ERNDIM
Urine mucopolysaccharides
ANNUAL REPORT 2014

Scheme Organiser	Scientific Advisor	Website for reporting results
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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) in conjunction with SKML, the Dutch organisation for quality assurance in medical laboratories (MCA laboratory, Winterswijk, NL) and CSCQ, the Swiss organisation for quality assurance in medical laboratories.

2. Participants

In 2014 108 laboratories from many different countries participated in the Urine MPS scheme (Table 1). The number of participants has increased slightly compared to 2013 (105 participants).

Table 1. Number of participants in 2014 per country.

Country	No. of participants	Country	No. of participants
ARGENTINA	2	LATVIA	1
AUSTRALIA	6	LUXEMBOURG	1
AUSTRIA	1	MALAYSIA	2
BELGIUM	4	NETHERLANDS	5
BRAZIL	1	NEW ZEALAND	2
CANADA	4	NORWAY	1
CHINA	1	POLAND	1
COLOMBIA	1	PORTUGAL	3
CROATIA	1	REPUBLIC OF SINGAPORE	1
CYPRUS	1	RUSSIA	1
CZECH REPUBLIC	1	SLOVAKIA	1
DENMARK	1	SOUTH AFRICA	2
ESTONIA	1	SPAIN	4
FINLAND	1	SWEDEN	1
FRANCE	9	SWITZERLAND	2
GERMANY	6	TURKEY	2
HONG KONG S.A.R.	1	UK	17
INDIA	3	UKRAINE	1
ITALY	3	USA	12

3. Design of the scheme and logistics

The samples used in 2014 were authentic human urine samples, 5 from MPS patients and 1 from a healthy individual (Table 2). Samples were selected by the Scientific Advisor and tested for suitability in the Scientific Advisor's laboratory (Erasmus Medical Centre, Rotterdam, Netherlands). while aliquoting and dispatch of the samples was done by the Scheme organiser. Sample preparation is performed by lyophilisation of 5 mL aliquots. After preparation by the scheme organiser, one set of samples is checked in the Scientific Advisor's laboratory.

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

The scheme format was kept identical to that of 2011-2013. 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. *Please see item 7 (end) for a note on the use of check boxes and the comments box for reporting results.*

Table 2. Samples included in the 2014 ERNDIM Urine MPS scheme

Survey, reporting deadline	Sample no.	Sample type
2014-1, April 30, 2014	MPS27	MPS II (m, 11 y)
	MPS28	MPS I H/S (m, 3 y)
	MPS29	Normal control (m, 6 y)
2014-2, June 30, 2014	MPS30	MPS III (f, 7 y)
	MPS31	MPS VI (m, 20 y)
	MPS32	MPS II (m, 42 y)

In 2014 website reporting of results was started. Results were submitted to the CSCQ website https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php. Two reporting deadlines were chosen: April 30 and June 30. The website also included a section to specify methods.
In 2014 a total of 95 reports were received for samples MPS27 to MPS29 and 94 reports for samples MPS30 to MPS32. Nine participants did not submit any report, while 9 other participants submitted one of the two reports. In 2013 the average number of reports was 99 per sample.
The CSCQ website manager has extracted results from the website and has sent this to the Scientific Advisor. Results were analysed and scored by the Scientific Advisor using Excel.

4. Scoring of results

A scoring system was developed in 2012 and approved by the ERNDIM Scientific Advisory Board. 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 3).
Qualitative results and diagnostic proficiency of the 2014 samples were scored using the criteria given in Table 4 and 5. 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 (SAB) during the spring meeting (March 2015 for the 2014 schemes). Satisfactory performance required at least 12 points out of the maximum 24 in the 2014 scheme.

Starting with the 2014 schemes the concept of 'critical error' is introduced to the assessment of the qualitative schemes. Labs failing to make a correct diagnosis of a sample considered as eligible for

this category will be deemed not to have reached a satisfactory performance even if their total points for the year exceed the limit set at the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on March 19, 2015. Critical errors were identified in the 2014 Urine MPS scheme for samples MPS27, 28, 30, 31 and 32. Details are given under item 7 'Results of individual samples and evaluation of reporting'.

Table 3. 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 4)	1
	Incorrect: minimally required results not reported	0
Diagnostic proficiency	Correct according to criteria set for the sample as defined by scientific advisor (Table 5)	2
	Partially correct	1
	Unsatisfactory or misleading	0
	Maximum total score	4

Table 4. Criteria used for scoring qualitative results of 2014 samples

Sample	To obtain 1 point the report should state (minimally)
MPS27	Increased DS
MPS28	Increased DS
MPS29	Normal results for all GAG types, or increased CS only
MPS30	Increased HS
MPS31	Increased DS
MPS32	Increased DS

Table 5. Criteria for scoring of diagnostic proficiency of 2014 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
MPS27	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
MPS28	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 Any other (combination of) MPS No diagnosis
MPS29	Normal	-	Any (combination of) MPS No diagnosis
MPS30	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS31	MPS VI MPS VI or VII	MPS I or II or VI MPS I or II or VI or VII	Normal Any other (combination of) MPS No diagnosis
MPS32	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 Any other (combination of) MPS No diagnosis

Please see item 7 (end) for a note on the use of check boxes and the comments box for reporting results.

5. Communication of results

Interim reports with diagnoses and summaries of the results submitted were sent June 2nd, 2014 (survey 2014-1) and September 19th (survey 2014-2). Scores have been sent to individual participants by email in January 2015..
The annual report summarises scheme organisation and results.

ERNDIM provides a single certificate for all its schemes with details of participation and performance.

Seven Performance Support letters will be send for the 2014 surveys. Six were sent for the 2013 scheme.

6. Proficiency of the 2014 surveys

Distribution of scores in 2014 is depicted in Figure 1. In 2014, 97% (87/90) of the participants that submitted both reports achieved satisfactory performance (≥12 points), while 78% had at least 18 points. Twelve participants did not accomplish satisfactory performance, including 9 due to incomplete submission of results (i.e. 1 survey report submitted instead of 2 reports).

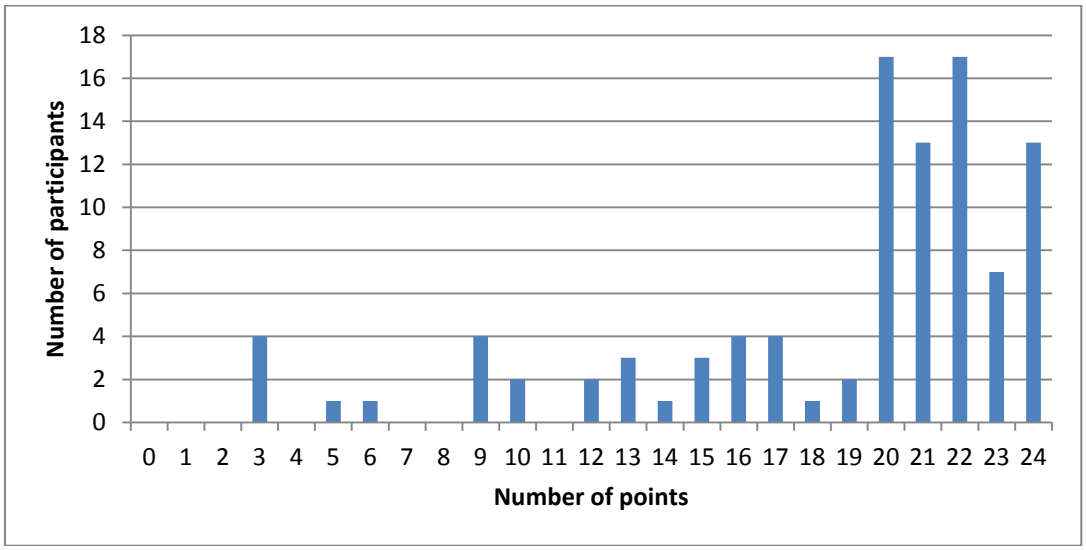

Fig. 1. Distribution of scores in 2014

7. Results of individual samples and evaluation of reporting

Results are summarised in Table 6.
Quantitative GAG results were evaluated separately for each method (DMB, Alcian Blue, Harmine/carbazole, CPC/turbidity). Most participants use DMB (approx. 80 %) for quantitative GAG analysis. The number of participants using the other 3 methods is small, which prohibits statistically meaningful interpretation. Interlaboratory CVs of DMB results were 21-31 % for the 6 different samples

Table 3. Summary of the results reported for samples MPS27 to MPS32

Sample ID	**MPS27**	**MPS28**	**MPS29**	**MPS30**	**MPS31**	**MPS32**
Diagnosis **Age of patient**	MPS II 11 y	MPS I 3 y	Normal 6 y	MPS III 7 y	MPS VI 20 y	MPS II 42 y
No. of reports	95	95	95	94	94	94
Creatinine (mmol/L)						
Average	5.51	1.90	3.89	1.85	5.24	3.49
SD	0.46	0.21	0.34	0.25	0.34	0.35
GAG (mg/mmol)						
DMB						
Average	19.1	103.4	9.2	59.5	15.2	25.3
SD	4.7	31.1	2.1	18.7	3.2	6.5
Median	18.7	105.2	8.8	61.3	14.7	25.0
n	71	72	69	72	71	71
Uronic/carb/harmine						
Average	2.9	15.0	0.9	11.1	2.4	3.7
SD	0.8	6.0	0.4	5.0	1.0	2.2
Median	3.2	12.5	0.9	10.9	2.0	3.0
n	7	7	7	6	6	6
Alcian Blue						
Average	21.4	103.9	12.0	48.6	15.5	24.1
SD	9.1	23.7	4.5	12.8	4.8	7.6
Median	20.9	102.5	11.0	47.6	14.3	22.3
n	4	4	4	4	4	4
CPC/turbidity						
Average	19.8	104.1	10.4	73.1	20.4	31.8
SD	6.2	34.1	0.7	29.9	6.5	12.0
Median	19.5	95.2	10.3	65	18	27.5
n	4	4	4	4	4	4
Quantitative GAG						
Increased (%)	95	99	14	99	97	98
Normal (%)	5	1	86	1	3	2
Diagnosis						
(Part.) Correct (%)	72	75	91	86	78	84
Not correct (%)	22	21	5	10	18	10
No diagnosis %)	6	4	4	4	4	6

Sample MPS27

Sample type. This was an MPS II sample with a relatively mild elevation of GAG.
Analytical proficiency. Although the GAG level was only moderately elevated, 95% of the participants reported the quantitative GAG result increased. In 2012 and 2013 we have noted that the CPC/turbidity method may produce too low GAG values in samples with relatively low GAG concentrations. This was not the case for sample MPS27; 3 out of 4 labs using CPC reported elevated GAG. From the 5 labs that reported normal results for quantitative GAG, 2 found abnormal results for electrophoresis/TLC and came to the correct diagnosis.
Almost all labs (98%) reported abnormal test results of GAG sub fraction analysis (i.e. electrophoresis or TLC). 89% reported elevated DS, while 63% found elevated HS.
Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 45% of the participants, while another 26% concluded MPS I, II or VI. Twenty-one labs (22%) did not mention MPS II as a possibility. Among the incorrect diagnoses MPS VI was frequent (n=9), but also MPS III was reported (n=4). In this sample the ratio DS/HS apparently was rather high, which may have led some labs to miss HS elevation and to conclude MPS VI (all 9 labs concluding 'MPS VI' had failed to detect elevated HS).
Overall proficiency (based on points) was 74%.
Reporting normal test results for both quantitative GAG analysis and GAG subtype analysis and consequently reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1).

Sample MPS28

Sample type. A sample of a 3-year old MPS I patient clinically typed as intermediate severity (Hurler-Scheie).
Analytical proficiency. GAG was grossly elevated in this sample; 99% of the participants reported the quantitative GAG result increased. A huge variation was apparent in the GAG concentrations reported for the DMB users: 19 to 193 mg/mmol with an interlaboratory CV of 30%. This suggests that details of the DMB methods used vary considerably.
The majority of the labs (98%) reported abnormal test results of GAG electrophoresis or TLC. 95% reported elevated DS, while 55% found elevated HS.
Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 51% of the participants, while 24% concluded MPS I, II or VI. Twenty labs (21%) did not mention MPS I. Among the incorrect diagnoses MPS VI was frequent (n=15). In 2011, 2012 and 2013 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.
Overall proficiency (based on points) was 78%.
Reporting normal test results for both quantitative GAG analysis and GAG subtype analysis and consequently reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1).

Sample MPS29

Sample type. Normal control, 6-year old male.
Analytical proficiency. 86% of the participants reported a normal result in quantitative GAG testing. Some participants stated that the GAG elevation was only borderline. Nine of the 12 labs that reported elevated quantitative GAG concluded that this was not an MPS sample on the basis of GAG electrophoresis/TLC.
Most participants reported normal test results of GAG electrophoresis/TLC. Three reported elevated DS, 2 found elevated HS and 1 lab reported elevated KS.
Interpretative proficiency. 91% correctly concluded that this was not an MPS sample. Five participants (5%) did conclude an MPS disorder: MPS I or II (n=2), MPS IV (n=2) and MPS VI (n=1).
Overall proficiency (based on points) was 89%.
This sample was not considered eligible for critical error.

Sample MPS30

Sample type. An MPS III sample, severe phenotype.
Analytical proficiency. Clearly elevated GAG concentration in this sample; 99% of the participants reported an increased quantitative GAG result. Again a large variation was observed in the GAG concentrations reported for the DMB users: 18 to 123 mg/mmol, interlaboratory CV 31%.
95% of the participants reported elevated HS in this sample.
Interpretative proficiency. This was a straightforward MPS III sample; 86% of the participants came to the correct diagnosis. Six labs concluded various other (combinations of) MPS types, while 3 participants reported 'normal' as the most likely diagnosis.
Overall proficiency (based on points) was 89%.
Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=2).

Sample MPS31

Sample type. Twenty-year old MPS VI.
Analytical proficiency. Although the GAG concentration was not extremely high, for an adult the value was clearly abnormal and 97% of the participants reported an abnormal test result. All 3 labs that interpreted the quantitative GAG test result as normal did find elevated DS and came to a (partially) correct diagnosis.
Many labs (94%) reported elevated DS. In contrast to samples MPS27 and MPS28, HS was generally reported to be normal or not detected (87%).
Interpretative proficiency. 48% concluded MPS VI for this sample, while 30% was more cautious and also mentioned MPS I and MPS II as a possibility. Seventeen participants (18%) did not mention MPS VI as a possible diagnosis. In 9 out of 17 incorrect diagnoses, MPS I or II was reported, mostly related to elevated HS (n=7).
Overall proficiency (based on points) was 77%.
Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1).

Sample MPS32

Sample type. Adult MPS II patient, aged 42, with a mild phenotype.
Analytical proficiency. The very high GAG concentration in this sample obtained from an adult was reflected in a high percentage of participants reporting an elevated quantitative GAG test result (98%). Virtually all labs (99%) reported abnormal test results of GAG sub fraction analysis (i.e. electrophoresis or TLC). 93% reported elevated DS, while 80% found elevated HS. The DS/HS ratio in this sample apparently was lower and more typical for MPS II.
Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 63% of the participants, while another 21% concluded MPS I, II or VI. Nine labs (10%) did not mention MPS II as a possibility.
Overall proficiency (based on points) was 82%.
Reporting normal test results for both quantitative GAG analysis and GAG subtype analysis and consequently reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1).

On average, 5 % of the laboratories did not report a diagnosis (range 4-6 for the 6 different samples). This was mainly due to the fact that these laboratories did not perform qualitative analysis of GAG.

The use of check boxes and the comment box.
For reporting the interpretation of results the check boxes should be used to indicate the most likely diagnosis. The use of the **'comments' box** in the website form is recommended to explain your interpretation of results. For example in the case of increased DS with normal or undetectable HS, one could check the MPS VI box and explain in the comments box that MPS I (and II) cannot be excluded

on the basis of the results . Or alternatively the boxes for MPS I, II and VI could be checked with a comment entered explaining that MPS VI is more likely.

8. Preview of the scheme in 2015

The format of the MPS 2015 scheme will be similar to that of 2014.

The following changes will be introduced in the 2015 scheme:

- Sample numbering will be changed to 2015.01 to 2015.06 in line with sample numbering of other schemes.
- Reporting deadlines will be **April 30, 2015** and **September 30, 2015**, in response to requests to spread surveys more evenly over the year (previously reporting deadline were April 30 and June 30).

Website reporting to submit results was successfully introduced in 2014 and will be maintained in the Urine MPS scheme in 2015. The URL is https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php, choose 'Urine Mucopolysaccharides'.

Tentative planning:

Shipment of samples by SKML (all 6 samples in one box):	February 2015
Analysis start survey 1 (website open):	April 1, 2015
Deadline for reporting results of survey 1:	April 30, 2015
Interim report survey 1 available:	June 2015
Analysis start survey 2 (website open):	September 1, 2015
Deadline for reporting results of survey 2:	September 30, 2015
Interim report survey 2 available:	October 2015
Annual report 2015	April 2016

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