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Congenital Disorders of Glycosylation
Final Report 2018

[This final report was ratified by the Scientific Advisory Board in November 2018]

Date of issue: 15 July 2019

Amended reports issued: 21 August 2019[1]

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

Samples were selected by the Scientific Advisor and tested for suitability in the Scientific Advisor's laboratory (Laboratory of Genetic, Endocrine and Metabolic Disease, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands). Preparation and dispatch of the EQA samples was done by the Scheme organiser (MCA Laboratory, Winterswijk, Netherlands). All EQA materials are lyophilised plasma or serum samples (35 µl). Laboratories that need a larger sample volume due to their analysis method (e.g. HPLC) were extra sample sets 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 66 registered laboratories in one parcel on 13th February 2018. Twenty-two laboratories requested a total of 30 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 2018.01 - CDG 2018.03) and second round (samples CDG 2018.04 - CDG 2018.06) were received by the due date from 62 (94%) and 60 (91%) laboratories respectively. An additional 2 (3%) and 4 (6%) labs submitted their results for the first round and second round, respectively, after the submission deadline. Five labs only submitted results for the first round and 1 lab only submitted results for the second round. There were two 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 the presence of a secondary cause in light 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 sample .02, PMM2-CDG should have been mentioned and for sample .03 PGM1-CDG should be mentioned for full scores. For sample .04, further diagnostic tests should be indicated that result in identification of the diagnosis MAN1B1-CDG should be indicated. These could include glycan analysis tests or specific mentioning of MAN1B1. For sample .06, exclusion of liver disease as secondary cause can be mentioned.

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

Version Number (& Date)	Amendments
[1] version 2 (21 August 2019)	• Page 2: Table 2 updated to reflect changes to scores in table 4 (see below). • Page 5: In table 4 scores for samples 2018.04-06 have been inserted for lab 4.

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 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 2018 CDG scheme, no critical errors were identified. This has been agreed at the meeting of the Scientific Advisory Board on 29th November 2018.

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 (34), followed by HPLC (11) and CE (15), mass spectrometry (4) and western blot (1).

The shipped samples were from (CDG) patients and from controls. The final results of the six samples with respect to CDG are summarized in Table 1 below.

Table 1: Samples in the 2018 scheme

Sample	Clinical information (age, sex, phenotype)	Diagnosis
2018.01	F, 8 year, hepatomegaly, intellectual disability	Control
2018.02	M, 1 year, hypotonia, nystagmus, cerebellar hypoplasia	PMM2-CDG
2018.03	F, 14 year, short stature, past episodes of rhabdomyolysis, increased transaminases	PGM1-CDG
2018.04	M, 7 year, intellectual disability, obesitas	MAN1B1-CDG
2018.05	M, 5 year, low blood glucose, intellectual disability, hypotonia	Control
2018.06	F, 45 year, hepatitis	Chronic alcohol abuse

Table 2: Scoring of samples in the 2018 scheme

Sample	No of returns	Technical Aspects (%)	Diagnostic Suggestions (%)	Total (%)
CDG2087.01	62	98%	99%	99%
CDG2018.02	58	98%	86%	92%
CDG2018.03	63	80%	64%	72%
CDG2018.04	59	94%	77%	86%
CDG2018.05	61	99%	99%	99%
CDG2018.06	60	98%	79%	88%

Table 3: Distribution of scores (for labs that submitted results)

Total Score	No of labs
<60%	1
60 – 69.9%	3
70 – 79.9%	10
80 – 89.9%	13
90 – 99.9%	14
100%	24
Total	**65**

The full anonymised results for all labs that submitted results are given in Table 4 on page 5-6 at the end of this report.

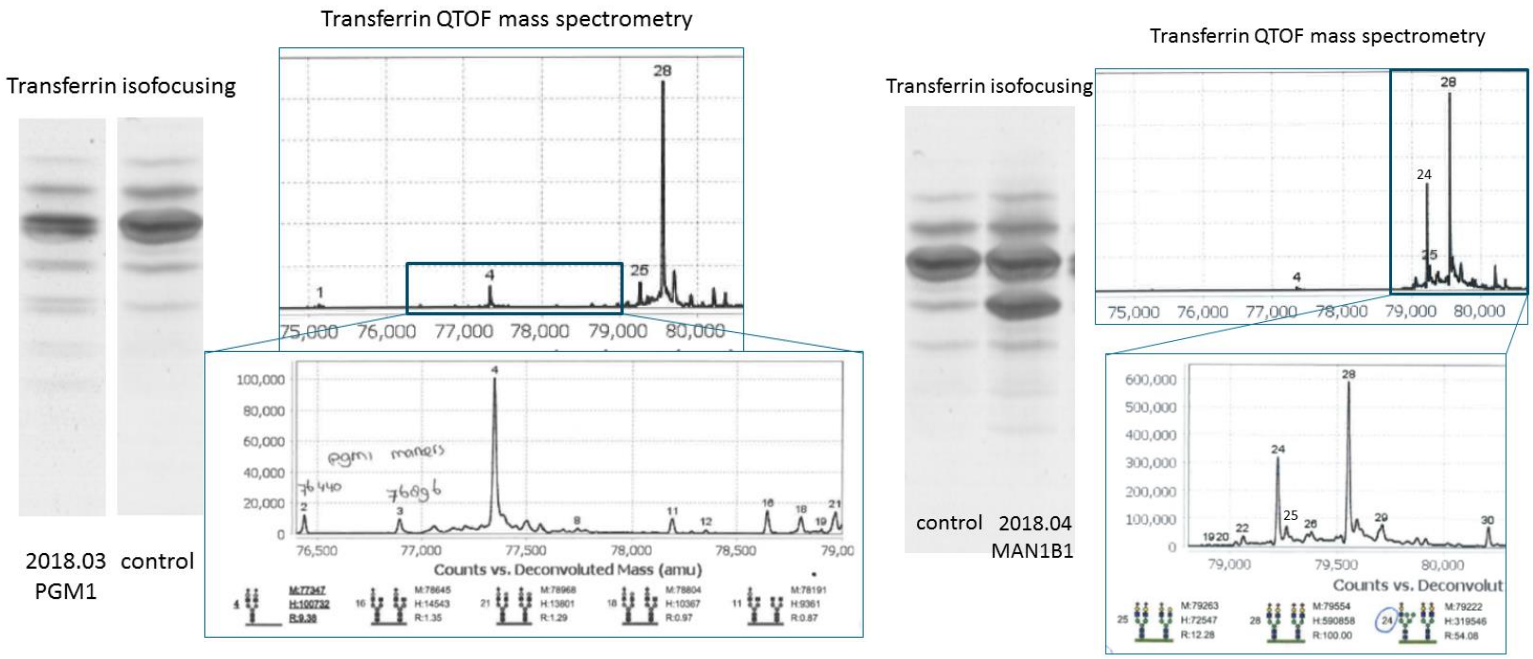


Figure 1. Illustrations of the diagnostic CDG patient samples CDG2018.03 and CDG2018.04. Shown are the results of transferrin isofocusing and of transferrin mass spectrometry. For PGM1-CDG (left, sample .03), diagnostic glycan structures include the combination of a lack of glycans (peaks 2, 3, and 4) and a lack of galactose (peaks 2, 3, 11, 16, 18, and 21). For MAN1B1-CDG, the main diagnostic glycan structure is peak 24, consisting of a so-called hybrid N-glycan (hybrid meaning a glycan with 1 antenna as complex glycan, ending with sialic acid, and 1 antenna as a mannose containing glycan).

ERNDIM CDG 2018.01: Control
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 99%.

ERNDIM CDG 2018.02: PMM2-CDG
Nearly all laboratories reported this sample as abnormal and correctly assigned the profile as type I. The sample is from a patient with a clinical suspicion for PMM2-CDG. This diagnosis was confirmed by identification of abnormal transferrin glycosylation (CDG type I), identification of enzymatic deficiency of phosphomannomutase and compound heterozygous mutations in the *PMM2* gene. The advice for further diagnostics should include the option of PMM2-CDG as most frequent CDG-I subtype. Proficiency score: 92%.

ERNDIM CDG 2018.03: PGM1-CDG
This is a bit more challenging sample as 8 out of 58 labs reported a normal profile. The sample is from a patient with PGM1 who was under treatment with galactose and therefore only mildly abnormal. The first sample of this patient showed much stronger (and diagnostic) glycosylation abnormalities, after which a diagnosis of PGM1-CDG was confirmed by enzymatic analysis of phosphoglucomutase and sequencing of the *PGM1* gene. In our experience, PGM1-CDG patients can present with similarly mild profiles upon diagnosis (i.e. before initiation of treatment). Nevertheless, since the sample was from a treated patient, no cricital error was defined for sample 2018.03. PGM1-CDG results in a mixed CDG-I/CDG-II with a lack of glycans and abnormal glycan structures lacking galactose. In most screening techniques, this mixed profile is not easily seen and quite a number of profiles (irrespective of the method) look like a mild CDG-II profile. In some methods, such as WB and MALDI-MS, the CDG-I aspect is observed more clearly. Therefore, quite a number of labs indicated a CDG-I profile.

Irrespective of the abnormal profile type, the clinical symptoms are highly suggestive for PGM1-CDG. 17 out of 58 centers did not suggest proper follow up diagnostics that would end up in a diagnosis of PGM1-CDG.

Identification of the profile as abnormal and advice for further diagnostics in the direction of PGM1-CDG should be included for full scoring. Proficiency score: 72%.

ERNDIM CDG 2018.04: MAN1B1-CDG
The majority of labs reported this sample as abnormal with a technical proficiency of 94%. A number of labs reported a normal profile in the assumption of a transferrin polymorphism. This sample is from a patient with MAN1B1-CDG. The diagnosis was made on basis of the glycan structural abnormalities (see Figure 1) and MAN1B1 sequencing. MAN1B1-CDG can be a challenge to diagnose, since the abnormal band in transferrin profiling co-migrates with a relatively common transferrin polymorphism variant. In addition, the abnormally

glycosylated transferrin can have a more or less equal intensity as compared to the tetrasialotransferrin band. In such cases, it is critical to definitely exclude a transferrin polymorphism by analysis of the parents or treating the sample with sialidase. Alternatively, high-resolution mass spectrometry of transferrin can solve the diagnosis. Proficiency score: 85%.

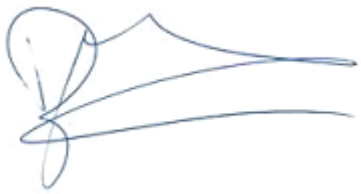
ERNDIM CDG 2018.05: control
A normal profile was identified by all centers and interpreted as normal by nearly all centers. Proficiency score: 99%.

ERNDIM CDG 2018.06: alcohol abuse
Many laboratories reported this sample as abnormal and indicated a mild type I profile. This sample is from an individual with chronic alcohol use. This is known as a secondary cause for (mild) CDG-I profiles. Liver pathology in some cases can result in a mild type II screening profile, but in this case the abnormal CDG-I profile can be explained by alcohol abuse. Proficiency score: 88%.

7. Preview of the 2019 scheme

The format of the scheme will remain the same, however, website reporting will be available for the first round of the 2019 scheme. The URL is **https://cscq.hcuge.ch/cscq/ERNDIM/** and details were included in the 2019 CDG scheme instructions.

For the 2019 scheme year the SAB have agreed to increase the level required for satisfactory performance to 70%. This implies that 17 out of 24 points should be scored for full submission and 8 out of 12 points for partial responders



Dirk Lefeber
Scientific Advisor

Note: This annual report is intended for the participants of the CDG scheme. The contents of this report or data derived from the use or analysis of ERNDIM EQA materials must not be used in written publications or oral presentations unless the explicit prior consent of ERNDIM has been granted.

Table 4: Detailed scores for submitting laboratories

2018	Technical, item C							Advice, item D							
Sample ID	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**		**.01**	**.02**	**.03**	**.04**	**.05**	**.06**		
Average score / **Lab ID**	1,97	1,97	1,60	1,88	1,98	1,95	**Total**	1,98	1,72	1,29	1,53	1,98	1,58	**Total**	**Total score (max 24)**
1	2	2	1	1	2	1	9	2	2	2	0	2	0	8	**17**
2	2	2	2	2	2	2	12	2	1	0	2	2	1	8	**20**
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	0	2	2	10	2	2	2	0	2	2	10	**20**
7	2	2	2	2	2	2	12	2	0	0	2	2	1	7	**19**
8	2	-	2	2	2	2	10	2	-	0	2	2	2	8	**18**
9	2	2	2	2	2	1	11	2	1	0	1	2	1	7	**18**
10	2	2	2	2	2	2	12	2	2	0	2	2	2	10	**22**
11	2	2	1	2	2	2	11	2	2	1	2	2	2	11	**22**
12	2	2	2	2	2	2	12	2	2	2	2	2	1	11	**23**
13	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
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	2	2	2	2	2	12	**24**
19	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
20	2	2	2				6	2	2	2				6	**12**
21	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
22	-	-	1	2	2	2	7	-	-	1	2	2	2	7	**14**
23	2	2	2	1	2	2	11	2	2	2	1	2	2	11	**22**
24	2	2	0	2	2	2	10	2	2	0	2	2	2	10	**20**
25	2	2	2	2	2	2	12	2	2	2	1	2	1	10	**22**
26	2	2	2	2	2	2	12	2	2	2	2	2	1	11	**23**
27	2	2	1	1	2	2	10	2	2	1	1	2	2	10	**20**
28	2	-	2	2	2	2	10	2	-	0	0	2	2	6	**16**
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	2	2	2	2	2	12	**24**
31	2	2	0	2	2	2	10	2	2	0	2	2	1	9	**19**
32	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
33	2	2	2				6	2	2	2				6	**12**
34	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
35	0	1	2	2	2	2	9	1	1	2	2	2	2	10	**19**
36	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
37	2	2	0	-	1	2	7	2	2	0	-	1	2	7	**14**
38	2	2	1	2	2	2	11	2	1	1	2	2	1	9	**20**
39	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
40	2	2	2	2	2	2	12	2	2	1	2	2	1	10	**22**
41	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
42	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
43	2	2	2	2	2	2	12	2	2	1	1	2	0	8	**20**
44	2	2	2	1	2	2	11	2	0	2	0	2	0	6	**17**
45	2	2	1	2	2	2	11	2	-	0	0	2	2	6	**17**

2018	Technical, item C							Advice, item D							
Sample ID	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**		**.01**	**.02**	**.03**	**.04**	**.05**	**.06**		
Average score / **Lab ID**	1,97	1,97	1,60	1,88	1,98	1,95	**Total**	1,98	1,72	1,29	1,53	1,98	1,58	**Total**	**Total score (max 24)**
46	2	2	1				5	2	2	2				6	**11**
47				2	2	2	6				2	2	1	5	**11**
48	2	-	2	2	2	-	8	2	-	0	1	2	-	5	**13**
49	2	2	2	2	2	2	12	2	2	2	2	2	1	11	**23**
50	2	2	0	1	2	2	9	2	1	0	1	2	0	6	**15**
51	2	2	2	2	2	2	12	2	1	2	1	2	2	10	**22**
52	2	2	2	2	2	2	12	2	2	0	2	2	1	9	**21**
53	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
54	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
55	2	2	0	2	2	1	9	2	2	0	1	2	2	9	**18**
56	2	2	1	2	2	2	11	2	2	1	0	2	1	8	**19**
57	2	2	2	2	2	2	12	2	2	1	2	2	2	11	**23**
58	2	2	2	2	2	2	12	2	2	1	2	2	0	9	**21**
59	2	2	2	2	2	2	12	2	0	2	1	2	2	9	**21**
60	2	2	0	-	2	2	8	2	2	0	-	2	2	8	**16**
61	2	2	0	2	2	2	10	2	1	0	0	2	0	5	**15**
62	-	-	-	2	2	2	6	-	-	-	1	2	2	5	**11**
63	2	2	0				4	2	1	0				3	**7**
64	2	1	1	2	2	2	10	2	0	0	0	2	2	6	**16**
65	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**

END OF REPORT