ERNDIM
Urine mucopolysaccharides
ANNUAL REPORT 2016

Scheme Organiser	Scientific Advisor	Website for reporting results
Dr. C. Weykamp Streekziekenhuis Koningin Beatrix Beatrixpark 1 7101 BN Winterswijk Netherlands e-mail: c.w.weykamp@skbwinterswijk.nl	Dr. G.J.G. Ruijter Erasmus Medical Center Dep. Clinical Genetics Ee2422 P.O. Box 2040 3000 CA Rotterdam e-mail: g.ruijter@erasmusmc.nl	Dr. Xavier Albe CSCQ Swiss Center for Quality Control 2 chemin du Petit-Bel-Air CH-1225 Chêne-Bourg Switzerland e-mail : Xavier.Albe@hcuge.ch

1. Introduction

The ERNDIM Urine Mucopolysaccharide scheme is aiming at (1) provision of urine samples obtained from confirmed MPS patients to enable laboratories to gain or maintain experience to identify MPS patients and (2) proficiency testing of 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 2016 99 laboratories from many different countries participated in the Urine MPS scheme (Table 1). The number of participants has decreased slightly compared to 2015 (105 participants). Two laboratories were educational participants. They take part in all aspects of the scheme, but performance is not indicated on the ERNDIM certificate of performance.

Table 1. Number of participants in 2016 per country.

Participant Country Name	number		Participant Country Name	number
ARGENTINA	2		LATVIA	1
AUSTRALIA	6		MALAYSIA	2
AUSTRIA	1		NETHERLANDS	3
BELGIUM	4		NEW ZEALAND	2
BRAZIL	1		NORWAY	1
CANADA	4		POLAND	1
COLOMBIA	1		PORTUGAL	3
CROATIA	1		REPUBLIC OF SINGAPORE	1
CYPRUS	1		SERBIA	1
CZECH REPUBLIC	1		SLOVAKIA	1
DENMARK	1		SOUTH AFRICA	2
ESTONIA	1		SPAIN	3
FINLAND	1		SWEDEN	1
FRANCE	8		SWITZERLAND	2
GERMANY	7		TAIWAN	1
HONG KONG	1		TURKEY	3
INDIA	1		UK	15
ITALY	4		USA	9
KINGDOM of SAUDI ARABIA	1			

3. Design of the scheme and logistics

Samples used in 2016 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 300-540 mL. Samples were prepared by lyophilisation of 2.5-4.5 mL aliquots. Preparation and dispatch of the samples was done by the Scheme organiser (MCA Laboratory, Winterswijk, Netherlands). 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 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.
Sample MPS2016.06, obtained from a mild MPS IV B patient, was included as an educational sample. The diagnosis was provided at the start of the scheme. Participants were asked to submit results, but the results were not scored.
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 2016 ERNDIM Urine MPS scheme. One sample was provide d by dr Filocamo, Genova, Italy and another sample by dr Levade, Toulouse , France. The other samples were made available by the sample repository at Erasmus MC, Rotterdam, The Netherlands.

Survey, reporting deadline	Sample no.	Sample type
2016-1, May 2, 2016	MPS2016.01	MPS II (m, 40 y)
	MPS2016.02	Normal control (m, 6 y)
	MPS2016.03	MPS I H/S (f, 9 y)
2016-2, October 3, 2016	MPS2016.04	MPS III (f, 2 y)
	MPS2016.05	MPS II (m, 5 y)
	MPS2016.06	MPS IV B (f, 51) Educational sample

Participants submitted results to the CSCQ website https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php.
The due dates for submitting results in 2016 were May 2 and October 3.
The website also included 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 2016 a total of 92 reports were received for survey 1 (samples MPS2016.01 to MPS2016.03) and 95 reports for survey 2 (samples MPS2016.04 to MPS2016.06). 91 labs submitted results for both surveys. Three participants did not submit any report, while 5 other participants submitted one of the two reports. In 2015 the average number of reports was 98 per sample.
The CSCQ website manager has extracted results from the website and has sent these 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. Scores are allocated to different elements of the results reported (Table 3).

Qualitative results and diagnostic proficiency of the 2016 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 30, 2016 for the 2016 scheme). Satisfactory performance required at least 12 points out of the maximum 20 in the 2016 scheme.

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 (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 results of 2016 samples

Sample	To obtain 1 point the report should state (minimally)
MPS2016.01	Increased DS
MPS2016.02	Normal results for all GAG types, or increased CS only
MPS2016.03	Increased DS
MPS2016.04	Increased HS
MPS2016.05	Increased DS
MPS2016.06	-

Table 5. Criteria for scoring of diagnostic proficiency of 2016 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
MPS2016.01	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
MPS2016.02	Normal	-	Any (combination of) MPS No diagnosis
MPS2016.03	MPS I (or VII) MPS I or II (or VII) MPS I or II or VI (or VII)	MPS VI	Normal Any other (combination of) MPS No diagnosis
MPS2016.04	MPS III	No diagnosis because of interference AND recommendation to analyse repeat urine	Normal Any other (combination of) MPS No diagnosis
MPS2016.05	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
MPS2016.06	-	-	-

Please see item 7 (end) 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 exceed the limit set by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on November 30, 2016. Samples MPS2016.01, MPS2016.03 and MPS2016.05 were eligible for critical error. One critical error was identified in sample MPS2016.05. Details are given under item 7 'Results of individual samples and evaluation of reporting'.

5. Communication of results

Interim reports with diagnoses and summaries of the results submitted were sent June 23, 2016 (survey 2016-1) and November 17, 2016 (survey 2016-2). Scores have been sent to individual participants by email December 20, 2016.
The annual report summarises scheme organisation and results.

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

Four Performance Support letters will be send for the 2016 surveys. For the 2015 scheme seven Performance Support letters were sent.

6. Proficiency of the 2016 surveys

Distribution of scores achieved in 2016 is depicted in Figure 1. 96% (87/91) of the participants that submitted both reports achieved satisfactory performance (score ≥12, no critical error), while 87% had at least 15 points. Twelve participants did not accomplish satisfactory performance, including 8 due to incomplete submission of results (i.e. no report or 1 survey report submitted instead of 2 reports) and 1 due to a critical error. One labs scored 12 points with one report submitted (maximum score).

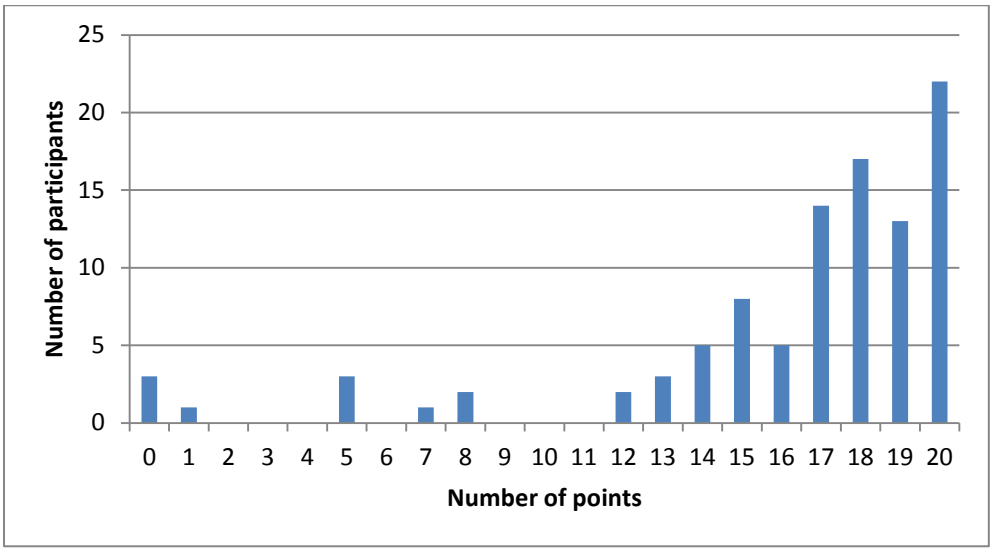

Fig. 1. Distribution of scores in 2016. Numbers include participants that have submitted no report (n=3) or one instead of two survey report (n=5).

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). No statistics are presented for LC-MS/MS-based GAG assays, since only 2 labs submitted results for these methods, one using methanolysis and one using enzymatic GAG hydrolysis. 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 CV values of DMB results were 24-59 % for the 6 different samples. Interlaboratory CVs tend to be lower in samples with relatively high GAG concentration.

Table 6. Summary of the results reported for samples MPS2016.01 to MPS2016.06

Sample ID	**MPS 2016.01**	**MPS 2016.02**	**MPS 2016.03**	**MPS 2016.04**	**MPS 2016.05**	**MPS 2016.06**
Diagnosis **Age of patient**	MPS II M, 40 y	No MPS M, 6 y	MPS I H/S F, 9 y	MPS III F, 2 y	MPS II M, 5 y	MPS IV B F, 51 y
No. of reports	92	92	91	95	95	95
Creatinine (mmol/L)						
Average	5.21	4.85	3.97	1.91	4.02	3.47
SD	0.30	0.31	0.28	0.18	0.35	0.32
GAG (mg/mmol)						
DMB						
Average	23.8	6.7	20.0	74.4	54.8	6.4
SD	5.7	2.6	4.9	31.3	17.4	3.8
Median	24.0	6.3	19.9	74.5	56.5	6.3
n	71	72	71	72	71	72
Uronic/carb/harmine						
Average	4.1	1.7	4.7	64.7	27.0	2.8
SD	2.9	0.8	2.2	66.0	33.9	3.2
Median	3.7	1.5	3.9	50.6	11.9	1.9
n	6	6	6	6	6	6
Alcian Blue						
Average	29.3	10.4	25.4	77.5	43.9	14.6
SD	8.3	3.1	8.1	53.7	27.6	5.2
Median	28.0	11.2	26.5	75.0	46.2	14.5
n	5	5	5	5	5	5
CPC/turbidity						
Average	26.7	6.8	19.0	54.1	37.8	16.1
SD	6.1	4.1	2.0	24.8	24.5	8.4
Median	28.1	5.0	20.0	46.9	30.7	17.0
N	3	3	3	3	3	3
Quantitative GAG						
Increased (%)	99	3	94	97	98	66
Normal (%)	1	97	6	3	2	34
Diagnosis						
(Part.) Correct (%)	93.5	96.8	91.2	76.9	82.1	
Not correct (%)	4.3	2.2	7.7	15.8	13.7	
No diagnosis %)	2.2	1.1	1.1	7.4	4.2	

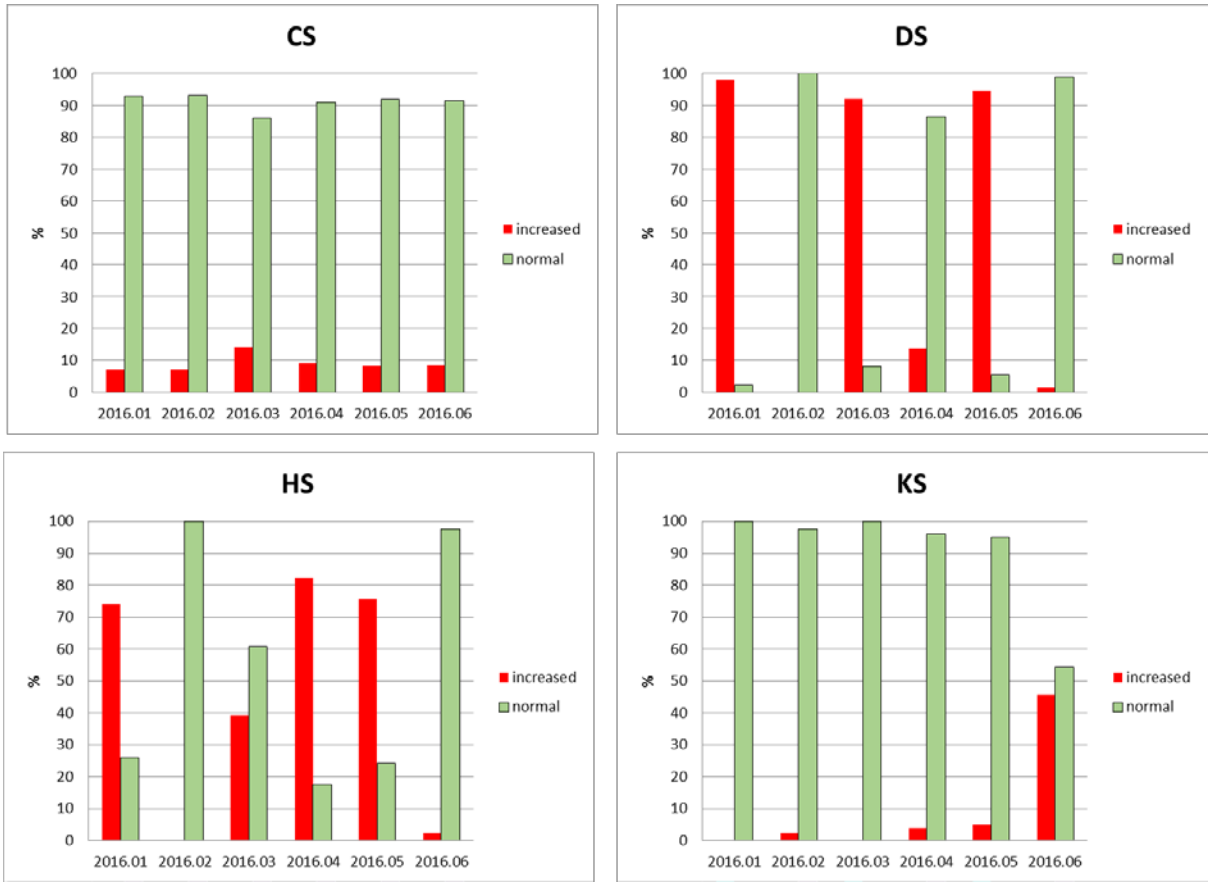

Fig.2. Results of GAG subtype analysis. For every sample and each of the GAG species (CS, DS, HS and KS) the percentages reported increased or normal is depicted. Qualifications 'Normal' and 'Not detected' have been combined. On average the number of reported results was 84 per sample-GAG combination (6 samples, 4 GAG species; total 2024 results reported).

Sample MPS2016.01

Sample type. An MPS II sample from an adult patient (40 y) not receiving ERT treatment.

Analytical proficiency. Total GAG was clearly elevated; 99% of the participants reported the quantitative GAG result increased. The single lab that reported normal results for quantitative GAG screening did not report results for electrophoresis and hence did not achieve diagnosis.
All labs reported abnormal test results of GAG sub fraction analysis (i.e. electrophoresis or TLC): 98% reported elevated DS, while 74% found elevated HS (Fig. 2).

Interpretative proficiency. MPS I or II (or VII) was reported as the most likely diagnosis by 67% of the participants, while another 26% concluded MPS I, II or VI (or VII). Nine labs (9%) did not mention MPS II as a possibility, including 3 that concluded MPS III as the most likely diagnosis. Two participants reported 'no diagnosis'.
The results of this sample were very similar to the results of sample 2015.01, which was also from an adult MPS II patient. A clear difference was observed in the number of participants that reported elevated HS, but included MPS VI in the most likely diagnoses. Elevated HS is not expected in an MPS VI urine. In 2015.01 25 labs included MPS VI in the possible diagnosis, and 11 out of these 25 reported elevated HS. Interestingly, in 2016.01 a comparable number of labs included MPS VI in the possible diagnosis (n=23), but only 6 out of 23 reported elevated HS.

Overall proficiency (based on points) 88.3%.

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

Sample MPS2016.02

Sample type. Normal control, 6-year old male.

Analytical proficiency. 97% (87/90) of the participants reported a normal result in the quantitative GAG test. All 3 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 (Fig. 2). Two labs reported elevated KS, while DS and HS were reported normal by all participants.

Interpretative proficiency. 97% correctly concluded that this was not an MPS sample. Two participants (2%) concluded MPS IV, while one lab reported 'no diagnosis'.

Overall proficiency (based on points) 95.7%. Given the high percentage of correct conclusions, this sample apparently was clearly normal with respect to MPS.

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

Sample MPS2016.03

Sample type. A sample of a 9-year old female MPS I patient clinically typed as intermediate severity (Hurler-Scheie).

Analytical proficiency. 94% of the participants (84/89) reported elevated total GAG concentration in this sample. 92% (80/87) reported elevated DS, but only 39% (34/87) found elevated HS. This once again illustrates that HS is generally less elevated in MPS I urine samples compared to MPS II samples (e.g. 2016.01 and 2016.05).

Interpretative proficiency. MPS I and MPS I or II (or VII) were reported as the most likely diagnosis by only 29% of the participants (n=26), while 41% concluded MPS I, II or VI (n=37). Twenty labs (22%) reported only MPS VI as the most likely diagnosis. In previous years other MPS I samples gave similar results and shows the difficulty to distinguish MPS I from MPS VI on the basis of urine mucopolysaccharide analysis with present technologies. Because of this we have decided to score combination of MPS I, II and VI with two points. Diagnosis MPS VI was scored with 1 point (Table 5).

Overall proficiency (based on points) 85.7%.

Critical error. 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=0).

Sample MPS2016.04

Sample type. MPS III A, female 2 year-old patient with a classical (severe) phenotype.

Analytical proficiency. Total GAG concentration in this sample was grossly elevated and reported increased by 97% of the participants (88/91). Elevated HS was reported by 82% of the participants

(70/85) (Fig. 2). Twelve participants reported the presence of an interfering/unknown band during electrophoresis/TLC. Some suggested that this may have originated from cream or bacterial growth. Because of this interference, 7 participants decided not to conclude on a diagnosis, but instead suggested to repeat the analysis in a new urine sample. The other five labs did conclude MPS III despite the presence of the interfering band.

Interpretative proficiency. MPS III was reported by 67%, while 16 labs (17%) reported 'no diagnosis' (including 7 due to interference noted). Scoring criteria included the possible presence of interfering compounds and effects of this on interpretation (Table 5). Fifteen labs (16%) stated an incorrect diagnosis, including various combinations of MPS I, II, VI and VII (n=10) and MPS IV (n=4).

Overall proficiency (based on points) 78.4%.

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

Sample MPS2016.05

Sample type. MPS II patient, aged 5.

Analytical proficiency. Similar to the other MPS II sample circulated this year (MPS2016.01) the percentage of participants reporting an elevated quantitative GAG test result was high (98%).
Most labs reported abnormal test results of GAG electrophoresis or TLC. 95% (86/91) reported elevated DS, while 76% (69/91) found elevated HS (Fig. 2). The DS/HS ratio in this sample apparently was comparable to sample 2016.01 for which 74% of the respondents reported elevated HS.

Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 63% of the participants, while another 19% concluded MPS I, II or VI. In total, 82% mentioned MPS II among the possible diagnoses. Thirteen labs (14%) did not mention MPS II as a possibility.

Overall proficiency (based on points) was 83.2%.

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

Sample MPS2016.06

Sample type. A sample of an 51-year old MPS IV B patient.
This was an educational sample. The GAG abnormalities were very mild and possibly not easily detected. The diagnosis was provided with the sample, i.e. before the survey was started.

Analytical results. Indeed, GAG excretion was mildly elevated in this sample; only 66% (60/91) of the participants reported the quantitative GAG screening result increased.
Elevated KS was reported by 46% (37/81) of the labs that reported results for this particular GAG (Fig. 2). Four labs mentioned determination of KS by LC-MS/MS and all 4 reported elevated KS. Although oligosaccharide analysis is not included in the urine MPS scheme, an abnormal oligosaccharide pattern was reported by 8 participants.

Interpretation. In sample 2016.06 the following diagnoses were reported:

MPS IV	n=51	(54%)
Normal	n=21	(22%)
MPS IV/normal	n=2	(2%)
No diagnosis/not performed	n=20	(21%)
MPS II	n=1	(1%)

Since this sample was educational, the results were not scored and the sample was not eligible for critical error.

On average, 3% of the laboratories did not report a diagnosis (range 1-7% for samples 2016.01 to 2016.05). This was partly due to the fact that a few 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.

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. Comments made in the sample reports are assessed in scoring of the results.

8. Preview of the scheme in 2017

The format of the MPS 2017 scheme will be similar to that of previous years.

Website reporting to submit results was successfully used in 2014-2016 and will be maintained in the Urine MPS scheme in 2017. The URL is https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php, choose 'Urine Mucopolysaccharides'. Currently software is developed to evaluate results and to generate interim reports. This will most probably be ready for use in the 2017 surveys. Interim reports including provisional scores will then be available for downloading from the CSCQ website.

Tentative planning:

Shipment of samples by SKML (all 6 samples in one box):	February 2017
Analysis start survey 1 (website open):	April 3, 2017
Deadline for reporting results of survey 1:	May 1, 2017
Interim report survey 1 available:	June 2017
Analysis start survey 2 (website open):	September 4, 2017
Deadline for reporting results of survey 2:	October 2, 2017
Interim report survey 2 available:	November 2017
Annual report 2017	December 2017

Rotterdam December 19, 2016

Dr George Ruijter
Scientific Advisor