ERNDIM
Urine mucopolysaccharides
ANNUAL REPORT 2017

Scheme Organiser	Scientific Advisor	Website for reporting results
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1. Introduction

The ERNDIM Urine Mucopolysaccharide scheme offers (1) urine samples obtained from confirmed MPS patients to enable laboratories to gain or maintain experience to identify MPS patients and (2) proficiency testing for laboratories providing urine screening of mucopolysaccharidosis. 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 2017 102 laboratories from many different countries participated in the Urine MPS scheme (Table 1). The number of participants is relatively stable over the years (2015: 105, 2016: 99 participants). Six laboratories were educational participants in 2017 (2 in 2016). They take part in all aspects of the scheme and receive interim reports with scores, but performance is not indicated on the ERNDIM certificate of performance.

Table 1. Number of participants in 2017 per country.

Country	no. participants	Country	no. participants
ARGENTINA	2	LATVIA	1
AUSTRALIA	6	MALAYSIA	2
AUSTRIA	1	NETHERLANDS	5
BELGIUM	5	NEW ZEALAND	2
BRAZIL	1	NORWAY	1
CANADA	5	POLAND	1
COLOMBIA	1	PORTUGAL	2
CROATIA	1	REPUBLIC OF SINGAPORE	1
CYPRUS	1	SERBIA	1
CZECH REPUBLIC	1	SLOVAKIA	1
DENMARK	1	SOUTH AFRICA	2
ESTONIA	1	SPAIN	4
FRANCE	8	SWEDEN	1
GERMANY	7	SWITZERLAND	2
GREECE	1	TAIWAN	1
HONG KONG	2	TURKEY	3
ITALY	6	UK	15
KINGDOM of SAUDI ARABIA	1	USA	6

3. Design of the scheme and logistics

As usual, the samples used in 2017 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). Bulk sample volumes were 420-860 mL. Samples were prepared by lyophilisation of 3.5-7.1 mL aliquots. Preparation and dispatch of the samples was done by the Scheme organiser (MCA Laboratory, Winterswijk, Netherlands). Integrity of the samples was checked in the Scientific Advisor's laboratory before shipment to participants.

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.

Table 2. Samples included in the 2017 ERNDIM Urine MPS scheme. Two samples were provided by dr Wijburg, Amsterdam, The Netherlands. One sample was donated by dr Sheth, Ahmedabad, India and another sample by drs Hahn and Nuoffer from Bern, Switzerland. The other two samples were made available by the sample repository at Erasmus MC, Rotterdam, The Netherlands.

Survey, reporting deadline	Sample no.	Sample type
2017-1, May 1, 2017	MPS2017.01	MPS III B (m, 18 y)
	MPS2017.02	MPS II (m, 3 y)
	MPS2017.03	MPS II (m, 46 y)
2017-2, October 2, 2017	MPS2017.04	MPS IV A (f, 14 y)
	MPS2017.05	Normal control (f, 12 y)
	MPS2017.06	MPS VI (f, 19)

The scheme format was kept identical to those of previous years. Samples were shipped by regular mail in February along with other ERNDIM samples. 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 4 (scoring of results) for a note on the use of check boxes and the comments box for reporting results

Participants submitted results to the CSCQ website https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php. The due dates for submitting results in 2017 were May 1 and October 2.
The website includes a section to specify methods. Method specification is required for correct evaluation of the quantitative results (method specific statistics for DMB, harmine, Alcian Blue, CPC, LC-MS/MS test results). Unfortunately, not all participants have specified their methods.
In 2017 a total of 99 reports were received for survey 1 (samples MPS2017.01 to MPS2017.03) and 98 reports for survey 2 (samples MPS2017.04 to MPS2017.06). 97 labs submitted results for both surveys. Two participants did not submit any report, while 3 other participants submitted one of the two reports. In 2016 the number of reports was 92-95 per sample.
In 2017 an evaluation program made by dr Albe from CSCQ was used for the first time to evaluate and score results submitted by participants. The use of this software enabled production of customised interim reports, i.e. including scores, for each individual participant. In previous years the website manager has sent results extracted from the database to the Scientific Advisor, who then analysed and scored results 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. Scores are allocated to different elements of the results reported (Table 3).
Qualitative results and diagnostic proficiency of the 2017 samples were scored using the criteria given in Table 4 and 5. These criteria have been set by the Scientific Advisor, approved by the Scientific Advisory Board, 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 Autumn meeting (November 23, 2017 for the 2017 scheme). Sample 2017.01, obtained from an attenuated MPS III B patient, appeared to be particularly challenging. Based on initial marking, overall proficiency of this sample was 57%. The Scientific Advisory board has decided to class sample 2017.01 as educational. For that reason, sample 2017.01 will not be included in the final scores of the 2017 surveys. As a result, satisfactory performance requires at least 12 points out of the maximum 20 in the 2017 scheme.

A note on scoring of diagnostic proficiency and the use of check boxes and the comment box:

To indicate the most likely diagnosis check boxes must be used to facilitate evaluation of results. The use of the 'comments' box in the website form is recommended to explain your interpretation of results. Comments will be taken into account to score interpretation.
For example we have noted in previous surveys that it may be hard to distinguish MPS I and VI. In the case of increased DS with normal or undetectable HS, checking just the MPS VI box may result in lower than maximum marks if this actually was a MPS I sample. In this case we advise to check the MPS VI box and explain in the comments box that MPS I (and perhaps 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.

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

Table 4. Criteria used for scoring qualitative GAG results (electrophoresis, TLC, LC-MS/MS) of 2017 samples

Sample	To obtain 1 point the report should state (minimally)
MPS2017.01	Educational sample; not scored
MPS2017.02	Increased DS
MPS2017.03	Increased DS
MPS2017.04	Increased KS
MPS2017.05	Normal results for all GAG types, or increased CS only
MPS2017.06	Increased DS

Table 5. Criteria for scoring of diagnostic proficiency of 2017 samples. Sample 2017.01 has been classed as an educational sample and interim scores have been retracted.

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
MPS2017.01	-	-	-
MPS2017.02	MPS II (or VII) MPS I or II (or VII)	MPS I or II or VI (or VII)	Normal Any other (combination of) MPS No diagnosis
MPS2017.03	MPS II (or VII) MPS I or II (or VII)	MPS I or II or VI (or VII)	Normal Any other (combination of) MPS No diagnosis
MPS2017.04	MPS IV	MPS IV or normal	Normal Any other (combination of) MPS No diagnosis
MPS2017.05	Normal	-	Any (combination of) MPS No diagnosis
MPS2017.06	MPS VI with any combination of MPS I, II and VII	Any combination of MPS I, II and VII	Normal Any other (combination of) MPS No diagnosis

Please see item 4 (scoring of results) for a note on the use of check boxes and the comments box for reporting results.

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 eligible for this category will be deemed not to have reached a satisfactory performance even if their total points for the year is sufficient according to the requirement set by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on November 23, 2017. Samples MPS2017.02, MPS2017.03 and MPS2017.06 were eligible for critical error. Amongst the reports of regular participants no critical errors were identified in 2017. Details are given under item 7 'Results of individual samples and evaluation of reporting'.

5. Communication of results

Interim reports with diagnoses, summaries of the results submitted and interim scores were made available July 3, 2017 (survey 2017-1) and October 25, 2017 (survey 2017-2). Sample 2017.01 has been classed as an educational sample and interim scores have been retracted. Scores of the other 5 samples have not been adjusted; scores provided in the interim reports are final scores.
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 2017 surveys. Two of these 7 participants have also received a performance support letter in 2015 or 2016. Unsatisfactory performance (either due to overall score or due to critical error) within an EQA scheme for at least 2 out of 3 years that the participant has subscribed for will result in a notification letter of unsatisfactory performance to the quality manager or head of department.
For the 2016 scheme four Performance Support letters were sent.

6. Proficiency of the 2017 surveys

In 2017, 97 participants submitted 2 reports including 6 educational participants. From the 91 ordinary (non-educational) participants 84 (92%) achieved satisfactory performance (score ≥12, no critical error). Twelve participants did not accomplish satisfactory performance, including 5 due to incomplete submission of results (i.e. no report or 1 survey report submitted instead of 2 reports).

Overall proficiencies of each sample are depicted in Table 6.

Table 6. Overall proficiencies of the 2017 surveys.

Sample ID	Sample type	Proficiency (%)
MPS2017.01	MPS III B (m, 18 y)	Educational sample
MPS2017.02	MPS II (m, 3 y)	86
MPS2017.03	MPS II (m, 46 y)	84
MPS2017.04	MPS IV A (f, 14 y)	65
MPS2017.05	Normal control (f, 12 y)	93
MPS2017.06	MPS VI (f, 19)	88

7. Results of individual samples and evaluation of reporting

Quantitative results of creatinine and total GAG are summarised in the two interim reports. Quantitative GAG results were evaluated separately for most methods (DMB, Alcian Blue, Harmine/carbazole, CPC/turbidity). No statistics are presented for LC-MS/MS-based GAG assays, since very few labs submitted results for these methods. Most participants use DMB (approx. 75 %) for quantitative GAG analysis. The number of participants using the other 3 methods is small.

Sample MPS2017.01

Sample type. MPS III B, attenuated phenotype, male aged 18 y.

This sample appeared to be particularly challenging. Based on initial marking, overall proficiency was 57%. Because of the low proficiency, the Scientific Advisory board has decided to class sample 2017.01 as educational. For that reason, sample 2017.01 will not be included in the final scores of the 2017 surveys.

Analytical proficiency. Only 49% of the participants (47/95) reported elevated total GAG concentration in this sample. 64% (58/90) reported elevated HS, while 22 participants stated that HS was normal or not detected.

Interpretative proficiency. MPS III was reported by 56 participants (57%), while 28 (28%) considered this a normal urine. Seven participants reported ‘no diagnosis’ with 2 of those mentioning MPS III as a possibility and another 2 stating the sample was abnormal, i.e. suggestive of an MPS disorder without being able to specify the type. Six labs reported various combinations of MPS I, II, IV, VI and VII as a diagnosis.

Overall proficiency (based on points): no marking; educational sample

Critical error. Sample not eligible for critical error.

2017.01	**GAG screening**	**CS**	**DS**	**HS**	**KS**
	(number)				
Normal	48	69	30	12	23
Increased	47	3	5	58	1
Not detected	-	12	54	20	57
N	95	84	89	90	81

2017.01	
Diagnosis	**n (total = 97)**
MPS III	56 (58 %)
Normal	28 (29 %)
No Diagnosis	7 (7 %)

MPS IV	2 (2 %)
MPS I/MPS II	1 (1 %)
MPS I/MPS II/MPS VII	1 (1 %)
MPS VI	1 (1 %)
MPS I/MPS II/MPS III/MPS VI	1 (1 %)

Sample MPS2017.02

Sample type. MPS II patient, aged 3 y. This patient was receiving ERT for about a year when the urine sample was collected. Since the GAG concentration was still grossly abnormal, treatment did not prohibit use of the sample in the scheme.

Analytical proficiency. The percentage of participants reporting an elevated quantitative GAG test result was high: 97% (92/95). Most labs reported abnormal test results of GAG electrophoresis or TLC. 96% (90/94) reported elevated DS, while 80% (74/92) found elevated HS.

Interpretative proficiency. This was a rather straightforward MPS sample. MPS I or II (or VII) was reported as the most likely diagnosis by 68% of the participants (65/96), while another 22% concluded MPS I, II or VI (or VII). In total, 90% mentioned MPS II among the possible diagnoses. Eight labs did not mention MPS II as a possibility.

Overall proficiency (based on points): 86%

Critical error. Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1; educational participant).

2017.02	**GAG screening**	**CS**	**DS**	**HS**	**KS**
	(number)				
Normal	3	65	3	15	22
Increased	92	16	90	74	3
Not detected	-	3	1	3	55
N	95	84	94	92	80

2017.02

Diagnosis	**n (total = 96)**
MPS I/MPS II	32 (33 %)
MPS I/MPS II/MPS VII	28 (29 %)
MPS I/MPS II/MPS VI/MPS VII	11 (11 %)
MPS I/MPS II/MPS VI	10 (10 %)
MPS II	4 (4 %)
MPS VI	2 (2 %)
MPS III	2 (2 %)
MPS II/MPS VII	1 (1 %)
MPS VII	1 (1 %)
MPS I/MPS II/MPS III/MPS VI	1 (1 %)
MPS IV	1 (1 %)
MPS I/MPS II/MPS III/MPS VI/MPS VII	1 (1 %)
No Diagnosis	1 (1 %)
Normal	1 (1 %)

Sample MPS2017.03

Sample type. An MPS II sample from an adult patient (46 y) **not** receiving ERT treatment.

Analytical proficiency. All 94 participants that submitted results of GAG screening in this sample reported an elevated concentration. Also, most labs reported abnormal test results of GAG

electrophoresis or TLC. 98% (92/94) reported elevated DS, while 71% (65/92) found elevated HS. The HS concentration in this sample apparently was slightly lower compared to sample 2017.02 for which 80% of the respondents reported elevated HS.

Interpretative proficiency. MPS I or II (or VII) was reported as the most likely diagnosis by 54% of the participants, while another 31% concluded MPS I, II or VI (or VII). In total, 85% mentioned MPS II among the possible diagnoses, a results that is slightly lower than the other MPS II sample circulated this year (MPS2017.02; 90%). MPS I (n=5) and MPS VI (n=7) were mentioned by more participants as the most likely diagnosis compared to sample 2017.02. This result is consistent with less apparent HS storage in 2017.03 compared to sample 2017.02, which was obtained from a severely affected patient. The results of this sample were similar to the results of samples obtained from adult MPS II patients (such as 2016.01, overall proficiency 88%).

Overall proficiency (based on points) 84%.

Critical error. Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=0).

2017.03	**GAG screening**	**CS**	**DS**	**HS**	**KS**
	(number)				
Normal	0	66	1	21	21
Increased	94	4	92	65	1
Not detected	-	15	1	6	57
N	94	85	94	92	79

2017.03

Diagnosis	**n (total = 96)**
MPS I/MPS II	30 (31 %)
MPS I/MPS II/MPS VII	20 (21 %)
MPS I/MPS II/MPS VI	16 (17 %)
MPS I/MPS II/MPS VI/MPS VII	13 (14 %)
MPS VI	7 (7 %)
MPS I	5 (5 %)
MPS III	2 (2 %)
MPS II	1 (1 %)
MPS II/MPS VII	1 (1 %)
MPS III/MPS VI/MPS VII	1 (1 %)

Sample MPS2017.04

Sample type. MPS IV A, 14 year-old female patient.

Analytical proficiency. GAG excretion was mildly elevated in this sample; 66% (61/92) of the participants reported an abnormal (increased) result of quantitative GAG screening.
Elevated KS was reported by 67% of the 82 labs that reported results for this particular GAG.
This once again confirms that KS detection is challenging with current routine electrophoresis/TLC methods. While chondroitin 6-sulfate may accumulate in urine from MPS IV A patients, this was not obvious in sample MPS2017.04, since only 19% (16/84) reported elevated CS.

Interpretative proficiency. MPS IV was reported as the most likely diagnosis by 63% of the participants, while another 3% concluded 'MPS IV or normal'. Most laboratories that did not come to the right diagnosis reported the sample as normal (22%).

Overall proficiency (based on points) 65%.
Proficiency was similar to results obtained with two other MPS IV A samples circulated in 2015 (proficiency 61%), 2013 (64%) and 2012 (64%).

Critical error. This sample was not considered eligible for critical error.

2017.04	GAG screening	CS	DS	HS	KS
	(number)				
Normal	31	60	23	33	4
Increased	61	16	3	5	55
Not detected	-	8	59	48	23
N	92	84	85	86	82

2017.04

Diagnosis	**n (total = 94)**
MPS IV	59 (63 %)
Normal	20 (21 %)
No Diagnosis	4 (4 %)
MPS III	4 (4 %)
MPS IV/Normal	3 (3 %)
MPS I/MPS II	1 (1 %)
No Diagnosis/Normal	1 (1 %)
MPS I/MPS II/MPS III/MPS IV/MPS VI/MPS VII/Normal	1 (1 %)
MPS I/MPS III/MPS IV/MPS VI	1 (1 %)

Sample MPS2017.05

Sample type. Normal control, 12-year old female.

Analytical proficiency. 96% (89/93) of the participants reported a normal result in the quantitative GAG test. Most participants reported normal test results of GAG electrophoresis/TLC. Two labs reported elevated DS, two others increased HS and one participant reported increased KS.

Interpretative proficiency. 90% correctly concluded that this was not an MPS sample. Four participants concluded a mucopolysaccharidosis in this sample. Two participants (2%) concluded MPS III, one 'MPS I or II' and one lab reported 'MPS I, II or VI'.

Overall proficiency (based on points) 93%.

Critical error. This sample was not considered eligible for critical error.

2017.05	GAG screening	CS	DS	HS	KS
	(number)				
Normal	89	78	23	32	19
Increased	4	2	2	2	1
Not detected	-	5	60	51	61
N	93	85	85	85	81

2017.05

Diagnosis	**n (total = 94)**
Normal	85 (90 %)
No Diagnosis	3 (3 %)
MPS III	2 (2 %)
MPS VII/Normal	1 (1 %)
MPS I/MPS II	1 (1 %)
MPS I/MPS II/MPS VI	1 (1 %)
MPS IV/No Diagnosis	1 (1 %)

Sample MPS2017.06

Sample type. 19-year old female MPS VI patient.

Analytical results. The GAG concentration was rather high for an adult and clearly abnormal. All but one of the participants (99%) reported abnormal GAG concentration. Almost all labs (98%) reported elevated DS. A surprisingly high number of labs (24/88; 27%) reported elevated HS. This is not expected in MPS VI urine samples. MPS VI patients have a deficiency of N-acetylgalactosamine 4-sulfatase (arylsulfatase B) which affects DS degradation but not HS degradation. In another MPS VI sample, circulated in 2014 (sample code MPS31) and obtained from a 20 y-old male, 11/84 (13%) of the participants reported HS elevated.

Interpretation. MPS VI was reported as the most likely diagnosis by 34% of the participants (n=31), while in total 42% concluded MPS VI in various combinations with MPS I, II or VII (n=39). Because of the relatively large number of labs reporting elevated HS and including MPS I and II in the possible diagnoses we have decided to score all combinations of MPS VI with MPS I, II and VII with two points. Combinations of MPS I, II and VII without mentioning MPS VI were scored with 1 point (Table 5).

Overall proficiency (based on points) 88%.

Critical error. Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=0).

2017.06	**GAG screening**	**CS**	**DS**	**HS**	**KS**
	(number)				
Normal	1	65	2	26	15
Increased	92	11	87	24	4
Not detected	-	7	0	38	61
N	93	83	89	88	80

2017.06	
Diagnosis	**n (total = 92)**
MPS VI	31 (34 %)
MPS I/MPS II/MPS VI/MPS VII	16 (17 %)
MPS I/MPS II/MPS VI	12 (13 %)
MPS I/MPS VI	7 (8 %)
MPS I/MPS II	5 (5 %)
MPS I/MPS II/MPS VII	5 (5 %)
MPS I	3 (3 %)
MPS VII	3 (3 %)
MPS VI/MPS VII	2 (2 %)
MPS III	2 (2 %)
MPS I/MPS VI/MPS VII	1 (1 %)
MPS I/MPS VII	1 (1 %)
MPS I/MPS II/MPS IV	1 (1 %)
MPS I/MPS III/MPS VI	1 (1 %)
MPS IV	1 (1 %)
MPS I/MPS II/MPS III	1 (1 %)

On average, 6% of the laboratories did not report a diagnosis (range 3-9% for samples 2017.01 to 2017.06). This was partly due to the fact that some laboratories did not perform qualitative analysis of GAG, but also inconclusive test results, e.g. for the MPS III sample, affected the number of diagnoses.

8. Preview of the scheme in 2018

The format of the MPS 2018 scheme will be similar to that of previous years.

Website reporting to submit results was used in 2014-2017 and in 2017 CSCQ software was used successfully to evaluate results and to produce interim reports. This will be maintained in the Urine MPS scheme in 2018. The URL is https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php, choose 'Urine Mucopolysaccharides'. Currently software is developed to produce annual reports. This will most probably be ready for use in the 2018 surveys. As for the interim reports, annual reports produced by the software will be customized for each participant.

Tentative planning:

Shipment of samples by SKML (all 6 samples in one box):	February 2018
Analysis start survey 1 (website open):	April 2, 2018
Deadline for reporting results of survey 1:	April 30, 2018
Interim report survey 1 available:	June 2018
Analysis start survey 2 (website open):	September 3, 2018
Deadline for reporting results of survey 2:	October 1, 2018
Interim report survey 2 available:	November 2018
Annual report 2018	December 2018

Rotterdam
January 4, 2018

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Please note:
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