ERNDIM
Urine mucopolysaccharides
ANNUAL REPORT 2015

Scheme Organiser	Scientific Advisor	Website for reporting results
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1. Introduction

The ERNDIM Urine Mucopolysaccharide scheme is aiming at (1) provision of urine samples obtained from confirmed MPS patients to enable laboratories of gaining or maintaining their 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 2015 105 laboratories from many different countries participated in the Urine MPS scheme (Table 1). The number of participants has decreased slightly compared to 2014 (108 participants).

Table 1. Number of participants in 2015 per country.

Country	No. of participants	Country	No. of participants
ARGENTINA	2	KINGDOM of SAUDI ARABIA	1
AUSTRALIA	6	LATVIA	1
AUSTRIA	1	MALAYSIA	2
BELGIUM	4	NETHERLANDS	4
BRAZIL	1	NEW ZEALAND	2
CANADA	4	NORWAY	1
COLOMBIA	1	POLAND	1
CROATIA	1	PORTUGAL	3
CYPRUS	1	REPUBLIC OF SINGAPORE	1
CZECH REPUBLIC	1	RUSSIA	1
DENMARK	1	SERBIA	1
ESTONIA	1	SLOVAKIA	1
FINLAND	1	SOUTH AFRICA	2
FRANCE	8	SPAIN	4
GERMANY	7	SWEDEN	1
GREECE	1	SWITZERLAND	2
HONG KONG S.A.R.	2	TURKEY	3
INDIA	3	UK	16
ITALY	4	USA	8

3. Design of the scheme and logistics

All samples used in 2015 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 420-600 mL. Samples were prepared by lyophilisation of 3.5-5 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 that of 2011-2014. 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 7 (end) for a note on the use of check boxes and the comments box for reporting results

Table 2. Samples included in the 2015 ERNDIM Urine MPS scheme

Survey, reporting deadline	Sample no.	Sample type
2015-1, April 30, 2015	MPS2015.01	MPS II (m, 44 y)
	MPS2015.02	MPS IV A (m, 18 y)
	MPS2015.03	MPS III A (m, 9 y)
2015-2, September 30, 2015	MPS2015.04	MPS III B (f, 20 y)
	MPS2015.05	Normal control (f, 13 y)
	MPS2015.06	MPS II (m, 15 y)

Results were submitted to the CSCQ website https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php.
The due dates for submitting results in 2015 were April 30 and September 30. Previously the reporting deadline of survey 2 was June 30. This was changed to September 30 in 2015 after comments by participants that the two surveys were too close together.
The website also included a section to specify methods.
In 2015 a total of 98 reports were received for survey 1 (samples MPS2015.01 to MPS2015.03) and 97 reports for survey 2 (samples MPS2015.04 to MPS2015.06). Four participants did not submit any report, while 7 other participants submitted one of the two reports. In 2014 the average number of reports was 95 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. Points are allocated to different elements of the results reported (Table 3).
Qualitative results and diagnostic proficiency of the 2015 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 spring meeting (March 17, 2016 for the 2015 schemes). Satisfactory performance required at least 12 points out of the maximum 24 in the 2015 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 2015 samples

Sample	To obtain 1 point the report should state (minimally)
MPS2015.01	Increased DS
MPS2015.02	Increased KS
MPS2015.03	Increased HS
MPS2015.04	Increased HS
MPS2015.05	Normal results for all GAG types, or increased CS only
MPS2015.06	Increased DS

Table 5. Criteria for scoring of diagnostic proficiency of 2015 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
MPS2015.01	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
MPS2015.02	MPS IV	MPS VI or normal	Normal Any other (combination of) MPS No diagnosis
MPS2015.03	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS2015.04	MPS III	Normal or MPS III	Normal Any other (combination of) MPS No diagnosis
MPS2015.05	Normal	-	Any (combination of) MPS No diagnosis
MPS2015.06	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

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 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 17, 2016. Critical errors were identified in the 2015 Urine MPS scheme for samples MPS2015.01 and MPS2015.06. 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 25, 2015 (survey 2015-1) and November 9, 2015 (survey 2015-2). Scores have been sent to individual participants by email February 15, 2016.
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 2015 surveys. For the 2014 scheme also seven Performance support letters were sent.

6. Proficiency of the 2015 surveys

Distribution of scores in 2015 is depicted in Figure 1. In 2015, 94% (88/94) of the participants that submitted both reports achieved satisfactory performance (≥12 points), while 73% had at least 18 points. Fifteen participants did not accomplish satisfactory performance, including 9 due to incomplete submission of results (i.e. no report or 1 survey report submitted instead of 2 reports). Two labs scored 12 points with one report submitted (maximum score).

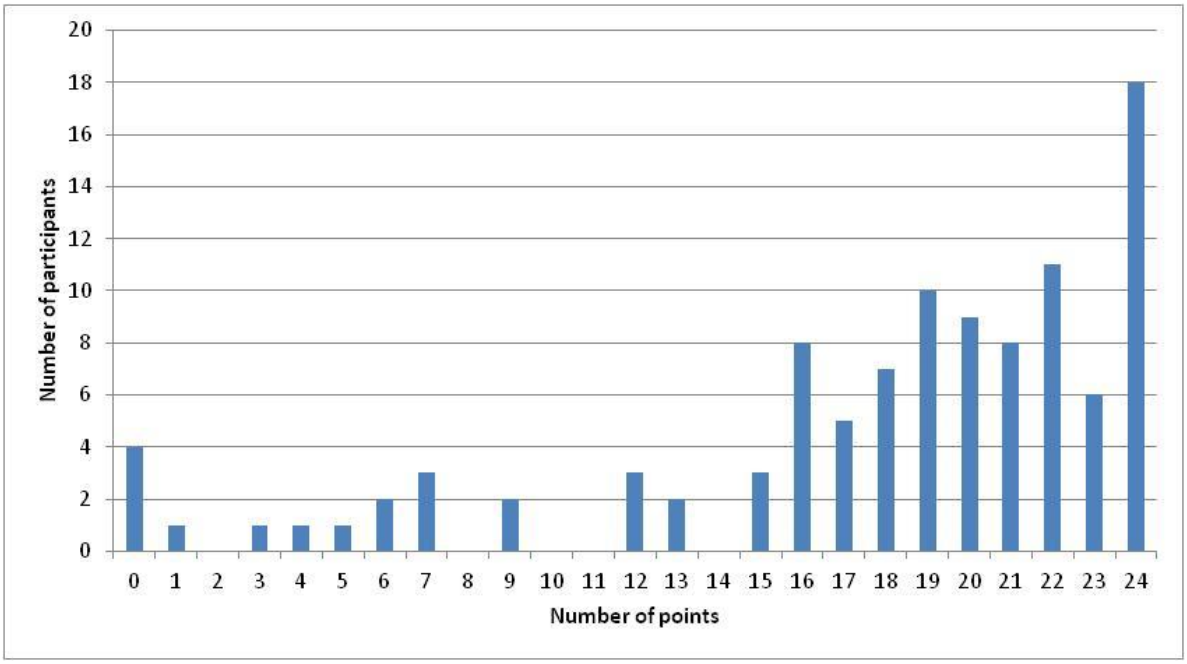

Fig. 1. Distribution of scores in 2015. Numbers include participants that have submitted no report (n=4) or one instead of two survey report (n=7).

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 CV values of DMB results were 25-47 % 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 MPS2015.01 to MPS2015.06

Sample ID	**MPS 2015.01**	**MPS 2015.02**	**MPS 2015.03**	**MPS 2015.04**	**MPS 2015.05**	**MPS 2015.06**
Diagnosis **Age of patient**	MPS II M, 44 y	MPS IV M, 18 y	MPS III M, 9 y	MPS III F, 20 y	Normal F, 13 y	MPS II M, 15 y
No. of reports	98	98	98	97	97	97
Creatinine (mmol/L)						
Average	6.91	1.85	2.38	2.48	3.62	2.75
SD	0.47	0.17	0.25	0.20	0.25	0.21
GAG (mg/mmol)						
DMB						
Average	17.2	14.8	25.4	14.7	5.5	25.0
SD	4.6	5.4	8.2	5.6	2.6	6.4
Median	17.1	13.9	24.4	15.1	5.3	25.8
n	73	72	74	74	74	75
Uronic/carb/harmine						
Average	3.9	3.2	5.2	2.0	1.1	3.5
SD	2.3	2.5	3.8	1.4	0.9	1.7
Median	3.5	2.4	4.9	1.9	0.9	3.0
n	6	6	6	6	6	6
Alcian Blue						
Average	17.0	16.4	24.0	16.6	9.6	21.8
SD	8.1	11.3	13.8	8.0	5.3	9.6
Median	13.4	15.2	26.1	17.4	9.3	23.2
n	6	6	6	6	6	6
CPC/turbidity						
Average	17.8	10.3	20.4	8.1	3.6	27.5
SD	6.9	3.4	5.6	6.8	2.6	12.8
Median	20.0	10.0	20.0	7.1	3.5	21.9
n	3	3	3	4	4	4
Quantitative GAG						
Increased (%)	99	81	91	87	12	99
Normal (%)	1	19	9	13	88	1
Diagnosis						
(Part.) Correct (%)	89	60	83	78	87	81
Not correct (%)	5	29	12	13	4	14
No diagnosis %)	6	11	5	8	9	4

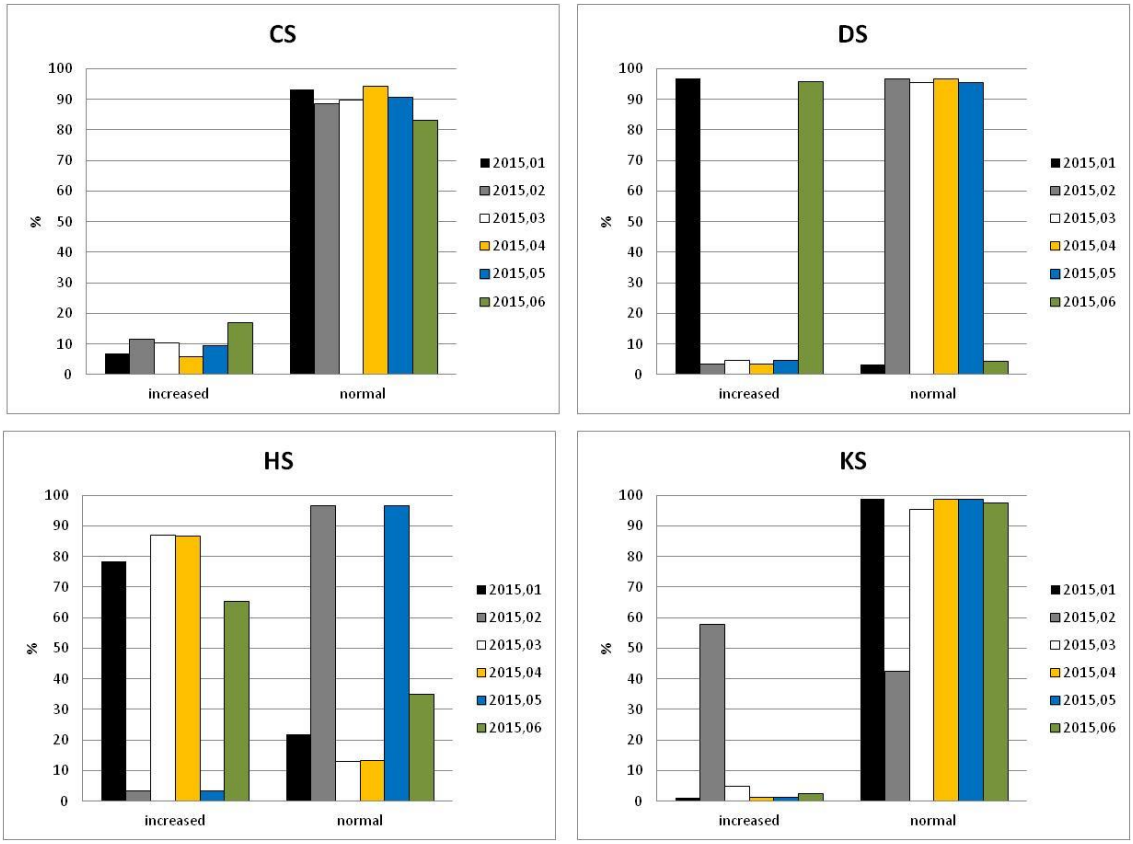

Fig.2. Results of GAG subtype analysis. For every sample and each of the GAG species (CS, DS, HS and KS) the percentages reported increased or normal is depicted. Qualifications 'Normal' and 'Not detected' have been combined. On average the number of reported results was 87 per sample-GAG combination (6 samples, 4 GAG species; total 2090 results reported).

Sample MPS2015.01

Sample type. This was an MPS II sample from an adult patient (44 y) not receiving ERT treatment.
Analytical proficiency. Total GAG was clearly elevated, and 99% of the participants reported the quantitative GAG result increased. The single lab that reported normal results for quantitative GAG screening reported abnormal results for electrophoresis and came to the correct diagnosis.
Almost all labs (98%) reported abnormal test results of GAG sub fraction analysis (i.e. electrophoresis or TLC): 97% reported elevated DS, while 78% found elevated HS (Fig. 2).
Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 65% of the participants, while another 24% concluded MPS I, II or VI. Eleven labs (11%) did not mention MPS II as a possibility. Interestingly, among the labs that included MPS VI in the possible diagnosis (n=25), 11 did report elevated HS, which is not expected in an MPS VI urine.
Overall proficiency (based on points) was 85%.
Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=0).

Sample MPS2015.02

Sample type. A sample of an 18-year old MPS IV A patient.
Analytical proficiency. Apparently, GAG excretion was mildly elevated in this sample; only 81% (76/94) of the participants reported the quantitative GAG screening result increased.
Elevated KS was reported by 58% (49/85) of the labs that reported results for this particular GAG (Fig. 2). This confirms that KS detection remains 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 MPS2015.02, since only 11% (10/87) reported elevated CS.
Interpretative proficiency. MPS IV was reported as the most likely diagnosis by 48% of the participants, while another 12% concluded 'MPS IV or normal'. The majority of the laboratories that did not come to the right diagnosis in sample MPS2015.02, reported this sample as normal (22/28).
Sample 2015.02 was also circulated in 2012 (sample code: MPS15). In 2012, a total of 64% of the participants mentioned MPS IV as the most likely diagnoses, which is slightly higher compared to 2015 (60%).
Overall proficiency (based on points) was 61%.
This sample was not considered eligible for critical error.

Sample MPS2015.03

Sample type. An MPS III A sample, male 9 year-old patient with a mild phenotype.
Analytical proficiency. Total GAG concentration in this sample was reported elevated by 91% of the participants (86/94), and 87.0% of the participants (80/92) reported elevated HS in this sample (Fig. 2).
Interpretative proficiency. Although this sample was from a mild MPS III patient, still 80 % reported the correct diagnosis. Three participants (3%) were not sure whether this was a normal or an MPS III sample. Twelve labs stated an incorrect diagnosis, including 'normal' (n=6).
Overall proficiency (based on points) was 83%.
This sample was not considered eligible for critical error.

Sample MPS2015.04

Sample type. An MPS III B sample, obtained from a female 20 year-old patient, also with a mild phenotype.
Analytical proficiency. 87% of the participants (83/95)reported elevated total GAG concentration in this sample. Elevated HS was reported by 86.7% of the participants (78/90; Fig. 2). These numbers are slightly lower compared to sample MPS2015.03, which was also from a mild MPS III patient, and this might be related to the lower GAG concentration in sample MPS2015.04 due to higher age.
Interpretative proficiency. The slightly lower analytical proficiency is reflected in the interpretative proficiency, which is 80% based on scores. From the 13 labs that reported an incorrect diagnosis, 9 concluded 'normal'.
This sample was not considered eligible for critical error.

Comparison of the proficiencies of the two MPS III samples 2015.03 and 2015.04 shows that 7 laboratories failed to identify MPS III in sample 2015.03 (MPS III A, 9 y), while 10 other labs failed to identify MPS III in sample 2015.04 (MPS III B, 20 y). Seven labs missed MPS III in both 2015.03 and 2015.04. This latter group included 4 participants that did not report electrophoresis/TLC results and diagnosis. In conclusion it is reassuring that most labs successfully identified both or at least one of the two mild MPS III patients, suggesting that MPS III detection in urine is functioning relatively well.

Sample MPS2015.05

Sample type. Normal control, 13-year old female.
Analytical proficiency. 88% (84/95) of the participants reported a normal result in the quantitative GAG test. Seven of the 11 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). Four reported elevated DS, 3 found elevated HS and 1 lab reported elevated KS.
Interpretative proficiency. 85% correctly concluded that this was not an MPS sample. Four participants (4%) did conclude an MPS disorder: MPS I (n=1), MPS I or II (or VI) (n=2), MPS III (n=1). Two participants stated that MPS IV could not be excluded.
Overall proficiency (based on points) was 86%.
This sample was not considered eligible for critical error.

Sample MPS2015.06

Sample type. MPS II patient, aged 15.
Analytical proficiency. Analogous to the other MPS II sample circulated this year (MPS2015.01) The percentage of participants reporting an elevated quantitative GAG test result was high (99%).
Most labs (97%) reported abnormal test results of GAG electrophoresis or TLC. 96% reported elevated DS, while 65% found elevated HS (Fig. 2). The DS/HS ratio in this sample apparently was higher than in sample 2015.01 for which 78% of the respondents reported elevated HS.
Interpretative proficiency. MPS I or II was reported as the most likely diagnosis by 57% of the participants, while another 25% concluded MPS I, II or VI. In total, 81% mentioned MPS II among the possible diagnoses. Fourteen labs (14%) did not mention MPS II as a possibility. The majority of these concluded MPS VI (n=8).
Overall proficiency (based on points) was 82%.
Reporting 'normal' as the most likely diagnosis was considered a critical error in this sample (n=1).

On average, 7 % of the laboratories did not report a diagnosis (range 4-11 for the 6 samples). This was partly due to the fact that these laboratories did not perform qualitative analysis of GAG, but also inconclusive test results, e.g. for the MPS IV sample, is likely to decrease 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 2016

The format of the MPS 2016 scheme will be similar to that of previous years.

The following change will be made in the 2016 scheme:

- The number of points required for adequate performance will be increased from 12 to 15. This is equivalent to 60% of the maximum score achievable in a year and is the level required in all other ERNDIM schemes. If the 60% cut-off level would have been applied to the 2015 surveys, the number of satisfactory performers would decrease from 88 to 85.

Website reporting to submit results was successfully used in 2014/2015 and will be maintained in the Urine MPS scheme in 2016. 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 2016
Analysis start survey 1 (website open):	April 1, 2016
Deadline for reporting results of survey 1:	May 2, 2016
Interim report survey 1 available:	June 2016
Analysis start survey 2 (website open):	September 1, 2016
Deadline for reporting results of survey 2:	October 3, 2016
Interim report survey 2 available:	November 2016
Annual report 2016	March 2017

Rotterdam March 19, 2016

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