ERNDIM
Diagnostic Proficiency Testing
DPT Centre: Netherlands

ANNUAL REPORT 2016

Scheme Organiser	Scientific Advisor	Deputy Scientific Advisor
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1. Introduction

The ERNDIM Diagnostic Proficiency Testing (DPT) Scheme is the ultimate external quality assessment scheme for biochemical genetics laboratories. The minimal required test panel for participation in any DPT scheme includes dip sticks, amino acids, organic acids and quantitative GAG. DPT-NL additionally requires the analysis of oligosaccharides and purines-pyrimidines. It is strongly recommended to have the following tests available for DPT-NL: qualitative GAG analysis (electrophoresis/TLC), sialic acid, creatine-guanidinoacetate and polyols-sugars. Please note that in DPT schemes it is allowed to obtain results from neighbouring laboratories if one does not offer a certain test, while such test is deemed necessary for a sample. It is required to indicate in the report that results were obtained from a cluster lab.

2. Participants

The 2016 scheme had 21 participating laboratories with the allocations listed in Table 1. Twenty participants submitted results for both surveys. One participant did not submit any results.

Table 1. Participants in DPT-NL 2016

Country	Number of participants
Australia	2
Belgium	5
Czech Republic	1
Germany	2
The Netherlands	8
South-Africa	1
Spain	1
Switzerland	1

3. Logistics of the scheme

The samples used in the DPT scheme are authentic human urine samples and were selected by the Scientific Advisors of the scheme (Table 2). Sample pre-treatment (heat-treatment) was performed in the Scientific Advisor's laboratory, while aliquoting and dispatch of the samples was done by the Scheme organiser. Two surveys were performed; 2016-1 (samples A, B, C) starting February 22, and 2016-2 (samples D, E, F) starting May 23. Before dispatch to participants one set of samples was sent to the Scientific Advisor and checked. In all six samples the typical metabolic profiles were preserved. Sample dispatch was done February 1, 2016 by DHL.
Reports of the samples were submitted electronically on the website of the Swiss organisation for quality control (CSCQ) (https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php). The time allotted for submitting reports was 3 weeks after opening of the website. Clinical information on the samples was provided through the website.

Table 2. The 2016 DPT NL samples. Samples were provided by dr Fowler (Zurich, Switzerland, DPT-CH), dr Heiner (Groningen, The Netherlands), dr Kozich / dr Chrastina (Prague, Czech Republic, DPT-CZ), dr Martens (Brussels, Belgium) and dr Onkenhout (Leiden, The Netherlands). One sample was acquired with help of the Dutch patient organisation, VKS.

Sample	Diagnosis
A	Hyperoxaluria type 2 (common sample)
B	3MCC
C	HGPRT deficiency (Lesch-Nyhan syndrome)
D	Fucosidosis
E	MCADD
F	Prolidase deficiency

4. Scoring of results

General scoring criteria are depicted in Table 3. Scoring of the 2016 samples was performed according to the criteria summarised in Table 4. In order to achieve harmonised scoring throughout the five European DPT schemes, the ERNDIM Board has instituted a second scoring officer belonging to one of the partner DPT schemes as of 2011. The external scores are discussed with the scheme's own scientific advisor(s). For the DPT-NL scheme, the second evaluation was performed by the scientific advisor of the DPT CH scheme in 2016 (Prof. B. Fowler).

Table 3. General criteria for scoring results.

Item	Criterium	Score
Analytical performance:	Correct results of the appropriate tests	2
	Partially correct or non-standard methods	1
	Unsatisfactory or misleading	0
Interpretative performance:	Good (diagnosis was established) and adequate recommendations were suggested	2
	Helpful but incomplete	1
	Misleading / wrong diagnosis	0
	Total maximal score for each sample	4

Table 4. Specific criteria for scoring results of the 2016 samples.

Sample	Analytical	points	Interpretation	points
A	Oxalate elevated Glycerate elevated	1 1	Hyperoxaluria type 2 Hyperoxaluria unspecified or wrong type Advice to measure oxalate when oxalate analysis is not performed and no diagnosis	2 1 1
B	3-Me-Crotonylglycine (and) 3-OH-isovaleric acid elevated	2	3MCC	2
C	Xanthine and hypoxanthine elevated	2	HGPRT def /Lesch-Nyhan syndrome Xanthine oxidase def	2 1
D	Abnormal oligosaccharide pattern	2	Fucosidosis Any other oligosaccharidosis or oligosaccharidosis unspecified	2 1
E	Elevated MCADD characteristic acylglycine(s)	2	MCADD	2
F	Elevated iminopeptides or dipeptides Interference or medication metabolites in amino acid analysis	2 0	Prolidase deficiency Anormal amino acid pattern and advice to further investigate or repeat	2 1

The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. In accordance with a previous decision by the board, participants who failed to achieve satisfactory performance were those who scored less than 15 points out of the maximum of 24 in this year. Starting with the 2014 schemes the concept of 'critical error' is introduced to the assessment of the DPT 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 by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on November 30, 2016. Possible critical errors identified in the 2016 scheme are listed in Table 5. Previously, critical errors were not applied in 'normal' samples (i.e. without IEM). This will be changed. In future surveys a normal sample may be eligible for critical error if (1) a patient is not suspected of an IEM, and (2) the majority of participants do not mention abnormalities leading to diagnosis, and (3) the reported diagnosis could lead to harmful treatment.

Table 5. Critical errors in the 2016 scheme.

Sample	Critical error	No. of occurences
A	Failure to report elevated oxalic acid and glyceric acid	0
B	Failure to report elevated 3-methyl-crotonylglycine and 3MCC deficiency	0
C	None	-
D	Failure to report abnormal oligosaccharides and no recommendation to perform oligosaccharide analysis	0
E	Failure to report MCADD metabolites and MCAD diagnosis	1
F	None	-

5. Communication of results

The CSCQ scheme evaluation programme was used to generate individual lab reports and these were distributed on April 25th and July 8th 2016. These individual participant reports included the scores obtained. Scores are still preliminary in interim reports, since the 2nd evaluation has not been done. Discussion of the results took place in Rome during the ERNDIM workshop held at the SSIEM conference on September 6, 2016. The meeting, open to participants only, was attended by representatives from 14 of the participating institutes. The scientific advisor of the scheme, George Ruijter (Erasmus Medical Centre Rotterdam) chaired the meeting and presented the analytical/diagnostic points of interest. The minutes of this meeting and the presentation have been sent September 15, 2016 to all DPT-NL participants attending the meeting.
Finally, the annual report summarises scheme organisation and results and also includes items discussed during the DPT meeting.

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

Two Performance Support letters will be send for the 2016 surveys. Five were issued for the 2015 surveys.

6. Proficiency of the 2016 surveys

Proficiencies (% of maximal achievable points for all labs) of the 2016 samples are summarized in Table 6. Distribution of scores is given in Table 7.
Maximal scores (24 points) were obtained by 2 out of the 20 participating labs, while 13 labs scores at least 20 p. Samples A, B and E were rather straightforward, while sample C, D and F were more challenging. Overall performance for all six samples was 85%, which is comparable to the performance in 2015 (also 85%), but considerably better than earlier years.

Table 6. Performance on the DPT 2016 samples.

Sample	Diagnosis	No. of reports	Proficiency (%)		
			analytical	interpretation	TOTAL
A	Hyperoxaluria type 2	20	80	98	89
B	3MCC	20	100	100	100
C	HGPRT deficiency (Lesch-Nyhan syndrome)	20	85	78	81
D	Fucosidosis	20	95	65	80
E	MCADD	20	95	95	95
F	Prolidase deficiency	20	58	70	64

Table 7. Distribution of final scores in 2016; for each sample the number of participants with score 0/1/2/3/4 points is given. Score 0 due to non-submission of results is not included.

Sample	0 points	1	2	3	4
A	0	0	1	7	12
B	0	0	0	0	20
C	3	0	1	1	15
D	0	1	1	11	7
E	1	0	0	0	19
F	5	2	1	1	11

7. Results of individual samples and evaluation of reporting

Sample 2016-A: Hyperoxaluria type 2 (GRHPR deficiency, OMIM 260000).

Clinical description: At the age of 5 years this boy was referred for the first time to a pediatric nephrologist, because of urolithiasis. At ages 7 and 10, again renal stones were found. At the time of the urine collection, he was 10 y old and in good health. He used no medication, had a normal diet and adequate renal function.

Sample A was the common sample distributed to participants of all 5 DPT centres and was discussed during the ERNDIM workshop at the SSIEM symposium in Rome, September 6, 2016. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Sample 2016-B: 3-Methylcrotonyl-CoA carboxylase deficiency (OMIM 210200, 210210).

Clinical description: A male, aged 30, investigated for rheumatic disease.

Analytical.
All participants reported elevated 3-methylcrotonylglycine (2 points) and all but one elevated 3-OH-isovaleric acid. Analytical proficiency was 100%. The following concentrations were reported:

Organic acid	median value	range	n
3-OH-isovaleric acid	1366	105-2183	15
3-methylcrotonylglycine	398	151-1650	10

The presence of allopurinol/oxypurinol was reported by many labs. Also elevated xanthine, low/decreased uric acid and elevated orotic acid were mentioned. This patient was apparently treated by allopurinol for his rheumatic disease.

Interpretation.
All participants concluded that 3MCC deficiency was the most likely diagnosis. Biotinidase deficiency (and holocarboxylase synthase deficiency) was considered a possibility by some participants. This cannot be excluded with certainty on the basis of merely urine organic acids, but is unlikely since no other organic acid abnormalities were present suggestive for multiple carboxylase deficiency (such as elevated lactate, methylcitrate, 3-OH-propionic acid). Further investigations to establish 3MCC or biotinidase deficiency is necessary. The possibility of dietary biotin deficiency was suggested and as a consequence the need to rule out biotinidase deficiency.
Recommendations for further investigations included: plasma acylcarnitine analysis, 3MCC activity in fibroblasts and mutation analysis of the MCC1 and MCC2 genes.

Overall proficiency (based on points) 100%
The diagnosis 3MCC deficiency in this sample was straightforward, but is an incidental finding. It does not explain the symptoms in the patient. Five participants did mention this in their report. Clearly, if this had been a real diagnostic request, the absence of a causal relationship between symptoms and the diagnosis must be explained.

Sample 2016-C: Lesch-Nyhan syndrome (HGPRT deficiency; OMIM 300332).

Clinical description: A 12 months old boy with muscular hypotonia, dystonia and slight global retardation. The sample was taken at 5 years while under treatment.

At 12 months of age this patient had elevated xanthine, hypoxanthine and uric acid in urine and high plasma uric acid. HGPRT activity in lymphocytes was deficient (Amsterdam) confirming Lesch-Nyhan syndrome. The diagnosis was additionally confirmed by DNA analysis. Treatment has been undertaken with allopurinol. Before the start of allopurinol treatment xanthine was 500 mmol/mol creat, hypoxanthine 459 and uric acid 1991.

Analytical.
Labs performing purine and pyrimidine analysis were able to detect increased hypoxanthine (n=16/20) and xanthine (n=17/20). Uric acid was reported normal (n=8) or low/decreased (n=8). The presence of allopurinol (or oxypurinol) was reported by 15 labs. Orotic acid was reported elevated by 2 labs, but normal by 5 others. Orotidine was detected in this sample by participants using LC-MS/MS for purine-pyrimidine analysis (n=5) as well as labs using HPLC-UV (n=6). Accumulation of orotidine, and to a lesser extent orotic acid, is explained by the allopurinol use.
Analytical proficiency was 85%. The following concentrations were reported:

Purines-Pyrimidines	median value	range	n
Xanthine	620	119-1649	15
Hypoxanthine	1271	702-1720	15
Uric acid (uricase)	208	160-266	8
Uric acid (LC)	224	174-228	4

Many labs reported slightly increased values of their GAG screening test (n=13). Electrophoresis or TLC was considered normal by 5 labs and 'borderline' by 1. Elevated GAG in this urine sample is unexplained. Oligosaccharides were stated as abnormal by 8 labs, but normal by another 5. The pattern is clearly abnormal for a 5-year old boy and by some participants interpreted as similar to Pompe's disease or α-mannosidosis. However, direct comparison of sample 2016 C to Pompe and α-mannosidosis samples shows differences (Fig. 1). The prominent band in sample 2016 C is lactose. The oligosaccharide abnormalities may relate to special nutrition.

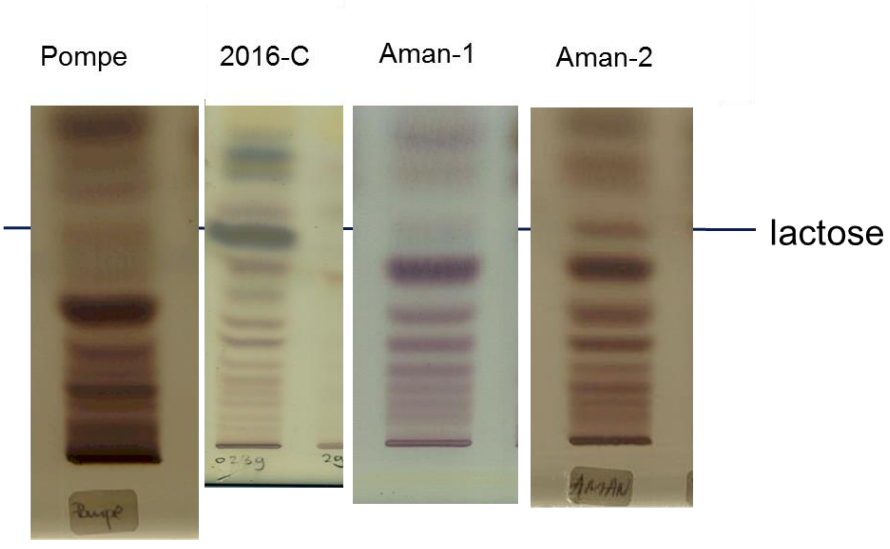

Fig. 1. Oligosaaccharide analysis of sample 2016 C compared to one Pompe and 2 different α-mannosidosis samples.

Interpretation.
From the 17 participants that determined purines-pyrimidines, 15 established the correct diagnosis. The following diagnoses were reported:

Diagnosis	Most likely	other possible	comment
HGPRT def	15	-	
Xanthine oxidase def	1	1	less likely based on clinical symptoms
PRPS superact	-	2	less likely based on clinical symptoms
Alpha-mannosidosis	1	1	oligosaccharide pattern not identical
MPS/mucolipidosis	1	1	GAG not clearly abnormal
ADSL def	1	-	SAICAr, S-Ado normal
Pompe disease	1	-	Oligosaccharide pattern not identical

Recommendations for further investigations included: HGPRT activity in erythrocytes or fibroblasts and mutation analysis of the HPRT gene.

Overall proficiency (based on points) 81%
Correct interpretation required purine analysis.

Sample 2016-D: Fucosidosis (α-L-fucosidase deficiency; OMIM 230000).

Clinical description: A boy aged 7 y with severe psychomotor retardation, coarse facies and dysostosis multiplex.

Analytical.
Oligosaccharide analysis (Fig. 2) was performed by 19/20 labs and all reported an abnormal profile, which was scored with 2 points. Almost all participants (18/20) reported (slight) elevation of total GAG, but only few reported abnormal excretion of GAG species (KS: 3/20, DS: 1/20). The occurrence of keratan sulfate in fucosidosis has been reported by Greiling et al (1978) J Clin Chem Clin Biochem 16:329. Four labs reported elevated conjugated sialic acid, while 2 reported elevated aspartylglucosamine.

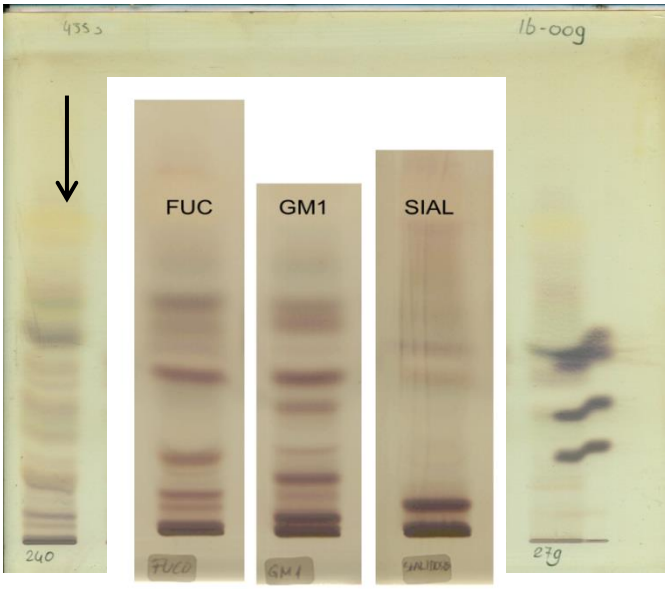

Fig. 2. Oligosacharide analysis by TLC. The arrow indicates sample 2016D. For comparison another fucosidosis sample, a GM1 sample and a sialidosis sample are also depicted.

Interpretation.
Seven participants interpreted the oligosaccharide pattern as characteristic for fucosidosis (2 points). Interpretation of the oligosaccharide pattern was apparently challenging and various other oligosaccharide disorders, mostly GM1 and sialidosis/galactosialidosis, were mentioned (1 point).

Diagnosis	most likely	other possible
Fucosidosis	7	-
GM1 gangliosidosis	5	3
Sialidosis/galactosialidosis	3	2
Mucolipidosis type II (III)	1	4
MPS IVB	1	2
MPS	2	-
Aspartylglucosaminuria	1	-
Oligosaccharidosis	-	1

Recommendations focused at measuring lysosomal enzyme activities consistent with the conclusions reported. α-Fucosidase activity testing is key in this patient. Mutation analysis of the FUCA1 gene is possible, but not widely available.
When interpretation of the oligosaccharide pattern is uncertain, several enzymes should be assayed in parallel. A mucolipidosis panel (several lysosomal enzymes in plasma) is usually available in labs performing LSD enzyme testing and α-fucosidase may be included in such panels.
Please note that an Oligosacharide kit containing positive urine samples is available at SKML (MCA laboratory, Winterswijk, The Netherlands).
A few reports have appeared in the literature describing oligosaccharide analysis by MALDI-TOF, such as Xia et al (2013) Clin Chem 59:1357-1368. In fucosidosis samples these authors report mono- and di-fucosylated oligosaccharides and, interestingly, some oligosaccharides characteristic for fucosidosis contain asparagine or sialic acid.

Overall proficiency (based on points) 80%
Abnormal oligosaccharides were reported by all participants performing oligosaccharide analysis, but interpretation was challenging.

Sample 2016-E: MCADD (OMIM 201450)

Clinical description: Girl diagnosed at the age of 2.5 years following an episode with coma. The urine sample was collected at age 19 y.

Sample E was obtained with the help of the Dutch Patient organisation VKS.

Analytical.
All, but one, of the participating labs found at least one of the acylglycines typically found in urine from MCADD patients (2 points). The following metabolites were reported elevated (sorted by decreasing number):

Organic acids	reported incr	median value	range
Hexanoylglycine	n=15	3.1	0-16
Phenylpropionylglycine	n=15	14.2	4-66
Suberylglycine	n=11	6.3	0-85
5-OH-hexanoic acid	n=3		
Suberic acid	n=2		
7-OH-octanoic acid	n=2		
Adipic acid	n=1		

In this sample of an MCADD patient in stable condition, the concentrations of the characteristic metabolites were not high, but yet clearly abnormal. In particular phenylpropionylglycine was easily detected (approx. 90 umol/L). Four labs reported abnormal acylcarnitines (e.g. C8, ratio C8/C10), while 3 considered the urine acylcarnitine pattern non-informative.

Interpretation.
Even though the hexanoyl-/phenylpropionyl-glycine concentrations were not very high, 19/20 participants identified these metabolites and established the correct diagnosis (2 points).
MADD was considered unlikely by 4 labs, but possible by 1 lab.
Recommendations included plasma free carnitine and acylcarnitine analysis, MCAD activity testing in lymphocytes and ACADM mutation analysis.

Overall proficiency (based on points) 95%
This was a straightforward sample, for both analysis and interpretation.

Sample 2016-F: Prolidase (PEPD) deficiency (OMIM 170100)

Clinical description: A 8 years old boy suspected of immunodeficiency. His history showed recurrent severe infections since neonatal period: sepsis, skin ulcers and upper respiratory tract infections leading to hearing problems with secondary mental retardation.

Analytical.
Iminopeptides/dipeptides characteristic for prolidase deficiency were reported by 12 participants (2 points). Five labs interpreted the amino acid abnormalities as interference/medication, while 3 participants reported normal or low concentrations of amino acids. The median of glycylproline concentrations reported was 548 mmol/mol (range 456-1153; n=4). Analytical proficiency was 58%.
Amino acids analysis following a procedure to hydrolyse iminopeptides was reported by 5 labs and recommended by another 2. Hydrolysis resulted in increases in proline (and hydroxyproline, glycine) and disappearance of iminopeptides.
GAG were reported slightly elevated by 8 labs, but normal by 6 labs.

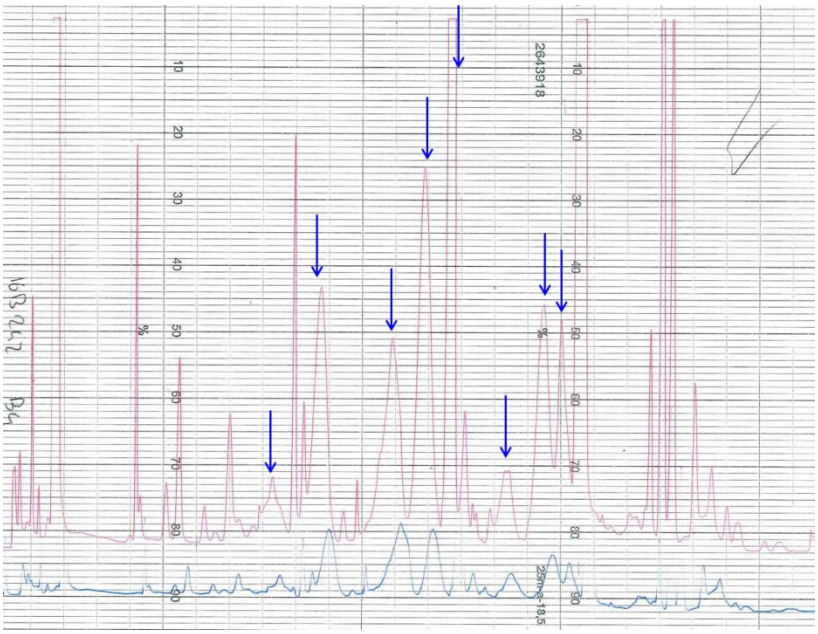

Fig. Amino acid analysis (Biochrom 30) of sample 2016 F.

Interpretation.
Prolidase was reported by 12 participants (2 points). The advice to repeat urine amino acid analysis was scored with 1 point. Interpretative proficiency was 70%.
PEPD activity tests in various cell types and PEPD mutation analysis were recommended. Hydrolysis of iminopeptides and repeating urine amino acid analysis in a new sample were also mentioned.

The following diagnoses were reported:

Diagnosis	Most likely	other possible	comment
Prolidase	12	1	
No diagnosis	5	-	
No diagnosis, repeat AA	2	-	
α-mannosidosis	1	-	oligosacch not typical for α-man

Overall proficiency (based on points) 64%
Recognition of the iminopeptide pattern in amino acid analysis was essential to establish diagnosis.

8. Preview of the 2017 scheme and changes in scheme organisation

The format and logistics of the DPT-NL scheme in 2017 will be identical to 2016.

Dr Marinus Duran has been the scientific advisor of DPT NL (or previously 'DPT Nijmegen') for many years. With his encyclopaedic knowledge on IEM he has thought us much about these rare disorders for which we are very grateful. Ries is retired now and will no longer be actively involved in DPT organisation. As of 2017 dr Willem Onkenhout (Erasmus MC) will be the deputy scientific advisor.

Tentative planning:

Shipment of samples by CSCQ (all six samples will be dispatched in one box):	February 6, 2017
Analysis start survey 1:	February 20, 2017
Deadline for reporting results of survey 1:	March 13, 2017
Interim report survey 1 available:	April 2017
Analysis start survey 2:	May 22, 2017
Deadline for reporting results of survey 2:	June 12, 2017
Interim report survey 2 available:	July 2017
Discussion of results (ERNDIM participant meeting, t.b.a.)	Autumn 2017
Annual report 2017	December 2017

Rotterdam, December 21, 2016

Dr George Ruijter
Scientific Advisor