ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Congenital Disorders of Glycosylation
Final Report 2016

[This final report was ratified by the Scientific Advisory Board in November 2016]

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1. Scheme Design

The scheme has been designed and planned by the Scientific Advisor (SA) and Scheme Organiser (SO, subcontractor on behalf of SKML), both appointed by and according to procedures laid down by the ERNDIM Board.

2. Samples

All EQA materials are lyophilised plasma or serum samples (25 µl). Laboratories that need a larger sample volume due to their analysis method (e.g. HPLC) were offered a 50 µl sample volume for a reduced scheme price. All samples are obtained following local ethical and consent guidelines.

3. Shipment

The six samples were sent out to the 62 registered laboratories in one parcel on 9th February 2016. Eighteen laboratories requested a total of 20 extra sample sets and were sent the larger sample volume.

4. Receipt of results

Returns were submitted by email to the SA. The returns for the first round (samples CDG 2016.01 - CDG 2016.03) and second round (samples CDG 2016.04 - CDG 2016.06) were received by the due date from 48 (76%) and 46 (73%) laboratories respectively.

There were three laboratories who failed to make a return on either submission round.

5. Scoring scheme

In agreement with ERNDIM rules, we applied a scoring system of 2+2:

Item C: technical aspects: 1 point for identification of an abnormal profile and 1 point for correct identification of the profile as type I or II.

Item D: diagnostic suggestions: This section should be filled for scoring. Just referring to a specialised lab is insufficient. If required, advice can be obtained from a reference laboratory or in collaboration with a clinical colleague. For normal profiles 2 points are scored. For abnormal profiles, comments should be made on the possibility of secondary causes in view of the clinical indication. In addition, the right suggestions should be made for the next step in the diagnostic process that eventually will lead to the genetic defect. Scoring for this part is not so straightforward, but we tried to keep it as consistent as possible. For samples .02 and .05, exclusion of secondary causes and standard work-up for CDG-I defects have to be mentioned. This includes PMM2 enzyme analysis or similar methods that lead to the diagnosis for these particular cases. For sample .06, the possibility of alcohol abuse for the CDG-I screening profile should be mentioned for full scoring.

The maximum score achievable with full submission for all samples is 24, while a maximum of 12 points are available for labs that only submitted results for the first or second round. The level for satisfactory performance is 15 points. Laboratories that participate only in one circulation are treated as partial-submitters and can achieve satisfactory performance with 8 points. For the 2014 scheme onwards, another

Version Date	Amendments
[1] 22 June 2017	• Page 1: Date of issue changed from 16 June 2016 to 16 June 2017 • Page 5: In table 3, 2 point score for technical analysis of sample 2016.06 inserted for lab 20 instead of a blank space

criterion for satisfactory performance will be the absence of any "critical error", which is defined as an error resulting from seriously misleading analytical findings and/or interpretations with serious clinical consequences for the patient. For the 2016 CDG scheme, no critical errors were identified. This has been agreed at the meeting of the Scientific Advisory Board on 30th November 2016.

6. Results of samples and evaluation of reporting

All submitted results are treated as confidential information and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting. For the purposes of evaluation, the Scientific Advisor's centre is not included in the following results.

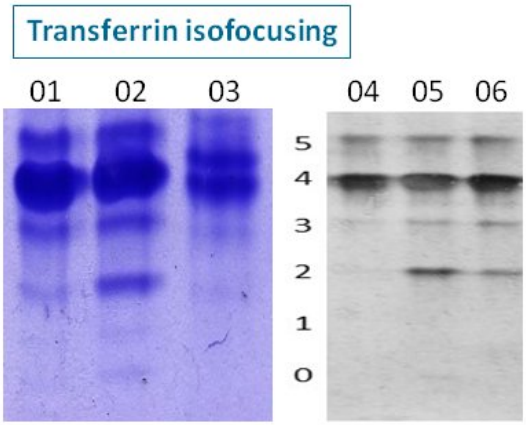
For the reporting laboratories, isofocusing was the method employed most often (31), followed by HPLC (12) and CE (11), mass spectrometry (2) and western blot (1).

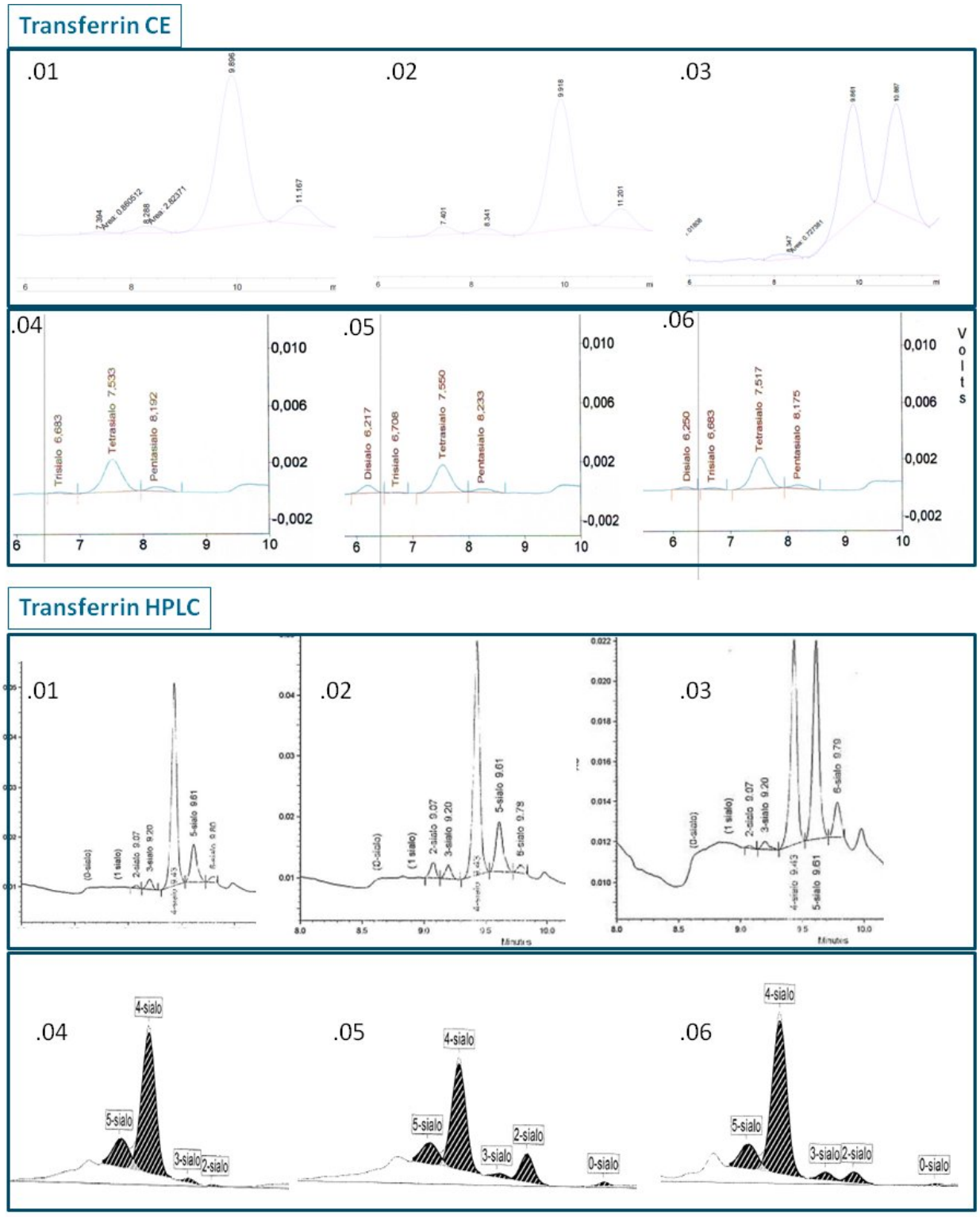
The shipped samples were from CDG patients, from controls and of patients with established excessive intake of alcohol. The final results of the six samples with respect to CDG are summarized in Table 1 below.

Table 1: Samples in the 2016 scheme

Sample	Clinical information (age, sex, phenotype)	Diagnosis
2016.01	F, 4 years, hepatomegaly, cataract, skeletal dysplasia	Control
2016.02	M, 10 yrs, deafness, epilepsy, intellectual disability	PMM2-CDG OMIM: 212065
2016.03	M, 4 months, cutis laxa, mental retardation	Control, transferrin polymorphism
2016.04	F, 8 yrs, frequent infections, liver fibrosis	Control
2016.05	F, 1 year, inverted nipples, hypotonia, strabismus coagulopathy, psychomotor retardation	PMM2-CDG OMIM: 212065
2016.06	M, 30 years, liver disease	Alcohol abuse

Figure 1. Example profiles of the six 2016 samples are shown below, as analysed by the most commonly employed methods: isofocusing, HPLC or CE. Examples were randomly selected from the submissions and shown anonimized.





ERNDIM CDG 2016.01
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 99%.

ERNDIM CDG 2016.02
The majority of labs reported this sample as abnormal and nearly all centers correctly assigned this profile as type I profile. A few labs missed the rather mild elevation of disialotransferrin as abnormal and suggestive of a CDG-I profile. The age and clinical presentation are not suggestive of a secondary cause for CDG-I (fructosemia, galactosemia, alcohol abuse) and not directly suggestive of a specific CDG-I subtype, although deafness has been reported as rather frequent in RFT1-CDG. The advice for further diagnostics should include the option of PMM2-CDG. Proficiency score: 86%.

ERNDIM CDG 2016.03
The majority of labs reported an abnormal profile of transferrin, suggesting a protein polymorphism. This transferrin polymorphism was clearly visible by IEF, CE, and HPLC (as shown in Figure 1) by the presence of two bands/peaks of equal intensity around the position of tetrasialotransferrin.

The clinical symptom of cutis laxa is well known within the context of CDG in ATP6V0A2-CDG. More recently, defects in ATP6V1E1 and ATP6V1A have also been linked to cutis laxa and variable glycosylation abnormalities (Van Damme et al AJHG 2017). Of note, transferrin N-glycosylation can be normal within the first 6 months of life, in which case the O-glycosylation of apoCIII is already reduced. Six laboratories commented on the possibility of ATP6V0A2-CDG and few suggested to perform ApoCIII isofocusing (no points were subtracted for not mentioning this possibility). The current sample was from a patient with unknown cause of cutis laxa.

Proficiency score: 93%.

ERNDIM CDG 2016.04
A normal profile was identified by all centers and interpreted as normal by nearly all centers. Proficiency score: 97%.

ERNDIM CDG 2016.05
Nearly all labs reported this sample as abnormal and correctly assigned this profile as type I. The age and clinical presentation could very well fit with the final diagnosis of PMM2-CDG, but other CDG-I subtypes are equally likely on basis of clinical symptoms only. The advice for further diagnostics should include the option of PMM2-CDG as most frequent CDG-I subtype and known to be associated with this clinical presentation. In view of age and symptoms, secondary causes are unlikely.

Proficiency score: 95%.

ERNDIM CDG 2016.06
Nearly all labs reported this sample as abnormal and correctly assigned this profile as type I. This sample was derived from an adult patient with excessive alcohol intake and liver fibrosis. In general, this results in abnormal CDG-I profiles. In view of age and clinical description, the presence of a secondary cause for the CDG-I profile is quite likely and should be mentioned.

Proficiency score: 93%.

Overview of scoring

Table 2: Proficiency per sample

Sample	No of returns	Technical Aspects (%)	Diagnostic Suggestions (%)	Total (%)
CDG2016.01	55	99%	100%	100%
CDG2016.02	55	93%	78%	86%
CDG2016.03	55	92%	94%	93%
CDG2016.04	58	97%	97%	97%
CDG2016.05	58	98%	92%	95%
CDG2016.06	58	96%	91%	93%

Table 3: Detailed scores for submitting laboratories

2016	Technical, item C							Advice, item D							
Sample ID	.01	.02	.03	.04	.05	.06	Total	.01	.02	.03	.04	.05	.06	Total	Total score
Average score	1,98	1,85	1,84	1,95	1,97	1,91		1,96	1,77	1,85		1,49	1,98		max 24
1	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
2	2	2	2	2	2	2	12	2	2	2	2	2	1	11	23
3	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
4	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
5	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
6	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
7	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
8	2	2	2	2	2	2	12	2	1	2	2	2	1	10	22
9	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
10	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
11	2	2	2	2	2	2	12	2	2	2	2	1	2	11	23
12	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
13	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
14	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
15	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
16	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
17	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
18	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
19	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
20	2	2	2	2	2	2	10	2	2	2	2	1	2	11	21
21	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
22	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
23				2	2	2	6				2	2	2	6	12
24	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
25	2	2	2	2	2	2	12	2	1	2	2	2	1	10	22
26	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
27	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
28	2	2	0	2	2	2	10	2	2	0	2	2	2	10	20
29	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
30	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
31	2	0	2	2	2	2	10	2	0	2	2	2	2	10	20
32	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
33	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23
34	2	2	2	2	2	2	12	2	2	2	2	2	1	11	23
35	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
36	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
37	2	2	2	1	1	2	10	2	1	2	0	0	0	5	15
38	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
39	2	0	2	2	2	2	10	2	0	2	2	2	2	10	20
40	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
41	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
42	2	2	0	2	2	1	9	2	2	0	2	2	1	9	18
43	2	0	0	2	2	0	6	2	0	2	2	0	0	6	12
44	2	2	2	2	2	2	12	2	1	2	2	2	2	11	23

2016	Technical, item C							Advice, item D							
Sample ID	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**	**Total**	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**	**Total**	**Total score**
Average score	1,98	1,85	1,84	1,95	1,97	1,91		1,96	1,77	1,85		1,49	1,98		**max 24**
45	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
46	2	2	1	2	2	2	11	2	2	1	2	2	2	11	22
47	2	2	2	2	2	2	12	2	0	2	2	1	2	9	21
48				2	2	2	6				2	1	1	4	10
49	2	2	2	2	2	2	12	2	0	2	2	2	2	10	22
50	2	1	2	2	2	2	11	2	1	2	2	2	2	11	22
51	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
52	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
53				2	2	2	6				2	2	2	6	12
54	2	2	2				6	2	2	2				6	12
55	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
56				2	2	2	6				2	2	2	6	12
57	1	1	2	2	1	0	7	2	1	2	2	1	1	9	16
58	2	2	0	0	2	2	8	2	1	0	0	2	2	7	15
59	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24