in 2013 compared to 2012 may be partly attributable to better proficiency as a result of participation in the ERNDIM Urine MPS scheme. From the 8 participants that did not accomplish satisfactory performance, 2 obtained a low score due to incomplete submission of results (i.e. 1 survey report submitted instead of 2 reports).

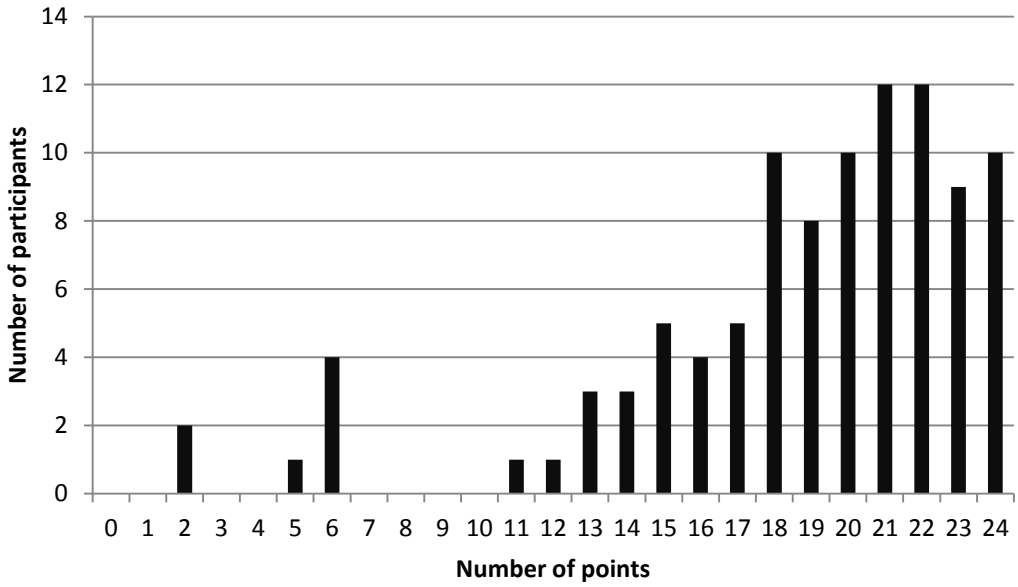

Fig. 3. Distribution of scores in 2013

9. Preview of the scheme in 2014

The format of the MPS 2014 scheme will be similar to that of 2013.

Website reporting to submit results will be used in the Urine MPS scheme in 2014. In 2013 we have started to develop website reporting for the Urine MPS scheme in collaboration with CSCQ, the Swiss organisation for quality control. The CSCQ has also developed website reporting for the ERNDIM Diagnostic Proficiency Schemes.

Starting with the 2014 schemes the concept of 'critical error' will be introduced to the assessment of all qualitative ERNDIM schemes, including the Urine MPS scheme.

A critical error is an error that would be unacceptable to the majority of labs and would have a serious adverse effect on patient management. The introduction of critical error is on the advice of the Genetic Services Quality Committee (GSQC) of the European Society of Human Genetics (ESHG), which wants to see harmonisation across all European genetic EQA providers. A confirmed critical error will mean automatic classification as a poor performer. The final scoring of all qualitative schemes will be discussed at the Spring meeting of the Scientific Advisory Board (SAB) and all proposed critical errors will need to be ratified by the SAB before being confirmed.

In order to explain the concept of critical error, a short description of possible critical errors in the 2013 samples is given below (reminder: critical error will not be applied for the 2013 samples). Please note

that criteria to establish critical error are context-dependent and hence criteria used for a particular sample in one survey may be different compared to a similar sample used in another survey.

Sample MPS21	Normal samples are currently not subject to critical errors.
Sample MPS22	The relatively low proficiency shows that it is challenging to establish diagnosis in this sample of a mild MPS III patient. Critical error is not applicable for this sample.
Sample MPS23	Failure to conclude an MPS disorder (i.e. identifying it as normal) in this sample of a severely affected MPS II patient would be classified as a critical error. All participants concluded 'MPS' in this sample.
Sample MPS24	An MPS IV sample with relatively low proficiency; critical error not applicable.
Sample MPS25	Out of the 98 participants that submitted results of this sample, 90 concluded 'MPS', 5 did not establish diagnosis and 3 concluded 'normal'. Proficiency of this sample was the highest amongst the 6 samples of this year. Failure to conclude an MPS disorder (i.e. identifying it as normal) would be classified as a critical error in this sample.
Sample MPS26	An MPS I Scheie sample with relatively low proficiency; critical error not applicable.

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).

Rotterdam, March 21, 2014

Dr George Ruijter
Scientific Advisor

This annual report is intended for participants of the ERNDIM Urine MPS scheme. The contents should not be used for any publication without permission of the scheme advisor